Clinical Nephrotoxins

Renal Injury from Drugs and Chemicals

Second Edition

Edited by

Marc E. **De Broe**
George A. **Porter**
William M. **Bennett**
Gert A. **Verpooten**

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Clinical Nephrotoxins

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- text processing
- secretarial work
- correspondence
- reference management

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- lay-out
- figure design
- index
- cover design
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This book is dedicated to:

Françoise ROCH-RAMEL MD, PHD † 26 June 2001

Salma SULEIMAN MD, PHD † 28 Sept. 2002

Preface

To you the reader, the joy of discovery begins, for us the job is done. In this edition, we have corrected past deficiencies, added new topics, expanded information regarding the pediatric age group, provided up to date (March 2003) references, while remaining true to our concept of a multi-national author book. We continue to believe that scientific information is an international commodity whose interpretation and application are strongly influenced by both the cultural and ethnic background of the observer. The opportunity to share in the rich diversity of the international scientific community remains a fundamental goal of this endeavor. To participate as equals leads to mutual respect and peer appreciation. The sharing of intellectual resources fostered by this effort should and has facilitated the advancement of sound science.

As society grows in complexity, so do the opportunities for adverse drug reactions to occur along with unexpected injury to organisms because of exposure to environmental/industrial toxins. Nephrotoxicity is truly a worldwide problem and we recognize this with the addition of a chapter dedicated to nephrotoxins unique to the African continent. As with the first edition, drugs were selected for inclusion based on both the frequency of use and current knowledge. Similar criteria were used for including environmental/industrial exposure. The nature of scientific inquiry has changed little in the past five years. One approach is the application of Koch's postulates, aided and abetted by various experimental animal models. Another involves population based epidemiologic associations to identify potentially causal relationships. Each has its advocates and disciples, and each provides valuable information that can be used by the clinician in better managing his/her patient. However, each technique yields data that must be interpreted with an understanding of the drawbacks and pitfalls inherent in each approach. By enlisting multiple authors for each chapter, plus rigorous editing we hope the final product is a balanced, rationale statement of the field, as it exists today.

We continue in our goal of providing a text which is useful, not only to the clinician, but of equal interest to the investigator. The selection of content has been directed at topics of current interest rather than those of historic contribution. We have stressed the contribution of cell biology and pathophysiology, were it exists, believing it provides both a better understanding of toxic injury when known, and a rational direction for therapy and prevention.

We are encouraged by the accumulation of recognized risk factors, which allow pre-treatment stratification of our patients' relative risk and allow us to focus our preventative techniques on the individuals most likely to gain the greatest benefit. As you read the various chapters, risk factors are prominently featured as a mean of reducing the incidence of nephrotoxic injury.

On a more personal note we confess that without the diligent technical contribution of Erik Snelders and Dirk De Weerdt, there would have been no preface for there would have been no book. We also applaud the timely contributions of our authors and their willingness to negotiate compromise when asked. Finally, to our wives Myriam and Marthel, two individuals whose gift of time made this labor possible, we are forever in your debt.

The future holds great promise for unraveling nephrotoxic mechanisms and understanding various aspects of clinical management. To enhance information transfer we decided to publish a CD as a companion to this book. Further into the future we plan to develop annual updates to supplement the text information and insure the reader is current in this rapidly evolving field.

Marc E. DE BROE
George A. PORTER
Spring 2003

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Clinical relevance

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General incidence and outcome

Drugs are an infrequent cause of community-acquired acute renal failure (ARF). However, drugs share the spotlight with renal hypoxia as the leading etiologic factors for hospital acquired ARF [1, 2]. With the increasing capacity of the medical establishment to treat the most serious life-threatening conditions, the in-hospital exposure to nephrotoxic drugs will increase as will the risk of drug-induced ARF.

The reported incidence of ARF varies depending on a number of independent variables. For example, is the patient population surveyed derived from a community wide database or is it restricted to hospitalized patients? What definition was adopted to designate acute renal failure (ARF)? For in-hospital surveys, were both post-surgical and medical patients enrolled, and if so what was the contribution from ICU patients with multi-organ failure? With what precision was the ARF diagnoses established? Were multiple centers involved in providing the information? These are the questions that complicate meaningful estimates of the incidence of ARF. In addition, as detailed by Turney et al. [3], significant changes have occurred in both the age of

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the ARF patients and also the etiologies. The incidence of in-hospital ARF attributed to drug nephrotoxicity is estimated at between 18 and 33% of cases [4-9], while from community-based statistics the incidence varied from 0 to 7% of cases [10, 11]. This observation is particularly important since the total number of cases of ARF, as derived from community based studies, is double that reported from in-hospital statistics [10]. This suggested that patients who are hospitalized are either exposed to more nephrotoxic agents and/or they are more vulnerable to the drugs inducing an adverse renal effect. Irrespective of which aspect of the drug interaction is more important, it has been observed that hospital-acquired ARF is usually associated with one of three renal insults, either a pre-renal event, exposure to nephrotoxins, or sepsis [1], and that nephrotoxins, alone or in combination, contribute to at least 25% of all cases of hospital acquired ARF [2].

A one-year survey of 2,175 cases of ARF, 398 (18.3%) were considered to be drug-induced [6]. Antibiotics were the most frequently cited drug followed by analgesics, NSAIDs and contrast media. More than half of the patients had non-oliguric ARF. The mortality rate was 12.6%, which is much lower than in patients who develop ARF in the setting of surgery or trauma [12]. At 6-month follow-up post-ARF, 47.7% were fully recovered, 15.3% had regained previous renal function, and 23.1% had some degree of residual renal impairment. Chronic hemodialysis was required in only 2 patients (0.5%). This is a better outcome than reported for a group of patients post-ARF due to multiple causes [13]. Of the 39% who survived ARF, 41% had residual renal insufficiency and 10% required chronic dialysis. Residual renal impairment was more frequent in older and oliguric patients, in those with previous chronic renal insufficiency, those who received antibiotics, and those whose ARF period was prolonged. The percentage of residual renal impairment is higher than that reported in the series of Davidman et al. [14] or Pru et al. [15], but is in accordance with that found 5 years later in the same country [16] and is supported by an earlier report from the European Dialysis and Transplant Association [17].

Table 1 summarizes the incidence of drug-induced ARF reported for the last two decades. As can be seen, the incidence of ARF due to contrast media and antibiotics has declined while, two new categories of offending agents have appeared, e.g. NSAIDs and ACE inhibitors. This trend has been confirmed in the recent survey of Ronco et al. [18].

The estimated incidence of 18-33% drug-induced ARF in hospitalized patients contrasts with the extremely low incidence of drug-induced renal disease in outpatients as reported by Beard et al. [20], i.e., 1:300,000 person/year. This low incidence may be partly due to the author's exclusion of chronic renal disease. On the other hand, acute iatrogenic renal disease developed in 1% of all patients admitted to a Canadian hospital and in as many as 5.6% of those admitted directly to the nephrology unit of the same institution [15]; the ARF was due to multiple etiologies in 50% of these patients.

Table 1. Incidence of acute renal failure due to drugs and contrast media.

Authors [ref.no.]	Year	N	% acute renal failure due to					
			Antibiotics	Contrast agents	Analgesics	NSAIDs	ACE-inhibitors	Total
Rasmussen and Ibels [4]	1982	143	11%	11%	-	-	-	**22%**
Hou et al. [5]	1983	129	7%	12%	-	-	-	**20%**
Frankel et al. [19]	1984	64	8%	5%	-	-	-	**19%**
Kleinknecht et al. [6]	1986	2,175	6%	2%	4%	3%	0.5%	**18%**
Fleury et al. [16]	1990	700	5%	2%	3%	3%	3%	**21%**
Kaufman et al. [7]	1991	100	3%	-	-	1%	6%	**19%**
Baraldi et al. [9]	1998	109	5%	2%	-	22%	7%	**36%**

Mechanisms of drug induced acute renal failure

Because of the rich blood flow to the kidney (25% of the resting cardiac output), plus the enormous oxygen supply required to support active ion and solute transport, the kidneys are vulnerable to any change in blood flow and/or oxygen deprivation. In particular, acute tubular necrosis involving the thick ascending limb (TAL) is a prominent manifestation of a sudden reduction in renal blood flow with accompanying hypoxia. This anatomic site is especially vulnerable to oxygen deprivation due to the marginal oxygen balance that results from a high oxygen consumption related to the active NaCl reabsorption and the limited blood supply due to the anatomic structure of the vasa recti [21]. A second important contributor to ARF occurs when the tubulo-glomerular feedback system fails. Tubulo-glomerular feedback is an autoregulatory mechanism that reduces glomerular filtration rate (GFR) and decreases the sodium load that is delivered to the TAL. The net result of the diminished sodium load is to minimize the oxygen required for active NaCl reabsorption [21, 22].

When considering the mechanism by which drug causes nephrotoxicity, two components of renal function are decisive. The first are the renal transport processes which are critical to recovering essential minerals and nutrients from the glomerular filtrate and the second are the renal enzyme systems which are essential to both detoxification of xenobiotics and maintaining the body's acid/base homeostasis [22, 23].

The principle renal transport systems, which contribute to drug nephrotoxicities, reside in the proximal tubule. An example is the organic ion transport system, which is instrumental in the intracellular accumulation of nephrotoxic cephalosporins due to the lower transport capacity of the lumenal membrane when compared to the basolateral membrane [24]. Another way in which proximal tubular transport is implicated in nephrotoxicity involves glutathione S-conjugates of xenobiotics. Once transported into the cell, these xenobiotic conjugates undergo biotransformation to electrophils, which then bind to macrophilic sites of intracellular macromolecules such as DNA. An example is tris(2,3 dibromopropyl) phosphate, which undergoes metabolic activation and eventually covalently binds to DNA [25]. Another example are cadmium-metallothionein complexes which are formed in the liver but eventually are filtered by the kidneys where they are reabsorbed in the proximal tubule by the same process as other low molecular weight proteins. Following lysosomal uptake, metallothionein production is stimulated, but once saturated, the inorganic cadmium is released within the renal cell causing cell death [26]. In a similar manner, aminoglycoside antibiotics, due to their cationic charge, attach to the proximal tubular membrane where they undergo pinocytotic uptake and accumulate within the cell inducing phospholipidosis, which leads to mitochondrial damage and cell death [27].

For more distal portions of the nephron, passive concentration of xenobiotics can occur due to the physiologic concentrating mechanism that provides a favorable gradient for the xenobiotic to undergo back-diffusion into the papillary region of the kidneys [23].

Another aspect of renal function, which contributes to drug nephrotoxicity, involves the renal enzyme systems that play key roles in maintaining body homeostasis. The flame retardant tris, which enters the proximal tubular cell conjugated with glutathione, undergoes bioactivation by glutathione-S-transferase resulting in reactive episulfonium ions that can cause cell death [25]. While the P-450 system of the liver is more abundant, substantial sex-linked renal P-450 activity causing bioactivation of xenobiotics, has been documented in animals [23]. A similar role in human nephrotoxicity has yet to be established. However, medullary prostaglandin synthetase has been assigned a prominent role in analgesic nephropathy where it is hypothesized to co-oxidize acetaminophen to the N-acetyl-p-benzoquinoneimine that then arylates cellular macromolecules to cause cell death [28]. Thus, the unique role of the kidneys in regulating body solute and water content, also make them targets for nephrotoxic drugs.

It is worth emphasizing that the same drug is capable of inducing several types of renal injury, e.g. NSAIDs may lead to intrarenal hemodynamic disturbances as well as to acute tubular necrosis, acute interstitial nephritis with or without nephrotic syndrome, and sometimes to various glomerular and arteriolar diseases [29, 30].

Traditionally, when searching for the etiology of ARF, the clinician's will subdivides the potential causes of a sudden decline of GFR into one of three general

pathophysiologic processes: prerenal failure, intrarenal failure or postrenal failure [1]. However, the actual renal injury may arises from a variety of insults such as, intrarenal and/or extrarenal hemodynamic changes that include endothelial injury or alteration of vascular reactivity, direct nephrotoxic damage (i.e. acute tubular necrosis), acute glomerulopathy, acute interstitial nephritis or angiitis due to hypersensitivity, intrarenal tubular obstruction, or a combination of the above mechanisms (Table 2). In two recent studies, prerenal failure was diagnosed in 14.5% [6] and 37.6% of patients with drug induced ARF [14]. In the latter series, NSAIDs and ACE inhibitors were responsible for three fourth of the cases, presumably by blocking the normal adaptive responses to renal hypoperfusion. Bridoux et al. [31] demonstrated that, in sodium depleted patients, azotemia could occur in response to ACE inhibitors therapy, without stenosis of the renal arteries. Usually, ACEI–induced renal failure rapidly reverses by discontinuing the drug.

In an analysis of 131 biopsies of drug-induced ARF [6, 16], acute tubular necrosis occurred in 61.1% of the cases while acute interstitial nephritis was the diagnosis in 16.8%. Most cases were due to aminoglycoside antibiotics, NSAIDs and analgesics. Interestingly, acute tubular necrosis may occur in a significant proportion of patients developed ARF due to ACE inhibitors. In a recent report, 5 of 10 biopsied patients with ARF related to ACE inhibitors had acute tubular necrosis [33]. Moreover, in a series of the Société de Néphrologie, a diagnosis of acute tubular necrosis was made in 30 of 50 cases following the use of various contrast agents. Most patients were oliguric, and in one-third of them serum creatinine values did not return to baseline level [33].

Acute interstitial nephritis has been an increasingly recognized cause of drug-induced ARF [32, 34-37]. Over 100 drugs have been implicated in kidney-related hypersensitivity reactions [38], the most common being listed on Table 2. For the other drugs, the number of cases reported is low and often anecdotal. In humans, cell-mediated immunity is probably involved with most cases of drug-induced acute interstitial nephritis [38].

The true incidence of acute interstitial nephritis is difficult to assess since renal biopsy is needed for definitive diagnosis [32, 38]. In a series of 976 patients presenting with ARF, renal biopsy was done in 218 cases for diagnostic purposes; drug-induced interstitial nephritis was found in only 8 patients, i.e. 0.8% of

Table 2. Classification of various drugs based on pathophysiologic categories of acute renal failure.

1. *Prerenal failure*	NSAIDs, ACE-inhibitors, cyclosporine, norepinephrine, angiotensin receptor blockers, diuretics, interleukins, cocaine, mitomycin C, Tacrolimus, Estrogen, quinine.
2. *Acute tubular necrosis*	Antibiotics: aminoglycosides, cephaloridine, cephalothin, amphotericin B, rifampicin, vancomycin, foscarnet, pentamidine. NSAIDs, glafenin, contrast media, acetaminophen, cyclosporine, cisplatin, IV immune globulin, dextran, maltose, sucrose, mannitol, heavy metals.
3. *Acute interstitial nephritis*	Antibiotics: ciprofloxacin, methicillin, penicillin G, ampicillin, cephalothin, oxacillin, rifampicin. NSAIDs, glafenin, ASA, fenoprofen, naproxen, phenylbutazone, piroxacam, tolemetin, zomepirac, contrast media, sulfonamides, thiazides, phenytoin, furosemide, allopurinol, cimetidine, omeprazole, phenindione.
4. *Tubular obstruction*	Sulfonamides, methotrexate, methoxyflurane, glafenin, triamterene, ticrynafen, acyclovir, ethylene glycol, protease inhibitors.
5. *Hypersensitivity angiitis*	Penicillin G, ampicillin, sulfonamides.
6. *Thrombotic microangiopathy*	Mitomycin C, cyclosporine, oral contraceptives.

Adapted from ref. [1] and ref [32]

all cases of ARF [34]. A similar frequency was found in the French collaborative study [6]. The proportion of patients with interstitial nephritis is higher in biopsied ARF patients, ranging from 2.5% [36] to 8.3% [35]. In the combined biopsy series [6, 19] a diagnosis of interstitial nephritis was recorded in 16.8% of patients with drug-induced ARF. More recently, Schwarz et al. [32] reviewed over 1000 diagnostic renal biopsies of which 6.5% were judged to be acute interstitial nephritis. Eighty-five percent of the cases of acute interstitial nephritis were drug-induced, with the majority being due to analgesics and NSAIDs.

While recovery of renal function following acute interstitial nephritis can be anticipated when the responsible drug is promptly withdrawn, certain drugs have a higher rate of permanent renal insufficiency [32]. When Schwarz et al. analyzed the outcome of drug-induced acute interstitial nephritis, 60% of NSAID induced acute interstitial nephritis were left with chronic renal insufficiency [32]. Naturally, persistent renal failure or even death is observed when the offending agent is continued or discontinued too late. Rossert [38] analysis of seven small, non-randomized, retrospective studies which compared patients who received corticosteroids and others who did not concluded that corticosteroids do not decrease the risk of chronic renal failure. While the pathologic similarities with acute transplant rejection support such a treatment approach, no controlled randomized studies have been reported. The best prognostic factors for recovery may be the duration of renal failure, while recovery is more frequent in non-oliguric than in oliguric patients [33]. Interestingly, a higher incidence of persistent renal impairment is found in cases with renal interstitial granulomas than in those without granulomas [37] (Table 3).

Acute renal failure caused by tubular obstruction can also occur with a number of drugs (Table 2), due to intratubular precipitation of the drug itself or of its metabolites. Of particular note have been the reports of obstruction associated with high-dose intravenous acyclovir used to treat systemic and genital herpes infections [39]. Only few cases of angiitis due to drugs are known, the most prominent being secondary to methamphetamine [40]. In adults, drug-induced thrombotic microangiopathy leading to hemolytic uremic syndrome have been associated with both the use of oral contraceptives [41], and cyclosporine [42].

Particular features due to specific drugs

Antibiotics, in combination with NSAIDs, ACE inhibitor and contrast media, are responsible for the majority of cases with drug-induced ARF. The antibiotic class most often implicated is the aminoglycosides [4, 5, 8]. Acute renal failure complicating treatment with aminoglycosides occurs in about 10% of therapeutic courses; most of these patients receive inappropriate regimens of the drug [7].

Over 30 billion tablets of NSAID were dispensed in the United States in 2000; approximately 16% represent prescriptions for NSAIDs [43]. These compounds enjoy a remarkable benefit/risk ratio when used in the treatment of acute self-limited pain syndromes. However, when taken chronically by the elderly or individuals with certain co-morbid conditions, the frequency of adverse reactions rises dramatically. Unfortunately, the real incidence of nephrotoxicity due to NSAIDs is unknown due to a lack of an accurate method of detection. The overall incidence could be very low, considering that up to 40 million people in the United States take NSAIDs on a regular basis [44]. In the 10 year-period 1972-1982, 8 million prescriptions for mefenamic acid were given in the United Kingdom, and only 23 cases of mefenamic acid nephropathy were observed [45]. This is in contrast to the higher incidence of nephrotoxicity in selected and prospective studies. Corwin and Bonventre [46] found that renal insufficiency secondary to NSAIDs accounted for approximately 6% of cases of ARF seen during a two-year period. In a prospective collaborative study, NSAIDs rep-

Table 3. Outcome of drug-induced acute interstitial nephritis with and without granulomas (modified from ref. [37]).

	Granulomas	No granulomas
Number of cases	12	31
Drugs involved		
NSAIDs	8%	29%
Analgesics	50%	19%*
Beta-lactams	17%	26%
Other	25%	29%
Oliguria	50%	29%
Permanent renal damage	50%	13%**

*$p<0.05$; **$p<0.01$

resented 15.6% of total patients with ARF [47]; half of prescriptions for NSAIDs could be considered as therapeutic errors, e.g. excessive or prolonged doses given in older and high-risk patients.

ACE inhibitors have emerged as a major cause of drug-induced ARF [9, 14, 31, 48, 49]. Only a minority of ACE-inhibitor related cases have associated renal artery stenosis [31]. However, these groups of patients are older than the usual patient with drug-induced ARF, and more frequently are afflicted with underlying chronic renal failure [49]. When a renal biopsy is performed, it shows either severe arteriosclerosis of small renal arteries or acute tubular necrosis [31]. ACE inhibition followed by ARF may sometimes result in severe irreversible renal damage [31, 49] and even death [31].

The frequency of contrast -media induced ARF is variable, but is judged to represent between 2 to 10% of all ARF patients. The incidence clusters in high-risk patients (see below) and cases of ARF that develop while hospitalized [5, 50, 51]. While the majority of patients with contrast-associated nephropathy present as non-oliguric renal failure, in diabetic patients this presentation may be one of oliguric renal failure [52]. The majority of cases result from parenteral administration of triiodinated agents, but oral contrast agents used for cholecystography have been implicated. In the French collaborative survey, acute tubular necrosis was diagnosed in 60% of contrast-associated cases, and mortality was double that in ARF due to other drugs [6].

As with other cases of ARF, ARF due to drugs is most often the consequence of multiple simultaneous insults. For example, Rasmussen and Ibels [4] found that 62% of 143 patients had more than one acute insult, including excessive aminoglycoside exposure and radiocontrast material administration. In the series of Davidman et al. [14], multiple causes of ARF were also present in 50% of 38 patients with drug-related renal disease.

Monitoring of renal function

The use of potentially nephrotoxic drugs requires close monitoring of renal function. The serum creatinine concentration is the most common method utilized to assess renal function but suffers from its lack of sensitivity. In patients with normal baseline renal function substantial renal injury can occur before there is a demonstrable rise in the serum creatinine concentration. A rise in the serum creatinine concentration that just exceeds the normal range may reflect as much as a 50 decline in the GFR. The failure of the serum creatinine to accurately reflect the degree of renal injury is particularly evident in patients with decreased muscle mass or those with chronic liver failure. Creatinine is produced from the metabolism of creatine in skeletal muscle. In turn, creatine is derived from the liver. In the setting of chronic liver disease or malnourished patients with decreased muscle mass creatinine synthesis becomes impaired. As a result more profound decreases in the GFR may occur before the serum creatinine concentration begins to rise above normal values [53]. By contrast, the serum creatinine concentration is a sensitive indicator of changing renal function in patients with chronic renal failure. In these patients a small decline in the GFR is associated with a large increase in the serum creatinine concentration.

Measurement of the creatinine clearance with a 24-hour urine collection is a more sensitive way to detect early impairment in renal function. At normal levels of renal function only a small percentage of creatinine appears in the urine by tubular secretion while the bulk of creatinine is filtered by the glomerulus. As a result, creatinine clearance is an accurate measurement of the GFR. However, the accuracy of creatinine clearance declines with advancing renal insufficiency. In this setting the percentage of creatinine, which reaches the final urine by tubular secretion, increases. As a result the creatinine clearance tends to overestimate the GFR in patients with renal insufficiency [54].

The most accurate way of assessing changes in the GFR is to measure the clearance of a compound that is freely filtered by the glomerulus but is neither secreted nor reabsorbed by the tubule. Radiolabeled sodium iothalamate and diethylenetriaminepenta-acetic acid (DTPA) are substances commercially available for this purpose.

Because of the problems with changes in creatinine production and secretion, other endogenous compounds have been evaluated in an effort to provide a more accurate estimation of GFR. Perhaps the most promising is cystatin C, a low molecular weight protein that is a member of the cystatin superfamily of cysteine protease inhibitors [55]. Cystatin C is produced by all nucleated cells and its rate of production is relatively constant, being unaltered by inflammatory con-

ditions or changes in diet. When compared to the GFR as determined by the clearance of radioactive iothalamate, serum cystatin C levels began increasing when the GFR was 88 ml/min while the serum creatinine concentration only increased when the GFR was 75 ml/min [56].

In summary, monitoring changes in the creatinine clearance or directly measuring the GFR using markers that are not acted upon by the tubule are the best tools to detect early drug-induced renal toxicity in patients with a normal baseline creatinine concentration. Serial monitoring of the serum creatinine concentration is usually adequate in those chronic failure patients whose creatinine is already increased. The role of other endogenous compounds such as cystatin C as a way to monitor renal function is not yet known.

Populations at risk

The changes in renal function of patients experiencing nephrotoxicity can be as dramatic as a sudden, acute deterioration requiring immediate dialysis to an insidious asymptomatic decline. This difference in presentation probably represents the level of exposure, i.e., dose and duration, to a drug or environmental toxin plus a component of genetic susceptibility. This formulation is supported experimentally since it has been shown that the rapidity with which renal failure occurs is dependent on the rate at which a known nephrotoxin is administered [57]. Similar observations regarding the influence of time-dosage effects have come from our laboratory using an experimental model of aminoglycoside nephrotoxicity [58]. The human counterpart is a study reported by Nicolau et al. [59] involving once daily aminoglycoside dosing in over 2000 patients. They found that in addition to dosing schedule, age and duration of treatment were factors in precipitating aminoglycoside-induced ARF. While this postulated dependency on time and *in vivo* drug concentration remains speculative for human renal disease, it provides a convenient approach to explaining many of the observations related to suspected environmental toxicants. The clinical course of renal failure has been defined to a great extent by observing the natural history of ARF [60]. It follows that much of our information regarding risk factors for the development of renal failure have also come from analysis of patients with ARF [1-5, 8]. However, with the advent of ESRD registers, i.e., EDTA and USRDS, data regarding risks factors for progressive renal failure are being accumulated [61, 62].

Population characteristics which are candidates to correlate with increased susceptibility to toxin induced renal injury include: 1) genetic susceptibility, 2) occupational or environmental exposure, 3) gender, 4) age, 5) race, 6) nutritional status, 7) socio-economic status, 8) addictive personality, and 9) co-existing chronic disease. By defining populations at increased risk it is hoped that greater care will be exercised in either drug prescribing or removal of subjects from offending environments.

Sharing importance with individual susceptibility is the previously mentioned concept of critical body burden of toxicant as a prerequisite for inducing renal cell injury. It is this concept of body burden that helps explain why the various clinical manifestations of toxin induced renal disease run the gamut from sudden deterioration of renal function to the insidious loss of function.

Genetic/hereditary susceptibility

Inherited renal disease is an infrequent cause of ESRD, cystic kidney disease being the most prevalent accounting for about 3% of all cases [61]. However, experimentally inbred strains of rats are selected because of their known susceptibility to toxic injury, an example of which is the Fischer 344 rat [63]. This selective animal susceptibility has led to speculation that a similar situation might exist for humans. A possible relationship between occupational exposure and genetic susceptibility comes from a study conducted by the Michigan Renal Registry [64]. The study design was a case-control involving 325 men with ESRD in which an occupational exposure was sought. The results found that the strongest association for ESRD patients was a family history of renal disease (odds ratio = 9.30). Patients with ESRD that were excluded from consideration included; diabetic nephropathy, polycystic kidney disease, heroin nephropathy, lupus nephropathy, nephropathy due to malignancy, Alport's syndrome, unspecified chronic renal failure, obstructive nephropathy and uncommon nephropathies, leaving only patients with diagnoses compatible with toxin-induced renal injury, i.e., glomerulonephritis, nephrosclerosis and interstitial nephritis, for evaluation.

More recently, O'Dea et al. reported a higher risk of renal failure in first-degree relatives of Canadian patients with ESRD [65]. Their case-control study bracketed the years from 1987 to 1993. Diseases with a Mendelian pattern accounted for 8.4% of all cases and were excluded from relative risk analysis. Twenty-seven percent of the probands had at least 1 relative with renal failure as compared to 15% of the controls. The Odds Ratio for have a first degree relative with renal failure was 3.0 (95% CL 1.7-5.2), while the risk for ESRD was highest in families of probands with hypertensive nephropathy, interstitial nephritis, and diabetic nephropathy. Being a first-degree relative of a proband increased the incidence of ESRD from 79 per million per year to 297 per million per year, nearly a four fold increase. Freedman et al. [66] using a case-control format investigated the incidence of familial clustering of ESRD in African-Americans suffering from HIV-associated nephropathy. After controlling for age, family size, and sex, they found a 5.4 fold increase in the incidence of ESRD in close relatives of HIV-associated nephropathy patients compared to HIV patients without evidence of nephropathy. Although they could not completely eliminate environmental factors as contributing to the significant difference (p=0.004), they raised the possibility of an inherited susceptibility in the families studied.

Genetic polymorphism has also been identified as possibly contributing to progression of renal disease. Because of the relationship that has been identified between endothelial nitric oxide synthase (ecNOS) polymorphism and abnormal hemodynamics, Wang and associated [67] investigated the frequency of gene polymorphism of ecNOS intron 4 in patients with ESRD as compared to health controls. These authors found that the frequency of the a allele of endothelial nitric oxide synthase (ecNOS) was significantly higher in cases of non-diabetic ESRD when compared to healthy controls. Using similar reasoning, that ESRD is a complex phenotype that combines pre-existing renal disease with environment and genetic factors, Gumprecht et al. [68] evaluated the role of gene polymorphism in the renin-angiotensin system as it occurs either during the development or progression of chronic renal failure. Two hundred and forty-seven CRF patients and their parents were enrolled in the study. The angiotensin 235 T allele was transmitted significantly more frequently to CRF patients than would be expected if no association existed. However, the significant transmission was limited to patients with interstitial nephritis. Nobilis et al. [69] examined the association between very low birth weight newborns and ARF. They reasoned that since the neonate requires high RAS activity to maintain GFR, genetic polymorphism could impair either ACE activity or ANGIOTENSIN II-type I receptor functions in very low birth weight newborns. Neither the frequency of the ACEI allele and/or ATIR CII66 variant differed between the ARF group (n=42) and these with normal renal function (n=68). Thus, no correlation was evident between genetic polymorphism and the development of neonatal ARF. Obviously, this represents a fertile area for ongoing research.

Occupational/environmental exposure

While there are well-recognized instances of drugs and toxins inducing ARF, the evidence supporting their causality in CRF /ESRD is circumstantial and thus less compelling. This is to be expected given the insidious nature of progressive renal failure, an observation that suggests a long latency between exposure and onset of disease. This problem is compounded by the superimposition of associated chronic conditions associated with and leading to renal failure. Additionally, the lack of a uniform system of classifying renal disease (mixture of clinical and pathologic terms) and the distinct possibility of multifactorial causes for renal failure because of the many potential nephrotoxins, which exist in our environment [70]. At the Workshop on Chronic Disease in the Workplace, conducted by the Workplace Health Fund in 1983, it was estimated that nearly 4 million workers were exposed to known or suspected nephrotoxins during the 1971-72 interval [71]. Of interest was the list of nephrotoxins cited which are identical to those we currently believe to be candidates to produce chronic renal failure and eventually lead to ESRD. They included: I) heavy metals, i.e., lead, mercury, uranium and cadmium; 2) solvents, especially light hydrocarbons; 3) silica; 4) beryllium; 5) pesticides; 6) arsenic.

Solvents have been implicated as inducers of glomerulonephritis [72], while the association between chronic interstitial nephritis and analgesic abuse is acknowledged [73] and the association between hypertensive renal disease (nephrosclerosis) and lead nephropathy continues to be explored [74, 75]. According

to data provided by USRDS for 1994 through 1998, glomerulonephritis accounted for 9.1% of ESRD, hypertensive renal disease was present in 25.2%, secondary glomerulonephritis and vasculitis involved 2.2%, 3.6% were unknown etiology and interstitial nephritis/pyelonephritis accounted for 3.8% of all cases of ESRD being treated in USA [61]. From EDTA [62], 24.1% of new ESRD patients in 1987 were due to glomerulonephritis, 16.6% due to pyelonephritis/interstitial nephritis, 2.8% due to analgesic and other nephrotoxic agents, 9.9% due to renal vascular disease and 14.4% due to chronic renal failure of unknown etiology for a total of almost 68%. Thus, there exist a substantial number of ESRD patients whose etiology could involve a component of long-term, low level exposure to either environmental or occupational toxicants.

In addition to workplace exposures, there are acknowledged geographic regions of environmental contamination that expose the general population and can increase their risk of chronic renal damage. An example of such an environmental contamination is methyl mercury poisoning by industrial effluents in Minimata Bay region of Japan, which lead to both neurological and renal impairment in several hundred adults who ingested tainted fish [76]. In evaluating the occurrence of lead nephropathy in the general public, Staessen et al. concluded that while such exposure could impair renal function they were unable to demonstrate a cause/effect relationship [77].

Recently, reports linking Chinese herb remedies to fatal renal failure have appeared [78, 79]. The remedies have been taken for weight loss or the treatment of eczema. The offending compound is thought to be aristolochic acid which is the acknowledged nephrotoxin that was identified as contributing to the progressive interstitial nephritis that lead to renal failure and death in the Belgian experience [78]. In a recent editorial, De Broe [79] speculates that combining a potentially nephrotoxic agent, such a Chinese herbs with a renal vasoconstrictive agent, may account for the observation that not all patients who use the herbal product develop ARF. In the UK it is estimated that over 3000 clinics were prescribing Chinese herbs in 1999 [80].

Gender

Experimentally gender predilection for various nephrotoxins is well recognized. Examples include the male rat's sensitivity to the nephrotoxic effects of both carbon tetrachloride and aminoglycosides [58]. Recently, Moore et al. [81] demonstrated an increased susceptibility of women to the nephrotoxic effects of aminoglycosides using multi variant analysis. These observations make two important points, first is that the extrapolation of animal results to predict human response must be done with caution since the experimental data predicted a male susceptibility. Secondly, gender can impart either susceptibility or resistance depending upon your point of view.

In searching for an explanation of the slower rate of progression to ESRD for female patients, Miller et al. [82] have identified a gender dependent difference in the renal hemodynamic effect of angiotensin II. The infusion of angiotensin II in women was associated with a parallel decline in GFR and effective renal plasma flow (ERPF), while in men GFR was maintained despite the angiotensin II-induced fall in ERPF. Thus, for women, the decline in filtration fraction (FF) paralleled the fall in GFR, while in men FF remained unchanged. The authors speculate that the gender difference in progression to ESRD is due to the angiotensin II associated sustained FF in men which is absent in women. It has been recognized for some time that glomerular hypertension, which would be required to sustain FF in the face of a fall in ERPF, is a known contributor to the progression of renal failure [83].

Race

While direct evidence linking a patients' race with the risk of toxin induced renal injury is lacking, an indirect association is suggested based on the clinical course of hypertensive renal disease (nephrosclerosis) in black versus white males. From incidence data provided by USRDS, ESRD occurs 8 times more frequently in black males with hypertensive renal disease than white males with a similar diagnosis [61]. This relationship has been defined in greater detail by the case-control study conducted by Freedman et al. [84]. Based on their results, the presence of a first-degree relative with ESRD increased the risk for developing ESRD ninefold, with OR of 9.13 (95% CL 2.6 to 31.8). Hypertensive nephrosclerosis and Diabetes, type II was more prevalent than chronic glomerulonephritis. In a recent retrospective survey of the incidence of ARF in African-Americans, Obialo et al. [85] found that while pa-

tients > 64 year of age had a high incidence of ARF, this was shared by the age group < 40 years of age. However, they noted that patients > 64 years of age were less likely to receive dialysis. If one were to pursue the concept of multitoxin injury as contributing to nephrotoxic chronic renal failure, then the hypertensive kidney would be receptive substrate upon which a toxic insult could be superimposed. There is evidence from clinical studies involving in-hospital cases of ARF that hypertension is a risk factor [1-5].

Nutrition

Glomerular hyperfiltration regularly follows the ingestion of a protein rich diet. Furthermore, experimentally induced hyperfiltration induces glomerulosclerosis and chronic renal failure in animals deprived of their renal reserve [86]. In addition, pathologic variations in the body's mineral content has been linked with chronic renal injury in the case of severe hypokalemia induced by eating disorders [87], and shown to augment toxin induced injury in the case of calcium depletion and lead nephropathy [88], or salt depletion and analgesic nephropathy [89].

The assessment of the nutritional state of renal patients has come under scrutiny in recent years. While serum albumin has served as the common index of nutritional status, evidence is mounting that interpretation of this parameter is influence by other factors. Recently, Ikizler et al. [90] provided evidence of an interaction between depleted nutritional status and active inflammatory disease as providing markers for increased risk of hospitalization for chronic hemodialysis patients. This awareness of the impact of inflammation on nutrition status requires further evaluation in order to properly assess the contribution of each to the progressive atherosclerotic disease, which characterized ESRD patients [91].

Alcohol consumption has been reported to potentate the nephrotoxicity of lead [92] and anti-inflammatory drugs [93]. However, in a recent case-controlled study reported by Perneger et al. [94], these authors found the OR for developing ESRD was 4.0 (95% CL 1.2-13.0) among persons who self-reported consuming an average of >2 drinks/day. This was after adjusting for age, race, sex, income, and history of hypertension, history of diabetes mellitus, acetaminophen intake, cigarette smoking and opiate use. Fortunately, alcohol intakes of 2 drinks/day or less did not increase the risk of renal failure.

Socio-economic status

The exposure to lead based paints and subsequent lead nephropathy and encephalopathy, in the USA, is concentrated in substandard housing [93]. Individuals at the lower end of our economic ladder often are denied access to preventative health care thus putting them at additional risk for toxin exposure. Another example is the consumption of "moonshine" whiskey that has been associated with the development of lead nephropathy [95].

Age

Age, along with pre-existing renal disease and volume depletion, are well-recognized risk factors for in-hospital ARF [1, 4, 5, 8-10]. The higher risk of nephrotoxicity associated with age can be traced to normal age-related changes in renal function [96]. Aging is associated with a progressive decline in the GFR and renal blood flow, which correlate with an increase in renal vascular resistance (Table 4). Importantly, the renal vasculature is less responsive to vasodilators, while the response to vasoconstrictors remains unchanged. In addition to age-related changes in renal function, changes in the rate and manner that drugs are metabolized by elderly patients also increase their susceptible to renal toxicity [97]. Elderly patients, particular those with chronic illness, often have lower albumin levels,

Table 4. Risk factors for drug-induced nephrotoxicity in the elderly.

Age-related changes in renal function
↓ in glomerular filtration rate
↓ in renal blood flow
↑ in renal vascular resistance
Age-related changes in pharmacokinetics
↑ free drug concentration
• hypoalbuminemia
• retained metabolites
↓ total body water
↓ hepatic metabolism with longer drug half-life

which reduces protein binding of drugs resulting in higher free drug concentrations. Protein binding is further interfered with by retained metabolites, which accumulate as a result of the normal age-related impairment in renal function. Increased drug levels also occur as a result of the age-related decrease in total body water. Finally, decreased hepatic metabolism, which is often present in the elderly, contributes to a longer half-life of drugs potentially resulting in unexpectedly high drug levels (Table 4). For all of these reasons a given dose of a potentially nephrotoxic agent might be well tolerated in a young person but result in marked renal injury in an older person. In the community based study reported by Feest et al. [11], the annual rate of severe ARF rose from 83 per million population (pmp) in the six decade of life to 949 pmp in the ninth decade of life, a nearly tenfold increase. In the hospital based study of Khan et al. [10], the annual incidence of ARF increased from 606 pmp for the age range of 50 to 69, to 4266 pmp for individuals older than 80 years. On the other hand, Kohli and co-workers prospectively examined the incidence of treatment-related ARF in elderly hospitalized patients [98]. During a one-year interval, these authors identified treatment-related ARF occurring in 1.2% of patients ≥ 60 years of age. Multiple insults were identified in most cases of ARF; however, drugs contributed 66% of the time, sepsis and hypotension 46% of the time, contrast media 17% of the time, and the post-operative state 25% of the time. The development of treatment-related ARF doubled the death rate (25.4% vs. 12.7%).

Since toxin induced chronic renal failure is theorized to occur after years of low-level toxin exposure, it stands to reason that the incidence would be clustered in elderly patients. The study of Chester et al. [99] provides indirect support that elderly patients may be at greater risk. Of the 79 patients with chronic renal failure who met age criteria of 70 year or more, 29% were classified as having chronic interstitial nephritis, a clinical diagnosis quite compatible with toxin induced renal failure and an incidence substantially higher than the 10.4% in accumulated series in which patients 50 year and older were included [100]. Furthermore, in the 2000 USRDS survey of causes of ESRD, the average age for patients with a diagnosis of interstitial nephropathy was 66 year compared to 63 years for the entire population reported [61].

Co-existing chronic diseases

Conventional wisdom dictates that pre-existing renal insufficiency is a well-established significant risk factor in the ARF of contrast-associated nephropathy [101]. However, a study by Levy et al. [102] questions this wisdom. These authors performed a cohort analytical study involving over 16,000 patients undergoing radiocontrast procedures. One hundred and seventy-four patients diagnosed with contrast-associated nephropathy were paired to a like number of nephropathy-free patients matched for age, baseline creatinine and similar radiocontrast procedures. Mortality in contrast-associated nephropathy patients was 34% compared to 7% in match control group. When the authors considered the contribution of co-morbid conditions, the mortality rate in the ARF group was consistently higher for patients with: diabetes, hypertension, cancer, lymphoma, liver disease, heart failure, acute myocardial infarction, sepsis and gastrointestinal bleeding. The authors concluded that the high mortality rate in ARF is not explained by co-morbid conditions; rather ARF increases the risk of fatal, non-renal complications. A similar misconception may exist for NSAID given to patients with chronic renal failure [103]. Evans et al. [103] conducted a case-control study involving a population base of 420, 600 individuals. These authors found that for patients hospitalized with ARF, the risk was doubled for patients who ingested NSAIDs during the 90 days prior to admission. Interestingly, they could not identify any interaction between NSAID use in patients with chronic renal failure and subsequent hospitalization for ARF. However, for patients requiring intensive care treatment, evidence continue to mount correlating increased mortality in ARF with the number of failed internal organs [103-105].

For chronic renal failure the information is again circumstantial. Patients with sickle cell disease have a high frequency of papillary necrosis which is assumed to be the result of the slugging effect of the abnormal red cells as they course through the vasa recti and are exposed to the high osmolarity of the renal papillae [108]. However, these same patients have significant pain associated with' 'sickle crisis', for which they often take analgesics that are also associated with papillary necrosis [109]. This may represent a case of multiple insults superimposed to give an additive patho-

logic effect. Another example is the supposed increased risk of contrast associated nephropathy in patients with myeloma kidney due to a physical interaction between Tamm-Horsfall protein and the radiocontrast media leading to intraluminal obstruction and ARF [110]. Diabetic nephropathy is a documented risk factor for acute contrast induced nephropathy; however, the review of Mudge suggests that in up to 25% of such cases serum creatinine does not return to pretreatment levels and these patients end up with further deterioration of their renal function as a result of the acute insult induced by the contrast media [101]. The role of hypertension as a risk factor has already been described in the section entitled Race. Recently, Rihal et al. [111] reviewed the incidence of ARF post-percutaneous coronary intervention. Stratifying pre-percutaneous serum creatinine provided ample evidence that once serum creatinine exceeded 2 mg/dl that ARF risk was high irrespective of co-existing diabetes.

Addictive behavior

With drug abuse being an increasingly common behavior for the younger generation, it is not surprising that it has been linked to renal injury. Heroin nephropathy is a well-described cause of focal sclerosing glomerulonephritis with associated nephrotic syndrome [112, 113]. This particular pathologic entity often progresses to ESRD and may account for up to 10% of such patients in cities with large addictive populations [114]. Although cardiac and/or cerebral ischemia is the more common acute presentations of cocaine inhalation, renal ischemia also occurs [115]. The most frequent cause of cocaine associated ARF occurs in the setting of rhabdomyolysis [116]; however, a more recent association between habitual cocaine abuse and accelerated or malignant hypertension leading to deterioration of renal function have been identified [117]. Intravenous amphetamine or "speed" can induce polyarteritis nodosa with progressive renal failure and severe hypertension [118]. Recently, Bingham and coworkers have reported a case of chronic renal failure due to habitual intake of oral methamphetamine and 'ecstasy' [119]. More disturbing is a report by Richards et al. [120] in which 43% of rhabdomyolysis patients entering an emergency room had a positive toxicologic screen for methamphetamines.

Summary

While much of the data concerning nephrotoxic chronic renal failure is circumstantial and based on epidemiologic surveys involving ESRD patients [61], for certain xenobiotics the evidence is substantial. The most obvious group at risk is individuals exposed to known or suspected nephrotoxins in the workplace. A similar conclusion is valid for people living in geographic regions of contamination. The possible link between a family history of renal disease and development of renal failure may represent an inherited susceptibility or could result from a common geographic exposure. Altered nutrition and certain co-existing diseases including addictive behavior are additional parameters by which relative risk to nephrotoxin, can be ascertained. While gender, race and socio-economic status provide tantalizing clues that these factors could contribute to risk stratification, solid confirmation is needed. Thus, targeting populations at risk for future evaluation and follow-up is the most efficient strategy for the identification of patients early in the course of their toxic renal injury plus introducing protective measures to impede the progression of patients into ESRD programs.

Individual risk factors

Individuals may be at increased risk for developing nephrotoxicity from various drugs based on unique circumstances. For example, several antibiotics are well recognized as having nephrotoxic potential [121] but it must be appreciated that they are often administered under clinical circumstances in which acute renal insults co-exist, i.e., hypotension, reduced cardiac output, depressed liver function, etc. Rasmussen & Ibels used multivariant analysis to determine the role of acute insults such as hypotension, dehydration, pigmenturia, liver disease, sepsis, aminoglycoside administration and contrast media for patients developing ARF without a prior history of renal disease [4]. In 41 of 121 patients a single insult was considered to be responsible for ARF, 80% of the time this was hypotension. The remaining 80 patients were exposed to 140 insults or 1.75/patient giving support to the concept of the multifactorial basis for inducing nephrotoxic renal injury. This same pattern of multiple insults causing ARF is evident from the report of Kohli et al. [98]. Contrast-associated nephropathy has been evaluated

extensively for possible clinical conditions in which patients are at additional risk for the induction of ARF [122] Swartz et al. [122] using a retrospective analysis of factors related to renal failure following major angiography, reported that in addition to renal insufficiency, abnormal liver function tests, hypoalbuminemia, diabetes mellitus and proteinuria all were significantly correlated with the patient group which developed renal failure. They also noted a prevalence of 2.5-risk factors/case of contrast-associated nephropathy. Cochran et al. [123] used an odds-ratio analysis of 28 clinical factors that might correlate with increased risk for the development of contrast-associated nephropathy. In addition to underlying renal disease and elevated serum creatinine, their data confirmed proteinuria as a risk factor but failed to substantiate diabetes mellitus or abnormal liver function. They did demonstrate that male gender, hypertension, and vascular disease all were associated with significant additional risk as well as the amount and type of contrast administered. In a review of 6 publications which analyzed risk factors for contrast-associated nephropathy in 1416 patients, renal insufficiency was the only uniformly consistent factor for all studies [110], however, in 3 of 5 studies in which it was tested, the amount of contrast was found to correlate in a positive way. Cigarroa et al. [124] have used a modified contrast media dosing scheme for patients at high risk for contrast-associated nephropathy and reported that virtually every case of post procedure contrast-associated nephropathy occurred when the recommended limits of the calculated dose of contrast were exceeded.

In a similar vein, Leehey et al. [125] have reported on the frequency of aminoglycoside-induced nephrotoxicity using three different dosing schemes, including two that were based on pharmacokinetic principles. It is noteworthy that despite careful calculation of the dosing scheme, this did not alter the incidence of nephrotoxicity. However, the duration of dosing correlated positively with nephrotoxicity incidence, as did treatment with furosemide, old age, and liver disease.

While cyclosporine is an intrinsically nephrotoxic drug due to a direct action on the kidney, under other circumstances it can become nephrotoxic in the presence of a second drug, exhibiting a so-called drug-drug interaction. For example, drugs that inhibit the hepatic P-450 drug-metabolizing enzymes can cause a significant change in cyclosporine pharmacokinetics and, thus, render an otherwise stable dose nephrotoxic [126]. Drugs that induce such changes in cyclosporine levels include: erythromycin, fluconazole, ketoconazole, cimetidine.

Another example of drug-drug interaction occurs when non-steroidal anti-inflammatory drugs are given to patients receiving anti-hypertensive drugs [127]. Due to the action of the NSAID to inhibit prostaglandin synthesis, the loss of endogenous induced vasodilatation causes the blood pressure to become uncontrolled often necessitating increasing the current anti-hypertensive drug dosage or prescribing additional anti-hypertensive drugs [128]. Recently, Mehta et al. [129] have called attention to the increased mortality and permanent residual renal impairment in ARF patients who are treated with diuretics. Once again we are reminded that empirical treatment, for which pathophysiologic rationale can be developed, must be evaluated in a controlled clinical trial before being universal accepted as standard therapy. So not only is it important to understand the patients diseases, but also a complete list of all medications that the patient takes on a regular basis including those purchased over the counter.

References

1. Nolan CR, Anderson RJ. Hospital –acquired acute renal failure. JASN 1998; 9: 710-718.
2. Vijayan A, Miller SB. Acute renal failure: prevention and non-dialytic therapy. Sem. Nephrol 1998; 18: 523-532.
3. Turney JH, Marshall DH, Brownjohn AM, Ellis CM and Parsons FM. The evolution of acute renal failure 1956-1988. Q J Med 1990; 73: 83-104.
4. Rasmussen HH, Ibels LS. Acute renal failure: multi variant analysis of causes and risk factors. Am J Med 1982; 73: 211-218.
5. Hou SH, Bushunsky DA. Wish BB, Cohen JJ, Harrington IT. Hospital-acquired renal insufficiency: a prospective study. Am J Med 1983; 74: 243-248.
6. Kleinknecht D. Landais P. Goldfarb B. Les insuffisances renales aigues associees 11 des medicaments OU 11 des produits de contraste iodes. Resultats d'une enquete cooperative multicentrique de la société de Néphrologie. Néphrologie 1986; 7: 41-46.
7. Kaufman J, Dhakal M, Patel B, Hamburger R. Community-acquired acute renal failure. Am J Kidney Dis 1991; 17: 191-198.
8. Bennett WM. Porter GA. Overview of clinical nephrotoxicity. In: Toxicology of the kidney. Hook IB, Goldstein RS (editors). Raven Press, New York 1993; p. 61-97.
9. Baraldi A, Ballestri M, Rapana R, Lucchi L, Borella P, Leonelli M, Furci L and Lusvarghi E. Acute renal failure of medical type in an elderly population. Nephol Dial Transpl 1998; 13(supp7): 25-29.
10. Khan IH, Catto GRD, Edward N, Macleod AM. Acute renal failure: factors influencing nephrology referral and outcome. Q J Med 1997; 90: 781-785.
11. Feest TG, Round A, Hamad S. Incidence of severe acute renal failure in adults: results of a community based study. Br Med J 1993; 306: 481-483.
12. Pruchnicki MD, Dasta JF. Acute renal failure in hospitalized patients: part I. Ann Pharmacother 2002; 36: 1261-1267.
13. Margrra S, Kraft AK, Siebert G, Luft FC, Neumayer HH. Long-term outcomes in acute renal failure patients treated with continuous replacement therapies. Am J Kidney Dis 2002; 40: 275-279.
14. Davidman M, Olsen P, Kohen J, Leither T, Kjellstrand C. Iatrogenic renal disease. Arch Intern Med 1991; 151: 1809-1812.
15. Pru C, Ebben J, Kjellstrand C. Chronic renal failure after acute tubular necrosis. Kidney Int 1982; 21: 176.
16. Fleury D, Vanhille P, Pallot IL, Kleinknecht D. Drug induced acute renal failure: a preventable disease linked to drug misuse. Kidney Int 1990; 38: 1238.
17. Wing AJ, Broyer M, Brunner FP, Brynger H. Challah S. Challah S, Donckerwolcke RA, Gretz N, Jacobs C, Kramer P, Selwood NH. Combined report on regular dialysis and transplantation in Europe XIII Acute (reversible) renal failure. Proc Eur Dial Transpl Assoc 1983; 20: 64-71.
18. Ronco C, Zanella M, Brendolan A, Milan M, Canato G, Zamperetti N, Bellomo R. Management of severe acute renal failure in critically ill patients: an international survey in 345 centres. Nephrol Dial Transplant 2001; 16(2): 230-237.
19. Frankel MC, Weinstein AM, Stenzel KH. Prognostic patterns in acute renal failure: the New York Hospital, 1981-1982. Clin Exp Dialysis Apheresis 1983; 7: 1457.
20. Beard K, Perera DR, lick H. Drug induced parenchymal renal disease in outpatients. J Clin Pharmacol 1988; 28: 431-435.
21. Heyman SN, Brezis M, Epstein FH, Spokes K, Silva P, Rosen S: Early renal medullary hypoxic injury from radiocontrast and indomethacin. Kidney Int 1991; 40: 632-642.
22. Dishart M, Kellum JA. An evaluation of pharmacological strategies fro the prevention and treatment of acute renal failure. Drugs 2000; 59: 79-91.
23. Dekant W, Vamvakas S. Biotransformation and membrane transport in nephrotoxicity. Crit Rev Toxicol 1996; 26: 309-334.
24. Tune B. The nephrotoxicity of cephalosporin antibiotics. Sturcture-activity relationship. Commun Toxicol 1993; 1: 145-157.
25. Pearson PG, Omichinski JG, Holme JA, McClanahan RH, Brunborg G, Soderlund EJ, Dybing E, Nelson SD: Metabolic activation of tris (2, 3, dibromopropyl) phosphate to reactive intermediates: Covalent binding, reactive metabolite formation and differental metabolite-specific DNA damage, in vivo. Toxicol Appl Pharmacol 1993; 118: 186-196.
26. Goyer RA. Mechanisms of lead and cadmium nephrotoxicity. Toxicol Lett 1989; 46: 153-176.
27. Kaloyanides GJ. Aminoglycoside-induced functional and biochemical defects in the renal cortex. Fundam Appl Toxicol 1984; 4: 930-942.
28. Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Mol Pharmacol 1985; 28: 306-315.
29. Whelton A. Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiological foundations and clinical implications. Am J Med 1999; 106: 13S-24S.
30. Kleinknecht D. Diseases of the kidney caused by nonsteroidal anti-inflammatory drugs. In. Stewart JH (editor). Oxford University Press, Oxford 1993; p. 160-173.
31. Bridoux F, Hazzan M, Pallot JL, Fleury D, Lemaitre V, Kleinknecht D, Vanhille Ph. Acute renal failure after the use of angiotensin-converting enzyme inhibitors in patients without renal artery stenosis. Nephrol Dial Transplant 1992; 7: 100-104.

32. Schwarz A, Krause PH, Kunzendorf U, Keller F, Distler A. The outcome of acute interstitial nephritis: Risk factors for the transition from acute to chronic interstitial nephritis. Clin Nephrol 2000; 54:179-190.
33. Kleinknecht D, Landais P, Goldfarb B. Pathophysiology and clinical aspects of drug-induced tubular necrosis in man. Contr Nephrol 1987; 55: 145-158.
34. Richet G, Sraer JD, Kourilsky O. La ponction-biopsic rénale dans l'insuffisance rénale aigue. Ann Med Interne 1978; 129: 335-337.
35. Linton AL, Clark W, Driedger AA, Turnbull DJ. Lindsey RM. Acute interstitial nephritis due to drug. Review of the literature with a report of nine cases. Ann Intern Med 1980; 93: 735-741.
36. Buysen JGM, Houthoff HJ, Kredict RT, Arisz L. Acute interstitial nephritis, a clinial and morphological study in 27 patients. Nephrol Dial Transpl 1990; 5: 94-99.
37. Grunfeld JP. Kleinknecht D. Droz D. Acute interstitial nephritis. In: Diseases of the kidney. Schrier RW, Gottschalk CW (editors). Little Brown, Boston 1993; p. 1331-1353.
38. Rossert J. Drug-induced acute interstitial nephritis. Kidney Int 2001; 60: 804-817.
39. Choudhury D, Ahmed Z. Drug-induced nephrotoxicity. Med Clin N Am 1997; 81: 705-717.
40. Richards JR, Johnson EB, Stark RW, Derlet RW. Methamphetamine abuse and rhabdomyolysis in the ED: A 5-year study. Am J Emerg Med 1999; 17: 681-685.
41. Lieberthal W, Levinsky NG. Acute renal failure. In: The kidney: physiology and pathophysiology. Seldin W, Giebish G (editors). Raven Press, New York 1992; p 3181-3225.
42. Wolfe JA, McCann RL, Sanfillipo F. Cyclosporine-assoicated microangiopathy in renal transplantation: a severe but potentially - reversible form of early graft injury. Transplantation 1986; 41: 541.
43. Personal communication, Evans, T, McNeil Consumer Health Care, 2000.
44. Henrich WL. Nephrotoxicity of non-steroidal anti-inflammatory agents. In: Diseases of the kidney. Schrier RW, Gottschalk CW (editors). Little Brown, Boston 1993; p 1203-1217.
45. Nicholls AJ. Shortland JR. Brown CB. Mefenamic acid nephropathy - A spectrum of renal lesions. Proc Eur Dial Transpl Assoc 1985; 22:991-996.
46. Corwin HL Bonventre JV. Renal insufficiency associated with non-steroidal anti-inflammatory agents. Am J Kidney Dis 1984; 4: 147-152.
47. Kleinknecht D, Landais P, Goldfarb B. Analgesic and nonsteroidal anti-inflammatory drug-associated acute renal failure. A prospective study. Clin Nephrol 1986; 25. 275-281.
48. Makdassi R, Andrejak M, Westeel PF, Schmit JL, Renaud H, Houhou S, Fournier A. Aetiology and prognosis of iatrogenic renal failure on the basis of a 6-year experience. Nephrol Dial Transplant 1990; 5: 660-661.
49. Boiskin M, Marcussen N, Kjellstrand C. ACE inhibitors are now the second most common cause of acute, iatrogenic renal failure. J Am Soc Nephrol 1992; 3: 720.
50. Schusterman N, Strom BL, Murray TG, Morrison G, West SL, Maislin G. Risk factors and outcome of hospital-acquired acute renal failure. Am J Med 1987; 83: 65-71.
51. Rudnick M, Berns JS, Cohen RM, Goldfarb S. Contrast media-assoicated nephrotoxicity. Curr Opin Nephrol Hyperten 1996; 5: 127-133.
52. Weisberg LS, Kurnik PB, Kurnik BR. Risk of radiocontrast nephropathy in patients with and without diabetes mellitus. Kid Internat 1994; 45: 259-265.
53. Levey AS. Measurement of renal function in chronic renal disease. Kidney Int 1990; 38: 167-184.
54. Shemesh 0, Golbetz H, Kriss JP, Myers BD. Limitations ofcreatinine as a filtration marker in glomerulopathic patients. Kidney Int 1985; 28: 830-838.
55. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T, Grubb AO, Price CP. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. Kidney Int 1995; 47: 312-318.
56. Coll E, Botey A, Alvarez L, Poch E, Quinto L, Saurina A, Vera M, Piera C, Darnell A: Serum cystatin C as a new marker for noninvasive estimation ofglomerular filtration rate and as a marker for early renal impairment. Am J Kidney Dis 2000; 36: 29-34.
57. Aviv A. John E. Bernstein J, Goldsmith DI, Spitzer A. Lead intoxication during development. Its late effects on renal function and blood pressure. Kidney Int 1980; 17: 430-443.
58. Parker RA, Bennett WM. Porter GA. Animal models in the study of aminoglycoside nephrotoxicity. In: The aminoglycosides: microbiology, clinical use and toxicology. Whelton A, Neu HC (editors). Marcel Dekker Inc, New York, Basel, 1982; 235-268.
59. Nicolau DP, Freeman CD, Belliveau PP. Experience with once-daily aminoglycoside program administrated to 2184 adult patients. Antimicrob. Agent Chemother 1995; 39: 650-655.
60. Swan RC. Merrill JP. The clinical course of acute renal failure. Medicine 1953; 32: 215-292.
61. US Renal Data System, USRDS 2000 Annual Data Report: Atlas of end-stage renal disease in the United States. National Institutes of Health, Nathional Institute of Diabetes and Digestive and Kidney Diseases, Bethesda MD, 2000.

62. Combined report on regular dialysis and transplantation in Europe, XIX, 1988. Nephrol Dial Transplant 1989; 4(Suppl4): 5-29.
63. Porter GA. Bennett WM, Gilbert DN. Unraveling aminoglycoside nephrotoxicity using animal models. J Clin Pharmacol 1983; 23: 445-452.
64. Steenland NK, Thun MJ, Ferguson CW, Port FK Occupational and other exposures associated with male end-stage renal disease: a case/control study. Am J Pub Health 1990; 80: 153-159.
65. O'Dea DF, Murphy SW, Hefferton D, Parfrey PS. Higher risk for renal failure in first-degree relatives of white patients with end-stage renal disease: A population based study. Am J Kidney Dis 1998; 32: 794-801.
66. Freedman BI, Soucie M, Stone SM, Pegram S. Familial clustering of end-stage renal disease in blacks with HIV-associated nephropathy. Am J Kid Dis 1999; 34: 254-258.
67. Wang Y, Kikuchi S, Suzuki H, Nagase S, Koyama A. Endothelial nitric oxide synthase gene polymorphism in intron 4 affects the progression of renal failure in non-diabetic renal disease. Nephrol Dial Transplant 1999; 14(12): 2898-2902.
68. Gumprecht J, Zychma J, Grzeszczak W, Zukowska-Szczechowska E, and ESRD study group. Angiotension I converting enzyme gene insertion/deletion and angiotensiongen M235T polymorphism. Kidney Int 2000; 58: 513-519.
69. Nobilis A, Kocsis I, Toth-Heyn P, Treszl A, Schuler A, Tulassay T, Vasarhelyi B. Variance of ACE and AT1 receptor gene does not influence the risk of neonatal acute renal failure. Pediatr Nephrol 2001; 16(12): 1063-1066.
70. Sandler DP, Smith JC. Chronic renal disease risk associated with employment in industries with potential solvent exposure. In: Nephrotoxicity mechanisms: early diagnosis and therapeutic management. Bach PH, Gregg NJ, Wiks MF, Delacruz L (editors). Marcel Dekker Inc, New York 1991: p 261-266.
71. Landrigan PJ, Goyer RA, Clarkson TW, Sandler DP, Smith JH, Thun MJ, Wedeen RP. The work-relatedness of renal disease. Arch Environ Health 1984; 39: 225-230.
72. Bennett WM, EIzinga LW, Porter GA. Tubulointerstitial diseasc and toxic nephropathy. In: The kidney. Brenner BM, Rector FC Jr (editors). WB Saunders, Philadelphia 1991; p 1430-1496.
73. Gregg NJ, Elseviers MM, De Broe ME, Bach PH. Epidemiology and mechanistic basis of analgesic nephropathy Toxicol Lett 1989; 46: 141-151.
74. Sandler DP. Epidemiology in the assessment of nephrotoxicity. In: Nephrotoxicily in the experimental and clinical situation. Bach PH, Locke EA (editors). Martinus Nijhoff, Dordrecht 1987; 847-883.
75. Staessen J, Yeoman WB, Fletcher AE, Markowe HLJ, Marmot MG, Rose G, Semmence A, Shipley MJ, Bulpitt CJ. Blood lead concentration, renal function and blood pressure in London civil servants. Brit J Ind Med 1990; 47: 441-447.
76. Iesato K, Wakastin M, Wakastin Y. Renal tubular dysfunction in Minimata disease. Ann Intern Med 1977; 86: 731-733.
77. Staessen JA, Lauwerys RR, Buchet JP, Bulpitt CJ, Rondia D, Vanrenterghem Y, Amery A, Cadmibel Study Group. Impairment of renal function with increasing blood lead concentrations in the general population. N Engl J Med 1992; 327. 151-156.
78. Vanherweghem JL. Misuse of herbel rememdies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). J Altern Complement Med 1998; 4: 9-13.
79. De Broe ME. On a nephrotoxic and carcinogenic slimming regime. Am J Kidney Dis 1999; 33: 1171-1173.
80. Lord G, Tagore R, Cook T, Gower P, Pusey CD. Nephropathy caused by Chinese herbs in the UK. Lancet 1999; 354: 481-482.
81. Moore RD, Smith CR, Lipsky JJ, Mellitus ED, Lietman PS. Risk factors for nephrotoxicity in patients treated with aminoglycosides. Ann Intern Med 1984; 100: 352-373.
82. Miller JA, Anacta LA, Cattran DC. Impact of gender on the renal response to angiotensin II. Kidney Int 1999; 55: 278-285.
83. Anderson S, Rennke H, Brenner B. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest 1986; 77: 1993-2000.
84. Freedman BI, Spray BJ, Tuttle AB, Buckalew VM. The familial risk of end-stage renal disease in African Americans. Am J Kidney Dis 1993; 21: 387-393.
85. Obialo CI, Crowell AK, Okonofua EC. Acute renal failure mortality in hospitalized African Americans: age and gender considerations. J Natl Med Assoc 2002; 94(3): 127-134.
86. Porter GA. Risk factors for toxic nephropathies. Toxicol Lett 1989; 46: 269- 279.
87. Cremer W, Bock KD. Symptoms and causes of chronic hypokalemic nephropathy in man. Clin Nephrol 1977; 7: 112-123.
88. Wedeen RP. Poison in the pot. The legacy of lead. So Illinois Univ Press, Carbondale 1984.
89. Mudge GH. Analgesic nephropathy: renal drug distribution and metabolism. In: Nephrotoxic mechanisms of drugs and environmental toxins. Porter GA (editor). Plenum Med Book Co, New York, London 1982; p 209-225.
90. Itizler TA, Wingard RL, Harvell J, Shyr Y, Hakim RM. Association of morbidity with markers of nutrition and inflammation in chronic hemodialysis patients: A prospective study. Kidney Int 1999; 55: 1945-1951.
91. Stenvinkel P, Heimburger O, Paultre F, Diczfulusy U, Wang T, Berglund L, Jogestrand T. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int 1999; 55: 1899-1911.
92. Morgan JM, Hartley MW. Etiologic factors in lead nephropathy. South Med J 1976; 69: 1445-1449.
93. Wen SF, Parthasarathy R, Iliopoulos O, Oberley TD. Acute renal failure following binge drinking and nonsteroidal anti-inflammatory drugs. Am J Kidney Dis 1992; 20: 281-285.

94. Perneger TV, Whelton PK, Puddey IB, Klag MJ. Risk of end-stage renal disease associated with alcohol consumption. Am J Epidemol 1999; 150: 1275-1281.
95. Emmerson BT. Chronic lead nephropathy. Kidney Int 1973; 4: 1-5.
96. Rodriguez-Puyol D. The aging kidney. Kidney Int 1998; 54: 2247-2265
97. Bennett WM. Geriatric pharmacokinetics and the kidney. Am J Kidney Dis 1990; 16: 283-288.
98. Kohli HS, Bhaskaran MC, Muthukumar T, Thennarasu K, Sud K, Jha V, Gupta KL, Sakhuja V. Treatment-related acute renal failure in the elderly: a hospital-based prospective study. Nephrol Dial Transpl 2000; 15: 212-217.
99. Chester AC, Rakowski TI. Argy WP, Giacalone A, Schreiner GE. Hemodialysis in the eighth and ninth decade of lite. Arch Intern Med 1979; 139: 1001-5.
100. Marcias-Nunez IE, Cameron lS. Renal function and disease in the elderly. London, Boston, Durban, Singapore, Sydney, Toronto, Wellington: Butterworth 1987: p 503.
101. Mudge GH. Nephrotoxicity of urographic radiocontrast drugs. Kidney Int 1980; 18: 540-552.
102. Levy EM, Viscoli CM, Horwitz RI. The effect of acute renal failure on mortality: a cohort analysis. J Am Med Assoc 1996; 275: 1489-1494.
103. Evans JMM, McGregor E, McMahon AD, McGilchrist MM, Jones MC, White G, McDevitt DG, MacDonald TM: Non-steroidal drugs anti-inflammatory drugs and hospitalization for acute renal failure. Q J Med 1995; 88: 551-557.
104. Brivet FG, Kleinknecht DJ, Loirat P, Landais PJM, the French Study Group on Acute Renal Failure. Acute renal failure in intensive care units: causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. Crit Care Med 1996; 24: 192-198.
105. Liano F, Junco E, Pascual J, Madero R, Verde E, and the Madrid Acute Renal Failure Study Group. The spectrum of acute renal failure in the intensive care unit compared with that seen in other settings. Kidney Int 1998; 66: S16-S24.
106. Cole L, Bellomo R, Silvester W, Reeves JH. A prospective, multicenter study of the epidemiology, management, and outcome of severe acute renal failure in a "closed" ICU system. Am J Respir Crit Care Med 2000; 162: 191-196
107. deMendonca A, Vincent JL, Suter PM, Moreno R, Dearden NM, Antonelli M, Takala J, Sprung C, Cantraine FP. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. Intensive Care Med 2000; 26: 915-921.
108. De long PE, Statius van Eps LW. Sickle cell nephropathy: new insights into its pathophysiology. Kidney Int 1985; 27: 711-719.
109. Allen M, Lawson L, Eckman lR, Delaney V, Bourke E. Effects of nonsteroidal anti-inflammatory drugs on renal function in sickle cell anemia. Kidney Int 1988; 34: 500-506.
110. Porter GA. Contrast associated nephropathy. Am J Cardiol 1989; 64: 22E-26E.
111. Rihal CS, Textor SC, Grill DE, Berger PB, Ting HH, Best PJ, Singh M, Bell MR, Barsness GW, Mathew V, Garratt KN, Holmes DR Jr. Incidence and prognostic importance of acute renal failure after percutaneous coronary intervention. Circulation 2002; 105(19): 2259-2264.
112. Rao TK, Nicastri AD, Friedman EA. Natural history of heroin-associated nephropathy. N Engl J Med 1974; 290: 19-25.
113. Dubrow A, Mittman N, Ghali V, Flamenbaum W. The changing spectrum of heroin associated nephropathy. Am J Kidney Dis 1985; 5: 36-43.
114. Cunningham EE, Zielezny MA, Venuto RC. Heroin-associated nephropathy - a nationwide problem. JAMA 1983; 250: 2935-2938.
115. Warner E. Cocaine abuse. Ann Intern Med 1993; 119: 226-235.
116. Roth D, Alarcon FJ, Fernandez JA, Preston RA, Bougoignie JJ. Acute rhabdomyolysis associated with cocaine intoxication. N Engl J Med 1988; 319: 673-677.
117. van der Woude FJ. Cocaine use and kidney damage. Nephrol Dial Transplant 2000; 15: 299-301.
118. Citron BP, Halpern M, McCarron M. Lundberg GD, McCormick R, Pincus IJ, Tatter D, Haverback BJ. Necrotizing angitis associated with drug abuse. N Engl J Med 1970; 283: 1003-1007.
119. Bingham C, Beaman M, Nicholls AJ, Anthony PP. Necrotizing renal vasculopathy resulting in chronic renal failure after ingestion of methamphetamine and 3, 4-methylenedioxymethamphetamine ('ecstacy'). Nephrol Dial Transplant 1998; 13: 2654-2655.
120. Richards JR, Johnson EB, Stark RW, Derlet RW. Methamphetamine abuse and rhabdomyolysis in the ED: A 5 year study. Am J Emerg Med 1999; 17: 681-685.
121. Porter GA. Antimicrobial nephrotoxicity. In: Textbook of nephrology. Massry SG, Glassock JR (editors). Baltimore, Williams & Wilkins 1989; p 812-818.
122. Swartz RD, Rubin JE, Leeming BW, Silva P. Renal failure following major angiography. Am J Med 1978; 65: 31-37.
123. Cochran ST, Wong WS. Roe DJ. Predicting angiographic-induced acute renal impairment: clinical risk model. Am J Radiol 1983; 141: 1027-1033.
124. Cigarroa RG, Lange RA, Williams RH, Hillis LD. Dosing of contrast material to prevent contrast nephropathy in patients with renal disease. Am J Med 1989; 86: 649-652.

125. Leehey DJ, Braun DA. Tholl LS, Chung CA, Gross JA, Lentino JR. Can pharmacokinetic dosing decrease nephrotoxicity associated with aminoglycoside therapy? J Am Soc Nephrol 1993; 4: 81 -90.
126. Swann SK, Bennett WM. Nephrotoxic acute renal failure. In: Acute renal failure. Lazarus JM, Brenner BM (editors). Churchill Livingston, New York, 1993; p 357-392.
127. Levenson DJ, Simmons CE, Brenner BM. Arachidonic acid metabolism, prostaglandins and the kidney. Am J Med 1982; 72: 354-374.
128. Whelton A. Renal and related cardiovascular effects of conventional and COX-2 specific NSAIDs and non-NSAID analgesics. Am J Ther 2000; 7: 63-74.
129. Mehta RL, Pascual MT, Soroko S, Chertow GM. Diuretics, mortality, and nonrecovery of renal function in acute renal failure. JAMA. 2002; 288(20): 2547-2553.

02

Renal handling of drugs and xenobiotics

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Introduction

Pharmacology and clinical pharmacology define the desirable and undesirable effects of drugs and xenobiotics whereas pharmacokinetics defines the various processes that are involved in absorption - distribution - elimination of these agents. Needless to say that the former may strongly influence the latter.

The kidney and the liver have complementary functions in the elimination of drugs and xenobiotics. Lipophilic non-ionic substances of molecular weight higher than 300-500 dalton and highly bound to proteins appear to be eliminated by the liver, while the kidney prefers hydrophilic substances of molecular weight smaller than approximately 500 daltons. Metabolism occurs predominantly in the liver, transforming the original substance into more polar and more hydrophilic metabolites, which became dependent on the kidney for elimination. Consequently, the majority of all drugs and xenobiotics in one way or another have to pass through the kidney. In addition to this important "gateway" function of substances, which are not always without side-effects, the kidney itself is particularly sensitive to drugs and xenobiotics.

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Edition, 21-46.

This susceptibility of the kidney to nephrotoxic injury has several reasons (Table 1). Renal blood flow (25% of the resting cardiac output) exceeds 1000 ml/min = 3.5 ml/g of renal tissue/min. Compared to the majority of other tissues, except the brain, this results in a fifty times higher rate of drug delivery.

The kidney has the greatest endothelial surface per gram of tissue and possesses the highest capillary hydrostatic pressure favoring trapping of circulating antigen and *in situ* immune complexes formation. Tubular transport and other renal metabolic processes utilize considerable oxygen and are susceptible to the action of metabolic inhibitors. It is worthwhile to note that the S3-segment of the proximal tubule has the highest rate of oxygen delivery/oxygen consumption of all functional entities in the body [1]. The kidney is the only place where highly protein bound drugs dissociate, traverse the tubular cells and either accumulate within the proximal tubular epithelium and/or reach the tubular lumen. An abundance of tubular epithelial enzymes involved in the tubular transport systems can be blocked, particularly in view of the highly concentrated solutes in the tubular fluid that may reach urinary/plasma concentration ratios exceeding 1000 in some cases.

In the distal part of the nephron, urine is concentrated and the likelihood of crystalline precipitation increases substantially, particularly if urinary pH favors decreased solubility. As the urinary concentrating process also involves the counter-current mechanism, solute concentrations in the medullary interstitium can reach values several times higher than tissues elsewhere in the body. Finally during the process of renal excretion, a particular drug may undergo bioactivation resulting in reactive metabolites [2].

The kidney possesses several mechanisms for the renal handling/excretion of drugs and xenobiotics. They are listed in Figure 1 and each of them will be briefly discussed in this chapter. Numerous, if not the majority of drugs and xenobiotics, are handled-eliminated at least partly by the kidney. For their elimination by the kidney they use one, or in most cases, two or even more mechanisms (Figure 2). In addition many

Table 1. Vulnerability of the kidney.

Important blood flow (1/4 cardiac output)
High metabolic activity
Largest endothelial surface by weight
Multiple enzyme systems
Transcellular transport
Concentration of substances
Protein unbinding

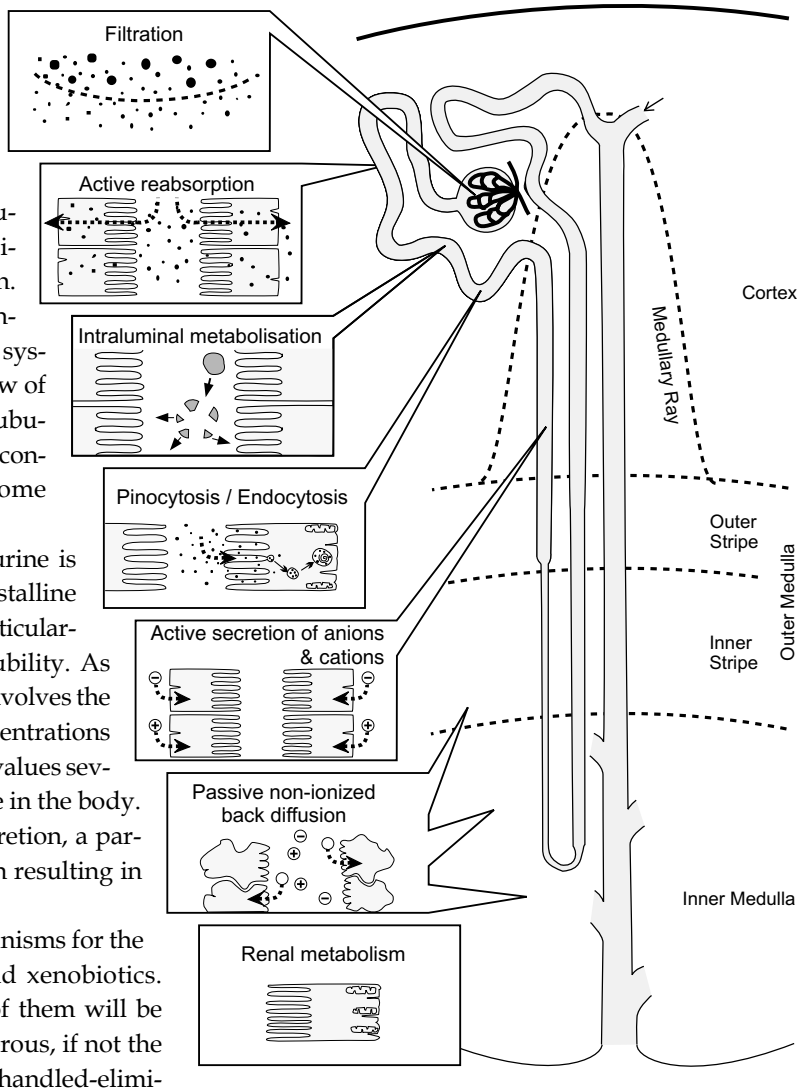


Figure 1. *Schematic representation and main localisation along the nephron of the various patterns of drug and xenobiotic handling by the kidney.*

other polar metabolites are formed by metabolism or conjugates by the liver, which are then excreted by the kidney. The use of various *in vitro* and *in vivo* techniques as models in studying drug transport in the kidney and/or renal toxicology is well documented in the literature [3-7]. Each approach possesses its own advantages and disadvantages and all have demonstrated their usefulness and application in renal pharmacokinetics/toxicology. A representative listing of these models, summarizing their most relevant characteristics, is presented in Table 2 [6, 8].

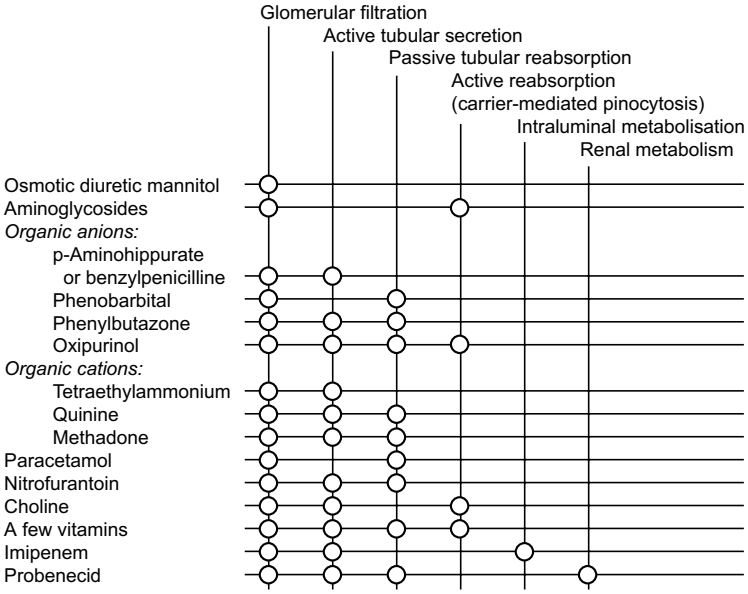


Figure 2. *Most drugs and xenobiotics have a renal handling consisting in more than one pattern.*

Table 2. *In vitro* methods for studying drug transport in the kidney

Method	Advantages	Disadvantages
Stop-flow	Easy to determine net direction of transport.	No precise anatomical localization.
Isolated perfused kidney	Morphologically identical to kidney *in vivo.* Can monitor renal function.	Short term use. In the process of degeneration.
Kidney slices	Easy technique. Good control of experimental conditions without concern for secondary effects due to hemodynamic changes.	Functional status of tubular lumen not clear. Tissue not homogenous and contains nontubular elements. Diffusion barrier for substrates to nephrons beneath the cut surface.
Proximal tubular suspensions	Relatively homogenous cell population.	Contribution of luminal uptake is dependent on luminal openings and can vary. Short term use.
Micropuncture	Can study transepithelial transport in surface portions of proximal and distal tubules.	Cannot study deep segments.
Cultures	Long-term storage. Precise control of growing environment. Cell population is relatively homogenous. Cells on filters permit study of bidirectional transport.	Dedifferentiation. Sterile conditions for culture.
Cell lines	Easily obtained and subcultured.	Origin ill-defined. Important dedifferentiation.
Primary cultures	Closely related to fresh tissue. Origin identified.	More difficult to prepare and maintain.
Vesicles	Transport in apical and basolateral membranes can be studied separately. No metabolism. No intracellular sequestration.	Membrane isolation may alter physiological function. Must correct for non-mediated transport.

Adapted from Williams & Rush [6] and Brater et al [8].

The maturation of renal drug elimination systems occurs at variable rates and may be influenced by a number of factors, including pre-or postnatal exposure to drugs. In addition, the mechanisms of drug uptake and storage in renal tubular cells are subject to maturational changes that may lead to age-related differences in intrarenal accumulation of a drug [8a].

Glomerular filtration

One fifth of the renal plasma flow (± 600 ml/min) is filtered at the glomeruli. This filtered fraction indicates that glomerular filtration can account for the plasma clearing of as much as 20% of a non-protein bound substance during one passage through the kidney. The determinants of a drug/xenobiotic to be filtered are protein binding, molecular size and charge, glomerular integrity and the number of filtering nephrons. Glomerular pores (± 75 A in diameter) allow passage of molecules up to the molecular weight of approximately 60, 000 dalton. The vast majority of drugs/xenobiotics are approximately two orders of magnitude smaller than this. For many drugs however, protein binding restricts filtration so that only the unbound fraction can be filtered (e.g. furosemide 95% and NSAID 98% bound to albumin), and in many cases depend on active tubular secretion for renal elimination. Drugs can bind to several serum proteins, however, by far the most important being albumin, followed by a α1-acid glycoprotein, an acute phase reactant. Acidic compounds preferentially bind to albumin [9] whereas for basic compounds binding to α1-acid glycoprotein is more important [10].

Nephrotic syndrome induces two important changes concerning protein binding. Hypoproteinemia causes a decrease in protein binding and the integrity of the glomerulus as a sieve is disrupted in this clinical condition. Drugs and xenobiotics can be carried with albumin into the urine enhancing renal elimination. Hypoproteinemia, however, induces simultaneously an increase in the distribution volume of numerous substances thus lowering their availability for filtration. The overall result on renal elimination being almost unperceptible.

Total plasma clearance and distribution volume of furosemide were much larger in analbuminemic rats compared to normals, whereas the urinary excretion was significantly lower. Injecting the albumin/furosemide complex markedly decreased the drug distribution volume, promoted diuresis in analbuminemic rats, in contrast to furosemide alone. Injection of the furosemide/albumin complex to furosemide resistant hypoalbuminemic nephrotic patients increased the urine volume. Another factor that may contribute to diuretic resistance in nephrotic patients is the presence of filtered albumin within the tubule lumen. Even when adequate amounts of diuretic are delivered to and secreted by the proximal tubule, much of the diuretic that reaches the lumen in a nephrotic patient will bind to filtered albumin; the protein/diuretic complex may not be effective in inhibiting the Na-K-2Cl pathway [11-14]. In rats with nephrotic syndrome, inhibitors of protein binding (warfarin and sulphisoxazole) restore the potency of furosemide [14].

Uncharged hydrophilic substances prefer glomerular filtration for their renal handling/elimination in contrast to the many ionized organic substances handled by additional nephron mechanisms, such as tubular secretion (e.g. penicillin).

Drugs and xenobiotics that have glomerular filtration as their major way of renal elimination will accumulate rapidly during acute or more chronic declines of glomerular filtration. If in addition the therapeutic/toxic window is narrow, the accumulation will result very quickly in toxic effects (e.g. aminoglycosides).

Renal tubular reabsorption

Reabsorption of weak acid and bases is generally passive, but in a few cases reabsorption can occur via facilitated reabsorption by carrier proteins or by endocytosis.

Reabsorption by simple diffusion

Passive reabsorption is driven by the progressive reabsorption of tubular fluid along the nephron. To penetrate the membranes of the tubular epithelium, whose main constituents are lipids, compounds should be liposoluble. As ionized compounds are in general hydrophilic, only the undissociated molecules of weak bases and acids will be rapidly reabsorbed by simple diffusion [15]. Consequently determinants for the rate of reabsorption are the pKa of the organic acid or base, the urinary pH, and the liposolubility of the undissociated base or acid. Another important determinant is

Table 3. Drugs and Xenobiotics with clinically important urine pH-dependent elimination.

Weak acids: *increased excretion at luminal pH > 7*	**Weak bases:** *increased excretion at luminal pH < 5*
Acetazolamide	Amitriptyline
Chlorthiazide	Amphetamine
Methotrexate (?)	Chloroquine
Penicillin G	Ephedrine
Phenobarbital	Imipramine
Phenylbutazone	Phencyclidine (Angel Dust)
Salicylates	Quinine
Sulfonamide derivatives	Tricyclic antidepressants

the contact time of the solute with the epithelium. In antidiuresis, this time is prolonged compared to diuresis, and thus passive reabsorption is increased along the whole nephron, as observed for salicylate (Table 3) [16].

Alkaline diuresis will favor the excretion of weak acids (anions) such as salicylate or phenobarbital. Indeed, the more the drug is ionized, the more it is trapped in the tubular lumen and consequently is not reabsorbed, hence eliminated in the urine. This mechanism can play a role in the treatment of severe intoxications. The reverse being true for weak bases (cations) such as methadone. Acidification of the urine facilitates the reabsorption of weak acids and will retard the reabsorption of weak bases. The magnitude of the effect obtained on organic acid excretion by urinary alkalinization will be smaller than that which may be achieved for organic cation excretion by urine acidification. Indeed, the achievable urinary proton concentration is up to three orders of magnitude higher than plasma concentration (pH 4.5 versus 7.4) (Figure 3). At the other end of the pH scale urinary proton concentration cannot exceed a value of one order of magnitude lower than plasma concentration (pH 8.5

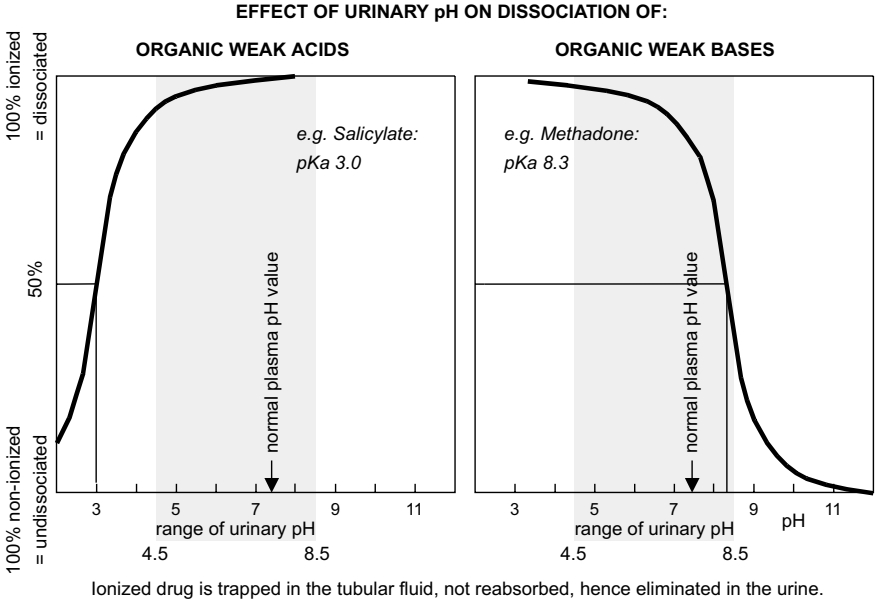


Figure 3. *Effect of urinary pH on dissociation of organic weak acids and organic weak bases. Lipid soluble compounds cross the cellular membranes preferentially in their undissociated form. The ionized form favours trapping and subsequent elimination by the kidney.*

versus 7.4) (Figure 3). The effect of urinary pH on the elimination of amphetamine may be better known to abusers of these drugs or particular sport trainers than to clinicians. Since amphetamine is a weak base, alkalizing the urine increases the non-ionized amount favoring reabsorption. Amphetamine abusers regularly ingest baking soda to prolong the "high". Therapeutically, it would be important to acidify the urine of a patient with an overdose of amphetamines or phencyclidine (angel dust) [17]. However, one has to take into account that the extent to which a change in urinary pH alters the rate of total body clearance depends on the contribution of renal clearance to the total body clearance. Weak acids like phenytoin and warfarin which are susceptible to a pH dependent elimination in the urine do not see a substantial effect of change in urinary pH on their total elimination since hepatic metabolism is the more important metabolic pathway [18].

There are examples of weak acids reabsorbed by simple nonionic diffusion which urinary excretion is not influenced by changes in urine pH. It is the case if the pKa is above or close to the upper limit of urine pH, as it is the case for barbital (pKa = 7.8), and a few other barbiturates. Also, if the pKa value is very low, such as it is the case for 2-nitroprobenecid (pKa=1.3), the acid remains mainly unionized in the physiological range of urine pH [15], and its excretion remains independent of tubular urine pH.

Reabsorption by facilitated mechanisms

A certain number of drugs and xenobiotics are reabsorbed by facilitated mechanisms. Some organic anions are transported at the apical membrane of proximal tubule by a sodium-cotransport mechanism. It is the case of vitamins, such as ascorbic acid, biotin, panthotenate, nicotinate, and pyridoxine (and its analogues) [19]. Pyrazinoate, a metabolite of pyrazinamide is reabsorbed by a sodium cotransport mechanism [20, 21], as well as by an anion-exchanger [20], which is implicated also in the reabsorption of urate. Oxypurinol, the metabolite of allopurinol might also be reabsorbed by the urate reabsorbing mechanism [22]. M-hydroxybenzoate and morphine-glucuronides are other organic anions reabsorbed by facilitated mechanisms that have yet to be identified [23, 24]. Little is known on the facilitated reabsorption of organic cations. The reabsorption of choline involved a sensitive pathway at the apical membrane [25].

Several peptide-like drugs such as β-lactam antibiotics (ceftibuten, cyclacillin) are substrates of the peptide transporters localized in the brush-border membrane, and are taken up into proximal cells. The peptide transporters mediate an electrogenic H^+-coupled cotransport of di- and tri-peptides, which is driven by the proton gradient and the negative transmembrane potential difference [26]. Two homologous peptide transporters have been identified by molecular cloning methods, PEPT1 and PEPT2. In the kidney, PEPT1 was localized to the brush-border membrane of S1 segments of proximal tubule, whereas PEPT2 was localized to the brush-border membrane of S3 segments [27]. Affinity of anionic cephalosporin without a-amino group (ceftibuten) and cyclacillin (aminopenicillin) is greater than that of aminocephalosporin, such as cephalexin, cefadroxil, cephradine. Because of their low affinity for the anionic cephalosporins PEPT1 and PEPT2, should not play a major role in cellular accumulation and potential toxicity of these cephalosporins when given at therapeutic doses. The peptide transporters might, however, be involved in the reabsorption of the nephrotoxin ochratoxin A [28]. The anticancer drug bestatin, and valacyclovir, a non-peptide antiviral agent, are also substrates for the peptide transporters [29].

The angiotensin-converting enzyme inhibitors, quinapril and enalapril, have affinity for the peptide transporters, however it is not known whether they are transported.

Endocytosis

One of the mechanisms of active reabsorption is endocytosis. Fluid phase endocytosis consists of the incorporation of fluid and solutes in vesicles formed at the base of the brush border membrane of the proximal tubular cells (Figure 1). A more efficient absorptive endocytosis involves first binding of a drug, such as the cationic aminoglycoside and/or may be cadmium [30, 31], to a carrier (phosphatidylinositol) located in the luminal membrane of the wall of the pinocytotic vesicle occurs followed by endocytosis and lysosomal fusion [32, 33].

Endocytosis is a normal mechanism for protein and insulin reabsorption at the proximal tubule of the kidney. A considerable amount of insulin (50%) is metabo-

lized by the kidney, which may account, at least in part, for the decreased insulin requirement that occurs in diabetic patients with decreased renal function. Furthermore, this uptake process allows highly hydrophilic lipid insoluble drugs such as aminoglycosides to enter a particular intracellular compartment (lysosomes) without crossing a membrane.

Renal tubular secretion of drugs/ xenobiotics

Most ionic xenobiotics are secreted by two transport mechanisms, one responsible for organic ion (or "organic acids") secretion, the other for organic cation (or "organic bases") secretion (for a non-exhaustive list, Tables 4 and 6) [34, 35, 35a]. Despite considerable advances in the understanding of basic transport pathways and mechanisms involved in the tubular secretion of organic compounds, there is still relatively little information on the regulation of this transport [35b]. The first step of secretion, transport across the basolateral membrane, of each of the two general mechanisms is performed by several subsystems which may correspond to different carrier molecules, for which substrates of rather unspecific molecular structure may have various affinities [19, 36, 37]. The molecular structure of several isoforms of these transporters has been identified by expression cloning. They are members of a newly identified transporter family, the organic ion transporters, which comprises OAT (organic anion transporter), and OCT (organic cation transporter) isoforms [29, 38, 39].

Our understanding of the organic ion secretory mechanisms derives essentially from investigations on a few transported compounds that are considered representative of other secreted organic ions. For organic anions the classical substrate is p-aminohippurate whereas for organic cations classical substrates are tetraethylammonium and N_1-methylnicotinamide

Both classical transport systems are located exclusively in the proximal tubule of the nephron. Several techniques such as visual observations, stop-flow experiments, tubular micropuncture, *in vivo* and *in vitro* tubular microperfusions have demonstrated this particular transport capacity of the proximal tubular segment of the nephron [19]. Secretion entails an increase of proximal cell concentration of transported substrates that may become higher than in interstitium, and in some case may result in nephrotoxicity [40-42].

Secretion is not uniform along the proximal tubule, and may differ between superficial and juxtamedullary nephrons. This heterogeneity of secretion, varying between species and substrates, might reflect different densities of carrier molecules along the tubule [19]. Since the number of carrier molecules is limited, secretion is saturable and subject to competition between substrates. Such competition may thus result in drug interactions some of them being of clinical relevance (see below) [43].

The transport mechanisms of the organic ion transport systems have been characterized at both membrane sides of proximal tubule, mainly by studies in brush-border and basolateral membranes purified from homogenates of renal cortex. Since a detailed review and a critical discussion of the present knowledge in this field was published by Pritchard [44], only the main conclusions are summarized here.

Beside these classical, long recognized secretory transport systems, other transport mechanisms are involved in the renal excretion of xenobiotics [45]. They are the basolateral oxalate/sulphate exchanger and the basolateral sodium-dicarboxylate transport system [37]. These transporters were identified by expression cloning, and named SAT1 [46] and NaDC3 [47], respectively. A number of other transporters has been cloned and identified in the renal brush border, but their functional role in the kidney has yet to be defined [45]. Among them are the multidrug resistance-associated proteins MRP and MDR/P-glycoprotein, which are ATP dependent primary active transporters for organic anions and organic cations respectively [48, 49]. They stimulate the active efflux from cell to lumen, of various organic ions. OATP1 is another transporter that mediates the apical transport of steroid conjugates and sulphobromophtalein [50], whereas OAT-K1 and OATK2 mediate methotrexate and folate transport [51, 52]. OCTN1 [53] and OCTN2 [54] are apical multispecific organic cation transporters. The functional characteristics of these transporters will be discussed below.

Transport mechanisms for tubular secretion of organic anions

The transport mechanisms of organic anions have been characterized mainly for p-aminohippurate.

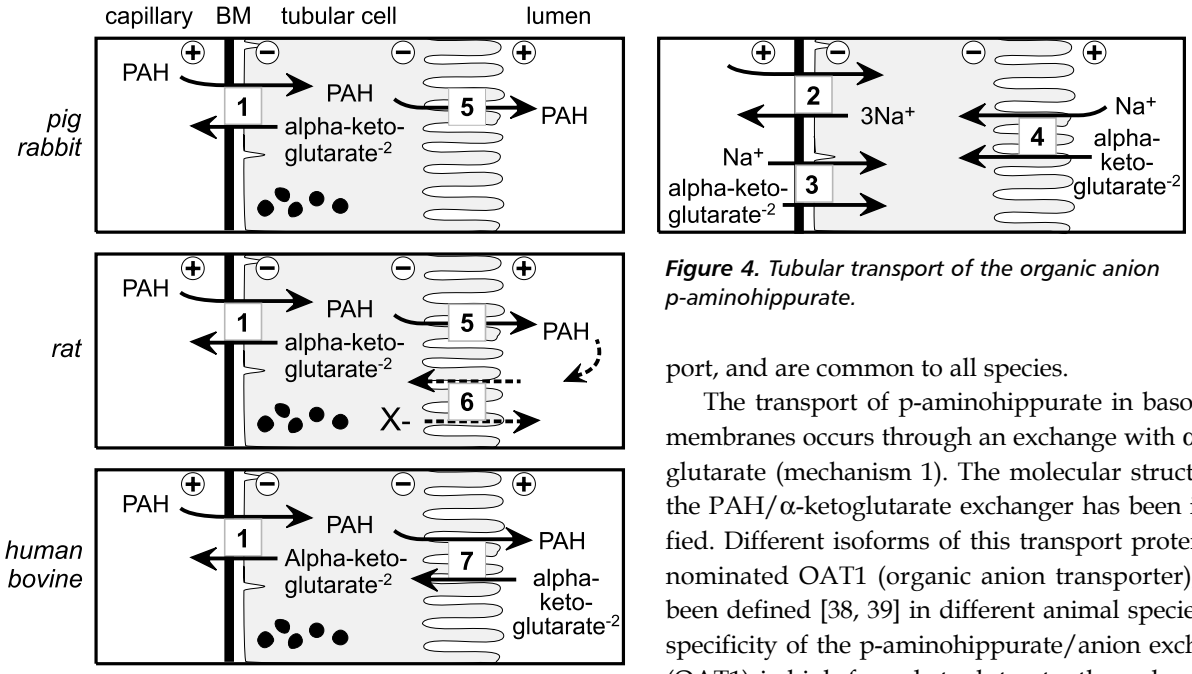


Figure 4. *Tubular transport of the organic anion p-aminohippurate.*

Owing to electro-negativity of the cell interior, resulting from Na-K-ATPase activity, a transfer of negatively charged molecules into cells occurs generally against an electrochemical gradient and requires energy ("active transport"). In contrast, efflux from cell to lumen takes place along a favorable electrochemical gradient and does not necessitate a direct energy supply. Large cell/interstitium concentration gradients, up to 40 in isolated perfused rabbit proximal tubules, can build up during secretion [44]. However, as only part of the p-aminohippurate accumulated in the cytoplasm might be free, the concentration gradient of diffusible p-aminohippurate, between peritubular interstitium and cell might be lower than estimated from total concentration. There exists strong evidence that part of p-aminohippurate might be sequestered in cytoplasmic organelles [55, 56].

As will be described below, the basolateral transport mechanism, which is the active step in p-aminohippurate secretion, is identical in all animal species investigated so far, whereas the apical mechanism, which does not require energy, differs between animal species.

The left side of Figure 4 shows a section of proximal tubule and the p-aminohippurate transport mechanisms identified in different mammalian species. On the right side of Figure 4, the mechanisms shown are indirectly implicated in p-aminohippurate transport, and are common to all species.

The transport of p-aminohippurate in basolateral membranes occurs through an exchange with α-ketoglutarate (mechanism 1). The molecular structure of the PAH/α-ketoglutarate exchanger has been identified. Different isoforms of this transport protein, denominated OAT1 (organic anion transporter), have been defined [38, 39] in different animal species. The specificity of the p-aminohippurate/anion exchanger (OAT1) is high for α-ketoglutarate, the only natural substrate showing affinity for the antiport. This p-aminohippurate basolateral transport mechanism has been found in all mammals (including humans) and lower vertebrates investigated so far. The energy source for p-aminohippurate transport in intact cells is provided by the low intracellular sodium concentration achieved by Na-K-ATPase activity (mechanism 2, right part of Figure 4), which expels 3 Na^+ from the cell in exchange of 2 K^+. This exchange creates the electronegativity of the cell. The transmembrane sodium gradient serves as energy source to drive α-ketoglutarate intracellularly from peritubular interstitium (mechanism 3) as well as from tubular lumen (mechanism 4), since both basal and apical membranes possess an α-ketoglutarate-sodium cotransport mechanism. They were identified by molecular cloning and named NaDC3 [47] and NaDC1 [57], respectively. Furthermore, α-ketoglutarate can also be produced by cell metabolism. In the dog, the intracellular concentration of α-ketoglutarate from transport and cell metabolism is about 5-10 times higher than in plasma [58], and is thus not rate limiting for p-aminohippurate transport. The α-ketoglutarate/p-aminohippurate exchange at the basolateral membrane (mechanism 1, left side of Figure 4) allows PAH to concentrate intracellularly by a tertiary active transport.

Intracellular traffic of secreted anions appears more complex than originally thought, and might proceed through accumulation into cell organelles (black dots

on the scheme), implying high local concentrations of the substrate, and involvement of a microtubular network [59, 60]. While the basolateral membrane transport system appears ubiquitous, the mechanisms involved in p-aminohippurate efflux from cell to lumen differ between animal species [44]. A voltage controlled pathway (mechanism 5) and/or anion exchanger (mechanism 6 and 7) might be implicated. The former, which is present in rabbits, pigs and rats (data are lacking for dogs) [44], is facilitated transport mechanism that, because of electronegativity of the cell, should drive p-aminohippurate efflux from cell to lumen. An anion exchanger, on the other hand, has been identified in rats (mechanism 6), and in dogs (not shown in the figure). This transporter accepts inorganic anions (Cl^-, HCO_3^-, OH^-), and several organic anions (X^- = lactate, succinate, α-ketoglutarate, etc.) and also uric acid [44]. The respective role of these two transport mechanisms observed in rats is not known. Indeed, in rat proximal tubule *in situ*, Ulrich did not observe any effect of changes in membrane potential on PAH cellular efflux, and did not observed any stimulation of PAH efflux by anion exchange [61]. The rat and dog anion exchanger which has affinity for urate, is most probably involved in urate reabsorption and might decrease the secretion of p-aminohippurate by recycling part of it into the cell. In humans, p-aminohippurate is not transported by a voltage-controlled pathway [62], nor by the anion exchanger that has urate as substrate [62]. The apical transport of p-aminohippurate is by a p-aminohippurate/anion exchanger, which accepts only α-ketoglutarate (mechanism 7), as it is the case in basolateral membranes [63]. The same mechanism has been found in bovine [64]. In human and bovine, because of the similarity of p-aminohippurate transport mechanism at each membrane, it is difficult to understand how vectorial transport occurs [64]. At present, these different apical transporters have not been identified at the molecular level.

Substrate requirements for the "PAH transport mechanism".

Many studies have been devoted to the characterization of the properties of substrates for the so-called "organic anion secretory mechanism", by measuring the ability of compounds to compete for p-aminohippurate transport across the basolateral membrane, the first step in secretion. In particular Ullrich et al. [61], investigated the interaction of all kinds of aliphatic and aromatic molecules on p-aminohippurate influx in rat proximal tubule cells *in situ*. The findings have been reviewed by the authors, and will not be detailed here. The main findings [37], which corroborate older data [36], are that the molecular structure of the transport substrates is rather unspecific, and that substrate affinity depends on the acidity and hydrophobicity of the substrate [37]. These authors demonstrated that, unexpectedly, the ionization of the substrate is not a prerequisite for interaction with the transporter [65]. In general, most anionic xenobiotics are secreted by the p-aminohippurate secretory mechanism (Table 4), and their secretion can be inhibited by probenecid. Substrates with high affinity are monovalent anions, containing a hydrophobic domain with a minimal length of about 4 A° (benzoyl derivatives). Ullrich et al. also characterized the substrate characteristics for the oxalate/sulphate transport mechanism, and the sodium-dicarboxylate cotransport mechanism, *in situ* in rat proximal tubules [37]. The oxalate/sulfate exchanger (SAT1) and the sodium-dicarboxylate transporter (NaDC3) have a much narrower substrate specificity than the PAH transporter (OAT1), which is the major basolateral organic anion transporter, and represents the classical "organic anion secretory mechanisms". Biphosphonates [66], and in particular alendronate [67] might be secreted by SAT1. Fewer studies have been devoted to the characterization of substrate requirement for the apical p-aminohippurate transport. The general substrate characteristics appear similar to that of PAH basolateral transport, i.e. hydrophobicity and acidity, and lack of molecular structure requirement [68, 69].

The molecular biology of the OAT transporter family

Several isoforms of OAT1 (rat, human, mice OAT1) have been cloned and their functional properties examined in different cultured cell systems and Xenopus oocytes. Detailed molecular structures and functional characteristics have been recently reviewed [29, 38, 39] (Figure 5). OAT1 belongs to a subgroup of a newly identified transporter family, the organic ion transporter family, which comprises other OAT isoforms, OAT2, and OAT3. These proteins possess a common structural feature, i.e. 12 putative transmembrane domains, with large hydrophobic loops between the first and second, and the sixth and seventh trans-

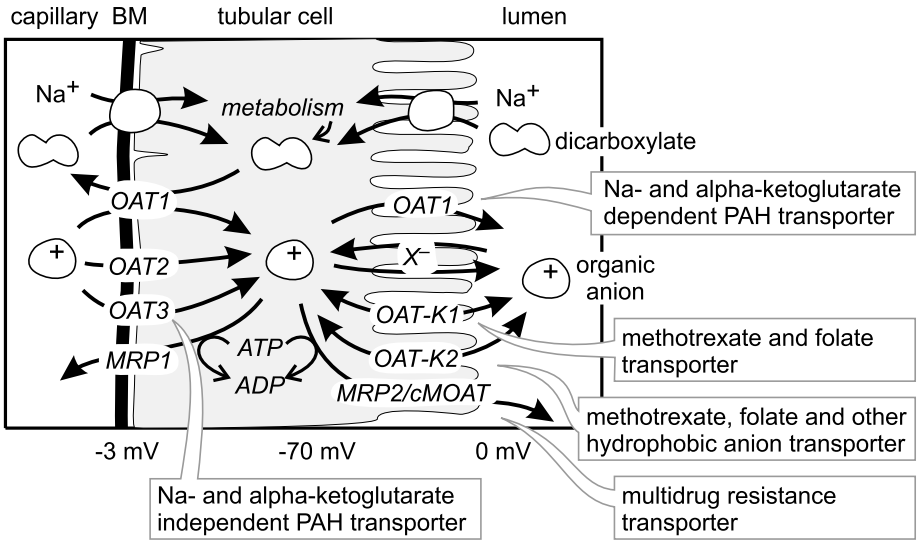


Figure 5. *Mechanisms of organic anion transport in renal tubular cells. Cellular uptake of organic anions across basolateral membranes (BLM) is mediated by OAT1 (1), which is an organic anion/dicarboxylate exchanger, and by OAT2 (2) and OAT3 (3). Anionic drug conjugates with glutathione may be extruded from cells into blood by MRPI (4). Exit of cellular organic anions across brush border membranes (BBM) is mediated by unidentified transmembrane potential-dependent organic anion transporter (5) and organic anion/anion (X^-) exchanger (6). Bidirectional transport of hydrophobic anions such as methotrexate and folic acid in the brush-border membranes is mediated by OAT-K1 (7) OAT-K2 (8) may also participate in tubular reabsorption and/or secretion of hydrophobic anions such as bile acids, methotrexate. and prostaglandin E_2. MRP2/cMOAT (9) may contribute to tubular secretion of anionic conjugates of hydrophobic compounds. Adapted from [29].*

membrane domains. The organic cation transporters, OCT and OCTN are members of the same organic ion transporter family [39]. Human and rat OAT1 has been localized exclusively to the basolateral membrane of S2 segments of proximal tubules, and when transfected in cell systems such as Xenopus oocytes or epithelial cultured cells, OAT1 has the ability to transport a wide variety of organic anions which are known to be secreted *in vivo* (Table 4). Transport is through an α-ketoglutarate/organic anion exchanger, which is dependent on the presence of chloride in the extracellular medium [70]. These are the same requirements than for transport trough the basolateral membrane of proximal tubules. The rat, human, or flounder OAT1 isoforms were demonstrated to transport PGE_2, cAMP, cGMP, α-ketoglutarate, estradiol 17β-D-glucuronide, nonsteroidal anti-inflammatory drugs (salicylate, acetylsalicylate, indomethacin, etc.), antiviral drugs (azidothymidine, acyclovir, etc.), diuretic (thiazide, bumetanide, ethacrynic acid, tienilic acid, and the nephrotoxin ochratoxin A [38, 39, 71-74]. The different OAT1 isoforms have some differences in substrate affinities, which might correspond to species differences in transport. For example, urate is transported by the rat rOAT1 [75], but not by the flounder fOAT1 [76] and the human hOAT1 [77]. In human, urate is not secreted by the PAH transport mechanism [78]. This observation gives support to the role of hOAT1 in PAH secretion. Methotrexate and PGE_2 are transported by rat rOAT1 [75, 79], but they have no affinity for the human hOAT1 [80]. Probenecid is not transported by the rat and the flounder transporters despite its binding affinity [75, 79]. Because probenecid, PGE_2 and methotrexate are secreted in human and rat, this lack of transport suggests that other OAT isoforms or transport proteins are involved in their secretion.

Recently, OAT2 and OAT3, two OAT1 isoforms, have been identified. OAT2 mRNA is predominantly expressed in the liver, and weakly in the kidney. In contrast OAT3 mRNA is expressed in the kidney, as well as in the liver and the brain [80a]. The substrate spectrum of OAT2 and OAT3 is diverse like that of OAT1 [81], but in contrast to OAT1, transport is not dependent on α-ketoglutarate, and a concentration gradient is sufficient to allow transport. The nephron distribution, and the membrane localization of OAT2 and OAT3 have not been established. Rat rOAT3 mediates PAH transport [82] estrone sulfate, ochratoxin A. Substrates for human hOAT3 are still to be defined [77].

There exists clear evidences that OAT1 plays a major role in PAH and other organic anion secretion. It is localized in the basolateral membrane of proximal tubule, it has the transport characteristics of basolateral

Table 4.

A. Compounds known to be secreted by the renal organic acid (anion) transport system.

Endogenous compounds	Drug metabolites	Drugs		
Aromatic amino acids	Acetylated sulfonamides	Acyclovir	Guanethidine	Penicillins
Bile salts	N-Acetylcysteine conjugates	Acetazolamide	Gossypol	Pentopril
Bilirubin	Gentisate	p-Aminohippurate	Hippuran	Phenobarbital
cAMP	Glucuronide conjugates	p-Aminosalicylate	Hydrochlorothiazide	Phenosulfonphthalein
cGMP	Gluthatione conjugates	ACE-inhibitors	Imipenem	Piretanide
Fatty acids	Glycine conjugates	Benzoates	Iodipamide	Probenecid
Folic Acid	Pyrazinoate	Bumetanide	Iothalamate sodium	Quinolones
Hydroxyindoleacetate	Sulphate conjugates	Carbenicillin	Loop diuretics (Etacrynic acid, Furosemide, ...)	Salicylates
Oxalate		Cephalosporins	Methotrexate	Saccharin
Prostaglandins		Chlorthalidone	Nitrofurantoin	Sulphinpyrazone
Urate		Cisplatin	NSAIDs (Indomethacin, Ibuprofen, Phenylbutazone, ...)	Sulphonamides
		Diatrizoate	Ochratoxin A	Thiazide/diuretics
		Diflunisal	Oxypurinol	Thymidine
		Diodrast		Tienilic acid
		Enoxacin		Zidovuine
		Enprofylline		

B. Compounds known to be secreted by or to have affinity for the renal organic base (cation) transport system.

Endogenous compounds	Drugs		
Acetylcholine	Acecainide (N-acetylprocainamide)	Ephedrine	Methadone
Creatinine	Amiloride	Ethambutol	Morphine
Dopamine	Atropine	Famotidine	Pindolol - various other β-blockers
Histamine	Cimetidine	Mecamylamine (Inversine)	Procainamide
Various catecholamines	Disopyramide	Mepacrine	Pseudoephedrine
Serotonin	Emepronium bromide	Metformin	Quinine

Adapted from: Brater et al. [8]; Weiner [15]; Roch-Ramel et al. [19]; VanGinneken and Russel [30]; Bessighir and Roch-Ramel [31].

Abbreviations: cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanidine monophosphate

membrane transport, i.e. it is an organic anion/α-ketoglutarate exchanger, and transport is dependent on the presence of chloride in the extracellular medium. The recent observation that the expression of rat OAT1 is strongly increased at birth fits well with the fact that the PAH secretory system develops post-natal [83].

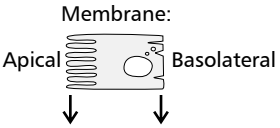
Molecular identification of apical putative PAH transporters.

NPT1 and MPR2 are two organic anion transporters which have been identified by molecular biology techniques, and which, in the kidney, were localized to the apical membrane of proximal tubule. They might be involved in organic anion secretion (Table 5).

Uchino et al. [84] cloned and characterized an apical PAH transporter isolated from human kidney, named NPT1. NPT1 was first identified as a low affinity sodium-dependent phosphate transporter, later it was characterized as an organic anion transporter. In human embryonic kidney cells transfected with NPT1, PAH, urate, benzyl penicillin, faropenem, estradiol-β-glucuronide are transported, and PAH uptake can be inhibited by various organic anions. NPT1 does not function as an organic anion exchanger, and thus is not the PAH/organic anion exchanger observed in rat and dog brush border membrane vesicles. Rabbit NPT1, a homologous of human NPT1, was demonstrated to mediate electrogenic penicillin transport [85], thus, NPT1 might be the PAH voltage sensitive pathway observed in rat and rabbit brush-border membrane vesicles. Further studies should confirm this hypothesis.

Another putative apical PAH transporter is the ATP-dependent export pump MRP2, a multidrug resistance protein isoform characterized by its apical localization in polarized cells such as hepatocytes [49]. In the kidney, MRP2 has been localized to the apical membrane of human and rat proximal tubule [86]. Substrates are anionic conjugates with glutathione (leukotriene C_4) or glucuronide (estradiol-17 β-D-glucuronide), as well as non-conjugated substrates such as probenecid, sulfinpyrazone, indomethacin, furosemide and penicillin [87]. Recently two research groups demonstrated that PAH and ochratoxin are transported substrates [88, 89]. MRP2 thus might contribute to the efflux of PAH and other organic anions at the apical membrane. MPR1 another member of the ATP-dependent export pumps that is associated with multidrug

Table 5. Proximal tubular ion transporters.



Transporters with affinity for PAH identified by molecular biology techniques		
NPT1		Might participate to the cellular efflux of PAH and other organic anions, second step of tubular secretion [85]
MRP2		ATP activated multidrug resistance transporter. Might also participate to PAH and organic anions luminal transport [88, 89]
	OAT1	PAH transporter, sodium and alpha-ketoglutarate dependent. Classical organic anion transporter [39]
	OAT3	PAH transporter independently from alpha-ketoglutarate and sodium [80a]
Transporters without affinity for PAH identified by molecular biology techniques		
OATP1		Transports bile acid, bromosulfophtalein, and conjugate and unconjugated steroid hormones, etc. The main basolateral transporter in hepatocyte, called also moat [50]
OAT-K1		Transports methotrexate and folate (anion exchanger)
OAT-K2		Transports methotrexate, folate, and many hydrophobic anionic compounds have affinity for it. It might participate to the apical transport of many hydrophobic anionic compounds
	SAT1	Sulfate-oxalate exchanger
	NaDC3	Na dependent alpha-ketoglutarate cotransport

resistance in cancer cell and is expressed in a few renal tubular segments, but not in the proximal tubule [90].

Both NPT1 and MRP2 appear to be involved in the apical efflux of organic anions, the second membrane step in secretion. Transport through NPT1 occurs down an electrochemical gradient, whereas MRP2 transport is primarily active.

Molecular identification of organic anion transporters without affinity for PAH

A number of transport molecules have been cloned from different tissues and identified in the renal proximal tubule, which do not transport PAH, but may contribute to the apical efflux of organic secretion [45].

OATP1. The S3 segment of proximal tubule expresses OATP1, an organic anion transporter cloned from rat liver, which transport bile acid, bromosulfophtalein, and conjugated and unconjugated steroid hormones, in a sodium independent manner. Although hepatic OATP1 is expressed in the basolateral membrane (blood side) of hepatocytes, in the kidney it is located in the apical membrane. In the rat renal OATP1 mRNA, but not the hepatic one, is strongly regulated by androgens and to a lesser extent by estrogens. OATP1 might play a role in the renal excretion of estrogens [50]. A homolog of OATP1, OATP3 was isolated from a rat retina and found to be expressed specifically in the retina and in the kidney. It transports taurocholate as well as thyroid hormone (T3 and T4) [91]. A homologous transporter, OATP2, a liver specific transporter, is not expressed in the kidney [91].

OAT-K1 and OAT-K2. These transporters are two homologous organic anion transporters specific to the kidney, which have been identified by molecular cloning strategy [51, 52]. In rats, OAT-K1 was localized in the apical membrane of straight proximal tubules. When expressed in cultured renal epithelial cells, OAT-K1 mediates both uptake and efflux of methotrexate through the apical membrane, and appears to be specific for methotrexate and folate [51]. Non-steroid anti-inflammatory drugs (indomethacin, ketoprofen, ibuprofen, flufenamate, phenylbutazone) inhibit methotrexate OAT-K1 mediated uptake, but are not transported themselves. OAT-K1 appears to be a site for methotrexate and non-steroidal anti-inflammatory drugs interaction [92].

In rats, OAT-K2, as OAT-K1, was localized in the apical membrane of straight proximal tubule [52]. When transfected in cultured epithelial cells, it mediates not only the apical transport of methotrexate and folate but also that of taurocholate and prostaglandin E_2. In cis-inhibition studies, steroids, bile acid analogs, and cardiac glycosides were shown to have a high affinity for OAT-K2, suggesting that it participates to the apical transport of hydrophobic anionic compounds in the kidney [52].

Conclusions

The molecular identification of various organic anion transport proteins, and the characterization of their transport mechanisms in various cell systems, gives an insight in the complexity of the renal secretion of organic anions (Figure 5). Among these numerous transport systems characterized at the molecular level, only OAT1 has a clearly established role, being the most likely candidate of the PAH secretory mechanism. The identification of the apical transporter for the PAH secretory mechanism remains to be established. However, in contrast to a main basolateral transporter, several apical organic anion transporters appear to facilitate the transport of the various substrates accumulated in the proximal cells by OAT1. The respective role of the apical transporters, need to be demonstrated *in situ*. *In vivo* models, such as transgenic mice, will allow the elucidation of the physiological and pharmacological roles of these transport proteins.

Tubular transport of organic cations

Transport mechanisms for organic cations have been investigated not only for the classical substrates, tetraethylammonium and N_1-methylnicotinamide, but also for a few other organic cations, mainly drugs.

Owing to electro-negativity of cell interior, a transfer of positively charged molecules from peritubular interstitium into cells occurs along a favorable electrochemical gradient and does not require energy. In contrast, energy is necessary for the efflux from cell to lumen which takes place against the electropositivity of the lumen. The situation is opposite to that of organic anions for which the active step is the basolateral transport. The mechanisms involved in tubular secretion of organic cations are schematically summarized in Figure 6. Transport of organic cations at the basolateral membrane occurs by a voltage sensitive pathway (mechanism 1), which was described for N_1-

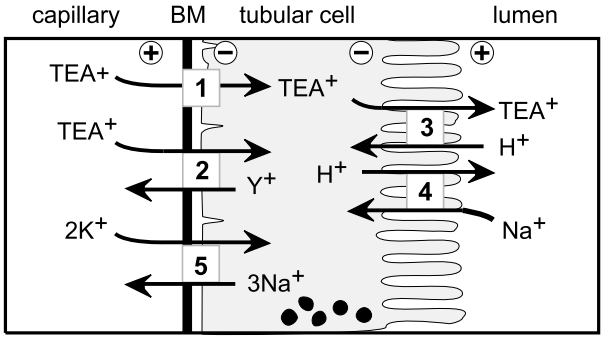


Figure 6. *Model of the organic cation tetraethylammonium transport in proximal tubule.*

methylnicotinamide, tetraethylammonium and/or procainamide in rats, dogs and rabbits. Because of the electronegativity of the cell this facilitated pathway drives organic cations into cells. In rabbits, an organic cation exchanger has also been observed (mechanism 2), the role of which in tubular secretion is unclear. As is described below, the molecular structure of a few isoforms of an organic cation transporter (OCT) has been defined, some of which might be the basolateral transporter of proximal tubule.

The Nernst equation predicts that because of the cell electronegativity, passive facilitated diffusion should allow a concentration ratio cell water/external medium approximating 10 to 15 at steady state. In isolated unperfused proximal tubules from rabbits, ratios exceeding 100 for tetraethylammonium, have been measured [44] and one can wonder if another mechanism exists, for example a cation exchanger, as demonstrated in rabbits (mechanism 2), but which has generally not been observed in rats and dogs [44], which might be implicated in basolateral uptake. However, as reported for anions, there is evidence that part of the tetraethylammonium accumulated into cells is bound to cytoplasmic organelles and that only part of tetraethylammonium is freely diffusible [44, 93]. It has also been demonstrated that endosomal membrane vesicles isolated from rat renal cortex can accumulate tetraethylammonium by an ATP-dependent mechanism [93]. It is conceivable that the favorable transmembrane potential is the principal or single driving force required for cellular uptake.

The efflux from cell to lumen is the active step of organic cation secretion transport being against the transmembrane potential. This active transport occurs through an exchange with protons (mechanism 3), maintained by the proton concentration gradient resulting from the Na^+/H^+ exchange at the same membrane (mechanism 4), mechanism energized itself by the low Na^+ concentration resulting from the Na-K - ATPase activity (mechanism 5). Thus, in all species investigated so far (rats, dogs, rabbits [44], pig [94], humans [95]), tetraethylammonium and N_1-methylnicotinamide were demonstrated to be transported in brush-border membrane vesicles by an electroneutral organic cation exchange system where one organic cation molecule is transported against one proton. Amiloride, cimetidine, morphine, procainamide, disopyramide, gentamicin and verapamil [96] are also transported by such an electroneutral proton exchanger, in rats and/or rabbits. Larger and more hydrophobic compounds (quinidine, quinine, d-tubocurarine, vecuronium) are inhibitors of organic cation transport, but are not transported by the proton-organic cation exchanger. They are transported by another transport mechanism, probably by the MDR1/P-glycoprotein (see below).

Many studies have been performed to characterize the requirements for a substrate to be transported by the "organic cation transport mechanism" [97-100]. As for organic anions, the molecular structure of substrates is rather unspecific. Hydrophobicity and basicity are the general characteristics of substrates, but their ionization is not a prerequisite for interacting with the basolateral carrier [65]. Similar properties were found in brush border membrane [97-100]. Although the ratio of basolateral to apical membrane affinities may vary with substrates and animal species [97].

More than one organic cation/proton exchanger appears to be involved in the transport of organic cations through the apical membrane, and substrates show overlapping affinities for these different exchangers [101]. Thus, proton-stimulated guanidine uptake by brush border membrane vesicles is only minimally inhibited by tetraethylammonium, N_1-methylnicotinamide and choline, whereas amiloride, clonidine, imipramine and harmaline are more potent inhibitors [101]. There are also species differences. Cephalexin, for instance, shows an affinity for the N1-methylnicotinamide or tetraethylammonium transporter in human brush border membranes [95], while in rats it has no affinity for the tetraethylammonium transport mechanism. At present the molecular identity of the organic

cation/H^+ remains unknown, although, as discussed below, two organic cation transporters were recently identified in the kidney, which might be the apical transporters of organic cations.

Multidrug transporter/P-glycoprotein (MDR or Pg)

The apical membrane of proximal tubules is particularly rich in MDR-glycoprotein ("multidrug transporter"), a membrane ATPase that mediates the active efflux of a wide variety of drugs across the plasma membrane of several cell types. This property explains the resistance of some cancer cells to hydrophobic cationic drugs [102]. It was demonstrated that MDR/P-glycoprotein can extrude many organic compounds (e.g. vinblastine, vincristine, colchicine, cyclosporine analogues) from renal proximal cell [103-105]. P-glycoprotein transport mechanism differs from the proton/organic cation exchanger since it does not transport tetraethylammonium [94, 105], but the more lipophilic substrate, and vinblastine, a substrate of MDR/P-glycoprotein, is not exchanged against protons in pig brush border membrane vesicles [93].

MDR/P-glycoprotein, which transports organic cations is the analogous of MRP2, which transport lipophilic organic anions. Both are responsible for the multidrug resistances of cells to anticancer drugs. Since some substrates of the P-glycoprotein system are also transported by the proton/organic cation exchanger, it is often difficult to clearly distinguish between the two systems at the functional level. Compounds transported by both the MDR/P-glycoprotein and the organic cation transporter, include daunomycin, colchicine, verapamil, quinidine and vinblastine [106]. On the other hand, secretion of digoxin, which is not an organic cation, is restricted to P-glycoprotein only [107].

Molecular Identification of putative basolateral organic cation transporter belonging to the OCT family

Expression cloning allowed the identification of several isoforms of a polyspecific organic cation transporter OCT. The molecular biology of these various OCTs have been described in detail by Koepsell et al. [108], Zhang et al. [109] and Burckardt and Wolf [76]. After the cloning of the first organic cation transporter (rOCT1) isolated from a rat kidney [110], a number of homologous cation transporters have been identified [108]. When expressed in various cell systems, OCT1 and OCT2 isoforms demonstrate a broad substrate affinities and a voltage dependent transport. These transport characteristics made them candidates to be the organic cation basolateral transporter of proximal tubule [29, 108, 111]. The HIV protease inhibitors, indinavir, nelfinavir, ritonavir, saquinavir inhibit TEA transport by hOCT1 but they are probably not transported [112]. Inhibitor potency for OCT1 and OCT2 varies with species [108, 109]. In general human hOCT1 interacts with the n-tetraalkylammonium compounds with a lower affinity than that of rats, mice, or rabbits [113]. Among OCT isoforms, rat rOCT1, rOCT2, rOCT3, human hOCT2 and hOCT3, and mice mOCT3 are involved in the renal transport of organic cations. In rats, rOCT1 and rOCT2 are expressed primarily in the kidney, and are localized in the basolateral membrane of proximal tubule [114, 115]. Both probably play a role in organic cation secretion [111]. The expression level of rOCT2 mRNA and protein in males is much higher than in females, which correlates with the higher transport of TEA in male basolateral membrane vesicles and cortical slices [116]. Because no gender differences were observed for rOCT1 expression in the kidney, rOCT2 and not rOCT1 might represent the main renal organic cation transporter in rats. Another isoform, rOCT3, which transport TEA and guanidine, is expressed in many organs including the kidney. However, because its tubular localization is still unknown, its functional role remains to be defined [117]. In the mice mOCT3 mRNA was found to be expressed in the proximal and distal tubule, but the membrane localization is unknown.

In human hOCT1 is expressed in the liver and not in the kidney, whereas hOCT2 is present predominantly in the kidney. However hOCT2, being restricted to the distal convoluted tubule, does not represent the organic cation secretory transporter in human [29](Figure 7). Human OCT3 is expressed in the kidney and also in other organs, its nephron localization has not been determined [117]. There are discrepancies between Gründeman et al. [118] and Wu et al. [117] concerning the substrate affinities for hOCT3. Wu et al demonstrated a broad substrate affinities for hOCT3, which transports various organic cations including, TEA, clonidine, imipramine, procainamide, endogenous amine, etc., whereas Gründeman et al. concluded that hOAT3 is limited to the transport of endogenous organic cations, such as dopamine, histamine, and that

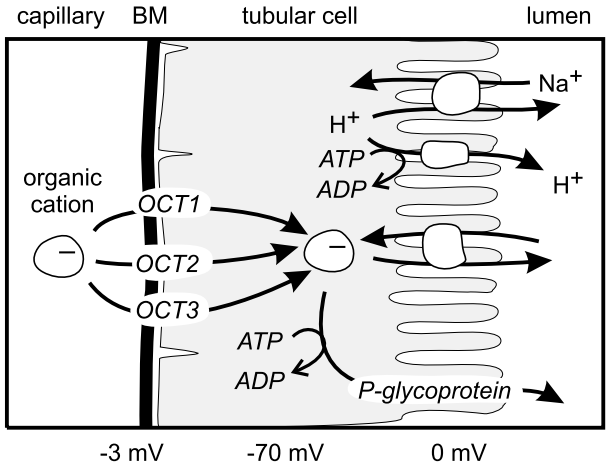


Figure 7. *Mechanisms of organic cation transport in renal tubular cells. Cellular uptake of organic cations across the basolateral membranes (BLM) is mediated primarily by membrane potential-dependent organic cation transporters such as OCT1 (1) and OCT2 (2). OCT3 (3) may contribute in part to the cellular uptake of organic cations. Exit of cellular organic cations across brush border membranes (BBM) is mediated principally by unidentified H^+/organic cation antiporter (4). P-glycoprotein (5) is involved in tubular secretion of hydrophobic drugs such as digoxin, anticancer agents, and some immunosuppressants (cyclosporine and tacrolimus). Adapted from [29].*

it does not transport TEA. At present human OCT3 is the only transporter isoform that has been implicated in renal transport of organic cations. Its presence in the basolateral membrane of proximal tubule must be demonstrated before one can conclude it is the human basolateral secretory transporter of organic cations.

In conclusion, substantial evidence exist that rOCT1 and rOCT2 are involved in the secretion of organic cations in rat proximal tubule. More precise localization of transporters are needed before determining the role of rOCT3 in rats, and hOCT3 in human.

Molecular identification of putative apical organic cation transporters

A few transport mechanisms have been identified in the kidney by expression cloning, which might be involved in the apical step of organic cation secretion, although their function *in situ* has not been established.

OCTN1, OCTN2. Two organic cation transporters, OCTN1 and OCTN2, were identified in the kidney and other organs of rats, mice, rabbits and human, by their homology to the basolateral transporter OCT [53, 54, 119]. When expressed in human embryonic kidney cells and Xenopus oocytes, human OCTN1 mediates the transport of TEA in a pH dependent manner, transport being higher at neutral or alkaline pH than at acidic pH. The transport of TEA was observed to be bidirectional and inhibited by various organic cations, such as choline, clonidine, cimetidine, quinidine, verapamil, etc., and by zwitterionic compounds such as L-carnitine, cephaloridine, levoflaxin. The transport of a few of these inhibitors, quinidine, verapamil, and L-carnitine, was demonstrated [53]. In summary, OCTN1 is a multispecific, bidirectional and pH-dependent organic cation transporter, which is probably energized by a proton antiport mechanism. Although its subcellular localization in the kidney is unknown, the functional characteristics of OCTN1 suggest that it might be involved in the apical step of organic cation secretion.

In the kidney, OCTN2 is expressed predominantly in cells of proximal and distal tubules, as well as in glomeruli. OCTN2 has the same functional characteristics than OCTN1, but the substrate affinities for the transporter differ [54]. Human, rat and mouse OCTN2 has the additional peculiarity of transporting L-carnitine and other zwitterions such as cephalosporins that contain quaternary nitrogen, in a-sodium dependent manner [54, 120, 121]. Site directed mutagenesis experiments provided evidence that the transport sites for organic cations and for carnitine are distinct [122]. OCTN2 thus might play a role in organic cation secretion, and in the reabsorption of carnitine by a sodium carnitine cotransport. *In vivo* cephaloridine was reported to increase the fractional excretion of carnitine by interfering with its reabsorption [120]. The possibility exists that this type of cephalosporin might be inefficient in patients with primary carnitine deficiency, that are receiving carnitine supplementation, because of competition for carnitine transport [120]. The anionic cephalosporins are not substrates for OCTN2, but they are substrates for the peptides transporters PEPT1 and PEPT2 [45]. Conversely, the cephalosporins, which have affinity for OCTN2, are not substrate of the peptide transporters [120].

Effects of protein binding on organic ion secretion

It is generally recognized that the tubular secretory rate is proportional to the concentration of free drug or xenobiotic [123-126], and that plasma albumin bind-

ing is not rate limiting for tubular secretion of organic anions with high affinity for the transport system [19, 36], because the dissociation rate of the organic anion/albumin complex is much faster than the transtubular transit time [19, 127]. Such is the case for hydrochlorothiazide [36]. On the other hand, organic anions with lower affinity for the transporter (e.g. phenol red) have a reduced secretion when bound to plasma proteins [124, 128]. Although the secretion of furosemide in the perfused isolated rat kidney can be delayed by the addition of albumin to the perfusate [123], secretion in humans does not appears to be limited by protein binding. Thus, in spite of a binding of more than 95% to plasma proteins, the urinary clearance (uncorrected for plasma protein binding) of furosemide in therapeutic doses is somewhat higher than inulin clearance [36, 129, 130]. Because of the high protein binding of furosemide its filtration rate is negligible and its diuretic effect, which is related to its luminal concentration in the thick ascending limb of Henle's loop, depends on its tubular secretion. Thus, inhibition of furosemide secretion by probenecid inhibits its diuretic effect [131].

Interactions of xenobiotics/drugs for secretion

Probenecid, which was first developed to delay penicillin excretion, is now generally used (besides its use as a uricosuric) to inhibit secretion of organic anions. Thus, it is generally considered that a compound whose secretion or transport across the proximal basolateral membrane is inhibited by probenecid is a substrate of the organic anion secretory mechanism. Probenecid has also been used as a tool to investigate the role of cellular accumulation of xenobiotics in nephrotoxicity. Inhibition of basolateral uptake of cephalosporins, such as cephaloridine and cephaloglycin, by probenecid, can prevent their cellular toxicity. These cephalosporins have a low extrusion rate through the apical membrane, resulting in a rather high concentration, which is a major contributing factor to their nephrotoxicity. However, it is worth noting that cell accumulation is necessary but not sufficient for cytotoxicity, as shown by cephalexin that has a low nephrotoxic potential despite marked cortical accumulation [40] (see also chapter 9).

The nephrotoxicity of cisplatin is reduced in humans [132], mice [133] and dogs [134] by co-administration of probenecid, suggesting that cisplatin is transported by the p-aminohippurate transport system. It has been proposed that platinum, like other nephrotoxic metal ions such as mercury and potassium dichromate, are taken up by tubular cells as sulphydryl conjugate through a probenecid-sensitive pathway [133]. However, cisplatin might also be transported by the organic cation transport system, since quinidine, cimetidine and ranitidine inhibited its net secretion flux in the dog kidney [134].

In human, methotrexate is largely cleared unchanged from the body by renal excretion through glomerular filtration and tubular secretion. Rises in serum methotrexate levels accompanied by life-threatening increases in methotrexate toxicity can occur if aspirin, salicylates or non-steroidal anti-inflammatory drugs are given concurrently. The increased methotrexate toxicity observed by concomitant administration of ibuprofen [135], salicylates [135], or flurbiprofen [136] might be in part the result of interaction at the basolateral membrane [137], resulting in a decrease in methotrexate renal excretion.

Excretion of digoxin is primarily renal, by glomerular filtration and tubular secretion and reabsorption. Competition studies have shown that the "classic" anion or cation transport systems are not involved. The secretory process as studied *in vivo* and in a renal epithelium *in vitro*, may be carried out by the apical membrane P-glycoprotein. It is well known clinically, that several drugs (most notably quinidine, verapamil, nifedipine, propofenone, spironolactone, and amiodarone) reduce the renal (tubular) clearance of digoxin and increase the plasma concentration and toxic risks of the cardiac glycoside [63, 138, 139]. Accordingly, these interactions may be explained by a competition at the secretory step controlled by P-glycoprotein at the luminal membrane. Such a possibility has received experimental support for several of these compounds [139-143]

Concurrent use of drugs that reduce renal blood flow in patients with renin-angiotensin prostaglandin dependent renal perfusion (e.g. NSAID), that are weak organic acids competing for tubular secretion [144] and/or nephrotoxic (cisplatin) can delay drug excretion [145] and lead to severe myelosuppression.

Interactions of cimetidine and other H_2-receptor antagonists with the renal secretion of several drugs have been repeatedly described, and comprehensively listed [146]. Thus, cimetidine inhibits renal secretion

of procainamide in humans and prolongs its elimination half-life [147, 148]. Similar inhibitory effects have been shown on creatinine, ranitidine and many other cationic compounds [149].

Interactions between organic anion and organic cation secretion

The lack of strict structural requirements for substrates in organic anion and cation transport systems, the prominent role of substrate hydrophobicity in the interaction with both classes of carriers, and the ability of non-ionized substrates to interact with the transporters, are all factors explaining that some substrates might be transported by both transport systems [37]. For example, the renal excretion of cimetidine and famotidine, two organic cations, is reduced by probenecid [150, 151]. *In vitro* also, cimetidine uptake by brush border membrane vesicles is inhibited by probenecid or furosemide, and cimetidine in turn can inhibit p-aminohippurate uptake, demonstrating the existence of some link between organic anion and cation transport [152-155]. Such observations appear to overturn the dogma of distinct transport systems for organic ions. Some compounds have chemical characteristics ("zwitterions"), which account for their particular substrate behavior: creatinine [156], amino-cephalosporins (e.g. cephaloridine) and gyrase inhibitors [157] bear both positive and negative charges, and are therefore "bisubstrates" [37]. Cimetidine has affinity for the organic cation basolateral transporter through its imidazole group, while hydrophobicity of the molecule and electronegativity of the cyanoguanidine group explain the affinity of the drug for the organic anion transporter [158]. Famotidine and ranitidine have a guanidine group and a nucleophilic side-chain accounting for the affinity for both transport systems [158]. Clonidine and pilocarpine are other imidazole derivatives interacting with both transporters [158]. Zidovudine secretion in rats might also proceed through both transport mechanisms [159], though the anion transporter appears to predominate [160, 161]. As reported above, cisplatin also appears to be transported by both transport systems.

Many other compounds interact at the basolateral membrane with the p-aminohippurate and the organic cation transport systems as was demonstrated by the systematic studies by Ullrich et al. [37].

Metabolism of drugs/xenobiotics in the kidney

Metabolic transformation is the biological conversion of a drug to another chemical form, occurring mainly in the liver, although many other tissues, among them the kidney are also capable of drug metabolism. Microsomal enzymes are responsible for oxidation, acetylation, conjugation (acylglucuronidation, N-glucuronidation, glycination) hydrolysis of drugs and xenobiotics. The usual result of this enzymatic conversion is drug metabolites, which are more polar, and less lipid soluble than the parent compound and consequently favoring renal excretion. The same enzymatic pathways for drug metabolism present in the liver are also found in the kidney, although the specific activity of these pathways in the kidney is substantially lower than those in the liver [2, 162]. In contrast to the liver, the metabolic pathways in the kidney are not uniformly distributed throughout the kidney, they are localized to specific nephron segments (Table 6). Examples of drug metabolism by the isolated perfused kidney are oxidation of bumetanide [163], acetylation of sulphisoxazole [164], conjugation of salicylic acid [165], and esterolysis of enalapril to enalaprilat [166].

The role of renal enzyme systems involved in the metabolism of drugs and their potential nephrotoxicity is well documented in the case of analgesic mixtures containing acetylsalicylic acid, acetaminophen and/or phenacetin combined with addicting compounds such as caffeine and codeine [167]. The kidney can metabolize acetaminophen to glucuronyl and sulphate conjugates but also to an arylating intermediate via the cytochrome P-450 mixed function oxidase system [168, 169]. The intra-renal distribution of this enzyme system explains the proximal tubular localization of acute acetaminophen toxicity [170]. Several observations in the Fischer rat suggests that this acute renal toxicity is mediated through the cytochrome P-450 mechanism [168].

Renal metabolism of isoproterenol [171], bumetanide [163], cimetidine [172] and N-methylnicotinamide [173] has been reported. Renal metabolites may have different mode of excretion [174], and may be more nephrotoxic than the original substance [175]. Renal glucuronidation may be substantial as in the case of morphine [176]. Xenobiotic glucuronidation can proceed by linkage through an ether or an ester bound.

Table 6. Intrarenal distribution of enzymes that participate in the biotransformation of xenobiotics.

	Renal cortex or proximal tubule	Outer medulla	Inner medulla
Cytochrome P-450 MFO	++++	++	trace
NADPH cytochrome reductase	++++	++	++
UDP-glucuronosyl transferase	++++	++	-
Sulfotransferase	++++	++	-
GSSG reductase	++++	++	++
Gamma-glutamyltranspeptidase	++++	++	trace
Glutathione peroxidase-I	++++	++	trace
Glutathione peroxidase-II	++++	++	+
Prostaglandin endoperoxide synthetase	trace	++	++++

++++ = highest activity among the three regions of the kidney irrespective of the absolute activity.
Adapted from Kaloyanides [32] with permission.

The latter process is called "acyl-glucuronide" characterized by instability under physiological conditions such that the glucuronide can deconjugate back to the parent compound (futile cycle). In patients with normal renal function, acyl-glucuronides are readily eliminated in the urine. In patients with renal insufficiency, the conjugate accumulates in plasma where it can spontaneously hydrolyse to reform the parent compound. This phenomenon, demonstrated for clofibrate [177, 178] diflunisal [179, 180] and some NSAID [181, 182], leads to a paradox in which a drug may accumulate in patients with renal insufficiency even through negligible amounts of parent drug are eliminated in the urine of patients with normal renal function.

The main role of the kidney in the process of drug metabolism consists in the excretion of the many, more or less pharmacologically active metabolites formed in the liver [8]. Needles to say that renal insufficiency may result in the accumulation of metabolites and, if pharmacological active, may result in serious side effects/toxicity [33]. Renal metabolism of drug-xenobiotics and its contribution to elimination has been inadequately explored so that clinical implications are for the most part inferred from animal models or speculative.

The impact of knowledge of renal handling on drugs and xenobiotics on their clinical use is clearly demonstrated with the aminoglycosides (see chapter 8).

The development of imipenem is another convincing demonstration of how insight into the renal handling/metabolism of a particular drug has succeeded in maintaining its interesting pharmacological activity and preventing its nephrotoxic effect. Indeed during the development of the carbapenem's, imipenem (N-formidoyl derivative of thienamycin) had the highest potency broad spectrum activity and lack of cross-resistance. However the product was nephrotoxic and furthermore there was an extensive renal metabolism of imipenem resulting in low urinary concentrations of the antibiotic [183]. A renal dipeptidase, dehydropeptidase-I, was responsible for hydrolysing imipenem. The strategic subcellular localization of this dehydropeptidase-I at the luminal membrane of the proximal tubular epithelium, accounts for its impact on the renal handling of imipenem. Indeed, the enzyme has access to the antibiotic, both in the glomerular ultrafiltrate and the transcellular flux between the blood and the lumen mediated by anionic secretion. In other words, systemic persistence of the antibiotic is insulated from renal metabolism, whereas urinary tract bioavailability is drastically reduced. In order to counter these effects, a specific dehydropeptidase-I-inhibitor, cilastatin was developed. Once cilastatin was combined with imipenem, the renal clearance rate of the antibiotic was dramatically elevated well above the glomerular filtration rate. This indicates that there is a significant secretory component of imipenem that is revealed only when its metabolism is blocked by the dehydropeptidase-I inhibitor. When probenecid was added, it caused an immediate drop in renal clearance to the level of the glomerular filtration rate, it induced a significant reduction in the plasma clearance rate [183]

and nephrotoxicity was prevented. Probenecid is presumed to inhibit the active uphill transport of imipenem into the tubular epithelium, transport that is evidenced when intracellular metabolism of imipenem by dehydropeptidase-I is simultaneously blocked by cilastatin. The disappearance of the nephrotoxic effect of imipenem when associated with cilastatin, is due to the combined effect of cilastatin reducing the secretory transport of imipenem at proximal tubular level [184] and the mitochondrial protection of cilastatin towards the powerful acylating potential of imipenem [185]. Furthermore, cilastatin blocks intracellular and luminal metabolism of imipenem by dehydropeptidase-I increasing dramatically the renal clearance rate - urinary concentration of the antibiotic.

The combination of imipenem and cilastatin overcame the pharmacokinetic [184], metabolic and toxicological challenges presented by this powerful antibiotic.

References

1. Brezis M, Rosen S, Silva P, Epstein FH. Renal ischemia: a new perspective. Kidney Int 1984; 26: 374-383.
2. Rush GF, Smith JH, Newton JF, Hook JB. Chemically induced nephrotoxicity: role of metabolic activation. CRC Crit Rev Toxicol 1984; 13: 99-160.
3. Hook JB, Hewitt WR. Toxic responses of the kidney. In: Casarett & Doull's Toxicology. The basic science of poisons, 3rd edition. Klaassen CD, Amdur MO, Doull J (editors). Macmillan, New York 1986; p. 310-329.
4. Tay LK, Bregman CL, Masters BG, Williams PD. Effects of cis-diamminedichloroplatinum (II) on rabbit kidney *in vivo* and on rabbit renal proximal tubule cells in culture. Cancer Res 1988; 48: 2538-2543.
5. Williams PD. The application of renal cells in culture in studying drug-induced nephrotoxicity. *In vitro* 1989; 25: 800-805.
6. Williams PD, Rush GF. An evaluation of *in vitro* models for assessing nephrotoxicity. In: *In vitro* toxicity testing. Frazier JM (editor). Marcel Dekker Inc, New York/Basel/Hong Kong 1992; p. 85-110.
7. Kastner S, Soose M, Stolte H. Human kidney in *in vitro* pharmaco-toxicology. In: Human cells in *in vitro* pharmaco-toxicology: present status within Europe. Rogiers V, Sonck W, Shephard E, Vercruysse A (editors). VUBPress, Brussels 1993; p. 197-238.
8. Brater DC, Sokal PP, Hall SD, McKinney TD. Disposition and dose requirements of drugs in renal insufficiency. In: The kidney: physiology and pathophysiology. Seldin DW, Giebisch G (editors). Raven Press Ltd, New York 1992; p. 3671-3695.

8a. John EG & Guignard JP. Development of renal excretion of drugs during ontogeny. In: Neonatal and fetal medicine. Polin RA & Fow WW Eds. Saunders WB, Philadelphia. pp 188-193, 1997.

9. Kragh-Hansen U. Molecular aspects of ligand binding to serum albumin. Pharmacol Rev 1981; 33: 17-53.
10. Routledge PA. The plasma protein binding of basic drugs. Brit J Clin Pharmacol 1986; 22: 499-506.
11. Green TP, Mirkin BL. Resistance of proteinuric rats to furosemide: urinary drug protein bindings as a determinant of drug effect. Life Sci 1980; 26: 623-630.
12. Green TP, Mirkin BL. Furosemide disposition in normal and proteinuric rats: urinary drug protein bindings as a determinant of drug excretion. J Pharmacol Exp Ther 1981; 218: 122-127.
13. Kirchner KA, Voelker JR, Brater DC. Intratubular albumin blunts the response to furosemide - a mechanism for diuretic resistance in the nephrotic syndrome. J Pharmacol Exp Ther 1990; 252: 1097-101.
14. Kirchner KA, Voelker JR, Brater DC. Binding inhibitors restore furosemide potency in tubule fluid containing albumin. Kidney Int 1991; 40: 418-424.
15. Weiner IM. Organic acids and bases and uric acid. The kidney: physiology and pathophysiology. Seldin DW, Giebisch G (editors). Raven Press Ltd, New York 1985; p. 1703-1724.
16. Roch-Ramel F, Roth L, Arnow J, Weiner LM. Salicylate excretion in the rat; free flow micropuncture experiments. J Pharmacol Exp Ther 1978; 207: 737-747.
17. Brater DC. Drugs and the kidney: renal contribution to handling of drugs. In: Handbook of renal therapeutics. Martínez-Maldonado M (editor). Plenum Medical Book Company, New York/London 1983; p. 191-206.
18. Goodman LS, Gilman A, Rall TW, Nies AS, Taylor P (editors). The pharmacological basis of therapeutics. Pergamon Press, New York 1990.
19. Roch-Ramel F, Besseghir K, Murer H. Renal excretion and tubular transport of organic anions and cations. In: Handbook of physiology, section 8: renal physiology. Windhager EE (editor). Oxford University Press, New York/Oxford 1992; p. 2189-2262.
20. Guggino SE, Aronson PS. Paradoxical effects of pyrazinoate and nicotinate on urate transport in dog renal microvillus membranes. J Clin Invest 1985; 76: 543-547.
21. Bessighir K, Roch-Ramel F. Pyrazinoate transport in the isolated perfused rabbit proximal tubule. Pflügers Arch 1986; 407: 643-648.
22. Elion GB, Yü TF, Hitchings GH. Renal clearance of oxipurinol, the chief metabolite of allopurinol. Am J Med 1968; 45: 69-77.

23. May DG, Weiner IM. The renal mechanisms for the excretion of m-hydroxybenzoic acid in cebus monkeys: relationship to urate transport. J Pharmacol Exp Ther 1971; 176: 407-417.
24. Van Crugten JT, Sallustio BC, Nation RL, Somogyi A. Renal tubular transport of morphine, morphine-6-glucuronide, and morphine-3-glucuronide in the isolated perfused rat kidney. Drug Metab Disp 1991; 19: 1087-1092.
25. Wright SH, Wunz TM, Wunz TP. A choline transporter in renal brush-border membrane vesicles - energetics and structural specificity. J Membrane Biol 1992; 126: 51-65.
26. Daniel H, Herget M. Cellular and molecular mechanisms of renal peptide transport. (review). Am J Physiol 1997; 273: F1-F8.
27. Shen H, Smith DE, Yang T, Huang YG, Schnermann JB, Brosius FCI. Localization of PEPT1 nand PEPT2 proton-coupled oligopeptide cotransporter mRNA and protein in rat kidney. Am J Physiol 1999; 276: F658-F665.
28. Keppler D, Leier I, Jedlitschky G. Transport of glutathione conjugates and glucuronides by the multidrug resistance proteins MRP1 and MRP2 (review). Biol Chem 1997; 378: 787-791.
29. Inui K, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int 2000; 58: 944-958.
30. Foulkes EC. Transport of heavy metals by the kidney. Toxicology Letters 1990; 33: 29-31.
31. Templeton DM. Cadmium uptake by cells of renal origin. J Biol Chem 1990; 265: 21764-21770.
32. De Broe ME, Paulus GJ, Verpooten GA, Roels F, Buyssens N, Wedeen RP, Van Hoof F, Tulkens PM. Early effects of gentamicin, tobramycin and amikacin on the human kidney. A prospective, comparative study. Kidney Int 1984; 25: 643-652.
33. Laurent G, Tulkens PM. Aminoglycoside nephrotoxicity: cellular and molecular aspects. ISI Atlas of Science - Pharmacology 1987; 1: 40-44.
34. Van Ginneken CAM, Russel FGM. Saturable pharmacokinetics in the renal excretion of drugs. Clin Pharmacokinetics 1989; 16: 38-54.
35. Besseghir K, Roch-Ramel F. Renal excretion of drugs and other xenobiotics. Renal Physiol 1987; 10: 221-241.
35a. Dresser, M. J., Leabman, M. K., and Giacomini, K. M. Transporters involved in the elimination of drugs in the kidney: Organic anion transporters and organic cation transporters [Review]. Journal of Pharmaceutical Sciences 90(4), 397-421. 2001.
35b. Berkhin, E. B. and Humphreys, M. H. Regulation of renal tubular secretion of organic compounds [Review]. Kidney International 59, 17-30. 2001.
36. Møller JV, Sheikh MI. Renal organic anion transport system: pharmacological, physiological, and biochemical aspects. Pharmacol Rev 1983; 34: 315-358.
37. Ullrich KJ. Specificity of transporters for organic anions and organic cations in the kidney. Biochim Biophys Acta 1994; 1197: 45-62.
38. Burckhardt G, Wolff NA. Structure of renal organic anion and cation transporters (review). Am J Physiol Renal Physiol 2000; 278(6): F853-F866
39. Sekine T, Cha SH, Endou H. The multispecific organic anion transporter (OAT) family (review). Pflügers Arch 2000; 440(3): 337-350.
40. Tune BM. Renal tubular transport and nephrotoxicity of β-lactam antibiotics: structure-activity relationships. Miner Electrolyte Metab 1994; 20: 221-231.
41. Dantzler WH, Evans KK, Groves CE, Welborn JR, North J, Stevens JL, Wright SH. Relation of cysteine conjugate nephrotoxicity to transport by the basolateral organic anion transport system in isolated S2 segments of rabbit proximal renal tubules. J Pharm Exp Ther 1998; 286: 52-60.
42. Berndt WO. The role of transport in chemical nephrotoxicity (review). Tox Pathol 1998; 26: 52-57.
43. Kosoglou T, Vlasses PH. Drug interactions involving renal transport mechanisms: an overview. CICP Ann Pharmacother 1989; 23: 116-22.
44. Pritchard JB, Miller DS. Mechanisms mediating renal secretion of organic anions and cations. Physiol Rev 1993; 73: 765-796.
45. Roch-Ramel F. Renal transport of organic anions (review). Curr Opin Nephrol Hypertens 1998; 7(5): 517-524.
46. Karniski LP, Lotscher M, Fucentese M, Hilfiker H, Biber J, Murer H. Immunolocalization of sat-1 sulfate/oxalate/bicarbonate anion exchanger in the rat kidney. Am J Physiol 1998; 275(1 Pt 2): F79-F87.
47. Wang H, Fei YJ, Kekuda R, Yang-Feng TL, Devoe D, Leibach FH, Prasad PD, Ganapathy V. Structure, function, and genomic organization of human Na^+-dependent high-affinity dicarboxylate transporter. Am J Physiol 2000; 278: C1019-C1030.
48. Klein I, Sarkadi B, Varadi A. An inventory of the human ABC proteins (review). Biochim Biophys Acta 1999; 1461: 237-262.
49. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins (review). J Nat Cancer Inst 2000; 92: 1295-1302.
50. Lu R, Kanai N, Bao Y, Wolkoff AW, Schuster VL. Regulation of renal OATP mrna expression by testosterone. Am J Physiol 1996; 270(2 Pt 2): F332-F337.
51. Masuda S, Ibaramoto K, Takeuchi A, Saito H, Hashimoto Y, Inui KI. Cloning and functional characterization of a new multispecific organic anion transporter, OAT-K2, in rat kidney. Mol Pharmacol. 1999; 55(4): 743-752
52. Masuda S, Takeuchi A, Saito H, Hashimoto Y, Inui K. Functional analysis of rat renal organic anion transporter OAT-K1: bidirectional methotrexate transport in apical membrane. FEBS Letters 1999; 459: 128-132.

53. Yabuuchi H, Tamai I, Nezu J, Sakamoto K, Oku A, Shimane M, Sai Y, Tsuji A. Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. J Pharmacol Exp Ther 1999; 289: 768-773.
54. Wu X, Huang W, Prasad PD, Seth P, Rajan DP, Leibach FH, Chen JW, Conway SJ, Ganapathy V. Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. J Pharmacol Exp Ther 1999; 290: 1482-1492.
55. Miller DS, Stewart DE, Pritchard JB. Intracellular compartmentation of organic anions within renal cells. Am J Physiol 1993; 264: R882-R890.
56. Masereeuw R, vandenBergh EJ, Bindels RJ. Characterization of fluorescein transport in isolated proximal tubular cells of the rat: evidence for mitochondrial accumulation. J Pharmacol Exp Ther 1994; 269: 1261-1267.
57. Pajor AM. Sequence and functional characterization of a renal sodium/dicarboxylate cotransporter. J Biol Chem 1995; 270(11): 5779-5785.
58. Lemieux G, Junco E, Perez R, Lemieux C, Allignet E. The metabolic response of the kidney to acute sodium lactate alkalosis. Can J Physiol Pharmacol 1985; 63: 687-692.
59. Miller DS, Barnes DM, Pritchard JB. Confocal microscopic analysis of fluorescein compartmentation within crab urinary bladder cells. Am J Physiol 1994; 267: R16-R25.
60. Miller DS, Pritchard JB. Nocodazole inhibition of organic anion secretion in teleost renal proximal tubules. Am J Physiol 1994; 267: R695-R704.
61. Ullrich KJ, Rumrich G. Luminal transport step of para-aminohippurate (PAH) - transport from pah-loaded proximal tubular cells into the tubular lumen of the rat kidney *in vivo*. Pflügers Arch 1997; 433(6): 735-743
62. Roch-Ramel F, Werner D, Guisan B. Urate transport in brush-border membrane of human kidney. Am J Physiol 1994; 266: F797-F805.
63. Roch-Ramel F, Guisan B, Schild L. Indirect coupling of urate and p-aminohippurate transport to sodium in human brush-border membrane vesicles. Am J Physiol 1996; 270: F165-F272.
64. Schmitt C, Burckhardt G. p-Aminohippurate/2-oxoglutarate exchange in bovine renal brush-border and basolateral membrane vesicles. Pflügers Arch 1993; 423: 280-290.
65. Ullrich KJ, Rumrich G. Renal contraluminal transport systems for organic anions (p-aminohippurate, p-aminohippurate) and organic cations (N1-methyl-nicotinamide, NMeN) do not see the degree of substrate ionization. Pflügers Arch 1992; 421: 286-288.
66. Troehler U, Bonjour JP, Fleisch H. Renal secretion of diphosphonates in rats. Kidney Int 1975; 8: 6-13.
67. Lin JH, Chen IW, Deluna FA, Hichens M. Renal handling of alendronate in rats. An uncharacterized renal transport system. Drug Metab Dispos 1992; 20: 608-613.
68. Russel FGM, Vermeulen WG. Effect of substituted benzoylglycines (hippurates) and phenylacetylglycines on p-aminohippurate transport in dog renal membrane vesicles. Pharmaceut Res 1994; 11: 1829-1833.
69. Russel FGM, Heijn M, De Laet RC, Van Ginneken CAM. Effect of substituted benzoates on p-aminohippurate transport in dog renal membrane vesicles. Naunyn-Schmiedeberg's Arch Pharmacol 1991; 343: 102-107.
70. Hosoyamada M, Sekine T, Kanai Y, Endou H. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. Am J Physiol 1999; 276: F122-F128.
71. Van Aubel RAMH, Masereeuw R, Russel FGM. Molecular pharmacology of renal organic anion transporters (review). Am J Physiol Renal Physiol 2000; 279(2): F216-F232.
72. Uwai Y, Saito H, Hashimoto Y, Inui KI. Interaction and transport of thiazide diuretics, loop diuretics, and acetazolamide via rat renal organic anion transporter rOAT1. J Pharmacol Exp Ther 2000; 295: 261-265.
73. Wada S, Tsuda M, Sekine T, Cha SH, Kimura M, Kanai Y, Endou H. Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir, and other antiviral nucleoside analogs. J Pharmacol Exp Ther 2000; 294: 844-849.
74. Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, Sweet DH. The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. Mol Pharmacol 1999; 56: 570-580.
75. Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H. Expression cloning and characterization of a novel multispecific organic anion transporter. J Biol Chem 1997; 272: 18526-18529.
76. Burckhardt BC, Wolff NA, Burckhardt G. Electrophysiologic characterization of an organic anion transporter cloned from winter flounder kidney (fROAT). J Am Soc Nephrol 2000; 11(1): 9-17.
77. Race J E, Grassl SM, Williams WJ, Holtzman EJ. Molecular cloning and characterization of two novel human renal organic anion transporters (hOAT1 and hOAT3). Biochem Biophys Res Comm 1999; 255: 508-514.
78. Boner G, Steele TH. Relationship of urate and p-aminohippurate secretion in man. Am J Physiol 19973; 225: 100-104.
79. Uwai Y, Okuda M, Takami K, Hashimoto Y, Inui K. Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. FEBS Letters 1998; 438: 321-324.
80. Lu R, Chan BS, Schuster VL. Cloning of the human kidney PAH transporter: narrow substrate specificity and regulation by protein kinase C. Am J Physiol 1999; 276: F295-303.

80a. Cha, S. H., Sekine, T., Fukushima, J., Kanai, Y., Kobayashi, Y., Goya, T., and Endou, H. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. Molecular Pharmacology 59(5), 1277-1286. 2001.
81. Sekine T, Cha S, Kanai Y, Endou H. Molecular biology of multispecific organic anion transporter family (OAT family). Clin Exp Nephrol 1999; 3: 237-243.
82. Kusuhara H, Sekine T, Utsunomiya-Tate N, Tsuda M, Kojima R, Cha SH, Sugiyama Y, Kanai Y, Endou H. Molecular cloning and characterization of a new multispecific organic action transporter from rat brain. J Biol Chem 1999; 274: 13675-13680.
83. Nakajima N, Sekine T, Cha SH, Tojo A, Hosoyamada M, Kanai Y, Yan K, Awa S, Endou H. Developmental changes in multispecific organic anion transporter 1 expression in the rat kidney. Kidney Int 2000; 57: 1608-1616.
84. Uchino H, Tamai I, Yamashita K, Minemoto Y, Sai Y, Yabuuchi H, Miyamoto K, Takeda E, Tsuji A. p-aminohippuric acid transport at renal apical membrane mediated by human inorganic phosphate transporter NPT1. Biochem Biophys Res Comm 2000; 270: 254-259.
85. Busch AE, Schuster A, Waldegger S, Wagner CA, Zempel G, Broer S, Biber J, Murer H, Lang F. Expression of a renal type I sodium/phosphate transporter (NaPi-1) induces a conductance in Xenopus oocytes permeable for organic and inorganic anions. Proc Natl Acad Sci USA 1996; 93(11): 5347-5351.
86. Schaub TP, Kartenbeck J, Konig J, Spring H, Dorsam J, Staehler G, Storkel S, Thon WF, Keppler D. Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. J Am Soc Nephrol 1999; 10: 1159-1169.
87. Bakos E, Evers R, Sinko E, Varadi A, Borst P, Sarkadi B. Interactions of the human multidrug resistance proteins MRP1 and MRP2 with organic anions. Mol Pharmacol 2000; 57: 760-768.
88. Leier I, Hummel-Eisenbeiss J, Cui YH, Keppler D. ATP-dependent para-aminohippurate transport by apical multidrug resistance protein MRP2. Kidney Int 2000; 57: 1636-1642.
89. Van Aubel RAMH, Peters JGP, Masereeuw R, van Os CH, Russel FGM. Multidrug resistance protein Mrp2 mediates ATP-dependent transport of classic renal organic anion p-aminohippurate. Am J Physiol Renal Physiol 2000; 279(4): F713-F717.
90. Peng KC, Cluzeaud F, Bens M, Van Huyen JP, Wioland MA, Lacave R, Vandewalle A. Tissue and cell distribution of the multidrug resistance-associated protein (MRP) in mouse intestine and kidney. J Histochem Cytochem 1999; 47: 757-768.
91. Abe T, Kakyo M, Sakagami H, Tokui T, Nishio T, Tanemoto M, Nomura H, Hebert SC, Matsuno S, Kondo H, Yawo H. Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate and comparison with oatp2. J Biol Chem 1998; 273: 22395-22401.
92. Masuda S, Saito H, Inui KI. Interactions of nonsteroidal anti-inflammatory drugs with rat renal organic anion transporter, OAT-K1. J Pharmacol Exp Ther 1997; 283: 1039-1042.
93. Pritchard JB, Sykes DB, Walden R, Miller DS. ATP-dependent transport of tetraethylammonium by endosomes isolated from rat renal cortex. Am J Physiol 1994; 266: F966-F976.
94. Dutt A, Heath LA, Nelson JA. P-glycoprotein and organic cation secretion by the mammalian kidney. J Pharmacol Exp Ther 1994; 269: 1254-1260.
95. Ott RJ, Hui AC, Yuan G, Giacomini KM. Organic cation transport in human renal brush-border membrane vesicles. Am J Physiol 1991; 261: F443-F451.
96. Takahashi Y, Itoh T, Kobayashi M, Sugawara M, Saitoh H, Iseki K, Miyazaki K, Miyazaki S, Takada M, Kawashima Y. The transport mechanism of an organic cation, disopyramide, by brush-border membranes: comparison between renal cortex and small intestine of the rat. J Pharm Pharmacol 1993; 45: 419-424.
97. Wright SH, Wunz TM, Wunz TP. Structure and interaction of inhibitors with the tetraethylammonium/H^+ exchanger of rabbit renal brush border membranes. Pflügers Arch 1992; 429: 313-324.
98. David C, Rumrich G, Ullrich KJ. Luminal transport system for H+/organic cations in the rat proximal tubule. Kinetics, dependence on pH; specificity as compared with the contraluminal organic cation-transport system. Pflüugers Arch 1995; 430(4): 477-492.
99. Wright S H, Wunz TM. Influence of substrate structure on turnover of the organic cation/H+ exchanger of the renal luminal membrane. Pflügers Arch 1998; 436: 469-477.
100. Wright SH, Wunz TM. Influence of substrate structure on substrate binding to the renal organic cation H+ exchanger. Pflügers Arch 1999; 437: 603-610.
101. Miyamoto Y, Tiruppathi C, Ganapathy V, Leibach FH. Multiple transport systems for organic cations in renal brush-border membranes vesicles. Am J Physiol 1989; 256: F540-F548.
102. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. Pharmacol Rev 1990; 42: 156-199.
103. Speeg KV, Maldonado AL, Liaci J, Muirhead D. Effect of cyclosporine on colchicine secretion by the kidney multidrug transporter studied *in vivo*. J Pharmacol Exp Ther 1992; 261: 50-55.
104. De Lannoy IAM, Mandin RS, Silverman M. Renal secretion of vinblastine, vincristine and colchicine *in vivo*. J Pharmacol Exp Ther 1994; 268: 388-395.

105. Schramm U, Fricker G, Wenger R, Miller DS. P-glycoprotein-mediated secretion of a fluorescent cyclosporin analogue by teleost renal proximal tubules. Am J Physiol 1995; 268: F46-F52.
106. Horio M, Pastan I, Gottesma MM, Handler JS. Transepithelial transport of vinblastine by kidney-derived cell lines. Application of a new kinetic model to estimate *in situ* Km of the pump. Biochim Biophys Acta 1990; 1027: 116-122.
107. Koren G. Clinical pharmacokinetic significance of the renal tubular secretion of digoxin. Clin Pharmacokin 1987; 13: 334-343.
108. Koepsell H, Gorboulev V, Arndt P. Molecular pharmacology of organic cation transporters in kidney (review). J Membr Biol 1999; 167: 103-117.
109. Zhang L, Brett CM, Giacomini KM. Role of organic cation transporters in drug absorption and elimination (review). Annual Rev Pharmacol Toxicol 1998; 38: 431-460.
110. Grundemann D, Gorboulev V, Gambaryan S, Veyhl M, Koepsell H. Drug excretion mediated by a new prototype of polyspecific transporter. Nature 1994; 372: 549-552.
111. Okuda M, Urakami Y, Saito H, Inui K. Molecular mechanisms of organic cation transport in OCT2-expressing Xenopus oocytes. Biochim Biophys Acta 1999; 1417: 224-231.
112. Zhang L, Gorset W, Washington CB, Blaschke TF, Kroetz DL, Giacomini KM. Interactions of HIV protease inhibitors with a human organic cation transporter in a mammalian expression system. Drug Metab Dispos 2000; 28: 329-334.
113. Dresser MJ, Gray AT, Giacomini KM. Kinetic and selectivity differences between rodent, rabbit, and human organic cation transporters (OCT1). J Pharmacol Exp Ther 2000; 292: 1146-1152.
114. Karbach U, Kricke J, Meyer-Wentrup F, Gorboulev V, Volk C, Loffing-Cueni D, Kaissling B, Bachmann S, Koepsell H. Localization of organic cation transporters OCT1 and OCT2 in rat kidney. Am J Physiol Renal Physiol 2000; 279(4): F679-F687.
115. Sugawara-Yokoo M, Urakami Y, Koyama H, Fujikura K, Masuda S, Saito H, Naruse T, Inui K, Takata K. Differential localization of organic cation transporters rOCT1 and rOCT2 in the basolateral membrane of rat kidney proximal tubules. Histochem Cell Biol 2000; 114: 175-180.
116. Urakami Y, Nakamura N, Takahashi K, Okuda M, Saito H, Hashimoto Y, Inui K. Gender differences in expression of organic cation transporter OCT2 rat kidney. FEBS Letters 1999; 461: 339-342.
117. Wu XA, Huang W, Ganapathy ME, Wang HP, Kekuda R, Conway SJ, Leibach FH, Ganapathy V. Structure, function, and regional distribution of the organic cation transporter OCT3 in the kidney. Am J Physiol Renal Physiol 2000; 279: F449-F458.
118. Grundemann D, Liebich G, Kiefer N, Koster S, Schomig E. Selective substrates for non-neuronal monoamine transporters. Mol Pharmacol 1999; 56(1): 1-10.
119. Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, Tsuji A. Cloning and characterization of a novel human ph-dependent organic cation transporter, OCTN1. FEBS Letters 1997; 419: 107-111.
120. Ganapathy ME, Huang W, Rajan DP, Carter AL, Sugawara M, Iseki K, Leibach FH, Ganapathy V. β-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. J Biol Chem 2000; 275: 1699-1707.
121. Wagner CA, Lukewille U, Kaltenbach S, Moschen I, Broer A, Risler T, Broer S, Lang F. Functional and pharmacological characterization of human Na^+-carnitine cotransporter hOCTN2. Am J Physiol Renal Physiol 2000; 279: F584-F591.
122. Seth P, Wu X, Huang W, Leibach FH, Ganapathy V. Mutations in novel organic cation transporter (OCTN2), an organic cation/carnitine transporter, with differential effects on the organic cation transport function and the carnitine transport function. J Biol Chem 1999; 274: 33388-33392.
123. Bowman RH. Renal secretion of [^{35}S] furosemide and its depression by albumin binding. Am J Physiol 1975; 229: 93-98.
124. Koschier FJ, Acara M. Transport of 2, 4, 5-trichlorophenoxyacetate in the isolated, perfused rat kidney. J Pharmacol Exp Ther 1979; 208: 287-293.
125. Webb DE, Edwards RM, Grantham JJ. Dependence of proximal tubule p-aminohippurate secretion on serum proteins and metabolic substrates. Am J Physiol 1986; 251: F619-F626.
126. Grantham JJ, Kennedy J, Cowley B. Tubule urate and p-aminohippurate transport: sensitivity and specificity of serum protein inhibition. Am J Physiol 1987; 252: F683-F690.
127. Rodrigues de Miranda JF, Hilbers CW. A nuclear magnetic resonance study of the kinetics of the binding of the renal contrast medium acetrizoate to albumin. Mol Pharmacol 1976; 12: 279-290.
128. Ochwadt BK, Pitts RF. Disparity between phenol red and diodrast clearance in the dog. Am J Physiol 1956; 187: 318-322.
129. Honari J, Blair AD, Cutler RE. Effects of probenecid on furosemide kinetics in man. Clin Pharmacol Ther 1977; 22: 395-401.
130. Homeida M, Roberts C, Branch RA. Influence of probenecid and spironolactone on furosemide kinetics in man. Clin Pharmacol Ther 1977; 22: 402-409.
131. Brater DC. Pharmacodynamic considerations in the use of diuretics. Annual Rev Pharmacol Toxicol 1983; 23: 45-62.
132. Jacobs C, Kaubisch S, Halsey J, Lum BL, Gosland M, Coleman CN, Sikic BI. The use of probenecid as a chemoprotector against cisplatin nephrotoxicity. Cancer 1991; 67: 1518-1524.
133. Ban M, Hettich D, Huguet N. Nephrotoxicity mechanism of cis-plantinum (II) diamine dichloride in mice. Toxicol Letters 1994; 71: 161-168.

134. Klein J, Bentur Y, Cheung D, Moselhy G, Koren G. Renal handling of cisplatin: interactions with organic anionas and cations in the dog. Clin Inv Med 1991; 14: 388-394.
135. Tracy TS, Krohn K, Jones DR, Bradly JD, Hall SD, Brates DC. The effects of a xalicylate, ibuprofen, and naproxen on the disposition in patients with rheumatoid arthritis. Eur J Clin Pharmacol 1992; 42: 121-125.
136. Frenia ML, Long KS. Methotrexate and nonsteroidal antiinflammatory drug interactions. Ann Pharmacother 1992; 26: 234-237.
137. Nierenberg DW. Competitive inhibition of methotrexate accumulation in rabbit kidney slices by nonsteroidal anti-inflammatory drugs. J Pharmacol Exp Ther 1983; 226: 1-6.
138. Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM. Inhibition of P-glycoprotein-mediated drug transport - A unifying mechanism to explain the interaction between digoxin and quinidine. Circulation 1999; 99: 552-557.
139. Verschraagen M, Koks CHW, Schellens JHM, Beijnen JH. P-glycoprotein system as a determinant of drug interactions: the case of digoxin-verapamil. Pharmacol Res 1999; 40: 301-306.
140. De Lannoy IAM, Koren G, Klein J, Charuk J, Silverman M. Cyclosporin and quinidine inhibition of renal digoxin excretion - evidence for luminal secretion of digoxin. Am J Physiol 1992; 263(4 Pt 2):F613-F622.
141. De Lannoy IAM, Mandin RS, Silverman M. Renal Secretion of Vinblastine, Vincristine and Colchicine *in vivo*. J Pharmacol Exp Ther 1994; 268: 388-395.
142. Okamura N, Hirai M, Tanigawara Y, Tanaka K, Yasuhara M, Ueda K, Komano T, Hori R. Digoxin-cyclosporin A interaction: modulation of the multidrug transporter P-glycoprotein in the kidney. J Pharmacol Exp Ther 1993; 266: 1614-1619.
143. Woodland C, Verjee Z, Giesbrecht E, Koren G, Ito S. The digoxin-propafenone interaction - characterization of a mechanism using renal tubular cell monolayers. J Pharmacol Exp Ther 1997; 283: 39-45.
144. Nierenberg DW. Drug inhibition of penicillin tubular secretion: concoidance between *in vitro* and clinical findings. J Pharmacol Exp Ther 1987; 240: 712-716.
145. Dupuis L, Loren G, Shore A, Silverman ED, Laxer RM. Methotrexate non-steroidal anti-inflammatory drug interaction in children with arthritis. J Rheumat 1990; 17: 1469-1473.
146. Banditt P, Meyer FP, Walther H. Influence of cimetidine on the pharmacokinetics of other drugs. Pharmazie 1990; 45: 11-16.
147. Somogyi A, McLean A, Heinzow B. Cimetidine-procainamide pharmacokinetic interaction in man: evidence of competition for tubular secretion of basic drugs. Eur J Clin Pharmacol 1983; 25: 339-345.
148. Christian CJ, Meredith CG, Speeg KJ. Cimetidine inhibits renal procainamide clearance. Clin Pharmacol Ther 1984; 36: 221-227.
149. Van Crugten J, Bochner F, Keal J, Somogyi A. Selectivety of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. J Pharmacol Exp Ther 1986; 236: 481-487.
150. Gisclon LG, Boyd RA, Williams RL, Giacomini KM. The effect of probenecid on the renal elimination of cimetidine. Clin Pharmacol Exp Ther 1989; 45: 444-452.
151. Inotsume N, Nishimua M, Nakano M, Fujiyama S, Sato T. The inhibitory effect of probenecid on renal excretion of famotidine in young, healthy volunteers. J Clin Pharmacol 1990; 30: 50-56.
152. Hsyu PH, Gisclon LG, Hui AC, Giacomini KM. Interactions of organic anions with the organic cation transporter in renal BBMV. Am J Physiol 1988; 254: F56-F61.
153. Ott RJ, Hui C, Giacomine KM. Mechanisms of interactions between organic anions and the organic cation transporter in renal brush border membrane vesicles. Biochem Pharmacol 1990; 40: 659-661.
154. McKinney TD, Myers P, Speeg JKV. Cimetidine secretion by rabbit renal tubules *in vitro*. Am J Physiol 1981; 241: F69-F76.
155. Brandle E, Greven J. Transport of cimetidine across the basolateral membrane of rabbit kidney proximal tubules: interaction with organic anions. Pharmacology 1992; 45: 231-240.
156. Schück O. Tubular secretion of creatinine and its plasma concentration. Int J Clin Pharmacol Ther Toxicol 1990; 28: 127-128.
157. Sokol PP. Effect of DQ-2556, a new cephalosporin, on organic ion transport in renal plasma membrane vesicles from the dog, rabbit and rat. J Pharmacol Exp Ther 1990; 255: 436-441.
158. Ullrich KJ, Rumrich G, David C, Fritzsch G. Bisubstrates-substances that interact with renal contraluminal organic anion and organic cation transport systems. A. amines, piperidines, piperazines, azepines, pyridines, quinolines, imidazoles, thiazoles, guanidines and hydrazines. Pflügers Arch 1993; 425: 280-299.
159. Chatton JY, Odone M, Besseghir K, Roch-Ramel F. Renal secretion of 3'-azido-3'-deoxythymidine by the rat. J Pharmacol Exp Ther 1990; 255: 140-145.
160. Griffiths DA, Hall SD, Sokol PP. Interaction of 3'-azido-3'-deoxythymidine with organic ion transport in rat renal basolateral membrane vesicles. J Pharmacol Exp Ther 1991; 257: 149-155.
161. Griffiths DA, Hall SD, Sokol PP. Effect of 3'-azido-3'-deoxythymidine (AZT) on organic ion transport in rat renal brush border membrane vesicles. J Pharmacol Exp Ther 1992; 260: 128-133.
162. Anders MW. Metabolism of drugs by the kidney. Kidney Int 1980; 18: 636-647.
163. Bekersky I, Popick A. Metabolism of bumetanide by the isolated perfused rat kidney. Drug Metab Dispos 1983; 11: 512-513.

164. Bekersky I, Colburn WA. Acetylation of sulfisoxazole by the isolated perfused rat kidney. J Pharm Sci 1980; 69: 1359.
165. Bekersky I, Colburn WA, Fishman L, Kaplan SA. Metabolism of salicylic acid in the isolated perfused rat kidney: interconversion of salicyluric and salicylic acids. Drug Metab Dispos 1980; 8: 319-324.
166. DeLannoy IAM, Nespeca R, Pang KS. Renal handling of enalapril and enalaprilat: studies in the isolated red blood cell-perfused rat kidney. J Pharmacol Exp Ther 1989; 251: 1211-1222.
167. Elseviers MM, De Broe ME. Epidemiology of analgesic nephropathy. J Nephrol 1992; 5: 94-98.
168. McMurtry RJ, Snodgrass WR, Mitchell JR. Renal necrosis glutathione depletion and covalent binding after acetaminophen. J Toxicol Appl Pharmacol 1978; 46: 87-100.
169. Mudge GH, Gemborys MW, Duggin GG. Covalent binding of metabolites of acetaminophen to kidney protein and depletion of renal glutathione. J Pharmacol Exp Ther 1978: 206: 218-226.
170. Kleinman JG, Breitenfield RV, Roth DA. Acute renal failure associated with acetaminophen ingestion: report of a case and review of the literature. Clin Nephrol 1980; 14: 201-205.
171. Szefler SJ, Acara M. Isoproterenol excretion and metabolism in the isolated perfused rat kidney. J Pharmacol Exp Ther 1979; 210: 295-300.
172. Rennick B, Ziemniak J, Smith I, Taylor M, Acara M. Tubular transport and metabolism of cimetidine in chicken kidneys. J Pharmacol Exp Ther 1984; 228: 387-392.
173. Bessighir K, Pearce LB, Rennick B. Renal tubular transport and metabolism of organic cations by the rabbit. Am J Physiol 1981; 241: F308-F314.
174. Watrous WM, May DG, Fujimoto JM. Mechanism of the renal tubular transport of morphine and morphine ethereal sulfate in the chicken. J Pharmacol Exp Ther 1970; 172: 224-229.
175. Hook JB, MacCormack KM, Kluwe WM. Biochemical mechanisms of nephrotoxicity. In: Reviews in biochemical toxicology, vol 1. Hodgson, Bend, Philpot (editors). Elsevier/North-Holland, New York 1979; p. 53-78.
176. Jacqz E, Ward S, Johnson R, Schenker S, Gerkens J, Branch RA. Extrahepatic glucuronidation of morphine in the dog. Drug Metab Dispos 1986; 14: 627-630.
177. Meffin PJ, Zilm DM, Veendendaal JR. Reduced clofibric acid clearance in renal dysfunction is due to a futile cycle. J Pharmacol Exp Ther 1983; 227: 732-738.
178. Gugler R, Kurten JW, Jensen CJ, Klehr U, Hartlapp J. Clofigrate disposition in renal failure and acute and chronic liver disease. Eur J Clin Pharmacol 1979; 15: 341-347.
179. Verbeeck R, Tjandramaga TB, Mullie A, Verbesselt R, Verberckmoes R, De Schepper PJ. Biotransformation of diflunisal and renal excretion of its glucoronides in renal insufficiency. Brit J Clin Pharmacol 1979; 7: 273-282.
180. Eriksson LO, Wahlin-Boll E, Odar-Cederlof I, Lindholm L, Melander A. Influence of renal failure, rheumatoid arthritis and old age on the pharmacokinetics of diflunisal. Eur J Clin Pharmacol 1989; 36: 165-174.
181. Aronoff GR, Ozawa T, DeSante KA, Nash JF, Ridolfo AS. Benoxaprofen kinetics in renal impairment. Clin Phamacol Ther 1982; 32: 190-194.
182. Advenier C, Roux A, Gobert C, Massias P, Varaquaux O, Flouvat B. Pharmacokinetics of ketoprofen in the elderly. Brit J Clin Pharmacol 1983; 16: 65-70.
183. Birnbaum J, Kahan FM, Kropp H, MacDonald JS. Carbapenems, a new class of β-lactam antibiotics. Am J Med 1985; 78(suppl 6A): 3-21.
184. Verpooten GA, Verbist L, Buntinx AP, Entwistle LA, Jones KH, De Broe ME. The pharmacokinetics of imipenem (thienamycin-formamidin) and the renal dehydropeptidase inhibitor cilastatin sodium in normal subjects and patients with renal failure. Brit J Clin Pharmacol 1984; 18: 193-193.
185. Tune BM, fravert D, Hsu C-Y. Thienamyn nephrotoxicity: mitochondrial injury and oxidative effects of imipenem in the rabbit kidney. Biochem Pharmacol 1989; 38: 3779-3783.

03

Pharmacovigilance

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Government agencies, like the (FDA) in the US, re-view pharmacology and toxicology data from a products approval application and determine if the product is safe and effective for human use. Despite diligent studies prior to approval, adverse drug reactions occur unexpectedly or with greater frequency than in the clinical trials that cause agencies to recall re-label the product. The use of spontaneous reports from healthcare professional has provided useful information utilized to evaluate the safety of products in populations not in the clinical trials. Pharmacovigilance is an epidemiology approach using case reporting systems, published reports in the literature and other sources to continually monitor the safety of marketed products. It is the continual monitoring for unwanted effects and other safety issues related to drugs.

Pharmacovigilance has been defined as the continual monitoring for safety related aspects of drugs that have been approved and marketed to the public [1]. The reason for pharmacovigilance is to provide information to assist healthcare professionals in assessing the risk/benefit profile of a drug product and improve public health and safety. Pharmacovigilance is an epidemiology approach using passive case reporting systems and published reports in the literature [2]. Unusual or rare events that occur during initial or long term drug use are more likely to be detected by case reports from the literature or voluntary reporting systems than in randomized controlled clinical trials [3]. And, it is in the post-marketing phase of a products life that the true determination of the frequency of adverse effects is identified [4]. In the pharmaceutical industry, it was found that the majority of epidemiologic projects are devoted to drug safety, drug surveillance/pharmacovigilance or to health/pharmacoeconomic outcomes research [5].

Drug induced toxicity has been a concern as early as 1848, when quinine imported for the US army during the Mexican War was adulterated resulting in the prohibition of the import of drugs from foreign countries. In 1877 chloroform anesthesia was associated with sudden death. It was identified in the early part of the 20th century that arsenicals caused fatal hepatic events, and aplastic anemia was associated with chloramphenicol. It was the deaths associated with elixir of sulfanilamide that led to the first enactment of legislation addressing adverse reactions known as the Federal Food, Drug and Cosmetic Act of 1938. However, it was the worldwide thalidomide tragedy that brought the US Congress to act in 1962 passing the Kefauver-Harris Amendment to the Food Drug and Cosmetic Act which required not only proof of efficacy, but required drug manufacturers to report adverse drug reactions to the FDA [2]. Spontaneous reporting systems were developed after the Thalidomide tragedy in 1962. However it was not until 1993 that the FDA established the Med Watch System as a national initiative to educate healthcare professionals about the importance of reporting serious adverse drug reactions to the agency.

Today, pharmaceutical products undergo extensive controlled clinical trial testing and review prior to approval by regulatory agencies. Despite these vigilant efforts, once the drugs are marketed, they often reach millions of patients with characteristics not observed in the controlled clinical environment and rare events can occur that were not noted in the premarketing trials [4].

The clinical testing of products prior to approval involves 3 phases. Phase 1 involves determining the

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drug's pharmacologic profile in a small number of healthy volunteers. The kinetics of the product, and dose ranging for safety are tested. In Phase 2, a larger number of healthy volunteers are tested to look for efficacy and further safety studies may be added. About 2-300 patients are enrolled. In Phase 3, the drug is administered by physicians in clinical settings to patients with the condition the drug is intended to treat. Patients are often screened for comorbidities, allergies and other conditions that might not be true of the population who will eventually be using the product. Typically, premarketing studies exclude patients who have complicating factors such as renal or hepatic insufficiency, diabetes or heart failure [4]. These trials often do not include special populations such as pregnant women or children or who may be at risk for unique adverse drug reactions. The less commonly occurring side effects, rare but serious or common delayed adverse drug reactions and drug interactions, may not be detectable or become apparent until used in a larger population with multiple disease states [2].

Therefore pre-marketing trials are limited in information on a drugs full safety profile because they employ too few patients. The "rule of three" states that to detect an unintended drug effect that occurs with a particular frequency, the number of subjects needed to follow up is 3 times that of the estimated frequency of the event. Consequently if an ADR occurs in 1 in 10,000 users of the product, one would need to observe 30,000 patients in order to be 95% likely to detect it [2]. Even reducing the probability of detecting an ADR with an occurrence of 1 in 10,000 from 95% to 80% requires the evaluation of 16,000 drug exposures [6].

Phase 4 are those studies conducted after the drug is marketed. Although not mandated by the FDA they may be negotiated as a condition for a new drug approval or for obtaining approval for another indication for a drug. Phase 4 research may include clinical trials that investigate identified events from post marketing spontaneous reporting systems or pharmacoeconomic studies to establish cost effectiveness. Most often they are employed to expand a claim or indications or for differentiation from other competitors [2].

Post marketing surveillance conducted by drug regulators, the manufacturers and the medical community may be the preferred method of collecting information for the appropriate use of drugs. These adverse drug reactions may be detected shortly after initiation of drug therapy or after long-term administration or some later time after the drug is discontinued [2-4, 6].

The health care professional, pharmaceutical manufacturer and regulator all share a common interest in the development and use of drugs that are effective and have an acceptable level of undesirable effects. Medwatch and similar programs are important tools for detection of adverse events effects. However, they are limited by the reliance on voluntary reporting as an individual health care professional is not required by law to report adverse drug reactions to the FDA [4].

Conversely, all sponsors of drugs with an New Drug Application (NDA), Abbreviated New Drug Application (ANDA) or grandfathered prescription medication are required by regulation to report all adverse drug reactions of which they become aware to the FDA. The FDA requires manufactures to file any serious unlabeled reactions to the FDA within 15 days. All nonserious and serious labeled events are reported quarterly for new entities and annually for all other approved NDA and ANDA drugs. Manufacturers must also submit all serious non-labeled ADRs on all FDA approved drugs that occur outside the US. Manufacturers of over-the-counter drugs without NDAs are not currently required to report. The manufacturer must analyze the frequency of serious labeled reactions and if an increased frequency is found, a narrative must be submitted within 15 days [2].

Filed reports maintain the confidentiality of the patient and the reporter of the ADR. Healthcare professionals are encouraged to report serious ADRs suspected to be caused by medications, medical devices, special nutritional products and other approved products regulated by the FDA. Serious adverse drug reactions include death, hospitalization, significant or permanent disability, congenital anomaly or an outcome that requires medical or surgical intervention [3].

By the end of 1996 there were over 1 million reports in the system. Approximately 90% were through the manufacturers and the remaining 10% came directly to the FDA. Of those reported directly to the FDA, 46% were from pharmacists, 19% from physicians and 20% from hospitals with the remaining from a variety of sources including consumers and law firms [2].

The physician must then consider attributing the event to the assignment of causality [6]. The development of a symptom or adverse drug reactions does not

establish the drug as the cause of the problem. Determining which adverse drug reactions is caused by the drug with reasonable certainty is an essential and difficult part of the adverse drug reactions reporting system [3]. Some of the issues to be considered include whether the interval between taking a drug and the onset of the adverse drug reactions was possibly related, and the response to discontinuing therapy and rechallenging with the drug.

Physicians are more apt to report reactions that are severe or unique, especially on newer agents [6]. Thus, when one reviews the Medwatch web site, one might conclude that new products are associated with a high incidence of adverse drug reactions. Using epidemiology methods it is the agency's responsibility to assess the causality, seriousness and frequency of these events based on the number of patients exposed. The results of these analyses might result in adding information to the label, withdrawing the drug from the market or refuting unsubstantiated allegations about a particular agent [6].

In the last 30 years there have been continued instances of drug recalls or precautionary statements due to pharmacovigilance reports and some more notable examples include benoxaprofen and hepatic disorders/deaths in the elderly and temafloxacin associated hemolytic anemia. Other recent examples are cardiac valve disorders from fenfluramine and phentermine (Fen-Fen), anaphylaxis from zomepirac, rhabdomyolysis associated with cerivastatin and cardiac arrests from drug interactions with terfenadine and drugs which inhibit P450 3A4 like ketoconazole and erythromycin [4]. When taking into account the number of new chemical entities approved for each decade, the rate at which new drugs are being withdrawn due to safety concerns has decreased steadily [2].

The reporting of adverse drug reactions is critical to the health care industry and as a result several systems have been developed worldwide. In 1968, the World Health Assembly requested the World Health Organization (WHO) to address the safety of drugs in international markets, resulting in the establishment of the WHO International Drug Monitoring System. By 1992, 36 countries were regularly submitting reports [2]. Today, the WHO Collaborating Centre for International Drug Monitoring at Uppsala, Sweden maintains the database that currently contains over 2 million reports from over 60 participating countries including the FDA. Many other countries have their own systems in place: for example, The Irish Medicines Board maintains the adverse drug reactions information on a national basis in Ireland [7].

Despite important progress in evaluating adverse drug reactions, there is still no reliable method to identify potential delayed events that might occur well after the original course of therapy. For example Medwatch system did not detect the identification of diethyl stilbesterol (DES) associated clear-cell adenocarcinoma in female fetuses exposed in utero [3].

On the other hand, post marketing surveillance may reveal unintended beneficial consequences. Pre-marketing studies focus on the efficacy of a product in a specific disease or primary efficacy but don't always assess the usefulness in a secondary efficacy endpoint [2]. Randomized controlled clinical trials, developed to monitor blood glucose control of antidiabetic agents were not designed to assess the positive benefits of blood glucose control on the cardiovascular or renal complications of diabetes. These benefits were identified in post marketing pharmacovigilance studies. Another benefit detected in post marketing studies has shown a reduction in deaths from cardiovascular disease in postmenopausal women on hormone replacement therapy compared with non-users [3]. Many epidemiology studies have now shown the potential for aspirin and certain non-steroidal anti-inflammatory drugs to prevent certain cancers. This has promoted the randomized controlled clinical trials of COX-2 agents in colon cancer prevention.

Ironically, it is interesting to note that thalidomide, which was withdrawn because of the high risk of congenital malformations and the spark for post marketing surveillance, is now making a limited come-back for the treatment of certain cancers due to a re-appraisal of its risk/benefit ratio for this indication.

In addition to the pharmaceutical industry, there are other organizations that facilitate reporting to the FDA. Joint Commission on Accreditation of Healthcare Organizations (JCAHO) standards require hospitals to maintain an adverse drug reaction reporting system although hospitals are not required by law to submit reports to the FDA. The American Society of Health Systems Pharmacists has issued guidelines on ADR monitoring and reporting [2].

Other sources for Pharmacovigilance studies include the growing databases maintained by health sys-

tems such as computerized data from managed care programs, hospitals and medical centers. These data integrate pharmacy, laboratory and other outcomes data [3, 4].

Healthcare professionals may report ADRs by telephone 1800 FDA 1088 Fax 800 FDA 0178 or mail Med Watch, 5600 Fishers Lane, Rockville, MD 20852-9787or through the Medwatch internet site www.fda.gov/medwatch. Medwatch provides feedback to healthcare professionals though the FDA Medical Bulletin and the Medwatch home page (www.fda.gov/medwatch). In Europe and other countries individual Ministries of Medicine may have a system in place such as Irelands yellow card system. One might also contact The World Health Organization (WHO) Collaborating Centre for International Drug Monitoring. The WHO Headquarters is at Avenue Appia 20, 1211 Geneva 27, Switzerland, telephone +41 22 791 21 11 or at www.who.int.

It is the responsibility of governing bodies to require and review randomized controlled clinical trials for evaluating drugs prior to approval and marketing to the public. After marketing, it is the responsibility of the agencies and the industry to carefully monitor the drugs safety. It is incumbent on every healthcare practitioner to report serious and unexpected adverse drug reactions to the appropriate agency, such as Medwatch, to be part of the pharmacovigilance team for evaluating drug safety.

References

1. Neutel C, Walop W. What is the epidemiology in pharmacovigilance ? Pharmacoepidemiology and Drug Safety 2002; 9: 337-340.
2. Hartzema AG, M. Porta M, H.H. Tilson (Editors). Pharmacoepidemiology. An Introduction (3rd edition). Harvey Whitney Books Company, 1998.
3. Brewer T, Colditz GA. Postmarketing surveillance and adverse drug reactions: current perspectives and future needs. JAMA 1999; 281(9): 824-829.
4. Murray M. Pharmacoepidemiology. Remington: The Science and Practice of Pharmacy (20th edition). Alfonso L, Gennaro (editor). Gennaro; Lippincott Williams & Wilkins 2000; p. 1980-1988.
5. Roht Lewis. Epidemiology in the pharmaceutical industry. Pharmacoepidem Drug Safety 2000; 9; 139-148.
6. Faich G. Postmarketing surveillance of prescription drugs. Current Status; CMRI, NY NY 1986.
7. Irish Medicines Board, Earlsfort Centre, Earlsfort Terrace, Dublin 2, Ireland.

04

Immunologically-mediated toxin-induced renal disease

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Introduction

The last three years have expanded insight as to how lymphocytes respond to antigens or xenobiotics, and also the knowledge of the pathophysiology of renal diseases. This provides new clues on the mechanisms by which chemically induced immune responses trigger kidney lesions. First, we will discuss the general mechanisms that could be responsible for drug-induce immune nephropathies. The role of Th1 and Th2 CD4+ T-cell subsets in the development of nephropathies will be also debated. Then, we will describe the types of immune nephropathy than can be induced by xenobiotics in patients. Finally, we will focus on experimental models of chemical-induced systemic autoimmune diseases because they illustrate some mechanisms described before and the genetic control of these diseases.

Mechanisms by which xenobiotics may induce an immune response

As summarized in Table 1, xenobiotics may induce an immune reaction by at least four mechanisms: 1) by inducing an abnormal immune response due to activation of antigen presenting cells, 2) by promoting an immune response directed against an autoantigen modified by the toxin or its metabolite, 3) by activating a limited set of autoreactive T cells and 4) by inducing global immune disregulation.

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Table 1. Mechanisms of toxin-induced immunopathology.

• **Abnormal T cell response due to activation of dendritic cells:**	heavy metals
• **T response against an autoantigen modified by a chemical:**	$HgCl_2$, Au^{III}, Ni
• **Induction of a limited set of autoreactive T cells:**	$HgCl_2$, Au^{III}, Ni
• **Chemical-induced immune disregulation:**	
- Interference with central tolerance:	cyclosporine A, procainamide
- Activation of T cells at the periphery:	hydralazine, procainamide, heavy metals

1. Abnormal immune response due to activation of antigen presenting cells.

The recognition of peptides/MHC complexes at the cell surface of an antigen presenting cell by a specific T lymphocyte is not sufficient for triggering an immune response. Dendritic cells, that are the professional antigen presenting cells, need to be activated for initiating the immune response [1]. Signals delivered by pathogens through the toll receptors at the dendritic cell surface and signals generated in injured tissues activate dendritic cells. For example, platelets that constitutively express CD40L can interact with CD40 at the cell surface of dendritic cells leading to their activation in a bleeding tissue. Non immune tissue injury leads to necrotic death, release of reactive oxygen species, of heat shock proteins (HSP) and of lymphokines such as IL-1, TNFα, type I interferons, each being able to stimulate dendritic cells. Activation of dendritic cells results in upregulation of costimulatory molecules, intracellular formation of immunogenic MHC-class II peptide complexes and redistribution of MHC class II products from intracellular compartments to the plasma membrane [2]. Many chemicals, including heavy metals, can directly activate dendritic cells [3], which certainly favors the development of a T-cell response. In addition, many chemicals may promote an inflammatory reaction, which indirectly contributes to dendritic cells maturation.

2. Induction of a T cell response against an autoantigen modified by the chemical.

A toxin or one of its metabolites may bind an autoantigen. The complex will be internalized and processed by antigen presenting cells. The peptide modified by the toxin will activate specific T-cells. This type of response is responsible for hypersensitivity reactions. For example, it was shown that $HgCl_2$ binds two cysteines in the sequence of fibrillarin, a nuclear antigen that modifies its molecular properties and induces a T-cell response [4]. The fact that B10.S mice injected with $HgCl_2$ produce antibodies against toxin-modified fibrillarin with subsequent synthesis of antibodies specific for the native protein suggests that an autoimmune response may be secondary to the response against the antigen modified by $HgCl_2$ [5]. According to Janeway's group [6], it can be supposed that haptenized determinants stimulate specific T-cells which in turn activate antigen presenting cells that become able to deliver costimulatory signals allowing normally silent autoreactive T-cells to be activated.

3. Induction of a limited set of autoreactive T cells.

A chemical may trigger autoimmunity by inducing the expression of an antigen that is normally absent. For example, $CdCl_2$ triggers HSP70 expression in SJL/J renal tubular cells; since no T-cell tolerance towards this antigen has been acquired during ontogeny, heat shock proteins reactive T-cells are induced and transfer tubulointerstitial nephritis into normal mice [7]. A toxin may also induce, directly or indirectly, the expression of normally cryptic determinants of auto-antigens for which no T-cell tolerance has been achieved. This could trigger immunopathological manifestations. Antigen presenting cells from mice infected with Theiler's virus initially present virus determinants and then auto-antigens from the central nervous system, which is responsible for autoreactive T-cell activation and demyelinating disease [8]. Metals including Au and Hg may induce the presence of cryptic determinants from bovine RNase A [9, 10, 11] but the role of such a phenomenon in the development of autoimmunity has not been proven. Chemicals may also act indirectly. Recently, it has been reported that reactive oxygen species modify glomerular basement membrane, unmasking determinants for which there was no immune tolerance and thus leading to an au-

toimmune response [12]. This mechanism could be involved in some drug-induced autoimmune nephritides since drugs affecting the kidney can generate reactive oxygen species either directly or as a consequence of toxic renal damage.

4. Chemical-induced disregulation of the immune system.

Chemicals may also cause an autoimmune kidney disease in the context of B- and/or T-cell activation, independent of antigen-specific recognition. This will lead to B-cell polyclonal activation with production of autoantibodies since, normally, autoreactive B-cells exist but are not activated due to a lack of T-cell help. Autoreactive T-cells with a high affinity for auto-peptides are eliminated in the thymus or at the periphery while those with a low affinity for auto-peptides escape deletion and emigrate to the periphery (discussed in [13, 14]). Several mechanisms explain the absence of autoimmunity in normal individuals: auto-peptides do not deliver any signal (T-cells are ignorant) or are recognized by a T-cell in the absence of adequate costimulation, which induces functional inactivation (T-cells are anergic). Finally, regulatory cells exert a negative control on potentially deleterious autoreactive T-cells. In this respect, some anergic cells could represent a subset of regulatory cells due to their production of IL-10, an immunosuppressive cytokine [15]. In experimental models, the immunosuppressant cyclosporine A blocks T-cell signaling pathways by inhibiting the phosphatase calcineurin and may induce autoimmunity by impairing central tolerance [16]. Lethally irradiated mice reconstituted with bone marrow cells and treated with high doses of cyclosporine A develop inflammatory lesions in multiple organs after cyclosporine A administration has been stopped. This disease is transferable by T-cells and is attributed to the fact that cyclosporine A blocks thymic negative selection. A toxin may also act at the periphery. It may lower the threshold of T-cell activation and/or deliver costimulatory signals. Recent data showing that the T-cell membrane needs to be rearranged in order to allow full T-cell activation, may provide a better understanding as to how some toxic agents induce polyclonal T-cell activation. Indeed, in normal conditions, negatively charged glycocalyx of the antigen presenting cells and the T-cells are so large that they hamper optimal T-cell receptor/MHC peptide interactions [17]. Non-antigen dependent factors such as chemokines, inflammatory mediators, or factors present in lymph nodes, would play an important role in the rearrangement of surface molecules, initiating the formation of the immune synapse, the T-cell area that contacts the antigen presenting cells by scaffolding the signalling molecules that will transduce the signal. This process is likely to be calcium-dependent [18] and requires cytoskeleton reorganization. This is associated with the exclusion of CD43, a component of the glycocalyx, from the synapse and diffusion in the membrane of the integrin LFA-1 and of adhesion molecules such as CD2. Altogether, these events would optimize peptide-MHC class II T-cell receptor interactions. It is possible that chemicals such as $HAuCl_4$ or $HgCl_2$ activate T-cells by this bias. It is also possible that chemicals act on downstream step(s) of T-cell receptor-dependent signaling pathways.

What is the role of different CD4+ T-cell subsets in the development of nephropathies?

Characterization of Th1 and Th2 cells

CD4+ T-lymphocytes are heterogeneous in terms of production of cytokines, and have different functions [19, 20]. Th1 cells secrete IL-2, IFN-γ and lymphotoxin which explains their role in activating macrophages and cytotoxic cells and therefore in cell-mediated immune responses. Th1 cells also help B-cells in the production of some isotypes: IgG2a in mice and IgG2b in rats. Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, promote IgE and IgG1 switch (in rats and mice), and activate eosinophils and mast cells. Although there is no marker that allows to identify Th1 and Th2 cells, these subsets express different chemokine receptors [21] and display specific transcription factors: c-maf, NIP-45 and GATA-3 characterize Th2 cells and control IL-4 and IL-5 gene transcription while T-bet is expressed by Th1 cells and is essential for IFN-γ expression (reviewed in [22]). The Th1/Th2 cell dichotomy brought new insights in the pathogenic mechanisms responsible for immunopathological manifestations. Th1 cells are widely considered as crucial in inflammatory processes while Th2 cells play a role in asthma, atopy and immediate hypersensitivity. Factors that influence Th1/Th2 polarization include

the type of cytokines present during differentiation (IL-12 and IL-4 direct Th1 and Th2 maturation respectively), the nature of antigen presenting cells, the nature of the antigen and the strength of T-cell receptor-peptide/MHC interaction, the route of antigen administration and genetic factors. For example, Brown-Norway rats are prone to develop Th2 responses [23].

IFN-γ contributes to Th1 cell development by stabilizing the expression of the β2 chain of IL-12 receptor (RIL-12). IL-4 amplifies its own production. Therefore, there is a positive feedback reinforcing Th1 or Th2 cell maturation. At the other hand, Th1 and Th2 cell development is antagonistic. IL-4 inhibits the expression of β2RIL-12 chain and IL-10 suppresses IL-12 production. GATA-3 directly regulates IL-5 gene transcription and contributes to IL-4 gene expression probably by upregulating c-maf that binds proximal IL-4 gene promoter. GATA-3 also down regulates β2RIL-12 chain expression. The Th1 transcription factor, T-bet downgrades IL-4 and IL-5 secretion.

It has recently been shown that the phenotype of differentiated Th1 and Th2 cells is stabilized after about 3-5 cell divisions [24]. Grogan et al. showed that, early in the differentiation, IL-4 and IFN-γ loci are easily accessible and IL-4 and IFN-γ gene transcripts equally detected after stimulation of naive CD4+ T-cells with plate-bound anti-T-cell receptor mAb [24]. In addition, there is evidence that the cytokines are translated. It has also been shown by fluorescent *in situ* hybridization that IL-4 and IFN-γ genes are localized away from the silenced centromeric chromatin; conversely, in Th1 cells, 52% of IL-4 alleles are reorganized in apposition to centromeric heterochromatin and, in Th2 cells, 67% of IFN-γ alleles are directed to heterochromatic domains. Such physical gene repositioning contributes to silence genes. This means that, during early phases of differentiation, naive cells may give rise to Th1 or Th2 cells in a plastic manner. This process is independent of cytokines and even of the presence of the specific transcription factors GATA-3 and T-bet [24].

Role of Th1 and Th2 cells in the development of nephropathies

Th1 cells very probably play an important role in nephropathies associated with pauci immune deposits and with interstitial infiltrates of T-cells and macrophages [25]). Neutrophils and platelets are also found. The role of Th1 cells has been clearly demonstrated in experimental murine models of crescentic glomerulonephritis induced by immunization with glomerular basement membrane antigens or by immunization with heterologous sheep or goat immunoglobulin prior to injection of heterologous anti-glomerular basement membrane immunoglobulin (reviewed in [25]). In humans, crescentic glomerulonephritis is also presumed to be Th1-mediated [25]. It is noteworthy that immunization of the Th2 prone BALB/c mice, with heterologous immunoglobulin prior to injection of heterologous anti-glomerular basement membrane immunoglobulin induces a glomerulopathy that is different from the one observed in C57BL/6 Th1 prone mice. The role of Th1 cells in a model of tubulointerstitial nephritis induced by immunization with tubular basement membrane antigens has also been documented [26], (Table 2).

Th2 cells are pathogenic in gold-salt induced glomerulopathy in Brown-Norway rats [27] and in glomerulopathies associated with allogeneic reactions [28, 29]. Injection of semi allogeneic spleen cells into BALB/c neonates induces donor B cell polyclonal activation under the control of alloreactive BALB/c Th2 cells. This Th2 response is particularly prominent when both donors and recipients are β2m knockout which probably prevents a negative control of Th2 cells by MHC class I restricted regulatory cells [30]. In this case, mice display blood hypereosinophilia, massive eosinophilic infiltration in different organs including kidneys, and develop severe autoantibody-mediated glomerulonephritis [30]. Th2 cells could also play a role in human membranous glomerulopathies (Table 2) mainly because immune deposits contain essentially IgG4, a Th2 dependent isotype. Th2 cells are probably also involved in the idiopathic nephrotic syndrome of childhood associated with the development of minimal change disease that is thought to be associated with T lymphocyte dysfunction often triggered by viral infections and with the production of circulating factor(s) resulting in proteinuria. IL-4 [31] and/or IL-13 [32] may also play a role.

A better understanding of the physiology of glomerular epithelial cells (podocytes) may explain the mechanisms by which Th1 and Th2 cells induce proteinuria. Proteins pass freely through the endothelium fenestrae, and the principal barrier is at the site of the slit diaphragm present between the foot processes of

podocytes [33]. As shown in Figure 1, interaction between integrins and extracellular matrix components transduces a signal leading to correct actin assembly and anchoring of nephrin and CD2AP into the slit diaphragm. The role of CD2AP, nephrin, α-actinin and Rho small G proteins has been demonstrated [34-39]. Stimuli such as TNF, aggregated IgG4, attack complex of complement, can trigger rearrangement of the cytoskeleton, with redistribution of nephrin and CD2AP, which could result in proteinuria [40, 41]. IL-4 and/or IL13 could induce proteinuria by direct interaction with podocytes since these cells express receptors for both cytokines [42]. Alternatively, they may act on monocytes to produce vascular permeability factor(s) responsible for nephrotic syndrome.

Table 2. Role of Th1 and Th2 cells in the development of kidney diseases.

Th1-dependent nephritides
Glomerulonephritis induced by inmmunization with glomerular basement membrane or by sensitization to heterologous immunoglobulin prior to administration of heterologous anti-glomerular basement membrane immunoglobulin: severe crescentic glomerulonephritis (C57BL/6).
Tubulointerstitial nephritis induced by immunization of SJL with renal tubular antigens.
Human crescentic glomerulonephritis (especially pauci immune ANCA-associated glomerulonephritis, Wegener's granulomatosis, glomerulonephritis induced by IL-2 or IFNγ administration).
Th2-dependent nephritides
Glomerulonephritis induced by by sensitization to heterologous immunoglobulin prior to administration of heterologous anti-glomerular basement membrane immunoglobulin: (BALB/c).
Glomerulopathy induced by $HgCl_2$ and gold salts in Brown-Norway rats and glomerulopathy associated with allogeneic reactions in mice.
Human membranous glomerulopathies and possibly minimal change disease

Ig = immunoglobulins; GN = glomerulonephritis, GBM = glomerular basement membrane, ANCA = anti-neutrophil cytoplasmic antibodies. Adapted from [25].

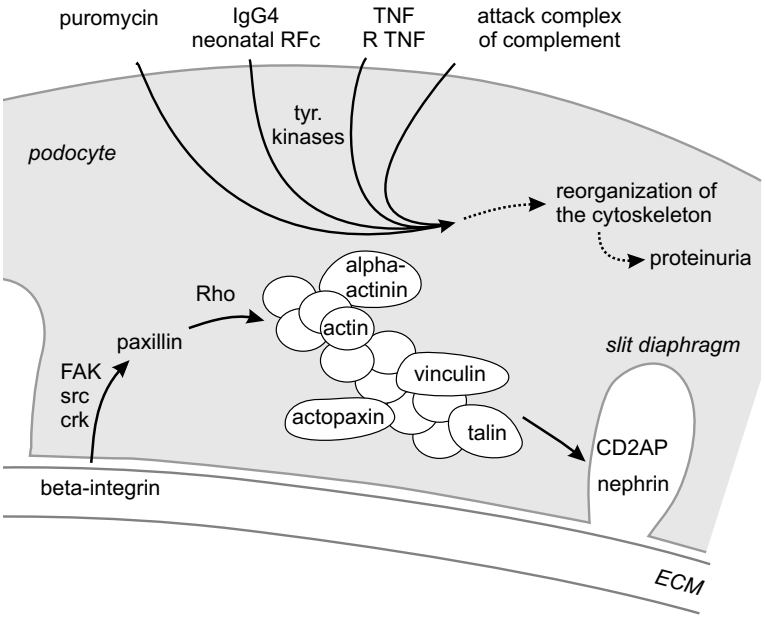

Figure 1. *Putative signalling pathways involved in the organization of the slit diaphragm in podocytes (From [93] and [94]). FAK, src and crk are tyrosine kinases involved in phosphorylation of paxillin which activates the small protein G Rho dependent signalling pathway, recruits actin-binding proteins actopaxin, talin, α actinin, vinculin, leads to actin polymerization with anchoring of nephrin into the slit diaphragm. Puromycin, aggregated IgG4, TNF, the attack complex of complement could trigger reorganization of the cytoskeleton, redistribution of nephrin and proteinuria [41]. At least for TNFα, this effect would be mediated by tyrosin phosphorylation with FAK, paxillin, and vinculin being phosphorylated after stimulation by TNF [40].*
ECM = extracellular matrix.

Immune nephropathies induced by xenobiotics

Glomerulopathies

Non-steroidal anti-inflammatory drugs are known to induce a nephrotic syndrome in addition to acute tubulointerstitial nephritis (discussed in [43]). Glomerulopathies include minimal change disease, focal glomerulosclerosis that could represent a continuum with the former entity and membranous glomerulopathy. A recent review of 97 patients with non-steroidal anti-inflammatory drugs-induced nephropathy reported the following incidence: minimal change disease (39.2%), acute tubulointerstitial nephritis (19.6%), membranous glomerulopathy (19.6%), focal glomerulosclerosis (13, 4%) and others (8, 2%) [43]. Lithium used to treat bipolar affective disorders (reviewed in [44]), gold salts or D-penicillamine used to treat rheumatoid arthritis patients, or mercurials as environmental pollutants, may also induce minimal change disease or membranous glomerulopathy. For example, gold salts cause proteinuria in about 10% of patients due to membranous glomerulopathy (89%) or minimal change disease (10%). This indicates that the same drug may induce several types of glomerulopathy with different immunopathological mechanisms leading to proteinuria. In the same way, a loss of nephrin expression was found in an experimental model of minimal change disease induced by injections of rats or mice with aminonucleosides (puromycin or adriamycin) and in a model of immune type glomerulopathy induced by injections of rats with $HgCl_2$ [45].

The occurrence of gold-induced membranous glomerulopathy is related neither to the cumulative dose of gold, neither to the duration of treatment nor to the type of gold salt used. Susceptibility is genetically controlled since patients with HLA-B8 or DRW3 are at higher risk [46]. The role of HLA DR3 in susceptibility to D-penicillamine-induced adverse reactions has been demonstrated since membranous glomerulopathy is 32 times more frequent in HLA DR3 positive patients than in those who are HLA DR3 negative [47].

Tubulointerstitial nephritides

Most often, tubulointerstitial nephritis is the consequence of glomerular lesions. However, they can be isolated and the causes include autoimmune responses towards tubular antigens, infections, and administration of pharmaceuticals [48].

Drug-induced tubulointerstitial nephritis represents 1-10% of cases of acute renal failure and is characterized by infiltrates of mononuclear cells with tubular cell injury [49]. Most of the patients exhibit enhanced serum IgE levels, hypereosinophilia and/or hypereosinophiluria, fever and skin rashes. Most often, withdrawal of the drug, with or without concomitant steroid administration, improves the renal functions.

Rifampicin-induced tubulointerstitial nephritis is interesting because, at least in some cases, a target might be identified [50]. There is often an association between renal failure and hematological abnormalities (hemolytic anemia and thrombopenia). Patients develop rifampicin-dependent IgG and IgM antibodies against the I antigen of red blood cells, which caused red blood cell lysis through interaction with the antigen on the erythrocyte surface. These antibodies, or a cell-mediated response against this antigen, could play a role in tubulointerstitial nephritis since the I antigen is also expressed on tubular epithelial cells.

The drugs that are most frequently responsible for tubulointerstitial nephritis are antibiotics, especially β-lactams, and non-steroidal anti-inflammatory drugs. Hypersensitivity reactions directed against the drug or its metabolites are usually found [43]. One study reported an association between tubulointerstitial nephritis and minimal change disease in 18 cases out of 27 patients with non-steroidal anti-inflammatory drugs-induced tubulointerstitial nephritis [51].

In one case of tubulointerstitial nephritis and nephrotic syndrome induced by Triazolam, a sleep inducer, numerous eosinophils were found to infiltrate glomeruli and interstitium [52] suggesting that eosinophils may be pathogenic in this situation.

An association with tubulointerstitial nephritis and nephrotic syndrome has also been occasionally reported for penicillin/amoxicillin-induced nephropathy [53]. Several reports have analyzed T-cells in penicillin-induced allergy. It was shown that 1) CD4+ T-cells specific for penicillin may be derived from the skin of patients and produce mainly IL-5, some of them being perforin positive with a cytolytic potential [54]; 2) β-lactams specific clones may be obtained only from patients with adverse drug reactions; the clones were Th2

whatever the type of clinical manifestation and whether or not specific serum IgE were present [55]; 3) Peni G impaired IFN-γ production in an antigen independent manner [56]. These studies support the view that β-lactams induce Th2-dependent hypersensitivity reactions. Such cells may recruit and activate eosinophils via their IL-5 production. It is also possible that direct-cellular contact between activated Th2 cells and tubular epithelial cells amplifies the local inflammatory reaction in the kidney because IL-4 and IL-13 increase the production of RANTES, a proinflammatory mediator by tubular epithelial cells [57].

Xenobiotic-induced immune disregulation

Some drugs such as hydralazine or procainamide may induce lupus like diseases with antinuclear antibodies and proteinuria. D-penicillamine not only causes glomerulopathies, but also myasthenia, polymyositis or lupus, suggesting that this compound provokes immune disregulation. Gold salts also are capable of inducing various immunopathological disorders such as pneumonitis, anemia, thrombocytopenia and hepatitis.

We shall discuss hydralazine or procainamide-induced autoimmunity and heavy metal-induced immunopathological disorders because these compounds have been extensively studied using multiple experimental approaches and in several experimental systems, and because it was shown that several mechanisms may contribute to the development of autoimmunity.

Hydralazine and procainamide-induced autoimmunity

Hydralazine and procainamide have been shown to interfere with central and peripheral mechanisms of tolerance and to lower the threshold of T-cell activation rendering them autoreactive [58, 59].

A metabolite of procainamide, injected into the thymus of (C57BL6 x DBA/2) F1 mice induces the emergence of autoreactive chromatin specific T-cells [58, 59]. In addition, procainamide impairs peripheral T-cell anergy [60].

Therapeutic concentrations of hydralazine and procainamide inhibit methyl transferase activity [61, 62, 63]. Methylation of deoxycytosine residues of gene promoters takes place during cell ontogeny and silences genes through fixation of methylcytosin binding proteins and changes in chromatin structures (developed in [64]); this pattern is maintained through subsequent mitoses by methyltransferases. It was shown that antigen specific T-cell clones, incubated with inhibitors of these enzymes, overexpressed LFA-1 and became able to proliferate in the presence of autologous antigen presenting cells, even in the absence of the nominal antigen. Autoreactivity is probably the consequence of the increase in LFA-1 expression since antigen specific T-cells transfected with LFA-1 also became autoreactive [63]. The injection of T-cells rendered autoreactive by incubation with procainamide [65] or with hydralazine [66] or of T-cells transfected with LFA-1 [63] into a non-irradiated syngeneic normal recipient triggers an autoimmune disease [66]. This disease is marked by anti-DNA antibody production, proliferative glomerulonephritis, pulmonary alveolitis, liver lesions resembling primary biliary cirrhosis, and histologic changes in the brain resembling central nervous system lupus [64].

Histone deacetylases are also important with respect to the accessibility of chromatin and gene expression; acetylation of histones is required for gene expression while deacetylation correlates with inhibition of transcription. Moreover, methylcytosine binding proteins associate with histone deacetylases directing the deacetylase activity to regions destined for inactivation [67]. This suggests a role for inhibitors of deacetylases in targeting gene expression including IFN-γ and IL-4 [68], which could contribute to the development of autoimmunity.

$HgCl_2$ and gold salt induced autoimmunity

Effect of these metals in mice

Injections of susceptible murine strains including B.10S and AS.W mice with non toxic amounts of $HgCl_2$ or gold salts trigger an increase in serum IgE and IgG1 concentrations, two Th2-dependent isotypes, and IgG1 antinucleolar antibody [69, 70]. Anti-IL-4 mAb administration inhibits the effect of $HgCl_2$ on serum IgE and IgG1 antibodies but does not modify the titer of antinuclear antibodies; this treatment is associated with the development of the Th1 dependent IgG2a and IgG3 antinuclear antibody isotypes [70].

Effect of $HgCl_2$, gold salts and D-penicillamine in rats

a) The model

Brown-Norway (BN) rats injected thrice a week with $HgCl_2$ (1 mg/kg bw, sc), $HAuCl_4$ (1 mg/kg bw, sc), aurothioropanolsulfonate sodium salt, the gold salt used in France for rheumatoid arthritis (allochrysine®, 20 mg/kg bw, sc) treatment, or D-penicillamine, develop an autoimmune disease while LEW rats are resistant. The disease is characterized by the production of numerous autoantibodies (anti-laminin, a component of the glomerular basement membrane, anti-dsDNA, anti-thyroglobulin antibodies …) and an increase in serum IgE and IgG1 (reviewed in [71-73]). Anti-laminin antibodies are found deposited in the kidneys; they are responsible for linear IgG deposits along the glomerular basement membrane and this is followed by the development of membranous glomerulopathy. The disease induced by $HgCl_2$ is more severe than the one caused by gold salts or D-penicillamine since rats display heavy proteinuria only in the former. Glomeruli appear to be normal at the light microscopy level and some infiltrates of mononuclear cells with predominantly CD4+ T-cells are occasionally found in the interstitium.

The disease is spontaneously regulated even if $HgCl_2$, $HAuCl_4$ or D-penicillamine administration is pursued and rats are resistant to rechallenge. This latter phenomenon appears to be dependent on CD8+ cells [74]. $HgCl_2$-induced autoimmunity is reduced in rats previously treated with D-penicillamine [72]. This suggests that mechanisms involved in the regulation of D-penicillamine-induced autoimmunity may also partially protect against the development of $HgCl_2$-induced autoimmunity.

b) Non-antigen specific lymphocyte activation

$HgCl_2$ and gold salts trigger a polyclonal activation of BN B- and T-cells. $HgCl_2$ does not induce the expansion of peculiar Vβ bearing cells, which rules out the possibility that this metal behaves as a superantigen [75]. $HgCl_2$ and $HAuCl_4$ increase the intracellular calcium concentration in BN and LEW T-cells ([76] and unpublished). $HAuCl_4$ triggers a calcium signal in up to 100% of CD4+ and CD8+ T- and in 70% of purified B-cells from both BN and LEW rats [76]. These data show that gold salts induce a panclonal activation of T-cells and that the resistance of LEW rats is not explained by an inability of metals to stimulate their lymphocytes.

Stimulation of T-cells through the T-cell receptor leads to a cascade of events [77], (Figure 2) initiated by activation of src kinases that phosphorylate tyrosine of the ITAMs (immunoreceptor tyrosine activated motives) in the T-cell receptor/CD3 chains. This results in recruitment and phosphorylation of the tyrosine kinase ZAP-70. Multiple adapter and effector molecules are then directed to signalling complexes and are activated, including phospholipase Cg1. The latter cleaves phosphatidylinositide 4, 5 biphosphate into inositol 3, 4, 5 triphosphate and diacylglycerol. Inositol 3, 4, 5 triphosphate releases intracellular calcium stores into the cytosol with an ensuing calcium entry responsible for a sustained increase in intracellular calcium concentration, while diacylglycerol activates protein kinase C. Finally, T-cell receptor-dependent signalling pathways converge to activate multiple transcription factors leading to proliferation, cytokine gene transcription etc. The calcium response elicited by $HgCl_2$ and $HAuCl_4$ in purified T-cells is the consequence of an effect of these metals on the early steps of T-cell activation. Indeed, the metals trigger a pattern of tyrosine phosphorylation similar to the one induced by T-cell receptor ligation, and PP2, an inhibitor of src kinases, abolishes $HAuCl_4$-induced calcium signal [76].

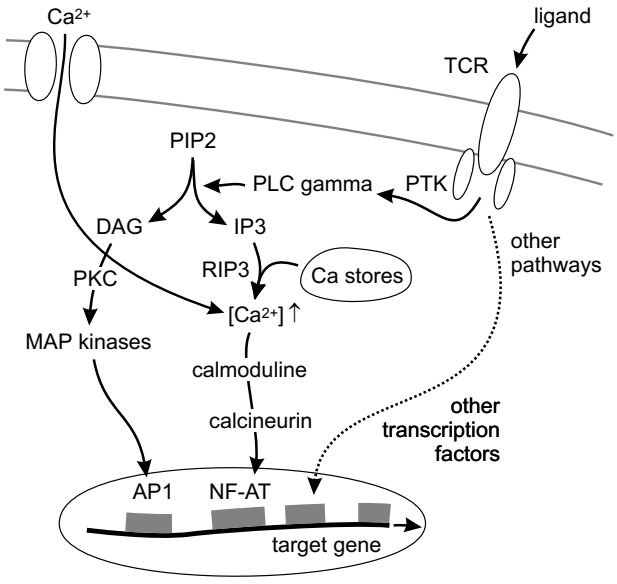

Figure 2. *Scheme of T-cell receptor-associated signalling pathways.*

TCR = T-cell receptor; PTK= protein tyrosine kinases; PLCg = phospholipase Cg; PIP2= phosphatidylinositide 4,5 biphosphate; IP3= inositol 3,4,5 triphosphate; RIP3 = receptor for IP3; DAG= diacylglycerol, PKC=protein kinase C.

$HAuCl_4$-induced T-cell activation results in early cytokine gene expression by lymphocytes from both Brown-Norway and LEW rats since two to four hour incubation with the metal is sufficient to detect an increase in IL-4 and IFN-γ mRNA [76, 78]. Nevertheless, the expression of IL-4 predominates in BN T-cells while the expression of IFN-γ is favored in LEW T-cells. The *in vitro* findings correlate quite well with the profile of cytokine expression in splenocytes of Brown-Norway and LEW rats injected with $HAuCl_4$, which gives relevance to the *in vitro* data. The pronounced IL-4 overexpression induced by gold in Brown-Norway rats is probably related to the fact that Brown-Norway rats mount preferential Th2 responses whatever the stimulus [79, 80]. It would be interesting to determine what is the frequency of IL-4 expressing T-cells upon stimulation with gold and whether this phenotype concerns a T-cell subset characterized by some particular markers.

It has been previously shown that $HgCl_2$ induces IL-4 gene transcription in BN but not in LEW T-cells [78]. In order to identify what are the molecular mechanisms at play, we used an IL-4 producing T-cell hybridoma, which allowed us to identify a new signalling pathway [81]. Indeed, in this hybridoma, $HgCl_2$ activates protein kinase C resulting in an entry of calcium sufficient to initiate IL-4 gene transcription [81, 82]. We then obtained evidence that this pathway was also turned on following stimulation through the T-cell receptor not only in T-cell hybridomas but also in Th2 clones [83]. There is a large body of evidence that naive T-cells, Th1 and Th2 cells differ not only by their expression in transcription factors, by the expression of receptors for cytokines or signalling pathways associated to these receptors but also with regard to T-cell receptor-dependent signalling pathways. This could mean that $HgCl_2$ or $HAuCl_4$ behave as polyclonal T-cell activators ([76] and unpublished) acting on the early steps of activation and switching on the T-cell receptor-dependent signalling pathways available in the cell studied, pathways that differ depending upon the state of differentiation.

$HgCl_2$ and $HAuCl_4$ are both B-cell activators but the mechanisms involved are probably different. Indeed the effect of $HgCl_2$ on B-cells depends upon IL-4 [76, 78], while $HAuCl_4$ directly acts on B cells, leading to an increase in MHC class II expression probably via the increase in intracellular calcium concentration [76].

c) Mechanisms responsible for autoimmunity

The fact that normal BN T-cells incubated with $HgCl_2$ transfer the disease [84] suggests that the effect of the metal is sufficient for inducing autoimmunity. Figure 3 schematizes some events by which $HgCl_2$ or $HAuCl_4$ may induce autoreactivity.

$HgCl_2$ or $HAuCl_4$-induced autoimmunity is probably not due solely to an overexpression of IL-4 since IL-4 transgenic mice have been described as autoimmune prone in one report [85]. In our model, $HgCl_2$ and allochrysine are found to induce the emergence of autoreactive anti-class II T-cells in both Brown-Norway and LEW rats. T-cell lines have been obtained from both strains; they are Th2 only when they originate from Brown-Norway rats [27]. Autoreactive Th2 cell lines transfer autoimmunity into CD8+ cell depleted Brown-Norway rats [27]. This suggests that the direct effect of metal on IL-4 gene expression in BN T-cells certainly favors the development of autoreactive Th2 cells that are pathogenic in this model. Another inter-

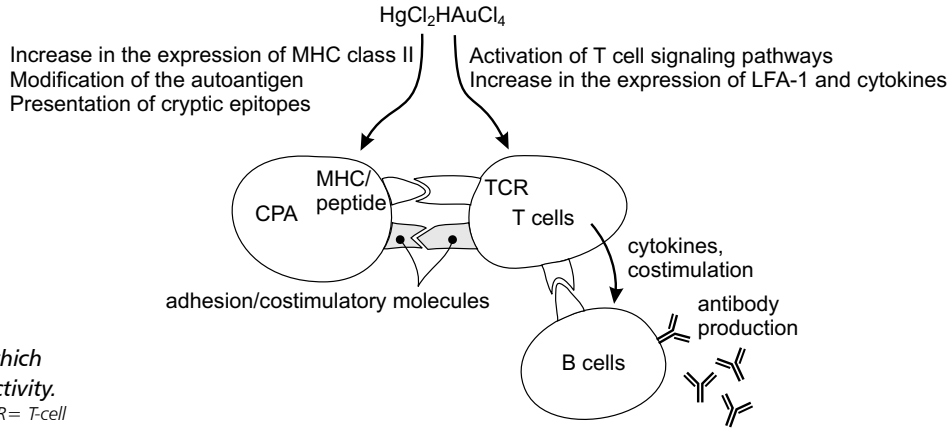

Figure 3. *Mechanisms by which metals may induce autoreactivity.*
APC = antigen presenting cells; TCR= T-cell receptor.

esting point is that the disease induced by injecting the T-cell lines into CD8+ cell depleted Brown-Norway rats is much more severe than the one induced by the administration of gold salts. Indeed, in the former case, rats display infiltration of many organs: liver, lung and kidneys by CD4+ cells. In addition, animals may die due to renal failure. This suggests that, in normal Brown-Norway rats, CD8+ cells exert a negative control on Th2 cells.

The expression of LFA-1 was found to be increased, among other markers, at the T-cell surface in Brown-Norway rats injected with $HgCl_2$ [86]. This could be sufficient for inducing autoreactivity as shown by Richardson's group [64].

Elements favoring the supposition that $HgCl_2$ and allochrysine would induce some specific responses come from experiments of Field et al. [87], who showed that neonatal administration of $HgCl_2$ does not induce autoimmunity, prevents the development of $HgCl_2$-induced autoimmunity at adult age, but does not modify the course of allochrysine-induced autoimmunity. The tolerance is transferable by splenocytes. Altogether, these data suggest that autoreactive T-cells may be controlled by regulatory cells that are specific either for mercury or for determinants specifically induced by mercury and not by gold salts.

d) Genetic control and resistance of LEW rats

Injection of Brown-Norway rats with gold salts provided a model to analyze the genetic control of the IgE response. A cohort of F2 progeny of susceptible Brown-Norway and resistant LEW strains has been studied to carry out a genome-wide search for loci controlling the IgE response. Genome scanning identified three loci, Atps1, Atps 2 and Atps3 on chromosomes 20, 10 and 9 respectively. Atps1 is linked to the MHC and Atps2 to the cytokine gene cluster that includes the IL-4 region [88, 89]. It is interesting to note that a region homologous to Atps2 is localized on human chromosome 5 in 5q31.1 and has been linked to serum IgE concentration in families of atopic patients from different ethnic origins [90]. A locus called Eae4 implicated in the susceptibility of Dark Agouti rats or the resistance of Brown-Norway rats to develop Th1-mediated autoimmune encephalomyelitis is mapped in the same region as Atps3 [91]. Regions implicated in the susceptibility of LEW rats and the resistance of Brown-Norway rats to develop experimental autoimmune encephalomyelitis also map in the same regions as Atps2 and probably Atps3 [92]. Finally, it has been shown in rats that CD4+T-cells with a high expression of the isoform CD45RC contain Th1 precursors while $CD45RC^{low}$ cells are enriched in Th2 precursors. The ratio CD4 $CD45RC^{low}$/ $CD45RC^{high}$ is 2:1 in the Th2 prone Brown-Norway strain and 1:2 in the Th1 prone LEW strain. Subra et al showed a linkage between this ratio and regions containing Atps1, 2 and 3 [80]. Altogether, these data suggest that Atps2 and Atps 3 contain genes that control the differentiation of T-cells into Th1 or Th2 cells.

The reasons why LEW rats are resistant to the development of gold-induced autoimmunity are unknown. However, two points are interesting: 1) LEW MHC is permissive since Brown-Norway.1L rats, which have the same MHC as LEW rats and non MHC genes from the BN background, develop gold-induced disease as Brown-Norway rats and 2) administration of anti-IFN-γ mAb render LEW rats partially susceptible to allochrysine-induced autoimmunity with the appearance of anti-laminin antibodies even if their titer is 5 times lower than in Brown-Norway rats [76]. This mild response could explain why IgG deposits are not found in the kidneys of LEW rats injected with allochrysine and anti-IFN-γ antibodies.

Conclusion

There is still a long way to go before all the mechanisms responsible for drug-induced immune kidney lesions will be explained. However, the notion that T-cell activation in addition to T-cell receptor-MHC peptide interactions also requires a tissue environment is an important concept for better understanding immunopathogenic mechanisms. For example, it is noteworthy that a toxic effect of the drugs on the kidney may initiate an immune response because they promote the presentation of haptenized determinants or even of self-peptides in inflammatory conditions. Th1 cells and probably Th2 cells may be pathogenic even if the effectors responsible for the lesions may be different. For example, in some patients, eosinophils, probably activated by Th2 cells could be pathogenic. Some drugs, such as hydralazine or heavy metals behave as T-cell polyclonal activators, which is sufficient for, or contributes to, the development of autoimmunity at least in some genetic backgrounds.

References

1. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Immunol 2001; 13: 114-119.
2. Inaba K, Turley S, Iyoda T, Yamaide F, Shimoyama S, Reis e Sousa C, Germain RN, Mellman I, Steinman RM. The formation of immunogenic major histocompatibility complex class II-peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. J Exp Med 2000; 191: 927-936.
3. Manome H, Aiba S, Tagami H. Simple chemicals can induce maturation and apoptosis of dendritic cells. Immunology 1999; 98: 481-490.
4. Pollard KM, Lee DK, Casiano CA, Bluthner M, Johnston MM, Tan EM. The autoimmunity-inducing xenobiotic mercury interacts with the autoantigen fibrillarin and modifies its molecular and antigenic properties. J Immunol 1997; 158: 3521-3528.
5. Kubicka-Muranyi M, Kremer J, Rottmann N, Lubben B, Albers R, Bloksma N, Luhrmann R, Gleichmann E. Murine systemic autoimmune disease induced by mercuric chloride: T helper cells reacting to self proteins. Int Arch Allergy Immunol 1996; 109: 11-20.
6. Mamula MJ, Lin R-H, Janeway Jr CA. Breaking T-cell tolerance with foreign and self co-immunogens A study of autoimmune B and T-cell epitopes of cytochrome c. J Immunol 1992; 149: 789-795.
7. Weiss RA, Madaio MP, Tomaszewski JE, Kelly CJ. T-cells reactive to an inducible heat shock protein induce disease in toxin-induced interstitial nephritis. J Exp Med 1994; 180: 2239-2250.
8. Katz-Levy Y, Neville KL, Girvin AM, Vanderlugt CL, Pope JG, Tan LJ, Miller SD. Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. J Clin Invest 1999; 104: 599-610.
9. Griem P, Gleichmann E. Metal ion-induced autoimmunity. Curr Opin Immunol 1995; 7: 831-838.
10. Griem P, Panthel K, Kalbacher H, Gleichmann E. Alteration of a model antigen by Au(III) leads to T-cell sensitization to cryptic peptides. Eur J Immunol. 1996; 26: 279-287.
11. Griem P, von Vultee C, Panthel K, Best SL, Sadler PJ, Shaw CF, 3rd. T-cell cross-reactivity to heavy metals: identical cryptic peptides may be presented from protein exposed to different metals. Eur J Immunol 1998; 28: 1941-1947.
12. Kalluri R, Cantley LG, Kerjaschki D, Neilson EG. Reactive oxygen species expose cryptic epitopes associated with autoimmune goodpasture syndrome. J Biol Chem 2000; 275: 20027-20032.
13. Stockinger B. T lymphocyte tolerance: from thymic deletion to peripheral control mechanisms. Adv Immunol 1999; 71: 229-265.
14. Klein L, Kyewski B. "Promiscuous" expression of tissue antigens in the thymus: a key to T-cell tolerance and autoimmunity ? J Mol Med 2000; 78: 483-494.
15. Buer J, Lanoue A, Franzke A, Garcia C, von Boehmer H, and Sarukhan A. Interleukin 10 secretion and impaired effector function of major histocompatibility complex class II-restricted T-cells anergized *in vivo*. J Exp Med 1998; 187: 177-183.
16. Bucy RP, Xu XY, Li J, and Huang G. Cyclosporin A-induced autoimmune disease in mice. J Immunol 1993; 151: 1039-1050.
17. Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T-cell signaling. Nat Immunol 2000; 1: 23-29.
18. Montes M, McIlroy D, Hosmalin A, Trautmann A. Calcium responses elicited in human T-cells and dendritic cells by cell-cell interaction and soluble ligands. Int Immunol 1999; 11: 561-568.
19. Goldman M, Druet P. The TH1/TH2 concept and its relevance to renal disorders and transplantation immunity. Nephrol Dial Transplant 1995; 10: 1282-1284.
20. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996; 383: 787-793.
21. MacKay CR. Follicular homing T helper (Th) cells and the Th1/Th2 paradigm. J Exp Med 2000; 192: F31-F34.
22. Glimcher LH, Murphy KM. Lineage commitment in the immune system: the T helper lymphocyte grows up. Genes Dev. 2000; 14: 1693-1711.
23. Fournié GJ, Cautain B, Xystrakis E, Damoiseaux J, Mas M, Lagrange D, Bernard I, Subra JF, Pelletier L, Druet P, Saoudi A. Cellular and genetic factors involved in the difference between Brown-Norway and Lewis rats to develop respectively type-2 and type-1 immune mediated responses. Immunol Rev. 2001 ; 184: 145-160.
24. Grogan JL, Mohrs M, Harmon B, Lacy DA, Sedat JW, Locksley RM. Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. Immunity 2001; 14: 205-215.
25. Holdsworth SR, Kitching AR, Tipping PG. Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. Kidney Int 1999; 55: 1198-1216.
26. Heeger PS, Smoyer WE, Saad T, Albert S, Kelly CJ, Neilson EG. Molecular analysis of the helper T-cell response in murine interstitial nephritis. T-cells recognizing an immunodominant epitope use multiple T-cell receptor V β genes with similarities across CDR3. J Clin Invest 1994; 94: 2084-2092.
27. Saoudi A, Castedo M, Nochy D., Mandet C, Pasquier R, Druet P, Pelletier L. Self reactive anti-class II Th2 cell lines derived from gold salt-injected rats trigger B cell polyclonal activation and transfer autoimmunity in CD8-depleted normal syngeneic recipients. Eur J Immunol 1995; 25: 1972-1979.

28. Powell TJ, Streilein JW. Neonatal tolerance induction by class II alloantigens activates IL-4 secreting, tolerogen-responsive T-cells. J Immunol 1990; 144: 854-859.
29. Schurmans S, Heusser CH, Qin H-Y, Merino J, Brighouse G, Lambert P-H. *In vivo* effects of anti-IL-4 monoclonal antibody on neonatal induction of tolerance and on an associated autoimmune syndrome. J Immunol 1990; 145: 2465-2473.
30. Coudert JD, Foucras G, Demur C, Coureau C, Mazerolles C, Delsol G, Druet P, Guery JC. Lethal host-versus-graft disease and hypereosinophilia in the absence of MHC I-T-cell interactions. J Clin Invest 2000; 105: 1125-1132.
31. Cho BS, Yoon SR, Jang JY, Pyun KH, Lee CE. Up-regulation of interleukin-4 and CD23/FcepsilonRII in minimal change nephrotic syndrome. Pediatr Nephrol 1999; 13: 199-204.
32. Yap HK, Cheung W, Murugasu B, Sim SK, Seah CC, and Jordan SC. Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increased IL-13 mRNA expression in relapse. J Am Soc Nephrol 1999; 10: 529-537.
33. Ruotsalainen V, Ljungberg P, Wartiovaara J, Lenkkeri U, Kestila M, Jalanko H, Holmberg C, Tryggvason K. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. Proc Natl Acad Sci USA 1999; 96: 7962-7967.
34. Shih NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O, Miner JH, Shaw AS. Congenital nephrotic syndrome in mice lacking CD2-associated protein. Science 1999; 286: 312-315.
35. Togawa A, Miyoshi J, Ishizaki H, Tanaka M, Takakura A, Nishioka H, Yoshida H, Doi T, Mizoguchi A, Matsuura N, Niho Y, Nishimune Y, Nishikawa Si, Takai Y. Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDIα. Oncogene 1999; 18: 5373-5380.
36. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR. Mutations in ACTN4, encoding α-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet 2000; 24: 251-256.
37. Pavenstadt H. Roles of the podocyte in glomerular function. Am J Physiol Renal Physiol 2000; 278: F173-F179.
38. Johnson RJ. New insights into the pathogenesis of proteinuria. Am J Kidney Dis 2000; 36: 214-219.
39. Putaala H, Soininen R, Kilpelainen P, Wartiovaara J, Tryggvason K. The murine nephrin gene is specifically expressed in kidney, brain and pancreas: inactivation of the gene leads to massive proteinuria and neonatal death. Hum Mol Genet 2001; 10: 1-8.
40. Koukouritaki SB, Vardaki EA, Papakonstanti EA, Lianos E, Stournaras C, Emmanouel DS. TNF-α induces actin cytoskeleton reorganization in glomerular epithelial cells involving tyrosine phosphorylation of paxillin and focal adhesion kinase. Mol Med 1999; 5: 382-392.
41. Doublier S, Ruotsalainen V, Salvidio G, Lupia E, Biancone L, Conaldi PG, Reponen P, Tryggvason K, Camussi G. Nephrin redistribution on podocytes is a potential mechanism for proteinuria in patients with primary acquired nephrotic syndrome. Am J Pathol 2001; 158: 1723-1731.
42. van den Berg JG, Aten J, Chand MA, Claessen N, Dijkink L, Wijdenes J, Lakkis FG, Weening JJ. Interleukin-4 and interleukin-13 act on glomerular visceral epithelial cells. J Am Soc Nephrol 2000; 11: 413-422.
43. Ravnskov U. Glomerular, tubular and interstitial nephritis associated with non- steroidal antiinflammatory drugs. Evidence of a common mechanism. Br J Clin Pharmacol 1999; 47: 203-210.
44. Bosquet S, Descombes E, Gauthier T, Fellay G, Regamey C. Nephrotic syndrome during lithium therapy. Nephrol Dial Transplant 1997; 12: 2728-2731.
45. Luimula P, Ahola H, Wang SX, Solin ML, Aaltonen P, Tikkanen I, Kerjaschki D, Holthofer H. Nephrin in experimental glomerular disease. Kidney Int 2000; 58: 1461-1468.
46. Wooley PH, Griffin J, Panayi GS, Batchelor JR, Welsh KI, Gibson TJ. HLA-DR antigens and toxic reaction to sodium aurothiomalate and D-penicillamine in patients with rheumatoid arthritis. N Engl J Med 1980; 303: 300-302.
47. Emery P, Panayi GS, Huston G, Welsh KL, Mitschell SC, Shah RR, Idle JR, Smith RL, Waring RH. D-penicillamine induced toxicity in rheumatoid arthritis : the role of sulphoxidation status and HLA-DR3. J Rheum 1984; 11: 626-632.
48. Neilson EG. Interstitial nephritis: another kissing disease ? J Clin Invest 1999; 104: 1671-1672.
49. Pelletier L, Druet P. Immunologically-mediated toxin induced renal diseases. In: Clinical nephrotoxins - renal injury from drugs and chemicals. De Broe ME, Porter GA, Bennett WM, Verpooten GA (editors). Kluwer Academic Publ, Dordrecht 1998; p. 31-38.
50. De Vriese AS, Robbrecht DL, Vanholder RC, Vogelaers DP, Lameire NH. Rifampicin-associated acute renal failure: pathophysiologic, immunologic, and clinical features. Am J Kidney Dis 1998; 31: 108-115.
51. Pospishil YO, Antonovych TM. NSAIDs associated nephropathy. Pol J Pathol 1998; 49: 35-39.
52. Makino H, Haramoto T, Sasaki T, Hironaka K, Shikata K, Takahashi K, Ota Z. Massive eosinophilic infiltration in a patient with the nephrotic syndrome and drug-induced interstitial nephritis. Am J Kidney Dis 1995; 26: 62-67.
53. Dharnidharka VR, Rosen S, Somers MJ. Acute interstitial nephritis presenting as presumed minimal change nephrotic syndrome. Pediatr Nephrol 1998; 12: 576-578.
54. Yawalkar N, Hari Y, Frutig K, Egli F, Wendland T, Braathen LR, Pichler WJ. T-cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug specific and cytotoxic. J Invest Dermatol 2000; 115: 647-652.

55. Brugnolo F, Annunziato F, Sampognaro S, Campi P, Manfredi M, Matucci A, Blanca M, Romagnani S, Maggi E, Parronchi P. Highly Th2-skewed cytokine profile of β-lactam-specific T-cells from nonatopic subjects with adverse drug reactions. J Immunol 1999; 163: 1053-1059.
56. Padovan E, von Greyerz S, Pichler WJ, Weltzien HU. Antigen-dependent and -independent IFN-γ modulation by penicillins. J Immunol 1999; 162: 1171-1177.
57. Deckers JG, De Haij S, van der Woude FJ, van der Kooij SW, Daha MR, van Kooten C. IL-4 and IL-13 augment cytokine- and CD40-induced RANTES production by human renal tubular epithelial cells *in vitro*. J Am Soc Nephrol 1998; 9: 1187-1193.
58. Kretz-Rommel A, Duncan SR, Rubin RL. Autoimmunity caused by disruption of central T-cell tolerance. A murine model of drug-induced lupus. J Clin Invest 1997; 99: 1888-1896.
59. Kretz-Rommel A, Rubin RL. Persistence of autoreactive T-cell drive is required to elicit anti-chromatin antibodies in a murine model of drug-induced lupus. J Immunol 1999; 162: 813-820.
60. Kretz-Rommel A, Rubin RL. A metabolite of the lupus-inducing drug procainamide prevents anergy induction in T-cell clones. J Immunol 1997; 158: 4465-4470.
61. Cornacchia E, Golbus J, Maybaum J, Strahler J, Hanash S, Richardson B. Hydralazine and procainamide inhibit T-cell DNA methylation and induce autoreactivity. J Immunol 1988; 140: 2197-2200.
62. Richardson B, Powers D, Hooper F, Yung RL, O'Rourke K. Lymphocyte function-associated antigen 1 overexpression and T-cell autoreactivity. Arthritis Rheum 1994; 37: 1363-1372.
63. Yung R, Powers D, Johnson K, Amento E, Carr D, Laing T, Yang J, Chang S, Hemati N, Richardson B. Mechanisms of drug-induced lupus. II. T-cells overexpressing lymphocyte function-associated antigen 1 become autoreactive and cause a lupuslike disease in syngeneic mice. J Clin Invest 1996; 97: 2866-2871.
64. Rao T, Richardson B. Environmentally induced autoimmune diseases: potential mechanisms. Environ Health Perspect 1999; 107 (S5): 737-742.
65. Quddus J, Johnson K, Gavalchin J, Amento E, Chrisp C, Yung R, Richardson B. Treating activated CD4+ T-cells with either of two distinct methyltransferase inhibitors, 5-azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. J Clin Invest 1993; 92: 38-46.
66. Yung RL, Quddus J, Chrisp CE, Johnson KJ, Richardson BC. Mechanisms of drug-induced lupus I. Clones Th2 cells modified with DNA methylation inhibitors *in vitro* cause autoimmunity *in vivo*. J Immunol 1995; 154: 3025-3035.
67. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 1998; 393: 386-389.
68. Bird JJ, Brown DR, Mullen AC, Moskowitz NH, Mahowald MA, Sider JR, Gajewski TF, Wang CR, Reiner SL. Helper T-cell differentiation is controlled by the cell cycle. Immunity 1998; 9: 229-237.
69. Pietsch P, Vohr HW, Degitz K, Gleichmann E. Immunopathological signs inducible by mercury compounds. II. HgCl2 and gold sodium thiomalate enhance serum IgE and IgG concentrations in susceptible mouse strains. Int Arch Allergy Appl Immun 1989; 90: 47-53.
70. Ochel M, Vohr H-W, Pfeiffer C, Gleichmann E. IL-4 is required for the IgE and IgG1 increase and IgG1 autoantibody formation in mice treated with mercuric chloride. J Immunol 1991; 146: 3006-3011.
71. Badou A, Saoudi A, Dietrich G, Druet E, Druet P, Pelletier L. Mercuric chloride-induced autoimmunity. Curr Prot Immunol 1999; 15: 1-18.
72. Tournade H, Pelletier L, Pasquier R, Vial M-C, Mandet C, Druet P. D-penicillamine-induced autoimmunity in Brown Norway rats: similarities with $HgCl_2$-induced autoimmunity. J Immunol 1990; 144: 2985-2991.
73. Tournade H, Guéry J-C, Pasquier R, Vial MC, Mandet C, Druet E, Dansette PM, Druet P, Pelletier L. Effect of the thiol group on experimental gold-induced autoimmunity. Arthritis Rheum 1991; 34: 1594-1599.
74. Mathieson PW, Stapleton KJ, Oliveira DBG, Lockwood CM. Immunoregulation of mercuric chloride-induced autoimmunity in Brown Norway rats: a role for CD8+ T-cells revealed by *in vivo* depletion studies. Eur J Immunol 1991; 21: 2105-2109.
75. Fillion J, Baccala R, Kuhn J, Druet P, Bellon B. Evidence for heterogenous TCRVβ expression in mercury-induced autoimmune disorders in rats. Int Immunol 1997; 9: 263-271.
76. Savignac M, Badou B, Delmas C, Subra JF, De Cramer S, Paulet P, Cassar G, Druet P, Saoudi A, Pelletier L. Gold is a T-cell polyclonal activator in BN and LEW rats but favors IL-4 expression only in autoimmune prone BN rats. Eur J Immunol 2001; 31: 2266-2276.
77. Cantrell D. T-cell antigen receptor signal transduction pathways. Ann Rev Immunol 1996; 14: 259-274.
78. Prigent P, Saoudi A, Pannetier C, Graber P, Bonnefoy Y, Druet P, Hirsch F. Mercuric chloride, a chemical responsible for Th2-mediated autoimmunity in Brown-Norway rats, directly triggers T-cells to produce IL-4. J Clin Invest. 1995; 96: 1484-1489.
79. Saoudi A, Guery JC, De Baets M. Is pathogenic humoral autoimmunity a Th1 response ? Immunol Today 2000; 21: 306-307.
80. Subra JF, Cautain B, Xystrakis E, Mas M, Lagrange D, van Der Heijden H, van De Gaar MJ, Druet P, Fournie GJ, Saoudi A, Damoiseaux J. The balance between cd45rc (high) and cd45rc (low) cd4 T-cells in rats is intrinsic to bone marrow-derived cells and is genetically controlled. J Immunol 2001; 166: 2944-2952.

81. Badou A, Savignac M, Moreau M, Leclerc C, Pasquier R, Druet P, Pelletier L. $HgCl_2$-induced IL-4 gene expression in T-cells involves protein kinase C-dependent calcium influx through L-type calcium channels. J Biol Chem 1997; 272: 32411-32418.
82. Badou A, Savignac M, Moreau M, Leclerc C, Foucras G, Druet P, Guéry JC, Pelletier L. Weak T-cell receptor (TCR) stimulation induces a calcium signal that triggers IL-4 synthesis while MAP kinases are activated for stronger TCR engagement and control IFNg production. Eur J Immunol. 2001; 2487-2496.
83. Savignac M, Badou A, Moreau M, Leclerc C, Guery JC, Paulet P, Druet P, Ragab-Thomas J, Pelletier L. Protein kinase C-mediated calcium entry dependent upon dihydropyridine sensitive channels: a T-cell receptor-coupled signaling pathway involved in IL-4 synthesis. Faseb J 2001; 15: 1577-1579.
84. Pelletier L, Pasquier R, Rossert J, Vial M-C, Mandet C, Druet P. Autoreactive T-cells in mercury-induced autoimmunity. Ability to induce the autoimmune disease. J Immunol 1988; 140: 750-754.
85. Erb KJ, Ruger B, von Brevern M, Ryffel B, Schimpl A, Rivett K. Constitutive expression of interleukin (IL)-4 *in vivo* causes autoimmune- type disorders in mice. J Exp Med 1997; 185: 329-339.
86. Roos A, Claessen N, Weening JJ, Aten J. Enhanced T lymphocyte expression of LFA-1, ICAM-1, and the TNF receptor family member OX40 in $HgCl_2$-induced systemic autoimmunity. Scand J Immunol 1996; 43: 507-518.
87. Field AC, Caccavelli L, Fillion J, Kuhn J, Mandet C, Druet P, Bellon B. Neonatal induction of tolerance to T(h)2-mediated autoimmunity in rats. Int Immunol 2000; 12: 1467-1477.
88. Kermarrec N, Dubay C, De Gouyon B, Blanpied C, Gauguier D, Gillespie K, Druet P, Lathrop M, Hirsch F. Serum IgE concentration and other immune manifestations of treatment with gold salts are linked to MHC and IL-4 regions in the rat. Genomics 1996; 31: 111-114.
89. Mas M, Subra JF, Lagrange D, Pilipenko-Appolinaire S, Gauguier D, Druet P, Fournié GJ. Quantitative trait locus analysis of immune responsiveness to gold salt in a F2 cohort of (Lewis x Brown Norway) rats identifies a new major susceptibility locus for IgE response on chromosome 9. Eur J Immunol 2000; 30: 1698-1705.
90. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum IgE concentrations. Science 1994; 264: 1152-1156.
91. Dahlman I, Jacobsson L, Glaser A, Lorentzen JC, Andersson M, Luthman H, Olsson T. Genome-wide linkage analysis of chronic relapsing experimental autoimmune encephalomyelitis in the rat identifies a major susceptibility locus on chromosome 9. J Immunol 1999; 162: 2581-2588.
92. Roth MP, Viratelle C, Dolbois L, Delverdier M, Borot N, Pelletier L, Druet P, Clanet M, and Coppin H. A genome-wide search identifies two susceptibility loci for experimental autoimmune encephalomyelitis on rat chromosomes 4 and 10. J Immunol 1999; 162: 1917-1922.
93. Smoyer WE, Mundel P. Regulation of podocyte structure during the development of nephrotic syndrome. J Mol Med 1998; 76: 172-183.
94. Turner CE. Paxillin interactions. J Cell Sci 2000; 113: 4139-4140.

05

Cellular mechanisms of nephrotoxicity

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Morphology of nephrotoxic injury

The changes in renal epithelial morphology that accompany acute renal failure are often subtle. At least four cellular fates can be identified in acute renal failure: cells may be necrotic; cells may become apoptotic; they may replicate and divide; or they may appear indifferent to the stress (Figure 1). Frank necrosis, as is often seen experimentally, is not prominent in the vast majority of human cases. Necrosis is usually patchy, involving small clusters of cells, sometimes resulting in small areas of denuded basement membrane. Less obvious injury is more often noted, including loss of brush borders, flattening of the epithelium, intratubular cast formation, and dilatation of the lumen. While proximal tubules show many of theses changes, injury to the distal nephron can also be demonstrated when human biopsy material is closely examined. The distal nephron is also the site of obstruction by desquamated cells and cellular debris.

Necrosis is a catastrophic breakdown of regulated cellular homeostasis and is accompanied by massive tissue damage leading to rapid collapse of internal homeostasis of the cell [1]. It is characterized by cell swelling with early loss of plasma-membrane integrity, major alterations of the organelles, and swelling of the

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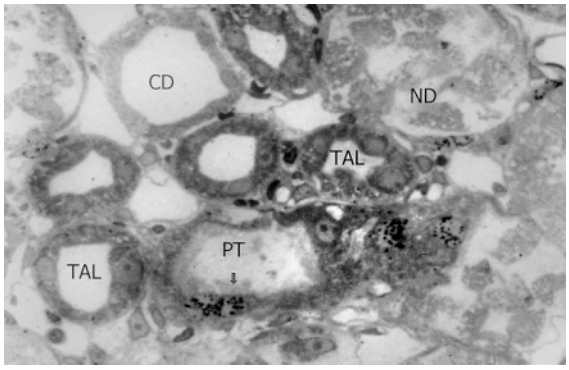

Figure 1. *Radiohistogram of outer stripe of outer medulla of rat kidney taken from animal 5 days after cisplatin, 5 mg/kg BW. Note three cell fates: 1) Necrosis (ND) of cells lining injured S3 segment; 2) apparent indifference of thick ascendary limb (TAL) and collecting duct (CD) epithelial cells; 3) cells of the proximal tubule (PT) undergoing DNA synthesis (arrow). Condensed nuclear debris may also be seen in such section indicating apoptotic bodies (not shown).*

nucleus with flocculation of the chromatin. Affected cells rupture, and the cellular components spill into the surrounding tissue space, evoking an inflammatory response. Apoptosis is also a feature of nephrotoxic injury and a distinction can be made between necrosis and apoptosis based on morphological criteria (Table 1).

In apoptosis, the most outstanding morphological and biochemical changes occur in the nucleus in which chromatin rapidly forms dense crescent-shaped aggregates lining the nuclear membrane [2-4]. The plasma membrane becomes convoluted, so that the cell separates into a cluster of membrane bound segments, "apoptotic bodies", which often contain morphologically normal mitochondria and other cellular organelles. The absence of inflammation is a crucial feature of apoptosis, and thus it permits cell death without damage to adjacent cells, and is thus advantageous for normal cell turnover, development and homeostasis of organs under physiological and pathological conditions. This form of cell death differs from frank necrosis in that it requires the activation of a regulated program that leads to DNA fragmentation, nuclear condensation, and cell loss without causing an inflammatory response.

Proximal tubule cells may undergo necrosis or apoptosis *in vitro* depending on the severity of insult [5,6]. Much of the evidence for the role of apoptotic mechanisms in renal tubular injury relates to the demonstration of chromatin condensation, the morphological hallmark of apoptosis, and endonuclease activation resulting in oligonucleosome-length DNA fragmentation (~200 bp), considered as the biochemical hallmark for apoptosis. Thus, apoptotic bodies in renal tubules have been shown in a variety of renal injuries including ischemia/reperfusion injury *in vivo* and hypoxia/reoxygenation *in vitro*, human allografted kidney, oxidant stress and compounds such as $HgCl_2$, cisplatin, and cyclosporine. Apoptosis has been noted after the administration of bacterial cell wall constituents such as lipopolysaccharides as well.

The site and relative contribution of apoptosis to the total loss of epithelial cell integrity in nephrotoxic damage is still a matter of dispute, but a consensus is emerging that apoptosis occurs in a minority of cells (<5% of the total cells in the renal cortex) and the majority of apoptosis is confined to the distal nephron. In large part the confusion stems from an over-reliance

Table 1. Different characteristics between apoptosis and necrosis.

Apoptosis	Necrosis
Affects scattered individual cells	Affects massive and contiguous cells
Chromatin marginates as large crescent aggregates	Chromatin marginates as small aggregates
A ladder of DNA fragmentation (-200 bp), sometimes no fragmentation	Dominant smear pattern of DNA
Cytoplasm and cell volume decrease	Cytoplasm and cell volume increase
Organelles retain integrity	Organelles swell (mitochondria, endoplasmicreticulum)
Cell breaks into small fragments	Cell ruptures
Cell fragments are phagocytized	Cell contents released
No inflammation	Extensive inflammation

upon the use of TUNEL staining rather than classic morphologic criteria to determine apoptosis. While endonuclease activation and resultant oligonucleosomal DNA fragments is a hallmark of apoptosis, several recent observations make equating DNA fragmentation with apoptosis problematic. Thus, chromatin condensation and DNA fragmentation are regulated by different metabolic pathways [7], apoptosis can occur without DNA fragmentation [7], and DNA fragmentation can be seen in necrotic cells. Indeed, rat renal proximal tubules subjected to hypoxia/reoxygenation result in DNA strand breaks and DNA fragmentation and, endonuclease inhibitors provided complete protection against DNA damage induced by hypoxia/reoxygenation and partial but significant protection against cell death. Iwata et al. [8] reported DNA fragmentation (indicative of endonuclease activation) *in vivo* ischemia/reperfusion injury associated with morphological features of necrosis rather than apoptosis. It is highly likely; therefore, that apoptotic and necrotic forms of cell death share many biochemical features together. The form of cell death initiated in any particular cell by a single toxin will depend on the dose of the toxin, the particular cell type, and whether the cell can mount an effective defense against the deleterious effects of the toxin (see below).

Pathophysiology of cell injury

The mechanisms of the changes in cell viability during renal injury are incompletely understood. Most of the experimental data have been derived from the ischemia-reperfusion model of acute renal failure and have focused on necrotic cell death. Because as many as 50% of patients have ischemia-induced acute renal failure, the observations should be relevant to a large portion of the patients at risk. Also, different stresses initiate common biochemical events, so that understanding the relevant pathways of one stress will most likely be applicable to others. What follows is a detailed analysis of some of the pathways currently thought to execute cell death in a variety of nephrotoxic insults.

Disruption of energy production

The two principal sites of energy production in proximal tubular cells reside within the mitochondria and peroxisomes. Both organelles oxidize short chain, medium chain, and long chain fatty acids to generate ATP [9-11]. Inhibition of fatty acid oxidation may represent a common pathophysiologic response of the kidney, and in particular the proximal tubule, during ischemia/reperfusion and cisplatin-induced acute renal failure. Ischemia/reperfusion injury and cisplatin inhibits fatty acid oxidation in mouse kidney and in proximal tubule cells in culture [12, 13]. In each of these insults there is reduced PPAR-a mediated transcription and activity. These DNA binding proteins induce mRNA of key enzymes in fatty acid oxidation. Another transcription factor, the PPAR-Gamma-Coactivator-1 (PGC-1) was also reduced by cisplatin. This latter nuclear protein has been shown to be a transcriptional co-activator of PPAR-a [14], PPAR-g [15], RXR [16], and other transcription factors like Nuclear Respiratory Factors (NRFs) that play critical roles in the regulation of oxidative metabolism, cellular respiration and adaptive thermogenesis [17,18]. *In situ* hybridization studies demonstrate the expression of PGC-1 in the mouse proximal tubule (PT) and the thick ascending limb (TAL), two nephron segments which also express high levels of PPAR-a and fatty acid oxidation enzymes and cisplatin inhibited the expression of PGC-1 in both nephron segments. The above studies suggest that the common underlying defect in proximal tubule energy production is a reduced PGC-1/PPAR-a function. The mechanisms for these changes in nuclear factor activity and how alterations in fatty acid oxidation metabolism lead to structural alterations of mitochondria and eventually cell death are not known.

Mitochondrial dysfunction in acute renal failure

Defects in energy generation

Mitochondrial dysfunction has long been considered to play a central role in the development of cell injury during ischemia-reperfusion and hypoxia-reoxygenation [19]. Besides the inhibition of fatty acid oxidation, mitochondrial energy generation is diminished because of defects in respiratory chain function. Inhibition of the F0-F1-ATPase leading to impaired function of respiratory complex I has been observed in I/R injury. Similar to ischemia, cisplatin has been shown to affect mitochondrial respiratory complexes and func-

tion [20]. Exposure of freshly isolated porcine proximal tubules to cisplatin resulted in loss of mitochondrial membrane potential as well and this decrease preceded cell death [21]. Cisplatin specifically inhibited complexes I to IV of the respiratory chain after 20 min incubation with 50 to 500 mM, respectively. As a result intracellular ATP was decreased to 70%.

Structural abnormalities

Two structural abnormalities in the mitochondria are considered important pathogenetic factors during ischemia. One is characterized by pore formation in the inner mitochondrial membrane and high amplitude swelling (mitochondrial permeability transition or MPT) [22, 23]. The second involves leakage of cytochrome C from the inter-membrane space into the cytosol [24]. Because of its role as an electron shuttle, dislocation of cytochrome *c* compromises respiration [25, 26], and as a cytosolic cofactor cytochrome C activates caspase 9, and triggers apoptosis [25-27] (see below).

Cytochrome C release may follow the MPT or occur independently. In a recent study of cisplatin toxicity [27] decrease in oxidative phosphorylation was due to the inhibition of mitochondrial F0-F1-ATPase activity, but the decrease in oxidative phosphorylation was accompanied by hyperpolarization of the mitochondrial membrane rather than a decrease in membrane potential that is usually associated with the MPT [28]. The studies also demonstrate a marked decrease in active Na transport and Na-K-ATPase activity that paralleled the decrease in F0-F1-ATPase activity and preceded increases in membrane potential in cisplatin treated renal proximal tubular cells. These studies would suggest that cytochrome C release into the cytoplasm and the subsequent formation of the apoptosome (see below) may occur independently of the MPT and that the initiation of cell death by disruption of energy metabolism can directly engage the caspase cascade.

Endonuclease activation is the final common pathway of cell death

Endonuclease activation is the final common executioner of the cell death pathways as both apoptotic and necrotic cell death initiated by renal ischemia/reperfusion injury *in vivo* and hypoxia/reoxygenation *in vitro* [7] is the generation of unrepairable double stranded DNA strand breaks. These lesions are generated by the activation of an endogenous DNase/endonuclease, a reaction that is considered a point of no return for cell death becomes irreversible [29]. Recent work from Basnakian et al. has established the activation of a 30 kDa endonuclease as the principal endonuclease activated in the kidney cortex following ischemia/reperfusion injury. Tyrosine phosphorylation has been implicated in the activation of the endonuclease in chemical hypoxia *in vitro* and inhibition of tyrosine kinase activity provided marked protection against DNA damage, inhibited the activation of the endonuclease, and limited cell death [30]. Interestingly, these effects were independent of any effect of the inhibitors on ATP depletion, indicating that kinase activity was not substrate limited. Two possible pathways of endonuclease activation exist. The first involves activation of endonuclease that requires caspase activation, especially that associated with ICAD (Inhibitor of Caspase-Activated DNase) proteolysis [31] and the other involves caspase-independent activation of endonuclease activity. JNK activation has been shown to be upstream in each of these pathways after UV exposure [32].

Caspases and cell death

Considerable evidence is accumulating to implicate the caspase pathway in the pathophysiology of acute renal failure. Caspases are a family of cell death proteases [33] that play an essential role in the execution phase of apoptosis and act upstream of DNA fragmentation [34-39]. The term 'caspase' for the cell death proteases embodies two distinct catalytic properties of these enzymes such that 'c' refers to the cysteine protease and 'aspase' refers to their specific ability to cleave after an Asp amino acid [33]. The role of caspases in apoptosis was first recognized in 1993 [40] when it was discovered that the cell death gene CED3 in Caenorhabditis elegans has sequence homology to caspase-1, which was then called interleukin-1 b converting enzyme [40].

Thus far, 14 members of caspase family have been identified from mammalian cells [34, 36, 41, 42]. They are divided into two main subfamilies based on sequence homology to caspase-1 and CED 3. Caspase-8, -10, -2, and -9 have larger prodomains and are termed

initiator caspases, while caspases with smaller domains, caspase-3, -7 and -6, are termed executioner caspases. Caspase-1, -4, -5, -11, -12, and -13 play a role in inflammation [35, 36, 43]. Over-expression of executioner and initiator caspases in transfected cells results in DNA fragmentation and cell death in a variety of mammalian cell lines [43-45]. Caspases share many common features, such as: i) they are synthesized as inactive proenzymes in the cytosol of living cells. Each proenzyme is composed of three structural domains: a variable prodomain, a large subunit of about 20 kDa size and a small subunit of about 10 kDa size. On receiving an apoptotic stimuli, these domains are cleaved and the large and small subunits oligomerize to form an active enzyme [34, 45], ii) they are capable of initiating an apoptotic response when transfected into recipient cells [35-37, 39, 46], iii) they are inhibited by substrate-specific synthetic peptide inhibitors and by the baculovirus protein, p35, or by the poxvirus serpin, CrmA. In cell culture, these inhibitors suppress mammalian cell apoptosis; iv) caspases are very specific proteases with an absolute requirement for cleavage after aspartic acid in the target substrates; and v) the active site contains the sequence QACxG in which C is a catalytic cysteine [34, 39, 41, 44].

At present, there are two relatively well-characterized cell death pathways that result in the activation of executioner caspases (Figure 2). One is receptor-mediated and the other is mitochondrial-dependent. On receiving an apoptotic stimulus, the receptor-dependent pathway is initiated by activation of cell death receptors such as Fas and tumor necrosis factor. The death receptor stimulation results in the formation of a death inducing signaling complex (DISC) that recruits and activates procaspase-8, which in turn cleaves and activates downstream caspases, caspases-3, -6 and –7 [47, 48]. Receptor-induced cell death and caspase-8 activation is inhibited by the cowpox virus protein Crm A [49-51] but not by Bcl-2 [52]. The other pathway is mitochondrial-dependent and is triggered by cytochrome c release from the mitochondria, which promotes the activation of procaspase-9 through Apaf-1 and dATP. Activated caspase-9 then cleaves and activates pro-caspase-3 [53-55] An active site mutant of caspase-9 is able to block activation of caspase-3 by caspase-9 [54]. Overexpression of Bcl-2/BclxL blocks cytochrome c release and the apoptosis-induced mitochondrial changes [23, 56, 57]. Recent data [58, 59] demonstrate that rat kidney cortex transcribes genes encoding caspases -1, -2, -3, -6, -8, and -9.

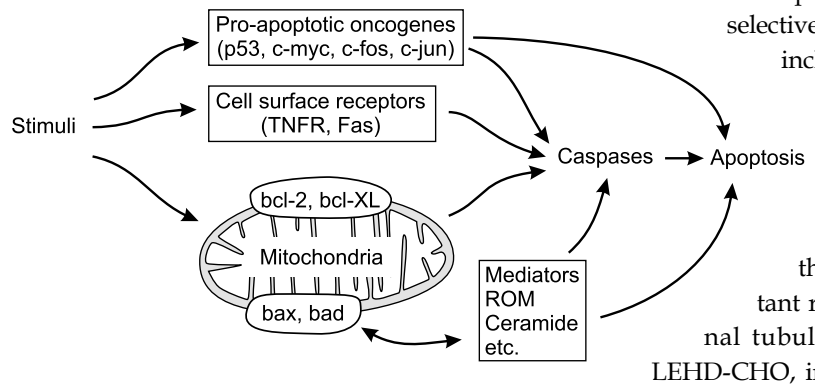

Figure 2. *Three pathways of caspases activation*

In vitro evidence of caspase activation in cytotoxicity

In studies *in vitro*, caspases are involved in hypoxic [58, 60] injury to RTE cells. Antimycin A-induced chemical hypoxia [60] or growth under hypoxic conditions results in increased caspase activity and pancaspase inhibition prevents hypoxia-induced DNA fragmentation and cell death in RTE cells. Partial ATP depletion of MDCK cells by antimycin A was also shown to result in apoptosis with marked increase in activation of caspase-8 and inhibition of caspases provided marked protection against antimycin A-induced cell death [61]. Exposure of freshly isolated RTE to hypoxia resulted in caspase activation and cell membrane damage [62]. In a related study, activation of caspase-3 during hypoxia or ATP depletion was shown to be accompanied by bax translocation and cytochrome c release [63]. As in ischemia, cisplatin activates the caspase cascade as well. Cisplatin induces selective and differential activation of caspases including executioner caspase-3 and initiator caspase-8 and -9 but not pro-inflammatory caspase-1 [64]. The activation of these caspases was markedly inhibited by their respective peptide inhibitors suggesting that these caspases may play an important role in cisplatin-induced injury to renal tubular epithelial cells. DEVD-CHO or LEHD-CHO, inhibitors of caspase-3 and caspase-9 respectively, provided partial protection against cispl-

atin-induced cell death and DNA damage in LLC-PK1 cells [64] indicating mechanisms other than caspase activation are also involved in cisplatin-induced cell death. Overexpression of crmA, a cowpox viral gene known to inhibit caspase-8, also provided protection against cisplatin-induced apoptosis in mouse proximal tubular cells [65] Thus, cisplatin-induced activation of caspase-8 and caspase-9 in renal proximal tubules indicate that both receptor and mitochondrial pathways participate in the activation process. In a recent study p53 inhibition was shown to partially protect cisplatin-induced cell death [66].

In vivo evidence of caspase activation in cytotoxicity

Renal ischemia/reperfusion injury *in vivo* activates caspase-1 and caspase-3 [58,67]. In a murine model of ischemia/reperfusion injury, ZVAD-fmk, a pancaspase inhibitor, was shown to attenuate reperfusion-induced DNA damage (as determined by TUNEL assay) and inflammation [67]. Recent studies by Edelstein et al. [62, 62a] help establish a link between the inflammatory aspects of the ischemic/reperfusion injury and caspase activation. In these studies it was observed that caspase 1 deficient mice were protected from ischemia-reperfusion injury. Aware that IL-18 is expressed after several cell stresses and is activated by caspase-1, the authors observed that IL-18 expression is increased in ischemia-reperfusion and caspase-1 converts IL-18 precursor to its active form. Furthermore in caspase-deficient mice the activity of IL-18 does not increase and the use of a neutralizing antibody to IL-18 offers protection in wild-type animals. The authors also demonstrate reduced leukocyte infiltration in caspase-1 deficient mice, completing a loop between cell injury, initiation of inflammation, and caspase-1 activation. Another study has demonstrated that caspase-3 activation during ischemia/reperfusion injury may be involved in the downregulation of calpastatin, an inhibitor of calpain [68] indicating a role of caspases for calpain activation during renal injury.

The Bcl-2 and the mitochondrial permeability transition: role in cell death

The essential role of cytochrome c release from injured mitochondria in the activation of caspase 9 has been alluded to above. This pathway is especially important in proapoptotic stimuli that are not initiated by surface receptors for apoptosis, such as UV irradiation, and may involve mitochondrial dependent pathways [69]. Continued respiration in the presence of an open mitochondrial pore may result in the generation of reactive oxygen species. Release of cytochrome c may be mediated by the opening of the mitochondrial PT pore, a non-selective channel whose composition is only partially defined [70]. Inhibitors of PT pore opening, such as cyclosporine, which binds to the adenine nucleotide translocator (ANT), a component of the PT pore, and bongkrekic acid, as well as Bcl-2, prevent cytochrome c release and inhibit apoptosis [71] whereas activators of the PT pore, such as atractyloside and Bax induce it [72]. Oxidants can rupture the outer membrane of mitochondria and release caspase-activating proteins [73]. Some studies have shown cytochrome c release before collapse of the mitochondrial membrane potential [69] suggesting alternate control of the PT pore. Many, but not all, of the members of the Bcl-2 family of proteins reside in the inner mitochondrial membrane, form ionic channels in lipid membranes and increase rates of proton extrusion in mitochondria [74] and thus may control the PT pore. The antiapoptotic and mitochondrial affects of Bcl-2 are independent of caspase activity as they occur in the presence of caspase inhibitors and also in yeast that lack caspases [72].

As pro- and anti-apoptotic members of the Bcl-2 family heterodimerize with each other, the relative concentration of the proapoptotic and pro-survival members may act as a rheostat for the suicide program [75]. Bax may have an independent role in apoptosis, as binding to Bcl-2 is controversial and it may damage organelles directly, a process that is inhibitable by Bcl-2 [53]. Bcl-2 has multiple antiapoptotic effects including binding to Apaf1 and preventing activation of Caspase 9 and inhibition of cytochrome c release from mitochondria [53]. The Bcl-2 proteins are regulated both transcriptionally and post transcriptionally. For example, Bad is induced transcriptionally as part of the p53-mediated damage response [76] and phosphorylated and sequestered after IL-3 addition to serum-starved hematopoietic cells. This pathway has been fully characterized and is mediated by activation of Akt/PKB [77].

The renal stress response determines whether cells survive or not

Molecular aspects

Exposure of renal cells to a hostile environment initiates a complex molecular response including the activation of phosphorylation cascades and the expression of many genes. Many of these molecular responses are not confined to areas of regeneration and in fact are localized to nephron segments not undergoing obvious injury or repair. For example, a typical immediate early gene response (IEG), as indicated by c-fos and c-jun activation, occurs most prominently in areas not undergoing an increase in DNA synthesis. Because the sites of increased DNA synthesis are spatially separated from those of IEG expression and many of the responses, including the expression of chemokine genes, resemble the response observed in cells exposed to adverse environmental conditions such as ionizing radiation, oxidants, and hypertonicity, the expression of these genes under these circumstances has been termed the Stress Response. This response is thought to be a major determinant of whether cells survive the insult or not, and might be necessary for the repair of injured cells. Thus, the stress response may ultimately determine much of the proinflammatory, reparative, cytoreductive, and perhaps functional aspects of renal failure, as well as which cells survive the stress or not. Several elements in the stress pathway have been manipulated to effect whether cells survive a particular stress or not.

Signal transduction pathways in the stress response

At least two pathways lead to the activation of c-Jun, as outlined in a simplified form in Figure 3. While both of these pathways converge on c-jun activation, their induction and effect on cell fate are quite different. Growth factors and phorbol esters activate c-Jun via the mitogen activated protein kinases (MAPKs), which include ERK-1 and 2. This pathway includes the activation of the MAPK kinases, MEK-1 and 2 and is most likely mediated through the activation of Ras and Raf 1. Although this cascade eventually leads to the activation of c-Jun, it does not appear to act directly on the c-Jun protein, but rather activates c-Fos, which in turn upregulates c-jun transcription via an AP-1 binding site. This pathway of activation is proliferative in nature. By contrast, oxidative stress and DNA damage, two stresses known to cause nephrotoxicity, increase c-fos and c-jun expression without provoking a proliferative response. The stress-associated expression of these genes is actually antiproliferative [78]. Analysis of the activation of c-Jun under these circumstances has led to the discovery of unique stress-induced protein kinases termed SAPKs (stress activated protein kinases) [78]. These kinases are comprised of the kinases JNK-1 and 2 (c-Jun N-terminal Kinase) and p38. Evidence to date suggests that the SAPKs are regulated by signal transduction paths separate from those that activate other MAPKs through distinct upstream regulators. JNK-1 and 2 have been shown to be the principal kinases responsible for c-Jun activation during oxidative stress and DNA damage while p38 seems to be the principal kinase activated by LPS and TNFa, all of which inhibit proliferation [79]. This activation of c-Jun is antiproliferative in nature and can lead to either cell survival or to cell death.

Studies on the effect of ERK and JNK activation on cellular outcome have revealed that the consequence of this activation is cell type specific. While the activation of ERKs is often proliferative in nature, it may not always be so. For example, the induction of ERK by nerve growth factor in PC12 cells leads to terminal differentiation and exit from the cell cycle [80]. In other cell types, the ability of the cell to survive an insult is often dependent on the balance between ERK and JNK

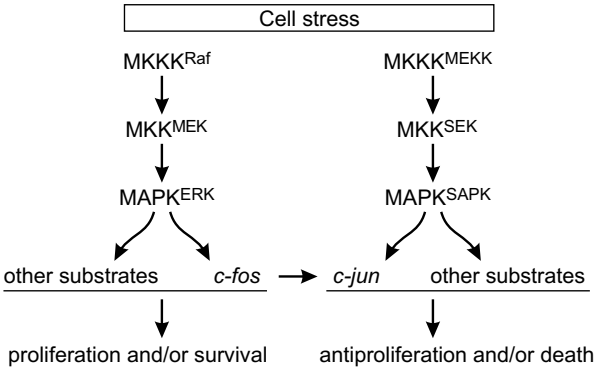

Figure 3. *Mitogen activated protein kinase pathways (MAPKs) initiated by cell stress. Note separation of $MAPK^{ERK}$ and $MAPK^{SAPK}$ activation by distinct upstream kinases (see text).*

induction so that predominant or sole JNK activation leads to apoptosis [81]. Manipulation of the ERK pathway by growth factors and inhibitors has demonstrated that ERK activation is cytoprotective in renal epithelial cells [19]. How ERK promotes survival is an active area of research currently.

Renal stress engages the cell cycle and is a determinant of cytotoxicity

Shortly after acute renal failure many normally quiescent kidney cells enter the cell cycle. Orderly progression through the cell cycle is regulated by sequential synthesis, activation, compartmentalization and degradation of proteins controlling both entry and exit from each of the four phases of the cycle: G1 (gap-1), S (DNA synthesis), G2 (gap-2) and M (mitosis) (Figure 4). Control of the various phases of the growth cycle is exerted by the cyclical activation and repression of the cyclin-dependent kinases. Two important regulators of the cell cycle have been now shown to participate in acute renal failure. One family of proteins, the cyclin-dependent kinase inhibitors, which bind to and inhibit assembled cyclin kinases, and in particular the cyclin-dependent kinase inhibitor protein p21 is expressed in all forms of renal failure studied, including ischemia-reperfusion, cisplatin and obstructive uropathy. This protein binds to and inhibits the CDKs that regulate the G1/S a G2/M transition. P21 expression is enhanced by hostile environmental conditions [82]. The function of these checkpoints is to monitor the fidelity of DNA replication and other macromolecular components of the cell necessary for accurate reproduction of the cell. The absence of these checkpoints leads to uncoordinated replication and leads ultimately to cell death, so that the expression of p21 is antiapoptotic in most situations studied. It has been shown that mice lacking the p21 gene are more sensitive to ischemic and cisplatin nephrotoxicity [83, 84]. In these studies a distinction could be made between regeneration and increased cell cycle activity such that large numbers of polyploid nuclei were associated with increased cell death and not regeneration [85]. Thus cell cycle control initially manifested as cell cycle inhibition is necessary for optimal recovery from acute renal failure (Figure 5). How the cell cycle machinery merges with the executioner pathways is an exciting new area of research in acute renal failure.

Summary

Cell death, survival and repair are intimately interrelated after renal injury. Disordered energy production is common to each of the models and may be part of the stress response. Of particular interest in this regard is the inhibition of fatty acid oxidation in mitochondrial and peroxisomal compartments. The caspases seem to play a key role in executing cell death whether the outcome is necrosis or apoptosis. The stress response characterized by transduction pathways and gene transcription that serve both positive and negative aspects of cell survival is intimately involved in the outcome of ischemic and nephrotoxic damage. The cell cycle and its regulation are key components of the life and death of the stressed cells throughout the kidney. Some cells participating in this response will sur-

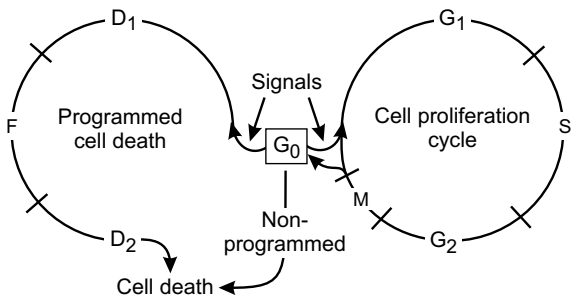

Figure 4. The cell cycle and stress. Note proliferative and apoptotic pathways are engaged by common stresses. The link between cell stress and these two pathways is an area of intense investigation.

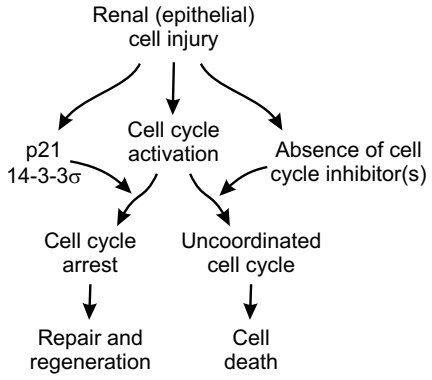

Figure 5. The regulation of the cell cycle is an important determinant of cell fate in acute renal failure.

vive and repair, whereas others will die (Figure 6). What determines whether a cell will recover from such injury or undergo cell death by necrosis or apoptosis is probably a function of the severity of the stress, the specific changes in gene regulation that the cell is capable of mounting, and the availability of survival factors in the cell's external milieu. Augmentation of the positive aspects of the stress pathway while carefully regulating the negative ones is a reasonable approach to altering the outcome of exposure to a nephrotoxic insult.

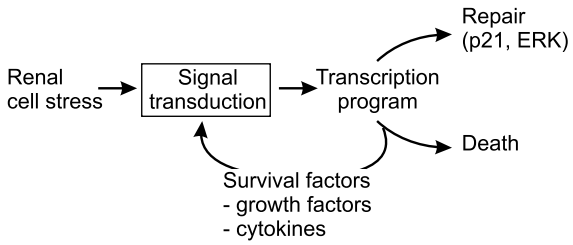

Figure 6. *Renal stress induces signal transduction and transcriptional programs that are modified by survival factors. The balance between the prosurvival or prodeath aspects of the stress response determines the ultimate fate of the cells.*

References

1. Nolan C, Anderson R. Hospital-acquired acute renal failure. J Am Soc Nephrol 1998; 9: 710-718.
2. Kerr J, Wyllie A, Currie A. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-257.
3. Wyllie A. Apoptosis (The 1992 Frank Rose Memorial Lecture). Br J Cancer 2001; 67: 205-208.
4. Morris R, Hargreaves A, Duvall E, Wyllie AH. Hormone-induced cell death: surface changes in thymocytes undergoing apoptosis. Am J Pathol 1984; 115: 426-436.
5. Lieberthal W, Menza S, Levine J. Graded ATP depletion can cause necrosis or apoptosis of cultered mouse proximal tubular cells. Am J Physiol 1998; 274: F315-F327.
6. Lieberthal W, Triaca V, Levine J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. Am J Physiol 1996; 270: F700-F708.
7. Ueda N, Walker P, Hsu S, Shah SV. Activation of a 15-kDa endonuclease in hypoxia/reoxygenation injury without morphologic features of apoptosis. Proc Natl Acad Sci USA 1995; 92: 7202-7206.
8. Iwata M, Myerson D, Torok-Storb B, Zager RA. An evaluation of renal tubular DNA laddering in response to oxygen deprivation and oxidant injury. J Am Soc Nephrol 1994; 5: 1307-1313.
9. LeHir M, Dubach U. Distribution of two enzymes of b-oxidation of fatty acids along the rat nephron; in AnonymousBiochemistry of kidney functions. Elsevier Biochemical Press, Amsterdam 1982, p. 82-94.
10. LeHir M, Dubach U. Peroxisomal and mitochondrial b-oxidation in the rat kidney. Distribution of fatty acyl coenzyme A oxidase and 3-hydroxyacyl-coenzyme A dehydrogenase activities along the nephron. J Histochem Cytochem 1982; 30: 441-444.
11. Bell D, Bars R, Elcombe C. Differential tissue-specific expression and induction of cytochrome P450IVA1 and acyl-CoA oxidase. Eur J Biochem 1992; 206: 979-986.
12. Portilla D, Dai G, Peters JM, Gonzalez FJ, Crew MD, Proia AD. Etomoxir-induced PPARa-modulated enzymes protects during acute renal failure. Am J Physiol 2000; 278: F667-F675.
13. Portilla D, Dai G, McClure T, Bates L, Kurten R, Megyesi J, Price P, Li S. Alterations of PPARa and its Coactivator PGC-1 in Cisplatin-induced acute renal failure. Kidney Int 2002; 62: 1208-1218.
14. Vega R, Huss J, Kelly D. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor a in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 2000; 20: 1868-1876.
15. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999; 98: 115-124.
16. Delerive P, Wu Y, Burris T. PGC-1 functions as a transcriptional coactivator for the retinoid X receptors. J Biol Chem 2002; 277(6): 3913-3917.
17. Scarpulla R. Nuclear control of respiratory chain expression in mammalian cells. J Bioenerg Biomembr 1997; 29: 109-119.
18. Virbasius J, Scarpulla R. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: A potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc Natl Acad Sci 1994; 91: 1309-1313.

19. Di Mari J, Davis R, Safirstein R. MAPK activation determines renal epithelial cell survival during oxidative injury. Am J Physiol 1999; 277: F195-F203.
20. Walker P, Shah S. Hydrogen Peroxide cytotoxicity on LLC-PK1 cells: a role for iron. Kidney Int 1991; 40: 891-898.
21. Kruidering M, Van de Water B, de Heer E, Mulder GJ, Nagelkerke JF. Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: Mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain. J Pharmacol Exp 1997; 280: 638-649.
22. Gunter T, Pfeiffer D. Mechanisms by which mitochondria transport calcium. Am J Physiol 1990; 258: C755-C786 (Abstract).
23. Lemasters JJ, Nieminen AL, Qian T, Trost LC, Herman B. The mitochondrial permeability transition in toxic, hypoxic and reperfusion injury. Mol Cell Biochem 1997; 174: 159-165.
24. Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. Ann Rev Physiol 1998; 60: 619-642.
25. Saikumar P, Dong Z, Weinberg JM, Venkatachalam MA. Mechanisms of cell death in hypoxia/reoxygenation injury. Oncogene 1998; 17: 3341-3349.
26. Varnes ME, Chiu SM, Xue LY, Oleinick NL. Photodynamic therapy-induced apoptosis in lymphoma cells: translocation of cytochrome c causes inhibition of respiration as well as caspase activation. Biochem Biophys Res Commun 1999; 255: 673-679.
27. Nowak G. Protein kinase C-a and ERK1/2 mediate mitochondrial dysfunction, decreases in Active Na^+ transport, and cisplatin-induced apoptosis in renal cells. J Biol Chem 2002; 277: 43377-43388.
28. Dzeja PP, Holmuhamedov EL, Ozcan C, Pucar D, Jahangir A, Terzic A. Mitochondria: gateway for cytoprotection. Circ Res 2001; 89: 744-746.
29. Wyllie A. Apoptosis: an endonuclease at last. Nature 1998; 391: 20-21.
30. Hagar H, Ueda N, Shah S. Tyrosine phosphorylation in DNA damage and cell death in hypoxic injury to LLC-PK1 cells. Kidney Int 1997; 51: 1747-1753.
31. Sakahira H, Enari M, Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature 1998; 391: 96-99.
32. Muhlenbeck F, Haas E, Schwenzer R, Schubert G, Grell M, Smith C, Scheurich P, Wajant H. TRAIL/Apo2L activates c-Jun NH2-terminal kinase (JNK) via caspase-dependent and caspase-independent pathways. J Biol Chem 1998; 273: 33091-33098.
33. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. Cell 1996; 87: 171.
34. Thornberry N, Lazebnik Y. Caspases: enemies within. Science 1998; 281: 1312-1316.
35. Fraser A, Evan G. A license to kill. Cell 1996; 85: 781-786.
36. Earnshaw W, Martins L, Kaufmann S. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 1999; 68: 383-424.
37. Cryns V, Yuan J. Proteases to die for. Genes Dev 1998; 12: 1551-1570.
38. Vaux D. Caspases and apoptosis-biology and terminology. Cell Death Differ 1999; 6: 493-494.
39. Nicholson D. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 1999; 6: 1028-1042.
40. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1b-converting enzyme. Cell 1993; 75: 641-652.
41. Wolf B, Green D. Suicidal tendencies: apoptotic cell death by caspase family proteinases. J Biol Chem 1999; 274: 20049-20052.
42. Ahmad M, Srinivasula SM, Hegde R, Mukattash R, Fernandes-Alnemri T, Alnemri ES. Identification and characterization of murine caspase 14, a new member of the caspase family. Cancer Res 1998; 58: 5201-5205.
43. Salvesen G, Dixit V. Caspases: intracellular signaling by proteolysis. Cell 1997; 91: 443-446.
44. Cohen G. Caspases: the executioners of apoptosis. Biochem J 1997; 326: 1-16.
45. Nicholson D, Thornberry N. Caspases: killer proteases. Trends Biochem Sci 1997; 257: 299-306.
46. Nagata S. Apoptosis by death factor. Cell 1997; 88: 355-365.
47. Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of Mach, a novel MORT1/FADD-interacting protease, in Fas/APO-1 and TNF receptor-induced cell death. Cell 1996; 85: 803-815.
48. Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 1995; 14: 5579-5588.
49. Takahashi A, Hirata H, Yonehara S, Imai Y, Lee KK, Moyer RW, Turner PC, Mesner PW, Okazaki T, Sawai H, Kishi S, Yamamoto K, Okuma M, Sasada M. Affinity labeling displays the stepwise activation of ICE-related proteases by Fas, staurosporine, and CrmA-sensitive caspase-8. Oncogene 1997; 14: 2741-2752.
50. Tewari M, Dixit V. Fas and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product. J Biol Chem 1995; 270: 3255-3260.

51. Miura M, Friedlander R, Yuan J: Tumor necrosis factor-induced apoptosis is mediated by a CrmA-sensitive cell death pathway. Proc Natl Acad Sci USA 1995; 92: 8318-8322.
52. Newton K, Strasser A. The Bcl-2 family and cell death regulation. Curr Opin Genet Dev 1998; 8: 68-75.
53. Green D, Reed J. Mitochondria and apoptosis. Science 1998; 281: 1309-1312.
54. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997; 91: 479-489.
55. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997; 90: 405-413.
56. Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT, Thompson CB. Bcl-xl regulate the membrane potential and volume homeostasis of mitochondria. Cell 1997; 91: 627-637.
57. Zamzami N, Marchetti P, Castedo M, Hirsch T, Susin SA, Masse B, Kroemer G. Inhibitors of permeability transition interfere with the disruption of the mitochondrial transmembrane potential during apoptosis. FEBS Lett 1996; 384: 53-57.
58. Kaushal G, Singh A, Shah S. Identification of caspase (ICE-like proteases) gene family in rat kidney and altered expression in ischemia/reperfusion injury. Am J Physiol 1998; 274: F587-F595.
59. Kaushal G, Singh A, Shah S. Caspases in kidney: cloning, expression and alteration in ischemia/reperfusion injury. J Am Soc Nephrol 1998; 9: 579A.
60. Kaushal G, Ueda N, Shah S. Role of caspases (ICE/CED 3 proteases) in DNA damage and cell death in hypoxic injury. Kidney Int 1997; 52: 438-445.
61. Feldenberg LR, Thevananther S, del Rio M, de Leon M, Devarajan P. Partial ATP depletion induces Eas-and caspase-medicated apoptosis in MDCK cells. Am J Physiol 1999; 276: F837-F846.
62. Edelstein C, Shi Y, Schrier R. Role of caspases in hypoxia-induced necrosis of rat renal proximal tubules. J Am Soc Nephrol 1999; 10: 1940-1949.
62a. Melnikov VY, Ecder T, Fantuzzi G, Siegmund B, Lucia MS, Dinarello CA, Schrier RW, Edelstein CL. Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. J Clin Invest 2001; 107: 1145-1152.
63. Saikumar P, Dong Z, Patel Y, Hall K, Hopfer U, Weinberg JM, Venkatachalam MA. Role of hypoxia-induced Bax translocation and cytochrome c release in reoxygenation injury. Oncogene 1998; 17: 3401-3415.
64. Kaushal GP, Kaushal V, Hong X, Shah SV. Role of caspases and their regulation by Akt/protein kinase B phosphorylation pathway in cisplatin-induced injury to renal tubular epithelial cells. Kidney Int 2001; 60: 1726-1736.
65. Takeda M, Kobayashi M, Shirato I, Osaki T, Endou H. Cisplatin-induced apoptosis of immortalized mouse proximal tubule cells is mediated by interleukin-1b converting enzyme (ICE) family of proteases but inhibited by overexpression of Bcl-2. Arch Toxicol 1997; 71: 612-621.
66. Cummings B, Schnellmann R. Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathways. J Pharmacol Exp 2002; 302: 8-17.
67. Daemen MA, van 't Veer C, Denecker G, Heemskerk VH, Wolfs TG, Clauss M, Vandenabeele P, Buurman WA. Inhibition of apoptosis induced by ischemia/reperfusion prevents inflammation. J Clin Invest 1999; 104: 541-549.
68. Shi Y, Melnikov VY, Schrier RW, Edelstein CL. Down-regulation of the calpain inhibitor protein, calpastatin, by caspases during renal ischemia. Am J Physiol 2000; 279: F509-F517.
69. Bossy-Wetzel E, Newmeyer D, Green D. Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. EMBO J 1997; 17: 37-49.
70. Qian T, Nieminen AL, Herman B, Lemasters JJ. Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes. Am J Physiol 1997; 273: C1783-C1792.
71. Zamzami N, Susin SA, Marchetti P, Hirsch T, Gomez-Monterrey I, Castedo M, Kroemer G. Mitochondrial control of nuclear apoptosis. J Exp Med 1996; 183: 1533-1544.
72. Xiang J, Chao D, Korsmeyer S. BAX-induced cell death may not require interleukin 1 b-converting enzyme-like proteases. Proc Natl Acad Sci USA 1996; 93: 14559-14563.
73. Susin SA, Zamzami N, Castedo M, Hirsch T, Marchetti P, Macho A, Daugas E, Geuskens M, Kroemer G. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. J Exp Med 1996; 184: 1331-1341.
74. Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC. Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci USA 1997; 94: 5113-5118.
75. Oltvai Z, Korsmeyer S. Checkpoints of dueling dimers foil death wishes. Cell 1994; 79: 189-192.
76. Han J, Sabbatini P, Perez D, Rao L, Modha D, White E. The E1B 19K protein blocks apoptosis by interacting with and inhibiting the p53-inducible and death-promoting Bax protein. Genes Dev 1996; 10: 461-477.
77. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC. Regulation of cell death protease caspase-9 by phosphorylation. Science 1998; 282: 1318-1321.
78. Ip Y, Davis R. Signal transduction by the c-Jun N-terminal kinase (JNK) from inflammation to development. Curr Opin Cell Biol 1998; 10: 205-219.

79. Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, Avruch J, Woodgett JR. The stress-activated protein kinase subfamily of c-Jun kinases. Nature 1994; 369: 156-160.
80. Gomez N, Cohen P. Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. Nature 1991; 353: 170-173.
81. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-38 MAP kinases on apoptosis. Science 1995; 270: 1326-1331.
82. Russo T, Zambrano N, Esposito F, Ammedol R, Cimino F, Fiscella M, Jackman J, O'Connor PM, Anderson CW, Appella E. A p53-independent pathway for activation of WAF1/CIP1 expression following oxidative stress. J Biol Chem 1995; 270: 29386-29391.
83. Megyesi J, Safirstein RL, Price PM. Induction of p21WAFI/CIP1/SDI1 in kidney tubule cells affects the course of cisplatin-induced acute renal failure. J Clin Invest 1998; 101: 777-782.
84. Megyesi J, Andrade L, Vieira J, Safirstein RL, Price PM. Positive effect of the induction of p21WAF1/CIP1 on the course of ischemic acute renal failure. Kidney Int 2001; 60: 2164-2172.
85. Megyesi J, Andrade L, Vieira JM, Safirstein RL, Price PM. Coordination of the cell cycle is an important determinant of the syndrome of acute renal failure. Am J Physiol 2002; 283: F810-F816.

06

Animal models for the assessment of acute renal dysfunction and injury

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Introduction

There are a variety of experimental models of acute renal dysfunction and injury for the study of nephrotoxicity. These models include whole animals, isolated perfused kidneys, preparations for the study of the renal microcirculation (including juxtamedullary nephron preparation, hydronephrotic kidney, isolated perfused afferent arteriole, isolated perfused juxtaglomerular apparatus), isolated proximal tubules and cultured tubular cells. In this chapter, three commonly used animal models: whole animals, isolated perfused kidneys, and preparations for the study of the renal microcirculation, will be reviewed. Because the functional effect of a nephrotoxin can include vascular, intraluminal, and direct tubular cell effects, no single experimental model is ideally suited to study the pathophysiology of nephrotoxic injury. Also, each technique also has major limitations which must be appreciated when interpreting the results. However certain models are useful for studying specific types of nephrotoxic injury. For example, techniques for study of the microcirculation are ideal to study drugs that cause acute renal dysfunction on a vascular basis. These drugs include prostaglandin inhibitors e.g. non-steroidal anti-inflammatory drugs (NSAIDs), direct vasoconstrictors e.g. cyclosporine and angiotensin II blockers e.g. angiotensin converting enzyme (ACE) inhibitors. Each model, when appropriately applied and interpreted, produces useful information. However, it should be emphasized that complementary models and approaches need to be used to study a particular nephrotoxin. For example, tubular damage caused by intramuscular injection of glycerol is caused by heme toxicity and is due to a combination of factors that include severe intrarenal vasoconstriction, heme-mediated oxidant injury to tubular cells and obstruction of distal tubules by casts of acid hematin.

A major difficulty in interpreting *in vivo* studies of nephrotoxic acute renal failure in whole animals is the amount and localization of nephrotoxin uptake by the kidney. It may be difficult to ascertain whether a given intervention alters pathways of nephrotoxin-mediated injury or merely the intracellular nephrotoxin burden. For example, the amount of drug which accumulates within tubular cells cannot be directly assessed because whole tissue analysis detects both intraluminal and intracellular drug content. Also, experimental conditions e.g., renal hypoperfusion, renal pedicle clamping may directly influence the amount of renal nephrotoxin uptake. Thus, a knowledge of the limitations of each *in vivo* experimental model is necessary. The use of complementary *in vitro* models e.g. isolated proximal tubules to assess direct tubular toxicity is often required.

The nature of tubular injury in acute renal failure (ARF) includes reversible sublethal injury (swelling, loss of apical brush border) and lethal injury (necrosis and apoptosis) [1, 2]. Proximal tubular cell death due to ischemic ARF *in vivo* and hypoxia *in vitro* results predominantly in necrosis, hence the term acute tubular necrosis or ATN. Apoptotic cell death in ischemic renal injury has been inconsistently demonstrated [3]. When apoptosis has been demonstrated in early ischemic ARF, it is present in the distal tubule [4, 5]. Apoptosis in proximal tubules may play a role in tubular regeneration and was demonstrated to occur at 3 days after ischemic injury in regenerating PT [6]. Thus, the nature of nephrotoxic injury, whether it is tubular dysfunction necrosis or apoptosis is also an important consideration.

Whole animal models

"Knockout" mouse models

Various animal models have been used to study the pathogenesis of ARF and develop therapeutic interventions that prevent or ameliorate the severity of tubular injury following an acute ischemic or toxic renal insult. Utilization of animal models has advantages over other *in vitro* models such as isolated perfused kidneys, isolated proximal tubules, or tubular cell culture. It reproduces the complex interactions of hemodynamics and local tubular factors seen in the whole animal with ARF.

In the early 1940s, the effect of induced ischemic myopathy on renal perfusion in the rabbit was studied. It was conclusively demonstrated in this model that there was extreme renal cortical vasoconstriction with preservation of the medullary circulation [7]. This early first demonstration of posttraumatic vasomotor nephropathy was independently confirmed 20 years later in the USA when 'preferential renal cortical ischemia' was demonstrated in acute renal failure in man. Since then, many animals have been used to study

pathogenesis of ARF. Rats and mice are the most popular experimental animals now. Dogs and rabbits are now less often used. Rats and mice are becoming more and more acceptable animals because they are easy to breed.

Compensatory responses

The ability to generate mice with a targeted mutation in a desired gene has made them a very attractive model. The first knockout mouse line was generated over 15 years ago. Many hundreds of genes have been targeted. In mice with a targeted mutation, it is possible to determine the function of gene product in various pathological conditions including renal ischemia. However, there may be discrepancies between results of studies in mice with a targeted mutation versus mice treated with an agent to neutralize the specific protein. For example, specific neutralization of interleukin-18 (IL-18) using anti-IL-18 antiserum, results in prolonged survival in lipopolysaccharide (LPS) lethality [8] whereas the IL-18 deficient mouse is often not protected against LPS lethality [9]. Also, neutralizing antibodies against IL-18 reduce disease severity in inflammatory bowel disease [10]. In contrast, IL-18 deficient mice exhibit enhanced disease severity [11]. In general, if neutralizing antibodies are available and if such reagents exhibit a high specificity for a particular cytokine, the use of such neutralizing antibodies is best to define the role of a cytokine compared to a deficiency of the cytokine from the time of conception as in the deficient mice. In any model involving production of several cytokines, neutralization of a single cytokine associated with a reduction in disease severity is a better test of the hypothesis because one is certain that several cytokines are produced in the wild-type mouse. In the case of the deficient mouse, one is not sure if several cytokines are produced or if some cytokines are being overproduced (compensatory). For example, it has been demonstrated in IL-18 deficient mice that levels of other cytokines such as IL-1β, IL-6 and TNF-α are overproduced as compared to wild type mice [9]. A mouse deficient of a specific cytokine can be very informative and highly useful in models of spontaneous disease activity. But in the case of a specific challenge such as nephrotoxic acute renal failure, the test of the hypothesis may be best served by specific blockade. The above principles may also apply to other proteins besides cytokines. Thus, in summary, when working with knockout animals, it is important to consider that the resultant phenotype is due to both loss of function of the targeted gene and the compensatory reaction that the animal develops to minimize that loss.

Another example of problems in interpreting results in knockout mice is demonstrated by the following: An important role of colony-stimulating factor (CSF) in hemopoiesis of myeloid lineage cells has been demonstrated. G-CSF was knocked out, animals were neutropenic and had decreased hemopoietic progenitors in bone marrow and spleen [12]. In contrast, mice with a null mutation in GM-CSF, which acts upstream to G-CSF in myeloid differentiation, demonstrated no impairment of hemopoiesis, but develop a characteristic pulmonary pathology [13]. Therefore, we have to interpret the results of experiments with knockout mice with caution.

Strain differences

Different strains of mice have different levels of susceptibility to ischemic kidney injury. For example, C57BL/6 mice, the major strain used in the development of genetically engineered mice, have greater susceptibility to renal ischemia/reperfusion injury than do the NIH Swiss mice [14]. The early knockout mice developed were in a mixed strain, C57BL/6 x SV129 F1. It is well known that the phenotype of a knockout mouse can depend critically on the background. For example, C57BL/6 x SV129 F1 mice have different coat colors ranging from black to white. The use of this mixed strain also runs the risk of genetic drift of background compared to the knockout mice being used. Thus, siblings from heterozygous crosses are better than "unrelated" controls. More recently, knockout mice are being "backcrossed" into the C57BL/6 background to eliminate strain differences. Rapid congenic protocols are available for the backcrossing [15, 16].

Gender differences

There are also gender differences in susceptibility to ischemic acute renal failure in mice. Male mice may be more susceptible to renal ischemia than females [17].

Table 1 demonstrates different lines of knockout mice that have been used to study ischemic ARF. These models are of potential value in the study of nephrotoxic injury from drugs and chemicals.

Table 1. Knockout mouse models of ischemic acute renal failure.

Deficient gene	ARF model	Protection against Ischemic ARF	Reperfusion period (hr)	Reference
Caspase-1	Bilateral renal pedicle clamping	Yes	24	[279]
Caspase-1	Unilateral renal pedicle clamping	No	24	[280]
iNOS	Bilateral renal pedicle clamping	Yes	24	[281]
Interleukin-1 receptor	Bilateral renal pedicle clamping	No	24	[282]
ICAM-1	Bilateral renal pedicle clamping	Yes	24, 48, 72	[283]
CD4/CD8 lymphocytes	Bilateral renal pedicle clamping	Yes	48	[284]
Osteopontin	Bilateral renal pedicle clamping	No	24	[285]

Types of renal injury

Ischemic, nephrotoxic, and septic rodent models of acute renal injury were developed to study mechanisms of acute renal failure. Decreasing renal blood flow is critical in the pathophysiology of ARF in humans. Ischemic and other animal models are used to reproduce the morphological features of human disease.

Ischemic

Ischemic ARF may be induced by intrarenal norepinephrine injection or by renal artery clamping. There are similarities between these two models of ischemic renal failure. In the norepinephrine model of renal failure, as in the arterial clamping model, there is the same degree of tubular injury except for a slightly greater frequency of tubular casts at 48 hours in ischemic model [18]. In both models, calcium channel blockers improve renal function [19, 20]. The major difference is that in the renal artery clamping model, morphology at 48 hours showed smooth muscle necrosis in half of the resistance vessels, but in less than 10% of those in norepinephrine-induced model.

There are bilateral and unilateral models of ischemic ARF. The bilateral model is used more often because it is more similar to the pathophysiology of the syndrome of acute renal failure in humans and the most likely to yield clinically relevant information. Moreover, uninephrectomy immediately before renal artery occlusion may offer protection from this insult [21, 22].

It is been known that proximal tubules are damaged more severely than distal tubules in ischemic ARF and that the S3 segment of proximal tubules is more susceptible to ischemia than the S1 segment [23, 24]. The greater susceptibility of the S3 segment to ischemia *in vivo* is suggested to be related to hemodynamic factors that result in persistent impaired perfusion of the outer medulla [25]. However, the degree of damage closely correlates with the severity of renal failure. For example, as the time of vascular obstruction or the dose of a nephrotoxin increases, injury also involves both S1 and S2 segments [26, 27]. Sections of kidney, stained with hematoxylin-eosin and periodic acid Schiff (PAS), show common features of ischemic damage: loss of proximal tubular brush border, congestion of the outer medulla, interstitial edema, proximal tubular injury, cast formation and interstitial leukocyte accumulation. The distal nephron is less affected, with mild damage in the thick limb of Henle and apoptotic cells in distal tubules. In contrast, tubular necrosis is less extensive in humans with ischemic ARF than in the rodent model. Morphological injury in humans is subtle and focal, affecting both proximal and distal tubules [28, 29]. The role of apoptotic cell death and the mechanisms of induction of apoptosis in ischemic ARF have been intensively discussed for the past decade. It has become apparent that apoptosis, a form of cell death, distinct from necrosis, may contribute to ischemic ARF [30] [31] [32]. Morphological and biochemical features that distinguish apoptosis from necrosis, as well as the role of apoptosis in ischemic ARF have been reviewed [33, 34]. Given the importance of the topic, we will again briefly summarize the features of necrosis and apoptosis and differences between them. In marked contrast to necrosis, apoptosis is an active, energy-dependent process. Even though ATP deficiency may be a signal both for apoptosis and for necrosis, cells with a rapid and

severe ATP deprivation die by necrosis rather than apoptosis. Other pro-apoptotic mediators have been shown to present in ARF, such as caspase-3 activation, expression of Fas and Fas ligand in renal epithelial cells and expression of tumor necrosis factor α (TNF-α) [35, 36]. The morphological characteristics of apoptosis and necrosis are quite different. The early loss of plasma membrane integrity, seen in necrosis, is associated with phospholipase activation, oxidant injury to cellular components and cell swelling, leading to the release of proteolytic enzymes into the extracellular space and subsequent inflammatory reaction. In contrast, in apoptosis the cell becomes progressively smaller in size, nuclear chromatin becomes condensed and fragmented, while the plasma membrane retains its integrity. Later on, the apoptotic cell disintegrates into "apoptotic bodies", which are ingested by macrophages, mesangial or epithelial cells and can be easily detected by light microscopy. Apoptotic cell death almost always occurs without marked inflammation and tissue injury.

Two waves of apoptosis during the reperfusion phase after ischemic ARF have been described. The first coincides with a maximum proliferative activity that is at 2-3 days post-injury. The second occurs on day 7-8 following injury [37]. Other investigators have demonstrated that apoptosis peaks between 4 and 14 days of post-ischemia [38]. The discrepancy may be due to different methods used to detect and quantify apoptosis or different animal models of ischemic ARF.

Cell death due to apoptosis or necrosis is not the only form of tubular injury in ARF. There is also sublethal injury causing cell dysfunction. For example, alterations in proximal tubular cell polarity occur during renal ischemia. Tubule polarity is essential for its primary function of selective reabsorption of ions from the tubular fluid. Sodium-potassium-ATPase (Na-K-ATPase), the enzyme, normally localized to the basolateral membrane, maintains tubular polarity by regulation of cellular transport sodium and potassium in proximal tubules. Na-K-ATPase is linked to the cytoskeleton/membrane complex by a variety of proteins including spectrin. It has been demonstrated that in early reperfusion period spectrin dissociates from the cytoskeleton and NaK-ATPase moves from the basolateral membrane into the cytoplasm and apical membrane [39-43].

The loss of gate function of the renal tubular cells prevents the renal epithelium from acting as a barrier to free movement of solute and water across the tubular epithelium. Thus, "backleak" of glomerular filtrate occurs. It has been demonstrated in humans that loss of proximal tubule cell polarity for Na-K-ATPase distribution is associated with enhanced delivery of filtered Na+ to the macula densa for seven days after allograft reperfusion [44].

As a result of the loss of cell-matrix adhesion, epithelial cells, normally attached to the underlying matrix, become detached from the basement membrane [45]. In the urine of patients with "acute tubular necrosis" in native and transplanted kidneys, there is significant tubular cell shedding with up to 100% viability of voided tubular cells.

Inflammation and kidney neutrophil accumulation in the post-ischemic period is another controversial issue. There is increasing evidence that leukocytes, particularly neutrophils, mediate tissue injury and play a deleterious role in the pathogenesis of renal failure [46-50]. Conversely, renal injury can occur by a neutrophil-independent pathway, as seen in neutropenic patients who develop ARF, indicating that neutrophils are not the only factor contributing to ARF [51]. Myeloperoxidase (MPO) activity is an accepted index for assessment of leukocyte accumulation in animal model. However, one should consider that hematoxylin-eosin staining (morphology of cell nucleus) remains the gold standard for identification of neutrophils. MPO assays or chloroacetate esterase staining should be regarded as tools to quantitate both neutrophils and monocytes/macrophages [27].

In summary, loss of brush border, presence of cast formation and predominant injury of the S3 segment of proximal tubules are similar in both human and experimental ARF. Reversibility of the reduction in GFR is another important similarity.

Nephrotoxic

From an epidemiological point of view, among the causes of ARF of a medical nature, drug-induced and toxic ARF are very important [52]. Nephrotoxic substances include a wide variety of compounds such as heavy metal ions, organic solvents, antibodies and natural toxins. Nephrotoxins induce ARF in humans by direct cellular toxicity, vasoconstriction, and crystal-mediated tubular obstruction. Acute interstitial inflammation is an important factor in pathogenesis of

acute interstitial nephritis. In general, a decrement of GFR is the result of combination of mechanisms rather than any single mechanism.

Table 2 shows the predominant mechanism of a decrease in GFR in different animal models of nephrotoxic ARF.

Septic

Sepsis is the most frequent cause of ARF in intensive care units [53, 54]. Moreover, when sepsis is associated with ARF the mortality increases dramatically [53]. The incidence of ARF increases even further in patients with septic shock. Also, the use of nephrotoxins e.g. aminoglycosides, amphotericin B in septic patients may precipitate or worsen the ARF.

In one prospective study, ARF occurred in 51% of septic shock patients [55]. The combination of ARF and sepsis is associated with a greater than 80% mortality [54]. Over the past 3 decades, sepsis and septic shock have been studied in various species including rats, dogs, pigs, primates. Only recently has a mouse model of septic ARF been developed. Administration of different doses of endotoxin (5-30 mg/kg intraperitoneal) to mice is associated with sepsis and septic shock, respectively [56].

The mouse peritonitis model of sepsis is also widely used. In this model, the cecum is isolated, ligated and punctured with a 25-gauge needle. The mortality, morbidity, and immunopathology in endotoxemic and peritonitis models of sepsis has been compared [57]. The models yield similar mortality and morbidity but have significant differences in the kinetics and magnitude of cytokine production. Also, the LPS model did not accurately reproduce the cytokine profile of human sepsis. As in humans, the septic shock mouse model has a higher incidence of ARF and mortality than the normotensive sepsis model.

It has been demonstrated that the early effects of sepsis in causing ARF primarily involve renal vasoconstriction. This primary vasoconstriction can be demonstrated in the absence of sepsis-mediated hypotension. Later events include apoptosis, leukocyte infiltration and morphological evidence of coagulation (e.g. glomerular fibrin) [58-61]. There is evidence that several vasoconstrictor and vasodilator pathways are activated during sepsis in various experimental models. During septic shock a hyperdynamic state occurs in which systemic vasodilation is associated with a secondary increase in cardiac output. The rise in cardiac output, however, may not be maximal for the degree of afterload reduction because of the myocardial depressant effect of cytokines such as tumor necrosis factor α (TNF-α). The arterial underfilling associated with systemic arterial vasodilatation is known to activate the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS) and the non-osmotic release of arginine vasopressin (AVP) [62, 63] [64]. While these events attenuate the degree of systemic hypotension, they also lead to renal vasoconstriction. The vasoactive events of septic shock are however more complex than initiated by arterial underfilling. The endotoxin-mediated increase in TNF-α is associated with an increase in inducible nitric oxide synthase (iNOS) [58, 65]. There is evidence in the

Table 2. Animal models of nephrotoxic ARF.

Animal model	Nephrotoxic agent	Predominant mechanism of decreased GFR	Primary site of injury	Reference
Dog	Uranyl nitrate 5-10 mg/kg	Backleak of filtrate Decreased ultrafiltration coefficient	S3 segment of proximal tubule	[286] [287]
Rabbit	Glycerol 7.5 g/kg	Tubular obstruction	Proximal tubules	[288]
Rat	Gentamycin 40-120 mg/kg/day	Decreased ultrafiltration coefficient Tubular obstruction	Proximal convoluted tubular	[289] [290, 291]
Dog, rats	Mercuric chloride 1-3 mg/kg	Increased preglomerular resistance Back leakage of filtrate	S3 segment of proximal tubule	[291, 292]
Rat	Cisplatin 6-10 mg/kg	Renal vasoconstriction Back leakage of filtrate	S3 segment of proximal tubule	[293, 294]

endotoxemic rat that the increased NO which results from the upregulation of iNOS exerts a negative feedback on the endothelial NOS (eNOS) in the kidney (See Table 3). Moreover, the secondary messenger of NO, cyclic GMP, has been shown to increase in the renal cortex during the initial 16 hours of sepsis but then at 24 hours to be down-regulated in spite of continued high plasma levels of NO [56]. Both of these events, namely NO-mediated decreased eNOS and down-regulation of cyclic GMP, would impair the normal counterregulatory vasodilator pathways which attenuate the renal vasoconstriction associated with activation of the RAAS, SNS and the non-osmotic release of AVP. There is also evidence against a role of iNOS in septic ARF (See Table 3). In this study, iNOS deficient mice or mice treated with a selective iNOS inhibitor were not protected against endotoxemic ARF.

Sepsis is also associated with increased reactive oxygen species. Antioxidants and superoxide scavengers have been suggested to attenuate the renal dysfunction of sepsis [66, 67]. Recent studies have further indicated that peroxynitrite, the product of the reaction between NO and superoxide, may be responsible for the renal oxidant injury associated with endotoxemia [68].

Some animal models of sepsis are shown in table 3.

Measurement of injury

Serum creatinine and blood urea nitrogen

Serum creatinine and blood urea nitrogen are accepted indicators of renal function in animal model of ischemic ARF, correlating well with GFR as measured by inulin clearance [69]. In the rat and mouse clamp model of ischemic ARF, the serum creatinine and BUN reach a peak in 24-48 hours of reperfusion and normalize by day 6-8 [70]. Thus, this is a reversible model of ischemic ARF.

Histology

Histologic changes remain to be an important marker of kidney injury in ARF both in human and in animals. Histological parameters of ARF include tubular necrosis, loss of proximal tubular brush border, casts in tubular lumens, neutrophil accumulation, Interstitial edema and vascular erythrocyte congestion. To assess the histological changes, a quantitative or semiquantitative approach should always be used. Areas of kidney damage such as cortex, outer or inner medulla should also be specified. Terms such as "extensive", "patchy", "widespread", "mild", or "severe" are not precise enough for evaluating the changes.

Table 3. Animal models of septic (endotoxemic) ARF.

Intervention	Septic model	Protection against septic ARF	Reference
TNF inhibition (TNFsRp55)	Mouse Lipopolysaccharide (5 mg/kg IP)	Yes	[295]
iNOS inhibitor (1400W)	Mouse Lipopolysaccharide (5 mg/kg IP)	No	[295]
iNOS deficient mice	Mouse Lipopolysaccharide (5 mg/kg IP)	No	[295]
Nonselective NOS inhibition (L-NAME)	Rat Lipopolysaccharide (0.5-50 mg/kg IP)	No	[296]
Selective iNOS inhibition (L-NIL)	Rat Lipopolysaccharide (10 mg/kg IV)	Yes	[296]
iNOS inhibition (agmatine aldehyde)	Rat Lipopolysaccharide (0.5-50 mg/kg IP)	Yes	[297]
Type IV phosphodiestrase inhibitor (RO 20-1724)	Rat Lipopolysaccharide (20 mg/kg IP)	Yes	[298]
Antioxidant (Dimethylthiourea)	Rat Lipopolysaccharide (0.5 mg/100 g IV)	Yes	[299]
Antioxidant (Superoxide dismutase)	Rat Lipopolysaccharide (0.5 mg/100 g IV)	Yes	[299]
Selective endothelin B receptor antagonist (BQ-788)	Rat Lipopolysaccharide (10 mg/kg IV)	Yes	[300]
Nonselective endothelin receptor antagonist (TAK-044)	Dog Lipopolysaccharide (250 ng/kg/min for 2 hr)	Yes	[301]

Inulin clearance

Inulin clearance has been used as the gold standard of GFR measurement. Accurate measurements of both urine and blood inulin concentrations are essential to get accurate results. Radiolabeled markers have been widely used, but they have a number of disadvantages such as cost and safety issues related to the use of radioisotopes. A new method of determining inulin clearance, representing a viable and accurate alternative to radioactive methods, has been described recently [71]. This method uses samples containing FITC-inulin that were stored between oil columns in constant-bore microcapillary tubes, which were then used as cuvettes to determine fluorescence on a microscope fluorometer. The authors report their method as simple to use, relatively inexpensive, and highly precise. The other advantage is that inulin concentration may be measured in nanoliter volumes of fluid, which make the method very important in micropuncture studies.

Molecular parameters

The effect of ischemia-reperfusion injury on activity, protein and m-RNA levels of proteins is also studied. For example, the enzymes that are involved in free radical detoxication (catalase, copper-zinc and manganese containing superoxide dismutase and glutathione peroxidase) were studied in rat kidney [72]. This study demonstrated that there was a significant decrease in the levels of mRNA coding for all the enzymes except manganese superoxide dismutase, which remained high. There was also structural and functional damage of peroxisomes and catalase-containing subcellular organelles [73]. The authors conclude that in ischemia-reperfusion, the antioxidant enzymes, providing protection by reducing the cellular level of free radicals, were downregulated at both the transcriptional and translocational level and may contribute to free radical species injury of intracellular molecules critical to cell homeostasis.

Proximal vs. distal tubular injury

The last segment of the proximal tubule (the S3 segment) and the medullary thick ascending limb (MTAL) are both located in the outer medulla of the kidney. This region of the kidney suffers the most severe ischemic damage because of the delayed return of blood flow after ischemia. In most *in vivo* experiments, cell injury and necrosis has been shown to be more severe in proximal than distal tubules [74]. The proximal tubule is also the main site of injury in the human kidney allograft with ARF [75]. The explanation for the increased vulnerability of the S3 segment to ischemia is that proximal tubules have little capacity for glycolysis compared to distal tubules [76]. Also, it has been shown that distal tubule cells demonstrate a very well-developed response to ischemia that is characterized by an alteration in the expression of many genes, that may be adaptive and result in decreased susceptibility of this segment to injury [77, 78]. Specifically, transcriptional downregulation of epidermal growth factor (EGF) as well as the activation of the immediate early gene response characterizes this segment's response to the ischemic insult [79, 80].

Mitogen-activated protein (MAP) kinase activation is regionally distributed in the postischemic kidney. It has been demonstrated that ischemia-reperfusion injury induces the activation of the c-Jun N-terminal kinases (JNKs) that occurred both in the cortex and inner stripe of the outer medulla [81, 82]. During ischemia, JNK activation has a deleterious effect and inhibition of JNK s ameliorated renal failure [81]. Proximal tubule cells are more sensitive than thick ascending limb (TAL) cells to oxidative stress as assessed by cell counting, light microscopy, propidium iodide uptake and fluorescence-activated cell sorting (FACS) analysis. Immunoprecipitation/kinase analysis revealed that JNK activation occurred in both cell types, whereas extracellular regulated kinase (ERK) activation occurred only in TAL cells. In TAL cells, ERK inhibition reduced cell survival nearly fourfold after oxidant exposure. In proximal tubule cells, activation of the ERK pathway by insulin-like growth factor I (IGF-I) increased survival by threefold and this IGF-I-enhanced cell survival was inhibited by a MAP kinase kinase (MEK)-1 inhibitor of the ERK pathway [83]. It is also possible that increased expression of some genes within the medullary thick ascending limb encode the production of paracrine growth factors that may contribute to the regenerative response in the cells of the adjacent S3 segment [77].

The isolated perfused rat kidney

Introduction

A huge number of studies of experimental renal injury have been performed using the isolated perfused rat kidney. Studies have explored vascular and tubular responses to toxic and hypoxic injury and to mediators thought to participate in regulation of normal renal function as well as in the biochemical and morphological changes accompanying renal injury.

Three main models have been used. The intact isolated perfused rat kidney (IPRK), is the most widely used model and first developed for the study autoregulation by Weiss et al in [84]. However, this prototype was little used initially because autoregulation and function declined after only 15-30 minutes. However, when simpler surgery and improved perfusion solutions were introduced by Ross, the model became useful for studies of renal biochemistry [85, 86]. With further improvements, including the addition of amino acids [87, 88] and sometimes erythrocytes [89-91] the model became useful for studies of physiology and pathophysiology [92-95].

The second model is usually known as the hydronephrotic rat kidney model, and was developed for *in vivo* visualization of the microcirculation by Steinhausen [96] and involves 60 min renal artery occlusion combined with 3 weeks of ligation of the ureter. Atrophy of tubular structures leaves the cortical vasculature relatively intact and visualizable using planar microscopy in an illuminated observation chamber with nerve and blood supply left intact. Absolute and relative changes in lumen diameter of the major resistance vessels-interlobular arteries, afferent and efferent arterioles can be monitored in response to vasoactive stimuli. This model was adapted for *in vitro* perfusion by Loutzenhiser et al [97], removing systemic neurohumoral influences.

The third model is the *in vitro* perfused juxtamedullary nephron, also developed to allow direct visualization of the renal microcirculation [98, 99]. Similarly to the IPRK, the kidney is perfused *in vitro* with albumin-containing physiological solutions with or without added erythrocytes. However, the preparation involves hemisection of the kidney, reflection of the papilla and ligation of the major branches of the renal artery until the vasculature perfusing a few glomeruli on the inner cortical surface is isolated. The microvasculature is then viewed by videomicroscopy and vessel lumen diameters measured by micrometer. Single nephron glomerular filtration rate and tubular perfusion can also be performed in this model and in contrast to the hydronephrotic model, tubuloglomerular feedback is intact in the juxtamedullary nephron preparation and can be investigated [100, 101].

Both the hydronephrotic kidney and the juxtamedullary model have been used primarily for physiological studies, including studies of drug action, whereas the IPRK has been used extensively for the studies of pathophysiology as well. Consequently the main focus of this section will be the intact IPRK, which is both the simplest and the most widely used model. The other two models will be discussed in greater detail in section 4 of this chapter.

Technique

The first useful version of the IPRK model [85, 86] recirculates Krebs bicarbonate buffer containing 6.7 g% bovine serum albumin (BSA) as an oncotic agent. Glucose and amino acids are added as substrates. Two peristaltic pumps (or two passes through the same pump) are used to drive the perfusate first through in-line filters into a cascading lung oxygenator, usually gassed with 95% O_2, 5% CO_2 (Fig 1). The 5% CO_2 serves to maintain the bicarbonate buffer pH at 7.4. Prior to perfusion, the right kidney of the anaesthetized rat is exposed and the ureter is cannulated using polyethylene tubing (0.28 mm (i.d.), 0.61 mm (o.d.)- known as PE10). Oxygenated and warmed perfusate is delivered into the rat kidney through a cannula introduced into the right renal artery via the mesenteric artery. The flow of warmed, oxygenated perfusate is commenced with the cannula in the mesenteric artery so that renal artery cannulation is initiated without even transient interruption of renal perfusion. After cannulation, the kidney is removed and mounted over a reservoir to collect the perfusate, which flows from the renal vein over the kidney, keeping the surface moist. The prewarmed perfusate keeps the temperature of the kidney constant, usually at 37°. While some researchers use a constant temperature cabinet for this purpose, we have found that thermostatic tubing and glassware is simpler, reducing surface drying and making the experimental setup more mobile (Figure 1).

After 20-30 min for equilibration, perfusate samples are collected to coincide with the start of urine collections at 5 to 15 min intervals. The urine volume depends on both perfusion pressure and oncotic pressure [102]. Perfusion pressure and flow are monitored continuously. Renal function is assessed from renal vascular resistance, urine flow, the ratio of ^{14}C-inulin in urine to plasma (U/Pinulin), glomerular filtration rate (GFR as inulin clearance UV/P) and the fractional excretion of sodium (FE_{Na}) and potassium (FE_K). Inulin measurement can also be performed chemically. In some situations GFR is misleading as an index of "good function" in this model. For example, in the absence of an oncotic agent, a "reasonable" GFR is over 1 ml/g/min, however U/Pinulin is rarely more than 2, FENa is over 0.1 and histological examination shows extensive proximal necrosis within 20-40 min of initiating perfusion. It appears that in this situation the massive urine volume artificially elevates the GFR. Similarly, dead space effects arising from urine in the renal pelvis, ureter and ureteric tubing reduce the real time relationship to a perturbation of GFR and other urine-based indices [95]. Because of these considerations, it is important to combine the various parameters of function and set minimum standards of function, which must be attained before results from individual perfused kidney experiments are utilized in data analysis (see below).

Many technical refinements have been added to this model over the years to help maintain renal physiological function as well as to assist with the technique of initiating perfusion. Technical modifications include the addition of albumin as an oncotic agent [85, 102, 103] addition of amino acids [86, 88], other substrates [104], addition of erythrocytes to improve outer medullary oxygenation [89-91], servo-control of perfusion

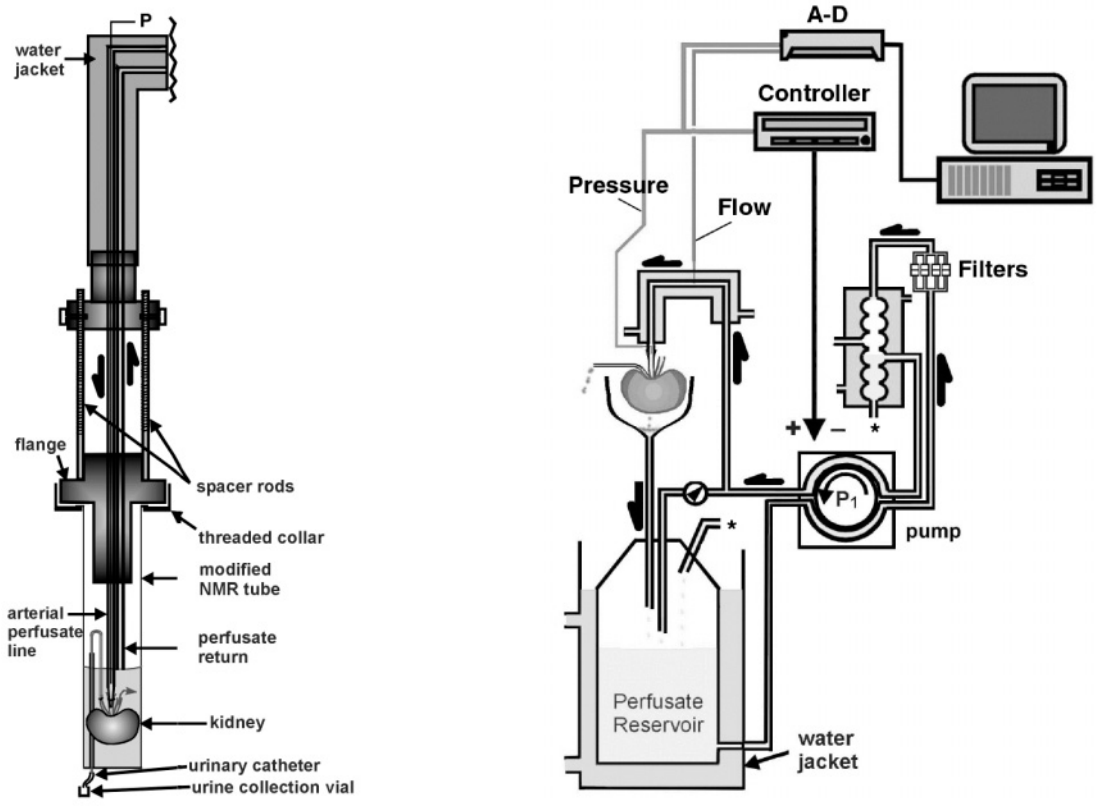

Figure 1. *The apparatus for isolated perfusion of rat kidneys is shown schematically. The typical circuit for bench experiments is shown on the right, while the extension using thermostatted tubing which is required for experiments conducted in the bore of a high field superconducting magnet for either magnetic resonance spectroscopy (MRS) or magnetic resonance microscopy is shown on the left (not to scale). Modified with permission from Endre et al [122].*

pressure or flow [94] and ultrasonic recording of flow [94]. While incremental improvements in renal physiological function followed the various modifications mentioned, especially the addition of BSA and amino acids, the most physiological preparation with longest viability for experimental work is the model with normal concentrations of erythrocytes, albumin, globulin plus supplementation with antidiuretic hormone to preserve concentrating capacity described by Lieberthal [105]. However, because of the additional effort and expense, this model is generally avoided where the presence of erythrocytes, especially in high concentration, is not required for interpretation of the results. This seems to be the case in many studies of vascular modulation. Oxygen uptake can be measured by the Fick method [106]. During perfusion, electrodes can be inserted into the renal tissue for measurement of tissue PO_2 [107-109] and nitric oxide [110]. Micropuncture studies have been applied to the IPRK [88]. At the end of perfusion the kidney can be fixed for morphological evaluation, optimally for tubular histology by perfusion with a flush solution (to remove precipitable albumin from within the kidney) followed by fixative. Immersion fixation after sectioning may be better for assessment of vascular pathology *in vivo*. This may not be essential after *in vitro* perfusion, since the vessels are presumably already well perfused and any dilatation induced by perfusion fixation is less likely to be artifactual.

Some modifications to the perfusion circuit (Fig 1) and equipment are required for perfusion of the IPRK in high field magnets for spectroscopic analysis of compartmental biochemistry. Such studies include measurement of ATP using ^{31}P magnetic resonance spectroscopy (^{31}P MRS) [92, 111-114] and of cations using ^{23}Na MRS [94, 115-118] for sodium and ^{87}Rb MRS [119] for potassium. They also include using ^{1}H MR for microscopy of the intact perfused kidney [120] or a combination of both imaging and regional spectroscopy of the cortex and medulla using image-guided volume-localized magnetic resonance microspectroscopy [95, 121]. Although earlier MR spectroscopy studies had yielded useful results, a major modification was required for MR microscopy to proceed, namely perfusion under buffer to eliminate air-fluid interfaces which produce magnetic susceptibility artifacts [95, 121, 122]. This also required a reduction in rat size to allow kidneys to be perfused inside an 18 mm i.d. glass cylinder, made from a modified "20 mm" MR sample tube (Fig. 1). For this it became necessary to perfuse kidneys from 130-170 gm rats, necessitating the use of smaller cannulas etc. The glass cannula originally used for renal artery cannulation limited perfusion of small kidneys from younger rats because glass cannulas have relatively large wall thickness to lumen ratios. Consequently, in our laboratory glass cannulas have been abandoned in favor of 21 to 26 gauge synthetic intravenous cannulas, which have improved and made reproducible the flow characteristics of the cannula, facilitated cannulation and reduced expense. Metal cannulas also have low wall thickness to lumen ratios, but we have avoided these since even non-magnetic metal cannulas are not usable in a strong magnetic field and our laboratory was committed to extensive application of magnetic resonance imaging and spectroscopy to the IPRK model [123]. Metal cannulas are also best avoided in studies involving free radicals including nitric oxide. Venous cannulation is usually not required and should be avoided unless necessary since small rises in venous pressure may dramatically diminish renal function. Occasionally, venous cannulation has been used successfully, for example where observation of the renal ^{87}Rb signal was used as a K congener in MR studies of K transport [119]. In this experiment, separation of the Rb-containing venous effluent from the kidney surface was required, since the MR spectroscopy measurements averaged the whole sample and venous cannulation allowed the perfusate to be separated from a warmed Rb-free bathing solution, which was used to fill the sample space around the kidney. Other refinements have recently allowed us to reduce animal size to the extent that perfusion of kidneys from mice of 20-25 g body weight has now become routine in our laboratory (M. Rahgozar, A. Matthias, ZH Endre, unpublished data).

In 1981, Alcorn et al [124] described a consistent artefact in MTAL cells in the inner stripe of the outer medulla in the cell-free perfused IPRK. This lesion was subsequently shown to be MTAL necrosis from hypoxia [125] and presumed to follow the diffusional shunting of oxygen from arterial to venous limbs of vasa recta entering the outer medulla [89]. Subsequent studies have confirmed that not only the medulla but even the cortical tissue oxygen tension is lower than venous PO_2 and that diffusional shunting of oxygen occurs *in vivo* as well as *in vitro* and in the presence as

well as in the absence of erythrocytes [108]. Necrosis of the MTAL cells is eliminated by the presence of even low concentrations of erythrocytes under both normal [89-91], and hypoxic conditions [91]. Concentrating capacity in the IPRK falls rapidly after initiation of perfusion and is presumed to follow the loss of systemic antidiuretic hormone (vasopressin) and washout of the medullary interstitial osmotic gradient [95]. The latter presumably accompanies high perfusate flow through the renal medulla and the gradient is absent within 20 min of initiating perfusion. While low concentrations of erythrocytes eliminate histological evidence of injury to MTAL, erythrocyte concentrations must be increased to near normal levels to normalize perfusate flow to the 5-8 ml/min/g seen *in vivo* and antidiuretic hormone must be added to restore concentrating capacity [90, 105]. This observation suggested that high flow in the cell-free IPRK model was a result of low viscosity or because of near maximal-vasodilatation in response to the low oxygen concentration of cell-free perfusate or a combination of both. However, recent experiments in our laboratory suggest an additional explanation for high flow, at least in kidneys from Sprague-Dawley rats. Our data suggest that excessive renal nitric oxide production under control conditions also contributes to the high flow and partially inhibits renal autoregulation (Z. Guan, A. Matthias, S. Manley, ZH Endre, unpublished data). The most pronounced improvements in IPRK physiological function followed the addition of erythrocytes as an oxygen carrier [89-91], although stroma-free lysates [126, 127] and alternative oxygen carriers have been used to maintain oxygenation [128, 129].

Although physiological function in the cell-free IPRK shows somewhat reduced Na reabsorption, with FENa levels of 1-5% in rats larger than 200g and values up to 10% in smaller rat kidneys (e.g., compare [91] with [95], GFR remains comparable to *in vivo* with values between 0.5 and 1.5 ml/gm kidney weight. Interestingly, little enhancement of physiological function accompanied elimination of the MTAL lesion under control conditions [91] suggesting that Na reabsorption in the MTAL contributes little to total Na reabsorption in this model. As highlighted already, renal vascular resistance, urine volume, FENa and the U/P inulin all need to be considered when evaluating function since GFR values may be distorted (inflated) by high urine volumes, even when extensive tubular injury is present, as in the most extreme case where kidneys are perfused with Krebs bicarbonate buffer alone. In the latter situation GFR values of 1-3 ml/mg kidney weight have often been recorded but the low oncotic pressure leads to rapid swelling of the kidney, perfusate flow rarely exceeds 10 ml/min and oxygen delivery is therefore inevitably and severely compromised. In this situation, FENa is often greater than 20-30% and histological examination reveals extensive proximal and MTAL injury within 60 to 90 min perfusion (PJ Ratcliffe, ZH Endre, JD Tange unpublished data).

Fortunately, these limitations have not inhibited important studies of renal vascular function in the IPRK perfused without albumin or erythrocytes, often with flow fixed at very low levels, ca. 5 ml/min. Many of these studies have contributed valuable data on renal vascular regulation in normal and hypertension affected kidneys [130-137]. This highlights the apparent lack of oxygen-dependence of some of these processes and certainly demonstrates the utility of even a relatively hypoxic IPRK model in addressing vascular regulatory mechanisms embracing nitric oxide, endothelin, endothelium-derived hyperpolarizing factor and antidiuretic hormone. These albumin-free perfusion studies were driven by the need for single pass perfusion instead of recirculating perfusion to eliminate possible changes in concentration of the various agents added to the perfusate and by the high cost of purified albumin which usually makes single pass perfusion prohibitive. While such studies are valuable, it would be helpful if some were repeated under more physiological conditions to ensure the validity.

Applications in ischemia-reperfusion injury

Ischemia-reperfusion injury has been studied in a number of ways using the IPRK. Hypoxia has been induced by switching the gas delivered to the oxygenator from 95% O_2, 5% CO_2 to 95% N_2, 5% CO_2 eg, [92, 94, 95, 138]. Alternatively, ischemia is produced by clamping the tubing and diverting flow over the kidney by a "Y' piece eg, [139-141], which has the benefit of maintaining kidney temperature and avoiding dehydration. Finally, chemical anoxia can be induced with metabolic inhibitors, eg, [142].

ATP depletion, cation shifts and oxygen-derived free radical injury

Early studies in the IPRK utilizing ^{31}P magnetic resonance spectroscopy (MRS) confirmed the rapid onset of ATP depletion with induction of hypoxia [92]. These studies also demonstrated that the extent of morphological injury during perfusion at different degrees of hypoxia was proportional to the extent of ATP deletion. More recent studies by Lieberthal in cultured mouse proximal tubule cells have confirmed that renal cells die after being subjected to ATP depletion; with severe ATP depletion the cells die by necrosis and with less severe ATP depletion the cells die by apoptosis [143].

Multinuclear MRS studies in the IPRK with ^{23}Na, ^{31}P and ^{87}Rb (a congener of potassium) MRS have demonstrated that increases in intracellular sodium and decreases in potassium accompany the decrease in ATP induced by hypoxia [144]. Multinuclear studies with ^{19}F, ^{35}Cl, ^{31}P and single, double and triple quantum ^{23}Na MR have also been performed in IPRK by the Gupta group. Brief (10 min) ischemia in an IPRK loaded with the membrane-impermeant intracellular calcium indicator, 5F-BAPTA, caused a partially reversible increase in the intracellular calcium from 256 to 660 nM as measured by ^{19}F [112]. They demonstrated that the increase in intracellular sodium approached extracellular levels after prolonged ischemia [112]. They also observed that kidneys exposed to higher (1.2 mM) extracellular Mg^{2+} showed better recovery of ATP and lower accumulation of inorganic phosphate compared to kidneys exposed to low Mg^{2+} (0.3 mM) during reperfusion after a 60 min of stopped flow ischemia [118]. Measurements of the ^{23}Na TQ signal following ischemia-reperfusion revealed that kidneys exposed to 1.2 mM Mg^{2+} exhibited significantly improved maintenance of low intracellular sodium as compared to those exposed to 0.3 mM Mg^{2+}. Consistent with results in isolated proximal tubules, glycine supplementation reduced the rate of sodium accumulation in the intact hypoxic kidney [145].

Interestingly, the rate and extent of increase in total renal sodium (largely intracellular) in the IPRK was also reduced by pretreatment with dimethylthiourea (DMTU) and dimethylsulfoxide (DMSO), both scavengers of oxygen-derived free radicals (OFR) [94]. These studies supported similar indirect evidence for OFR-induced injury during reperfusion [146, 147]. The source of these radicals has been debated. Studies in the IPRK have demonstrated that activated neutrophils produce OFR mediated injury after ischemia [148, 149], while other studies in isolated proximal tubules and in the IPRK (eg [94, 139, 140] indicated that OFR were generated and contributed to the injury process even in the absence of neutrophils. Furthermore, the role of infiltrating neutrophils in ischemia-reperfusion injury remains controversial [150, 151]. Studies in both isolated proximal tubules [152] and in the IPRK [139, 140] have identified that the specific OFR produced following ischemia-reperfusion include hydroxyl radicals and an other unidentified species, possibly an early lipid peroxidation product [140]. Pretreatment with either allopurinol, which acts both to inhibit xanthine oxidase and as an OFR scavenger, or DMTU reduced both the morphological features of injury the extent of DNA fragmentation in the MTAL [141], suggesting that hydroxyl radicals formed during reperfusion after ischemia play a significant role in both necrotic and apoptotic cell injury.

Site of renal ischemia-reperfusion injury

The target zone for hypoxic injury has also been extensively studied in the IPRK where it predominantly involves the S3 segment of the proximal tubule and distal tubules located within the outer stripe of the outer medulla and their cortical equivalent, the medullary rays. The debate over whether the proximal or distal nephron segment is the primary target for hypoxic injury continues. Many of the morphological events in ischemic injury are model dependent as are those in the IPRK. As discussed already, the consistent artifactual necrosis of MTAL cells first described by Alcorn [124] arises as a result of the absence of an oxygen carrier during erythrocyte-free perfusion. Many studies by Brezis and his colleagues in the IPRK demonstrated that the MTAL lesion resulted from hypoxia and found that the lesion could be reduced by factors inhibiting energy consumption in the presence of substrate (oxygen) limitation, such as loop diuretics and that factors further reducing regional oxygen delivery such as the reductions in medullary blood flow produced by inhibition of prostaglandins and/or nitric oxide inhibitors would exaggerate MTAL injury [109, 125, 153, 154]. However, while aggravation of MTAL injury clearly occurs under a number of defined circumstances, particularly where multiple insults are delivered to the

kidney, this usually occurs in association with increased proximal tubular injury as well [155]. Furthermore, Endre and colleagues in other studies in the IPRK showed that in the presence of erythrocytes in concentrations as low as 1% MTAL necrosis was prevented both under control conditions with high perfusate oxygen tension and in the presence of hypoxia [91]. The proximal tubule continued to be injured by hypoxia *in vitro*, confirming that MTAL necrosis was an artefact of cell-free perfusion in this model. In IPRK perfused with normal concentrations of erythrocytes, MTAL injury was similarly absent [156] and such lesions are absent during ischemia *in vivo* [157]. Thus, while the overwhelming data in IPRK and *in vivo* support the proximal tubule, especially the S3 segment, as the primary target for injury in hypoxia and ischemia, the broader focus on hypoxic injury in the IPRK has raised many useful questions and demonstrated that regional hypoxia in the kidney may be more widespread than previously appreciated.

A link between proximal and distal tubular injury and recovery

The studies in the IPRK and *in vivo* outlined in the previous section highlighted that both proximal straight tubules (S3) and MTAL were potential targets for hypoxic injury. The anatomical proximity of these tubular segments emphasizes the location of both segments in a region under constant threat of hypoxia. Outer medullary hyperaemia is a consistent phenomenon following renal artery clamping to induce acute renal failure first described by Mason and others [158, 159]. It was hypothesized that erythrocyte aggregation and stasis in the outer stripe was produced by oxygen-derived free radicals causing extravasation of plasma and local hemoconcentration, however free radical scavengers were of no benefit, whereas hemodilution and raised intraarterial pressure each reduced both medullary hyperaemia and tubular necrosis [158]. The phenomenon of medullary hyperaemia has not been as well described in the IPRK, probably because erythrocytes are usually not added to the perfusate and ischemia has been utilized by few groups in this model. When erythrocytes are present and flow is stopped for 20 min or more, a similar but less dramatic hyperaemia is observable although prolonged reperfusion reduces this further. Our MR microscopy studies of the IPRK demonstrated that hypoxia rapidly reduced flow through the vascular bundles passing through the inner stripe and through their cortical equivalents, the medullary rays [120, 122]. The enlargement of the tubules in these interbundle regions accompanying the reduction in flow through the vascular bundles suggested that a simpler explanation for the parallel *in vivo* phenomenon of medullary hyperaemia is cellular swelling leading to compression of the vascular bundles running between the clusters of proximal and TAL tubules. In any event, these observations in the IPRK provided anatomical support for the concept of local hypoxia in these regions.

A parallel phenomenon is the occurrence of DNA fragmentation by *in situ* end labelling (TUNEL) in MTAL cells after both brief or prolonged hypoxia in the IPRK or after brief ischemia *in vivo*, which is not accompanied by significant morphological evidence for apoptosis [141, 160]. Similarly, DNA fragmentation has been observed after 24 hours reperfusion following ischemia *in vivo* in rats, again with little or no morphological evidence of apoptosis [161]. DNA fragmentation has also been observed in human autopsy specimens after renal hypoperfusion [162]. Explanations for the dissociation between DNA fragmentation and apoptosis in MTAL cells include different pathways for these processes [163, 164] and also repair of damaged DNA and interruption of the apoptotic process. Studies by Gobé et al [161] [165] of the Bcl-2 multigene family 24 hours after 30 min bilateral arterial clamping *in vivo* have demonstrated a marked increase in expression of anti-apoptotic Bcl-2 and a moderate increase in anti-apoptotic Bcl-X(L) and pro-apoptotic Bax in distal tubules. Proximal tubules showed a marked increase in Bax expression and a moderate increase in Bcl-X(L). Twenty-four hours after expression of the Bcl-2 proteins was increased, IGF-1 and EGF protein levels were increased in the distal tubule, similar to the Bcl-2 anti-apoptotic proteins. These growth factors were also detected in the adjacent proximal tubules suggesting a paracrine action since the factors are apparently not synthesized in proximal tubules. TGF-β expression was moderately increased in regenerating proximal tubules, but no relationship to the pattern of expression of the Bcl-2 genes was seen. To reconcile the observations of proximal cell necrosis and DNA fragmentation without apoptosis in nearby MTAL, Gobé and colleagues [161] have proposed that the distal tubule is adaptively resistant to ischemic injury via

promotion of survival by anti-apoptotic Bcl-2 genes, which abort the apoptotic process in MTAL cells, leading to repair of the DNA fragmentation. Further studies by the same group [166] on the mechanism of this protection in cultured cells suggest that survival in distal tubular cells is associated with translocation of the Bcl-2 family proteins, Bcl-X(L) in the case of MDCK cells, to the mitochondrial membrane. Presumably, this prevents release of cytochrome c, which precedes activation of caspase 3 in the p53 cell death pathway [167]. Proximal tubular cells lack Bcl-2, but contain proapoptotic Bax and proceed to death by both apoptosis and necrosis. Survival of the distal tubular cells allows expression of EGF and IGF growth factors critical to the maintenance and regeneration of other distal tubular cells (autocrine action), and to survival and/or regeneration of the adjacent ischemia-sensitive proximal tubular cells (paracrine action). Since proximal cells are necrotic or have sloughed due to loss of cell adhesion, proximal recovery is delayed compared to the MTAL.

Thus studies in the IPRK have provided evidence that both ischemia and hypoxia produce reduced flow in the outer stripe of the outer medulla and that the resultant regional hypoxia affects nearby proximal and distal tubules leading to necrosis of proximal tubules and arrested apoptosis of the distal tubules. Follow up work *in vivo* and in cultured cells have suggested that the distal tubular cells are protected by Bcl-2 family upregulation and that survival of these cells allows growth factors to promote regeneration of the nearby injured proximal tubules. Interestingly these data fit with studies of localization of the early gene response and DNA synthesis in the kidney after ischemic injury. DNA synthesis occurs in the proximal tubule, whereas induction of the early gene response is restricted to the MTAL and collecting duct cells [168].

Tubuloglomerular feedback

Although direct observation of the microcirculation is not possible in the standard IPRK model, the use of hyperoncotic solutions to create a non-filtering kidney [169] allows a relatively clean interruption of tubuloglomerular feedback (by impairing distal tubular NaCl delivery) analogous to papillectomy in the perfused juxtamedullary nephron technique [101]. Frusemide can also be used to inhibit tubuloglomerular feedback in either preparation and *in vivo* [170]. However, the overlap with other actions of inhibitors (such as diuresis) produced by frusemide makes interpretation of the results more difficult especially in the study of ischemia-reperfusion injury [170].

Endothelin in ischemia-reperfusion injury

Many studies of endothelin action have been performed in the IPRK. The potential potent vasoconstrictor role of endothelin in acute renal failure was first noted in the IPRK by Firth [171], who also observed that endothelin-1 mRNA was upregulated for several days after renal pedicle clamping *in vivo* [172]. *In vivo* studies suggest that this upregulation of endothelin is modestly stimulated by hypoxia alone [173, 174] but that rapid and prolonged upregulation occurs after ischemia in renal medullary interstitial cells, damaged tubules at the corticomedullary junction and peritubular capillaries surrounding these damaged tubules [175-177]. IPRK studies showed that pretreatment with a selective endothelin (ETA) receptor antagonist), BQ-123, ameliorated the fall in inulin clearance and sodium transport in a renal artery clamp model of ischemic acute renal failure [178]. The benefit of endothelin antagonists in ischemic acute renal failure *in vivo* and *in vitro* is complicated by the different effects of ETA and ETB receptors, with protection arising from ETA antagonists and exacerbation from both non-selective and ETB-selective antagonists, the latter presumably because of impaired ETB-stimulated nitric oxide production [179].

Treatment of ischemic acute renal failure

Many experimental treatments for acute renal failure have been tested in the IPRK, either for efficacy or in the assessment of mechanisms leading to renal cell injury. One example leading to clinical application will be discussed. Lieberthal et al [180] observed that renal vascular resistance was increased during reflow in the isolated erythrocyte- perfused kidney subjected to 25 min of ischemia. Endothelium-independent vasodilators (atrial natriuretic factor, ANF, and sodium nitroprusside) prevented the increase. Acetylcholine and the calcium ionophore A23187, two vasodilators that act by releasing endothelium-derived relaxing factor, had no effect, while two inhibitors of EDRF, methylene blue and gossypol, increased RVR in nonischemic kidneys by an amount comparable to that found with ischemia alone. The increase in RVR found with the combination of EDRF inhibition and ischemia was the same as

that found with ischemia alone. In further studies [93], they found that ANF, administered alone after 25 min ischemia in the isolated kidney, reversed postischemic vasoconstriction but did not improve glomerular filtration rate (GFR). Mannitol alone had no effect on either renal vascular resistance or GFR. However, in isolated kidneys treated with the combination of both ANF and mannitol following reflow, GFR was markedly improved compared with GFR in the untreated ischemia group and was not different from GFR in the nonischemic controls. Comparable results were obtained in studies performed *in vivo*, suggesting that the combination of ANF and mannitol appear to act synergistically to improve GFR following ischemic injury. These studies provided the initial experimental basis for subsequent clinical studies of ANP in acute renal failure [181].

Nephrotoxic injury

The IPRK model has been helpful in assessing many potential nephrotoxins and in revealing mechanisms of toxicity and treatment. Given the hundreds of published studies, only selected areas will be cited. It should also be noted that many of the IPRK preparations have utilized cell-free perfusion and are therefore likely to have varying degrees of distal tubular injury. However, as already noted, this seems less of a problem where the primary interest is in vascular or proximal tubular function.

Cyclosporine

Nephrotoxicity arising from cyclosporine A has been extensively studied in the IPRK. Cyclosporine produces necrosis, vacuolization and lipid droplets of the proximal tubular cells, as well as glomerular afferent arteriolar constriction and granular juxtaglomerular cell hyperplasia. The mechanism of vasoconstriction is not well known, but there appears to be substantial impairment of endothelial cell function leading to enhanced release of vasoconstrictors such as endothelin and thromboxane [182]. L- propionylcarnitine, a potent analogue of carnitine, is able to correct and to prevent alterations in endothelial membrane permeability and has been identified in the kidney of various animal species. Pretreatment with L- propionylcarnitine before administering several doses of CSA in the IPRK reduced the vasoconstrictive effect of CSA on the glomerular and tubular capillaries and preserved the tubular epithelium both histologically and functionally with a reduction in cyclosporine-induced release of alanine aminopeptidase and N-acetyl- glucosaminidase [183].

Endothelin in hypertension and pro-atherogenic states

The role of renal endothelin receptors in diseases associated with hypertension appears to be critical. Hirata and colleagues [184] utilized the IPRK to demonstrate that ETB receptor stimulation induced release of nitric oxide. They found that ET-1 and ET-3 released nitric oxide via ETB receptors in renal vessels. ETB receptors were downregulated in deoxycorticosterone acetate (DOCA-salt) rat kidneys explaining why ETB-mediated NO release was reduced in DOCA-salt rats, an event which may modulate renal function and blood pressure regulation in DOCA-salt hypertensive rats. They subsequently observed that expression of ETB receptors in the endothelium was decreased in IPRK from 3 disease models (rats with hypertension, diabetes mellitus, and hypercholesterolemia) compared with that in the vascular smooth muscle cell [135]. Infusion of a highly selective ETB receptor agonist, BQ-3020, reduced renal perfusion pressure in Dahl salt-resistant rats but increased renal perfusion pressure in Dahl salt-sensitive rats. BQ-3020 caused a dose-dependent release of nitric oxide in both types of rats, although the level of nitric oxide release in salt-sensitive rats was lower. Similar effects of BQ-3020 were observed in Streptozotocin- induced diabetic rats and diet-induced hypercholesterolemic rats. Expression of endothelial NO synthase (eNOS) decreased in salt-sensitive rats but not in diabetic or hypercholesterolemic rats. They concluded that impaired NO release in response to stimulation of ETB receptors in these pathologic states is due, at least in part, to a decrease in endothelial ETB receptors and may play a role in vascular dysfunction usually associated with arteriosclerosis-related diseases.

Radiocontrast

Studies on the mechanisms of radiocontrast nephrotoxicity have been performed in IPRK with conflicting results. While some studies provided modest support for the contrast –induced renal vasoconstriction theory, perhaps resulting from reduced nitric oxide or

increased endothelin release, others demonstrated that different contrast agents had varying effects on the renal circulation. For example, pretreatment with BQ123 (a selective endothelin (ETA) receptor antagonist), but not with phosphoramidon (an endothelin-converting enzyme inhibitor), inhibited the sustained fall in renal perfusate flow produced by both iotrolan and diatrizoate. BQ123 markedly potentiated the renal vasodilatation produced by diatrizoate [185] and the AT1 receptor blocker, bosentan, prevented reduction in creatinine clearance after diatrizoate [186]. However, subsequent studies in the IPRK however suggest that NO and endothelin-mediated events are independent and not modulated by these radiocontrast agents. For example, in careful dose response studies in the IPRK, Morcos et al [187] observed that L-NAME did not interfere with the vasodilatation induced by diatrizoate in the presence of BQ123 and concluded that diatrizoate did not interfere with endothelium derived NO-dependent vasodilatation. They concluded that reduced production of NO in the vascular endothelium induced by contrast media was unlikely to play any role in the pathophysiology of the increase in renal vascular resistance produced by radiocontrast agents and specifically that the renal vasodilatation induced by diatrizoate was not dependent on endogenous production of NO. In this light it is perhaps not surprising that inhibition of ETB receptor stimulated nitric oxide release could have produced the exacerbation of radiocontrast nephrotoxicity by a non-selective endothelin antagonist in a recent prospective clinical trial [188]. Nevertheless the aetiology and treatment of radiocontrast nephropathy is far from clear. Further studies will need to account, inter alia, for the exacerbation in vasoconstriction produced by prostaglandin inhibition in the IPRK [189], for the role of adenosine suggested by amelioration by theophylline [190, 191]. One related experimental finding, at least, has changed clinical practice. Yoshioka et al [192] demonstrated that renal cortical antioxidant activity was reduced in water-deprived rats and that only water-deprived rats showed increased lipid peroxidation after contrast which was inhibitable by pretreatment with polyethylene glycol-coupled catalase. The link between water deprivation and, presumably free radical-mediated depletion of renal cortical oxidant activity is unclear. However, the observation has lead to the recent successful clinical trial of prevention of contrast nephropathy by acetylcysteine [193].

Mercuric chloride

A progressive fall in glomerular capillary plasma flow is observed in mercuric chloride-induced acute renal failure although the site of the main histological lesion is the proximal tubule. Studies in the IPRK showed that intense mercuric chloride-induced vasoconstriction could be inhibited by captopril but not enalapril arguing against renin-angiotensin system involvement, with binding of free Hg by the sulphydryl group of captopril suggesting a simpler explanation and supporting possible attack by Hg to tissue thiol moieties as the mechanism for vasoconstriction [194]. However, vasoconstriction was absent in the non-filtering IPRK, suggesting that intraluminal mechanisms might be involved [169]. Adenosine analogues selective for the A1 subclass of adenosine receptors, such as N6-cyclohexyladenosine (CHA), induce vasoconstriction in the IPRK [195]. However, theophylline failed to reverse mercuric chloride-induced vasoconstriction [196]. More recent studies *in vivo* suggest that increased endothelin and reduced nitric oxide may be involved in mercuric chloride-induced vasoconstriction. Both endothelin (ET-1) and eNOS proteins are expressed in the juxtaglomerular cells of afferent arterioles. The expression of ET-1 was significantly increased after mercuric chloride-induced acute renal failure whereas the expression of eNOS was markedly reduced [197]. Taken together, these data suggest that mercuric-chloride induced vasoconstriction is mediated by increased endothelin and reduced nitric oxide, the latter perhaps involving tubuloglomerular feedback.

Nitric oxide, endothelium-derived hyperpolarizing factor and prostaglandins

A role for endothelium-derived relaxing factor in renal vascular resistance and in glomerular and tubular function was first observed in the IPRK by Bhardwaj and Moore [198] and by Rademacher et al. [199-201]. Others have observed that manipulating nitric oxide can alter medullary oxygenation in the IPRK [109]. An increased endothelium-dependent vasodilator response to acetylcholine was observed in IPRK from cirrhotic rats [202]. Portal vein ligation also lowered renal vascular resistance that was not related to nitric oxide or prostaglandins, although increased nitric ox-

ide production interfered with the effects of the α-agonist methoxamine [203], further suggesting that nitric oxide plays an important role in the modulation of the renal vascular responses to vasoconstrictors in portal hypertension. Subsequent studies by Vargas et al [204, 205] in the IPRK pretreated with indomethacin and utilizing tetramethylammonium (a nonspecific blocker of potassium channels that inhibits acetylcholine-induced hyperpolarization) and varying potassium concentrations suggested that both nitric oxide and endothelium-derived hyperpolarizing factor are similarly involved in the endothelium-dependent vasodilatation induced by acetylcholine. Similarly, NO- and prostaglandin-independent, endothelium-dependent vasodilator responses to bradykinin are attributed to release of a hyperpolarizing factor. The contribution of K^+ channels to the renal vasodilator effect of bradykinin was assessed in the IPRK in the presence of ATP- and Ca^{2+}-activated K channel inhibitors [206, 207]. These studies implicated Ca^{2+}-activated K^+ channels in the renal vasodilator response to bradykinin and similarly support a role for a hyperpolarizing factor. Other studies in IPRK have demonstrated the biological importance of S-nitrosothiols (RS-NO) in the action and metabolism of endothelium-derived relaxing factor [208].

Studies in the IPRK utilizing single-pass perfusion have demonstrated a close interaction between renal nitric oxide and the cyclooxygenase pathway with inhibition of prostanoid production by nitric oxide [209]. The pressor effect of L-NAME also appears to partly rely upon the vasoconstrictor effect of TxA2 and PGH2. Other studies in the recirculating IPRK have shown interactions between bradykinin and ANP on both glomerular and tubular function [210], between estrogens and calcium-modulated endothelium-dependent dilatation [211], between angiotensin II and eicosanoid release stimulated through AT2 receptors [212], and that the NO-cGMP pathway is involved in adrenomedullin induced vasorelaxation [213]. Finally, other single pass studies suggest that impaired NO release in response to stimulation of endothelin ETB receptors may result from a decrease in endothelial ETB receptors, which may represent a mechanism for in vascular dysfunction usually associated with arteriosclerosis- related diseases [135].

Renal and cardiac fibrosis

Short-term pirfenidone and spironolactone treatment was recently found to reverse cardiac and renal fibrosis and to attenuate increased diastolic stiffness without normalizing cardiac contractility or renal function in STZ-diabetic rats [214].

Glomerular function in the IPRK

There is a significant literature exploring many aspects of glomerular function and models of glomerular injury in the IPRK. Examples include studies of albumin excretion, protein trafficking and vasoactive modulation of glomerular permselectivity [201, 215-219]; studies of glomerular immune injury including the role of complement [220], leukotrienes [221, 222], prostaglandins [223, 224] and clusterin [225] and studies of glomerular hemodynamics [226].

Disadvantages of the IPRK model

In common with most isolated organs, the disadvantages of the IPRK include some physiological limitations. There are also the unique anatomical artifacts arising from cell-free perfusion and there is a requirement for significant technical surgical skill. While the cell-free IPRK model exhibits much higher flow than *in vivo* kidney, it now appears that this vasodilated state arises from relative nitric oxide excess rather than simply low perfusate viscosity. Defining this more clearly will provide further insights into normal physiological responses. The model brings with it the expense and trouble involved in preparation and use of albumin-containing solutions and even more complexity when erythrocytes are added. Finally, despite extensive preparation and skilful handling, the model is only viable for a few hours - longer in the presence of erythrocytes, shorter in their absence. Thus, perturbations requiring longer than 2-3 hours for development can only be monitored after having first been induced *in vivo*, potentially negating at least part of the benefit of assessing some pathological or physiological events in the absence of non-renal or uncontrolled systemic influences.

Advantages of the IPRK model

The IPRK is an intact model of the kidney function, which is well suited to examining critical functions, which require an intact renal architecture to mimic the

in vivo situation. Such critical functions modified by the renal architecture encompass substrate delivery, including oxygen, and the links between vascular and tubular function. The model has the advantages of eliminating systemic hormonal and sympathetic nervous system influences, while still allowing tight control of pressure and flow and of permitting simultaneous measurement of renal vascular and tubular function with direct infusion of vasoactive agents into the renal artery. It allows quantifiable assessment of renal vascular, tubular and glomerular function and high quality morphological assessment. Finally the model permits more specialized assessment such as magnetic resonance microscopy and spectroscopy. With continuous modification for over 40 years, the IPRK technique has developed into a reliable and powerful method for studying many questions regarding renal physiology and function in both health and disease and utilizing most of the techniques applied *in vivo* and others that are too difficult to apply, such as magnetic resonance microscopy.

Methods to evaluate the renal microcirculation

Introduction

Studies of the pathophysiology of acute renal failure has classically considered both tubular and vascular mechanisms [227, 228]. *In vitro* techniques isolating either the vascular or tubular components have been developed. For example, the use of isolated proximal tubules in suspension or in culture allows the study of tubular mechanisms of injury in the absence of vascular factors [229] [230]. There are both *in vitro* and *in vivo* models to study vascular injury in the kidney. *In vitro* models include the study of vascular smooth muscle cells or endothelial cells in culture. In this section, the *in vivo* methods to evaluate the renal microcirculation will be discussed. This is of relevance as many nephrotoxins exert their deleterious effects through pharmacologic actions on the resistance vasculature with parenchymal injury occurring as a consequence of ischemia. In clinical practice nephrotoxins may cause prerenal azotemia as a result of increased renal vascular resistance. Nephrotoxins that cause acute renal failure on a vascular basis include prostaglandin inhibitors e.g. aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 (COX-2) inhibitors, vasoconstrictive drugs e.g. cyclosporine, tacrolimus, radiocontrast media and drugs that block angiotensin II e.g. angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists (ARBs) (Table 4). ACE inhibitors and ARBs are widely used for the treatment of hypertension, congestive heart failure and diabetic nephropathy. They preferentially dilate the efferent arteriole of the glomerulus and decrease intraglomerular pressure. Acute renal failure may occur in conditions where angiotensin plays a crucial role in maintaining the glomerular filtration rate e.g. volume depletion, bilateral renal artery stenosis, diuretic-induced sodium depletion and autosomal dominant polycystic kidney disease.

Control of vascular tone

Resting tone reflects basal properties intrinsic to the smooth muscle cells and the modulating influence of several well defined extrinsic factors [231]. These include the metabolic demand of the organ, neural and humoral factors, paracrine modulators and physical forces like stretch and shear. The myogenic response which increases smooth muscle tone in response to stretch is largely mediated by specialized, distinct stretch operated calcium channels [232]. The transduction of flow-related stimuli also intrinsically regulates blood vessels in the *in vivo* circulation. Flow produces shear that is readily detected by endothelial cells. Vasomotor responses to flow are predominantly dilator [233]. Nitric oxide (NO) derived from endothelial nitric oxide synthase (eNOS) [234] as well as endothelial cyclooxygenase products [235] have been implicated in the conversion of the endothelial shear response into smooth muscle relaxation. The role of NO in the resting circulation is indicated by the increases in basal vascular resistance after NOS inhibition [236]. The influence of humoral and neural factors like catecholamines, cholinergic mediators, prostanoids, angiotensin and aldosterone may be greater in stimulated or stress states than under basal conditions [231].

Vascular reactivity is the response to external and local stimuli that modify vascular tone. These factors are demonstrated in Table 5. The action of these factors varies among individual organs. For example, the kidney has greater vasoconstrictor sensitivity to endothelin-1 (ET-1) than other organs [237]. Integrity of the

vascular responses is crucial in the maintenance of organ function in the face of nephrotoxins. Loss of this normal responsiveness may result in vascular injury.

Experimental models to evaluate the renal microcirculation

Techniques have been developed for study of the renal microcirculation. These techniques have distinct advantages over *in vitro* endothelial and vascular smooth muscle cell preparations. They allow study of important anatomic and physiologic relationships that are lost in isolated cell systems. For example, the effects of both pressure and flow can be determined and the spatial relationship between the endothelium and smooth muscle is maintained. These techniques permit functional assessment of the resistance microvasculature without destroying vessel integrity while eliminating the confounding influence of undetected circulating, neural and parenchymal factors. The techniques are demonstrated in Table 6.

Table 4. Drugs that cause acute renal dysfunction on a vascular basis.

Prostaglandin inhibitors:
• Aspirin
• Non-steroidal anti-inflammatory drugs (NSAIDs)
• Cyclooxygenase-2 (COX-2) inhibitors
Vasoconstrictors:
• Cyclosporine
• Tacrolimus
• Radiocontrast media
Angiotensin II blockade:
• Angiotensin converting enzyme (ACE) inhibitors
• Angiotensin II receptor antagonists (ARBs)

Table 5. Endogenous agents that modify vascular tone and reactivity.

Endocrine or neural:
• Renal nerves
• Catecholamines
• Angiotensin II
• Natriuretic peptides
Paracrine:
• Endothelial derived e.g. nitric oxide, endothelin-1
• Angiotensin II
• Arachidonic acid metabolites e.g. thromboxane A2, prostaglandins, leukotrienes
• Purinoreceptors and vasoactive purine agonists e.g. P1 receptors and adenosine
• Dopamine and serotonin

Table 6. Techniques for study of renal microcirculation

• Juxtamedullary nephron preparation
• Hydronephrotic kidney
• Isolated perfused afferent arteriole
• Isolated perfused juxtaglomerular apparatus

In vitro perfused juxtamedullary nephron

In vivo micropuncture techniques have contributed greatly to the understanding of the forces governing glomerular hemodynamics and the ultrafiltration process [238]. The *In vitro* perfused juxtamedullary nephron first described in 1984 [239] allows study of a unique population of nephrons present in the inside cortex in apposition to the pelvic area of the kidney. These glomeruli have long afferent arterioles originating close to the main arcuate arteries. They are also located directly at the surface of the renal cortex normally covered by the pelvic mucosa. These superficial nephrons at the corticomedullary border have vascular characteristics of efferent arterioles breaking into the vasa recta which is typical for juxtamedullary nephrons. This technique does not involve microdissection or the isolation of glomeruli and their arterioles.

Methods

The method for the *in vitro* perfused juxtamedullary nephron technique in Sprague-Dawley rats is as follows: A common dissection procedure is used to expose the arteries and related nephrons located at the inside surface of the renal cortex lining the pelvic cavity. Rats are systemically heparinized. The right kidney is removed and the left kidney is perfused with physiologic solution containing albumin. The left kidney is then decapsulated, removed from the animal and placed in a Petri dish filled with physiologic solution containing albumin at room temperature. The kidney is cut longitudinally to expose the pelvic cavity with the papilla left intact. Most of the cortical tissue is resected. The arcuate arteries are dissected and ligated with tight ligatures. A microperfusion system allows

blood perfusion of the superficial nephrons via arcuate arteries.

Model

Anatomic and physiological studies can be performed in these kidneys [239]. Anatomically the models can be studied by light microscopy and scanning electron microscopy after infusion of the kidneys with resin. For physiological studies a perfusion system is used with donor blood or a cell free media. The microvasculature is viewed by videomicroscopy and vessel lumen diameters measured with a micrometer. It is also possible to simultaneously measure single nephron glomerular filtration rate or perform tubular perfusion with this technique. At a perfusion pressure of 100 mmHg, glomerular capillary pressure averaged 49 mm Hg, with most of the preglomerular pressure drop being localized to the terminal afferent arteriolar segment. Blood hematocrit can be reduced to approximately 30% with physiological solutions devoid of or containing albumin. In these conditions, the single nephron glomerular filtration rate averaged 34 nl/min (low plasma colloid osmotic pressure, PCOP) and 23.3 nl/min (maintained PCOP). Proximal tubule reabsorption ranges from 17 to 29%. In conclusion, the integrity of nephron function is maintained in this model, which provides insights into the dynamics of filtration and reabsorption processes of juxtamedullary nephrons. The procedure preserves the *in vivo* tubulovascular relationships, enables the use of a semi-microperfusion system and provides direct access to juxtamedullary nephrovascular units.

In early studies, microvascular reactivity of *in vitro* blood perfused juxtamedullary nephrons were studied in rats [240]. The effects of angiotensin II, epinephrine, and changes in perfusion pressure on glomerular capillary and afferent arteriolar pressures were assessed. At a perfusion pressure of 102 mm Hg, glomerular capillary pressure averaged 55 mm Hg. Afferent arteriolar pressure, measured at early-to-mid afferent locations, was 88 and decreased at the most terminal segments. In some nephrons, readjustments of glomerular capillary pressure occurred in response to step changes in perfusion pressure. Bolus injections of angiotensin II into the blood caused dose- dependent and reversible decreases in glomerular capillary pressure. Similar decreases in glomerular capillary pressure were observed in response to epinephrine. Epinephrine also consistently reduced afferent arteriolar pressures. In contrast, angiotensin II typically increased pressure in the early and mid segments of the afferent arteriole, but caused variable responses in the late afferent arteriole. The responses to vasoconstrictor agents were not mimicked by increases in perfusion pressure per se. This study demonstrates that the preglomerular vasculature of *in vitro* blood perfused juxtamedullary nephrons can exhibit autoregulatory behavior and is responsive to humoral vasoconstrictors.

Advantages

Advantages of the *in vitro* perfused juxtamedullary nephron preparation are 1) preservation of the circulatory network; 2) no microdissection trauma to vessels; 3) the elimination of neural and hormonal influences; 4) maintenance of tubulovascular relationship and 5) simultaneous evaluation of both vascular and tubular effects of toxic substances.

Limitations

Limitations of the *in vitro* perfused juxtamedullary nephron preparation include 1) the availability of only a select population of juxtamedullary glomeruli near the inner surface of the kidney; 2) underestimation of the contribution of flow in large vessels to preglomerular resistance and 3) lack of characterization of tubular transport.

Studies

To date, the *in vitro* perfused juxtamedullary nephron preparation has not been used to examine the effects of nephrotoxic agents. However the preparation has been used to examine the pathophysiology of tubuloglomerular feedback. It has also been used to study the effect of mediators like adenosine, oxygen radicals and nitric oxide. Some recent studies are discussed below.

The influence of neuronal nitric oxide synthase (nNOS) on renal arteriolar tone has been studied in the perfused juxtamedullary nephron preparation [241]. Superfusion with a specific nNOS inhibitor decreased afferent and efferent arteriolar diameters, and these decreases in arteriolar diameters were prevented by interruption of distal volume delivery by papillectomy. When volume delivery to the macula densa segment was increased, afferent arteriolar vasoconstrictor responses to the nNOS inhibitor were enhanced,

but this effect was again completely prevented after papillectomy. In contrast, the arteriolar diameter responses to a nonselective NOS inhibitor were only attenuated by papillectomy. Specific nNOS inhibition enhanced the efferent arteriolar vasoconstrictor response to angiotensin II but did not alter the afferent arteriolar vasoconstrictor responsiveness to angiotensin angiotensin II. In contrast, non specific NOS inhibition enhanced both afferent and efferent arteriolar vasoconstrictor responses to angiotensin II. This study demonstrates that the modulating influence of nNOS on afferent arteriolar tone of juxtamedullary nephrons is dependent on distal tubular fluid flow and that nNOS exerts a differential modulatory action on the juxtamedullary micro- vasculature by enhancing efferent, but not afferent, arteriolar responsiveness to angiotensin II. It has also been demonstrated that superoxide inhibits nNOS influences on afferent arterioles in spontaneously hypertensive rats [242]. The role of neuronal NOS on afferent arteriolar function has been demonstrated in enhanced tubuloglomerular feedback activity [243], angiotensin II induced hypertension [244] and chronic heart failure [245].

More recently, studies have been performed to determine the responsiveness of rat juxtamedullary afferent arterioles to receptor-selective P2- purinoceptor agonists [246]. Experiments were performed *in vitro* using the blood perfused juxtamedullary nephron technique, combined with videomicroscopy. The presence of multiple P2 receptors on juxtamedullary afferent arterioles and the classification of these receptors as members of the P2X- and P2Y2 (P2U)-receptor subtypes was demonstrated. In another study, the relative contributions of adenosine A1 and A2a receptors to the responsiveness of the renal microvasculature to adenosine was investigated [247]. The presence of adenosine A1 and A2a receptors on afferent and efferent arterioles of juxtamedullary nephrons was demonstrated. Also, adenosine A2a receptor-mediated vasodilation partially buffered adenosine-induced vasoconstriction in both pre- and postglomerular segments of the renal microvasculature.

The role of the cyclooxygenase pathway interaction with nNOS [248, 249], tyrosine kinase [250]. intracellular calcium [251] and insulin-like growth factor 1 (IGF-1) [252] on afferent arteriolar function has been demonstrated in the perfused juxtamedullary nephron.

Hydronephrotic kidney

The hydronephrotic kidney was developed for *in vivo* visualization of the glomerular microcirculation, the vasa afferens and the vasa efferens [96]. This preparation utilizes postischemic hydronephrosis (PIH) to destroy the renal tubular system while preserving a portion of the cortex. In this preparation, glomeruli and associated vasculature remain intact. Observations are made with either incident light or transillumination.

Model

Briefly, the model is as follows: Rats are anesthetized, the left kidney is exposed and the ureter is permanently ligated while the artery is clamped for 60 min. The rats develop a postischemic hydronephrotic kidney in about 3 weeks. Microcirculatory experiments are begun 3 to 12 weeks after initial surgery. The animals are placed on a heated operating table. A carotid artery catheter is placed for continuous arterial blood pressure monitoring. The left hydronephrotic kidney is suffused with an isotonic and isocolloidal solution warmed to 37°C. The kidney is split at the greater curvature with a cautery knife and the halves of the kidney are visualized with transillumination and fluorescence videomicroscopy. To visualize the flow velocity in single glomerular loops, fluorescent-labelled erythrocytes are injected so that only single erythrocytes pass through the glomerulus at one time. The flow velocity of fluorescent erythrocytes is monitored by analyzing sequences of single frames of video pictures. The geometry of glomerular structure is reconstructed. Capillary erythrocyte velocity and volume flow measurements are made. Blood flow velocity in afferent and efferent arterioles can be studied by the dual-slit method. Vessel internal diameter can also be measured electronically from a video image.

A high-speed video camera, recording up to 600 frames per second, can be incorporated in the setup and erythrocyte velocity *in vivo* can be measured offline with the line-shift-diagram method [253].

Methods

In the original description of this model by Steinhausen et al, the following measurements were made: The inner diameter of the vasa afferens, measured within 50 microns of the glomerular vascular pole, was 7.9 microns while that of the vas efferens was

7.7 microns. Both vessels were narrower adjacent to the glomerulus. A specialized round cell, which may act as a sphincter, was seen in the vasa efferens. Blood velocity, measured in the vasa afferens and efferens about 100 microns from the vascular pole, was 5.9 and 4.6 mm X sec-1, respectively. During angiotensin II infusion, the vasa efferens in the vicinity of the glomerulus constricted by 22% whereas the corresponding vas afferens showed no consistent response. During angiotensin II infusion, the filtration fraction (GFR/RPF) may, therefore, be elevated by an increased resistance in the vasa efferens, particularly at the outflow point of the glomerulus. Higher dosages of angiotensin II caused vasoconstriction of both vessels, especially at sites more distant from the glomerulus.

Advantages

The advantages of the hydronephrotic kidney preparation in examining the renal microvasculature are as follows: 1) It is possible to determine the real flow direction in both pre- as well as postglomerular elements of the microvasculature a three-dimensional way; 2) the circulatory network is preserved such that the pressure and flow effects of changes in resistances in one vascular segment can be determined in the adjacent upstream and downstream vessels; 3) the circulation can be examined without the trauma related to microdissection; 4) both outer and inner cortical microvessels can be studied.

Limitations

Limitations of the technique include: 1) The induction of hydronephrosis may alter vasoreactivity; 2) tubular atrophy eliminates tubular influences on the microcirculation such as those observed in the tubuloglomerular feedback phenomenon and 3) intrinsic vascular resistances are higher and blood flows lower in hydronephrotic compared to normal kidneys.

Many important observations regarding segmental changes in the renal microcirculation to physiologic, pharmacologic and toxic stimuli have been made with hydronephrotic kidney preparation.

Studies

The effect of various mediators on the renal microcirculation are summarized in Table 7. The effects of various drugs, including cyclosporine nephrotoxicity, on the renal microcirculation are summarized in Table 8.

Isolated renal microvessels

Methods

The isolated renal microvessel preparation was first described using adult female New Zealand White rabbits [254]. In this preparation, interlobular arteries and superficial afferent and efferent arterioles are dissected and mounted on micropipettes. Rabbits are sacrificed and the kidney is removed. The kidney is sliced along the corticomedullary axis and immediately placed in chilled artificial bath solution. A single microvessel is dissected using a sharpened forceps and transferred to a temperature-controlled chamber mounted on the stage of an inverted microscope. The vessel is viewed with a video camera fitted to the inverted microscope. Vessel lumen diameter is read directly off the video monitor. One end of the vessel is cannulated with concentric glass micropipettes. After the lumen is perfused to expel any residual erythrocytes, the other end of the vessel is occluded by sucking it into the tip of a constriction pipette. Intraluminal pressure is set with a syringe connected to the back of the perfusion pipette. Pressure is measured with a manometer or pressure transducer connected in series with the perfusion pipette. Intraluminal pressure is set at 90 mm Hg for intralobular arteries, 70 mm Hg for afferent arterioles and 20 mm Hg for efferent arterioles. After the pressure is set, the chamber temperature is gradually increased to 37^{o}C and the vessel is allowed to equilibrate for 30-45 min before an experiment is started. Some but not all afferent and efferent arterioles develop a degree of spontaneous tone as evidenced by a sustained decrease in lumen diameter upon warming the bath from 20 to 37^{o}C.

Model

The effect of acetylcholine, dopamine, and bradykinin on vascular tone has been examined in interlobular arteries and superficial afferent and efferent arterioles isolated from rabbit kidney [255]. Acetylcholine caused a dose-dependent relaxation of norepinephrine-induced tone in all three vessel types. Significant relaxation was observed with 10(-8) M acetylcholine and higher concentrations caused complete relaxation. In afferent and efferent arterioles dopamine caused a dose-dependent relaxation that was indistinguishable

Table 7. Hydronephrotic kidney: Effects of endogenous effectors.

Agent	Effect	Reference
Angiotensin II	Efferent arteriolar vasoconstriction. Blocked by saralasin	[302]
Dopamine	Afferent+efferent arteriolar vasodilation Dependence on balance of constrictors and dilators	[303]
Atrial natriuretic peptide	Reverses afferent arteriolar vasoconstriction peptide Potentiates efferent arteriolar vasoconstriction	[304, 305]
Perfusion pressure	Afferent arteriole constricts. Hypertension shifts response	[306]
Sex differences	Efferent arteriole unchanged Autoregulatory response is modified by prostaglandins especially in females	[307]
Endothelin	Decreases glomerular blood flow, dose-dependent Constricts afferent and efferent arterioles Constriction not affected by calcium channel blocker	[308, 309]
Adenosine	Needs a functioning angiotensin II receptor system for its vasoconstrictor action	[310]
Renal nerve stimulation	Afferent + efferent arteriolar constriction	[311]
Chloride channels	Ang II and NE induced afferent arteriolar Activation of voltage-dependent calcium channels	[312]
Insulin	Reverses norepinephrine/angiotensin II vasoconstriction Effect mediated by nitric oxide	[313]
Prostaglandin E2	Vasodilatory and vasoconstrictor-afferent arteriole	[314]
Nitric oxide	Modulates myogenic vasoconstriction of afferent arteriole in spontaneously hypertensive rats	[315]

Table 8. Hydronephrotic kidney: Effects of drugs.

Drug	Effect	Reference
Cyclosporine	Interlobular, afferent and efferent arteriolar constriction Vasoconstriction due to altered NO metabolism	[316] [317]
Calcium antagonist (aranidipine)	Dilates both afferent and efferent arterioles during norepinephrine-induced constriction	[318]
Lovastatin	Vasodilatory in partial nephrectomy model	[319]
Anti-oxidant	Prevents cyclosporine induced vasoconstriction	[320]
(Lazaroids)	Improves renal blood flow during sepsis	[321]
Cobra venom	Massive constriction of the interlobar and arcuate arteries Complement activation	[322]
Angiotensin II Receptor antagonist	Dilates afferent and efferent arteriole in hypertensive rats	[323]

from the one caused by acetylcholine. Dopamine was much less effective on interlobular arteries. In afferent arterioles atropine blocked the effect of acetylcholine, and metoclopramide selectively inhibited dopamine-induced relaxation. Bradykinin caused a dose-dependent relaxation of norepinephrine- induced tone only in efferent arterioles. Bradykinin, either in the bath or lumen, had no effect on the preglomerular microvessels. Acetylcholine and dopamine also caused relaxation of afferent arterioles with spontaneous tone while all three vasodilators relaxed efferent arterioles with spontaneous tone. This study demonstrates segmental heterogeneity for these vasodilators in the rabbit renal microvasculature, with acetylcholine causing re-

laxation in all three vessel types, dopamine acting primarily on the glomerular arterioles, and bradykinin affecting only the efferent arteriole.

The isolated renal vessel technique has been modified so that vessels can be isolated from rats instead of rabbits [256]. The preparation has also been modified for fluorescence measurements of cytosolic calcium in the smooth muscle cell layer [257]. Measurements of changes in smooth muscle calcium can be carried out simultaneously with determinations of changes in lumen diameters. The effects of various agonists and pressure stimulation in the presence and absence of pharmacologic agents can be determined. Angiotensin II-induced changes in lumen diameter and smooth muscle cell cytosolic calcium have been determined [236]. A maximal constricting concentration of angiotensin II caused abrupt and sustained increases in smooth muscle cell cytosolic calcium in afferent and efferent arterioles. When lumen pressure was reduced to zero, angiotensin II caused abrupt peak increases in smooth muscle cell cytosolic calcium in both afferent and efferent arterioles which declined rapidly thereafter – patterns distinctly different from pressurized vessels. With the calcium channel blocker, diltiazem in the bathing media, angiotensin II caused an abrupt rise and decline in smooth muscle cell cytosolic calcium in afferent arterioles, but a sustained elevation in efferent arterioles. This study demonstrates that maximal angiotensin II stimulates both Ca^{2+} entry and storage mobilization in afferent and efferent arterioles and that lumen pressure modifies the angiotensin II smooth muscle cell cytosolic calcium response profiles. Angiotensin II activates differing Ca^{2+} influx mechanisms in pre- and postglomerular arterioles [258]. In the afferent arteriole, Angiotensin II activates dihydropyridine-sensitive L-type Ca^{2+} channels, presumably by membrane depolarization. In the efferent arteriole, angiotensin II appears to stimulate Ca^{2+} entry via store-operated Ca^{2+} influx. Recent studies indicated that cyclic ADP-ribose (cADPR) serves as a second messenger for intracellular Ca^{2+} mobilization in a variety of mammalian cells including the renal vasculature [259]. Collectively, these studies demonstrate that the EP(4) receptor is the major receptor in preglomerular VSMC. E-prostanoid 4 receptors mediate prostaglandin E2-induced vasodilation in the rat kidney and signal through G proteins to stimulate cAMP and inhibit cytosolic calcium concentration [260].

Advantages

The advantages of the isolated vessel technique in defining microvascular physiologic and pathophysiologic mechanisms are: 1) The vessels are studied in the absence of a neurohumoral and parenchymal tissue environment, 2) it allows for direct assessment of vascular responses in defined segments, 3) transmural pressure is controlled, 4) hormones and drugs can be added to the bathing media or luminal perfusate, 5) intracellular ion concentrations can be measured by fluorescence microscopy and membrane potentials can be recorded with microelectrodes.

Limitations

The limitations of the preparation are: 1) autocoid production and vascular reactivity may be altered *in vitro*, 2) the absence of flow dynamics may alter endothelial cell function, 3) the small amount of tissue limits biochemical measurements, 4) isolated arterioles do not exhibit myogenic responses to changes in transmural pressure.

Studies

The isolated renal microvessel preparation demonstrates concentration-dependent sensitivity to a various constrictor and dilator substances. Early studies demonstrated the lack of an effect of atriopeptin II on rabbit arterioles [261]. In another study in rat arterioles [262], atriopeptin III dilated preconstricted afferent arterioles but constricted efferent arterioles that were either not pretreated or that were preconstricted with other agonists. The effect of atriopeptin III on preconstricted afferent arterioles did not require vasodilator prostaglandin mediation. The constrictor effect of atriopeptin III on efferent arterioles was not dependent on angiotensin, thromboxane, or α- adrenergic mediation.

Postsynaptic α-adrenoceptors have been characterized in afferent and efferent arterioles isolated from rabbit renal cortex [263]. In both the afferent and efferent arteriole selective α 1-adrenoceptor agonists produced concentration-dependent vasoconstrictor responses with the maximum responses being equal to that of norepinephrine. Selective α 2-receptor agonists had less of an effect. The α 1-receptor antagonist, prazosin, produced a rightward shift in the concentration-response curve to norepinephrine, while the selective α 2-receptor antagonist, rauwolscine had no

effect on norepinephrine-mediated vasoconstriction. This study confirms the presence of α-adrenoceptors, exclusively of the α 1-subtype, on the glomerular arterioles that mediated vasoconstriction.

The influence of arachidonate cyclooxygenase products on endothelin-evoked renal vasoconstriction has been assessed [264]. In microperfused rat afferent and efferent arterioles, indomethacin had no effects on the maximal contraction by endothelin, but reduced the duration of endothelin-induced constriction in both arterioles. Endothelin infusion to rats *in vivo* resulted in a selective increase in efferent but not afferent arteriolar resistance, leading to a dramatic increase in transcapillary hydraulic pressure difference. Glomerular filtration rate, which fell progressively during infusion of endothelin alone, was markedly preserved by cyclooxygenase inhibition, but not during selective thromboxane A2 antagonism. This study provides evidence that locally released cyclooxygenase products, play a key role in sustaining endothelin-induced renal arteriolar constriction.

The role of endothelin in mediating cyclosporine A related renal vasoconstriction has been studied [265]. Both the afferent and efferent arteriole exhibited concentration-dependent decreases in lumen diameter to increasing molar concentrations of cyclosporine A. The afferent arteriole was more sensitive to the vasoconstrictive effects of cyclosporine A than the efferent arteriole. These data suggest that cyclosporine A directly constricts renal microvessels and that this effect is mediated by endothelin in the afferent arteriole but not the efferent arteriole.

Angiotensin II receptors [266], endothelin receptors [267] and vasopressin V1a receptor (V1aR) and V2 receptor (V2R) receptors [268] have been isolated in rat arterioles. In isolated rabbit arterioles, the vasoconstrictor response of angiotensin II is counteracted by vasodilatory prostaglandins and nitric oxide [269]. The calcium response to angiotensin II in the isolated rabbit afferent arteriole shows tachyphylaxis [270]. This tachyphylaxis cannot be reversed by applying increasing doses of angiotensin II, protein kinase C does not seem to be involved in the tachyphylactic phenomenon and nifedipine and NO reduced the tachyphylaxis. In another study, the afferent arteriole had a higher sensitivity to luminal than interstitial angiotensin II in superficial but not juxtamedullary nephrons [271]. In this study, it was concluded that such heterogeneities in angiotensin II action may be important in the control of glomerular hemodynamics under various physiological and pathological conditions.

Hydroxyeicosatetraenoic acid (HETE) release in response to angiotensin II from preglomerular microvessels in rats has recently been demonstrated [272] indicating that an angiotensin II-phospholipase C effector unit is associated with synthesis of the vasoconstrictor product, 20-HETE, in these vessels. Angiotensin II directly downregulates the expression of G proteins in young spontaneously hypertensive rats but not in young control rat renal microvessels indicating that the diversity in its effect on G-protein expression may be important for enhanced renal sensitivity to angiotensin II in spontaneously hypertensive rats [273]. Cytochrome P450 hydroxylase and cyclooxygenase arachidonic acid metabolites contribute importantly to the afferent arteriolar diameter and renal microvascular smooth muscle cell calcium responses elicited by endothelin-1 [274].

Arginine vasopressin (AVP) is a potent vasoconstrictor that preferentially reduces renal medullary blood flow through the stimulation of the vasopressin V1a receptor (V1aR). Studies have also shown that the vasopressin V2 receptor (V2R) may modulate AVP-mediated vasoconstriction. The transcriptional and translational sites of the V1aR and V2R in microdissected intrarenal vascular segments from both the cortex and medulla was studied [268]. The results indicate that V1aR mRNA and proteins are present in the isolated cortical or medullary vasculature, but the V2R mRNA and proteins were not found. This study suggests that the vasoconstrictor action of AVP within the renal medulla is mediated through the V1aR and that the modulatory V2R- mediated vasodilation is probably through the release of paracrine hormones found within the renal interstitial or tubular cells.

Bradykinin plays an important role in the regulation of renal hemodynamics. The effects of bradykinin on isolated perfused rabbit afferent arterioles and the mechanisms of action of bradykinin was studied [275]. It was concluded that 1) bradykinin has a biphasic effect on afferent arterioles; 2) both dilation and constriction may be mediated by bradykinin B2 receptors and 3) the mechanisms of vasodilation and vasoconstriction are due to cyclooxygenase products, not nitric oxide.

Isolated perfused juxtaglomerular apparatus

Tubuloglomerular feedback, which operates between the tubule and the parent glomerulus, is important to renal autoregulation and homeostasis of body fluid and electrolytes. The juxtaglomerular apparatus (JGA) which has a close anatomical relationship between the specialized tubular cells of the macula densa and the afferent and efferent arterioles, is the anatomical site of tubuloglomerular feedback. To study the function of the JGA directly, an *in vitro* preparation in which both the afferent arteriole and macula densa (MD) of a microdissected rabbit JGA are microperfused simultaneously, has recently been developed [276]. This preparation has the advantage of allowing control of both pressure in the afferent arteriole and luminal fluid concentration at the macula densa. Real time images of the afferent arteriole, including luminal diameter, can be obtained. Increasing the NaCl concentration of the macula densa perfusate constricts the afferent arteriole in the segment close to the glomerulus. This constriction is blocked by furosemide, a loop diuretic known to inhibit tubuloglomerular feedback. Microperfusion of the afferent arteriole alone showed that they constrict significantly when intraluminal pressure is elevated, the so-called myogenic response. The myogenic response is the first to respond to changes in perfusion pressure. The anatomical relationship between the myogenic response and tubuloglomerular feedback may enable the kidney to achieve its extremely efficient autoregulation. The preparation will provide important insights into the mechanism by which the macula densa controls glomerular hemodynamics. In this regard, the modulatory role of the macula densa NO pathways in tubuloglomerular feedback has been demonstrated [277]. Advantages of the preparation include [276] the following: 1) the hemodynamic influence of the larger interlobular artery can be excluded, 2) preparations from different nephron populations can be studied and 3) hormonal manipulations can easily be performed. A disadvantage of the preparation is its technical difficulty [278].

References

1. Racusen LC: Renal histopathology and urine cytology and cytopathology in acute renal failure., chap. 9, in Acute renal failure. New concepts and therapeutic strategies, edited by Goligorsky MS, Stein JH, New York, Churchill Livingstone, 1995, p 194
2. Edelstein CL, Ling H, Schrier RW: The nature of renal cell injury. Kidney Int 51:1341-1351, 1997
3. Iwata M, Myerson D, Torok-Storb B, Zager RA: An evaluation of renal tubular DNA laddering in response to oxygen deprivation and oxidant injury. J.Am.Soc.Nephrol. 5:1307-1313, 1994
4. Nogae S, Miyazaki M, Kobayashi N, Saito T, Abe K, Saito H, Nakane PK, Nakanishi Y, Koji T: Induction of apoptosis in ischemia-reperfusion model of mouse kidney: possible involvement of Fas. J.Am.Soc.Nephrol. 9:620-631, 1998
5. Schumer M, Colombel MC, Sawczuk IS, Gobe G, Conner J, O'Toole KT, Olsson CA, Wise GJ, Buttyan R: Morphologic, biochemical and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. Am.J.Path. 140:831-838, 1992
6. Basile DP, Liapis H, Hammerman MR: Expression of bcl-2 and bax in regenerating rat tubules following ischemic injury. Am.J.Physiol. 272:640-647, 1997
7. Better OS: Josep Trueta (1897-1977): military surgeon and pioneer investigator of acute renal failure. AM.J.Nephrol. 19:343-345, 1999
8. Netea MG, Fantuzzi G, Kullberg BJ, Stuyt RJ, Pulido EJ, McIntyre RC, Jr., Joosten LA, Van der Meer JW, Dinarello CA: Neutralization of IL-18 reduces neutrophil tissue accumulation and protects mice against lethal Escherichia coli and Salmonella typhimurium endotoxemia. J.Immunol. 164:2644-2649, 2000
9. Sakao Y, Takeda K, Tsutsui H, Kaisho T, Nomura F, Okamura H, Nakanishi K, Akira S: IL-18-deficient mice are resistant to endotoxin-induced liver injury but highly susceptible to endotoxin shock. Int Immunol. 11:471-480, 1999
10. Siegmund B, Rieder F, Lehr HA, Eigler A, Endres S, Dinarello CA: Neutralization of IL-18 exerts anti-inflammatory activity in experimental colitis in mice. Eur Cytokine Netw 11: 2000
11. Pizarro T. Differential role of IL-18 in acute versus chronic experimental colitis. 2001. Conference proceeding: Immunogenetic mechanisms of intestinal inflammation: role of cytokines and chemokines. Univ. of Virginia Health System.
12. Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, Fowler KJ, Basu S, Zhan YF, Dunn AR: Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 84:1737-1746, 1994

13. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall JA, Maher DW, Cebon J, Sinickas V, Dunn AR: Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. Proc.Natl.Acad.Sci.U.S.A 91:5592-5596, 1994
14. Burne MJ, Haq M, Matsuse H, Mohapatra S, Rabb H: Genetic susceptibility to renal ischemia reperfusion injury revealed in a murine model. Transplantation 69:1023-1025, 2000
15. Markel P, Shu P, Ebeling C, Carlson GA, Nagle DL, Smutko JS, Moore KJ: Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. Nat Genet 17:280-284, 1997
16. Wakeland E, Morel L, Achey K, Yui M, Longmate J: Speed congenics: a classic technique in the fast lane (relatively speaking). Immunol Today 18:472-477, 1997
17. Kwon Moo Park, Bonventre JV: Castration protects male mice against ischemic acute renal failure. A critical role for testosterone and leukocyte trapping in the outer medulla. (abstract) J Am Soc Nephrol. 11:594A, 2000
18. Conger JD, Robinette JB, Hammond WS: Differences in vascular reactivity in models of ischemic acute renal failure. Kidney Int. 39:1087-1097, 1991
19. Malis CD, Cheung JY, Leaf A, Bonventre JV: Effects of verapamil in models of ischemic acute renal failure in the rat. Am.J.Physiol. 245:F735-F742, 1983
20. Burke TJ, Arnold PE, Gordon JA, Bulger RE, Dobyan DC, Schrier RW: Protective effect of intrarenal calcium membrane blockers before or after renal ischemia. Functional, morphological, and mitochondrial studies. J Clin Invest 74:1830-1841, 1984
21. Fried TA, Hishida A, Barnes JL, Stein JH: Ischemic acute renal failure in the rat: protective effect of uninephrectomy. Am.J.Physiol 247:F568-F574, 1984
22. Nakajima T, Miyaji T, Kato A, Ikegaya N, Yamamoto T, Hishida A: Uninephrectomy reduces apoptotic cell death and enhances renal tubular cell regeneration in ischemic ARF in rats. Am J Physiol 271:F846-F853, 1996
23. Tsukamoto T, Nigam SK: Tight junction proteins form large complexes and associate with the cytoskeleton in an ATP depletion model for reversible junction assembly. J Biol.Chem. 272:16133-16139, 1997
24. Donohoe JF, Venkatachalam MA, Bernard DB, Levinsky NG: Tubular leakage and obstruction after renal ischemia: structural-functional correlations. Kidney Int. 13:208-222, 1978
25. Thadhani R, Pascual M, Bonventre JV: Medical progress-acute renal failure. N Engl J Med 334:1448-1460, 1996
26. Shanley PF, Rosen MD, Brezis M, Silva P, Epstein FH, Rosen S: Topography of focal proximal tubular necrosis after ischemia with reflow in the rat kidney. Am.J.Pathol. 122:462-468, 1986
27. Ysebaert DK, De Greef KE, Vercauteren SR, Ghielli M, Verpooten GA, Eyskens EJ, De Broe ME: Identification and kinetics of leukocytes after severe ischemia/reperfusion renal injury. Nephrol.Dial.Transplant. 15:1562-1574, 2000
28. Jones DB: Ultrastructure of human acute renal failure. Lab Invest 46:254-264, 1982
29. Olsen TS, Hansen HE: Ultrastructure of medullary tubules in ischemic acute tubular necrosis and acute interstitial nephritis in man. APMIS 98:1139-1148, 1990
30. Lieberthal W, Levine JS: Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. Am.J.Physiol 271:F477-F488, 1996
31. Daemen MARC, Van t'Veer C, Denecker G, Heemskerk VH, Wolfs TGAM, Clauss M, Vandenabeele P, Buurman W: Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. J.Clin.Invest. 104:541-549, 1999
32. Edelstein CL: Editorial Comment: Calcium-mediated proximal tubular injury-what is the role of cysteine proteases? Nephrol.Dial.Transplant. 15:141-144, 2000
33. Lieberthal W, Koh JS, Levine JS: Necrosis and apoptosis in acute renal failure. Semin Nephrol 18:505-518, 1998
34. Lieberthal W: Biology of acute renal failure: Therapeutic implications. Kidney Int 52:1102-1115, 1997
35. Lau AH: Apoptosis induced by cisplatin nephrotoxic injury. Kidney Int 56:1295-1298, 1999
36. Kaushal GP, Singh AB, Shah SV: Identification of gene family of caspases in rat kidney and altered expression in ischemia reperfusion injury. Am.J.Physiol. 274:F587-F595, 1998
37. Shimizu A, Yamanaka N: Apoptosis and cell desquamation in repair process of ischemic tubular necrosis. Virchows Arch.B Cell Pathol.Incl.Mol.Pathol. 64:171-180, 1993
38. Gobe G, Zhang XJ, Willgoss DA, Schoch E, Hogg NA, Endre ZH: Relationship between expression of Bcl-2 genes and growth factors in ischemic acute renal failure in the rat. J Am Soc Nephrol. 11:454-467, 2000
39. Molitoris BA, Geerdes A, McIntosh JR: Dissociation and redistribution of Na^+, K^+ -ATPase from its surface membrane cytoskeletal complex during cellular ATP depletion. J Clin Invest 88:462-469, 1991
40. Van Why SK, Mann AS, Ardito T, Siegel NJ, Kashgarian M: Expression and molecular regulation of NaK-ATPase after renal ischemia. Am.J.Physiol. 267:F75-F85, 1994
41. Molitoris BA: Ischemia-induced loss of epithelial polarity: potential role of the actin cytoskeleton [editorial]. Am J Physiol 260:F769-F778, 1991
42. Molitoris BA, Wilson PD, Schrier RW, Simon FR: Ischemia induces partial loss of surface membrane polarity and accumulation of putative calcium ionophores. J.Clin.Invest 76:2097-2105, 1985

43. Molitoris BA: New insights into the cell biology of ischemic acute renal failure. J Am Soc Nephrol 1:1263-1270, 1991
44. Alejandro VSJ, Nelson WJ, Huie P, Sibley RK, Dafoe D, Kuo P, Scandling JDJr, Myers BD: Postischemic injury,delayed function and NaK-ATPase distribution in the transplanted kidney. Kidney Int. 48:1308-1315, 1995
45. Racusen LC, Fivush BA, Ll YL, Slatnic I, Solez K: Dissociation of tubular cell detachment and tubular cell death in clinical and experimental "acute renal failure". Lab.Invest. 64:546-556, 1991
46. Harlan JM: Neutrophil-mediated vascular injury. Acta Med.Scand.Suppl 715:123-129, 1987
47. Weiss SJ: Tissue destruction by neutrophils. N.Engl.J.Med. 320:365-376, 1989
48. Horl WH, Schafer RM, Horl M, Heidland A: Neutrophil activation in acute renal failure and sepsis. Arch.Surg. 125:651-654, 1990
49. Jennette JC, Falk RJ: Acute renal failure secondary to leukocyte-mediated acute glomerular injury. Ren Fail. 14:395-399, 1992
50. Lauriat S, Linas SL: The role of neutrophils in acute renal failure. Semin.Nephrol. 18:498-504, 1998
51. Melnikov VY, Faubel S, Siegmund B, Lucia MS, Ljubanovic D, Edelstein CL. Neutrophil-independent mechanisms of caspase-1 and interleukin-18-mediated ischemic acute tubular necrosis in mice. J. Clin. Invest. 110, 1083-1091, 2002.
52. Ronco C, Zanella M, Brendolan A, Milan M, Canato G, Zamperetti N, Bellomo R: Management of severe acute renal failure in critically ill patients: an international survey in 345 centres. Nephrol.Dial.Transplant. 16:230-237, 2001
53. Brivet FG, Kleinknecht DJ, Loirat P, Landais PJ: Acute renal failure in intensive care units—causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. French Study Group on Acute Renal Failure. Crit Care Med. 24:192-198, 1996
54. Liano F, Junco E, Pascual J, Madero R, Verde E: The spectrum of acute renal failure in the intensive care unit compared with that seen in other settings. The Madrid Acute Renal Failure Study Group. Kidney Int Suppl 66:S16-S24, 1998
55. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP: The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. JAMA 273:117-123, 1995
56. Knotek M, Esson M, Gengaro P, Edelstein CL, Schrier RW: Desensitization of soluble guanylate cyclase in renal cortex during endotoxemia in mice. J.Am.Soc.Nephrol. 11:2133-2137, 2000
57. Remick DG, Newcomb DE, Bolgos GL, Call DR: Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs. cecal ligation and puncture. Shock 13:110-116, 2000
58. Thijs A, Thijs LG: Pathogenesis of renal failure in sepsis. Kidney Int.Suppl 66:S34-S37, 1998
59. Ortiz-Arduan A, Danoff TM, Kalluri R, Gonzalez-Cuadrado S, Karp SL, Elkon K, Egido J, Neilson EG: Regulation of Fas and Fas ligand expression in cultured murine renal cells and in the kidney during endotoxemia. Am.J.Physiol 271:F1193-F1201, 1996
60. Richman AV, Gerber LI, Balis JU: Peritubular capillaries. A major target site of endotoxin-induced vascular injury in the primate kidney. Lab Invest 43:327-332, 1980
61. Hickey MJ, Sharkey KA, Sihota EG, Reinhardt PH, MacMicking JD, Nathan C, Kubes P: Inducible nitric oxide synthase-deficient mice have enhanced leukocyte- endothelium interactions in endotoxemia. FASEB J. 11:955-964, 1997
62. Schrier RW, Abraham WT: Hormones and hemodynamics in heart failure. N.Engl.J.Med. 341:577-585, 1999
63. Schrier RW, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodes J: Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. Hepatology 8:1151-1157, 1988
64. Schrier RW: Pathogenesis of sodium and water retention in high-output and low- output cardiac failure, nephrotic syndrome, cirrhosis, and pregnancy (2). N.Engl.J.Med. 319:1127-1134, 1988
65. Khan RZ, Badr KF: Endotoxin and renal function: perspectives to the understanding of septic acute renal failure and toxic shock. Nephrol.Dial.Transplant. 14:814-818, 1999
66. Leach M, Frank S, Olbrich A, Pfeilschifter J, Thiemermann C: Decline in the expression of copper/zinc superoxide dismutase in the kidney of rats with endotoxic shock: effects of the superoxide anion radical scavenger, tempol, on organ injury. Br.J.Pharmacol. 125:817-825, 1998
67. Zacharowski K, Olbrich A, Cuzzocrea S, Foster SJ, Thiemermann C: Membrane-permeable radical scavenger, tempol, reduces multiple organ injury in a rodent model of gram-positive shock. Crit Care Med. 28:1953-1961, 2000
68. Zhang C, Walker LM, Mayeux PR: Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. Biochem.Pharmacol. 59:203-209, 2000
69. O'Donnel P, Burne M, Nemoto T, Keane WF, RabbH: Direct measurement of renal function in mice with ischemia-reperfusion injury; comparison of inulin clearance with serum creatinine. (abstract) J Am Soc Nephrol 11:606A, 2001
70. Chiao H, Kohda Y, McLeroy P, Craig L, Linas S, Star RA: A-melanocyte-stimulating hormone inhibits renal injury in the absence of neutrophils. Kidney Int 54:765-774, 1998
71. Lorenz JN, Gruenstein E: A simple, nonradioactive method for evaluating single-nephron filtration rate using FITC-inulin. Am.J.Physiol 276:F172-F177, 1999
72. Gulati S, Ainol L, Orak J, Singh AK, Singh I: Alterations of peroxisomal function in ischemia-reperfusion injury of rat kidney. Biochim.Biophys.Acta 1182:291-298, 1993

73. Gulati S, Singh AK, Irazu C, Orak J, Rajagopalan PR, Fitts CT, Singh I: Ischemia-reperfusion injury: biochemical alterations in peroxisomes of rat kidney. Arch.Biochem.Biophys. 295:90-100, 1992
74. Venkatachalam MA, Bernard DB, Donohoe JF, Levinsky NG: Ischemic damage and repair in the rat proximal tubule: differences among the S1, S2, and S3 segments. Kidney Int. 14:31-49, 1978
75. Lieberthal W, Nigam SK: Acute renal failure. Relative importance of proximal vs. distal tubular injury. Am.J.Physiol. 275:F623-F632, 1998
76. Bagnasco S, Good D, Balaban R, Burg M: Lactate production in isolated segments of the rat nephron. Am.J.Physiol 248:F522-F526, 1985
77. Witzgall R, Brown D, Schwarz C, Bonventre JV: Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. J Clin.Invest 93:2175-2188, 1994
78. Safirstein R: Gene expression in nephrotoxic and ischemic acute renal failure [editorial]. J Am Soc Nephrol. 4:1387-1395, 1994
79. Megyesi J, Di Mari J, Udvarhelyi N, Price PM, Safirstein R: DNA synthesis is dissociated from the immediate-early gene response in the post-ischemic kidney. Kidney Int 48:1451-1458, 1995
80. Safirstein R, Price PM, Saggi SJ, Harris RC: Changes in gene expression after temporary renal ischemia. Kidney Int 37:1515-1521, 1990
81. Dimari J, Megyesi J, Udvarhelyi N, Price P, Davis R, Safirstein R: N-acetyl cysteine ameliorates ischemic renal failure. Am.J.Physiol 272:F292-F298, 1997
82. Pombo CM, Bonventre JV, Avruch J, Woodgett JR, Kyriakis JM, Force T: The stress-activated protein kinases are major c-Jun amino-terminal kinases activated by ischemia and reperfusion. J Biol.Chem. 269:26546-26551, 1994
83. di Mari JF, Davis R, Safirstein RL: MAPK activation determines renal epithelial cell survival during oxidative injury. Am.J.Physiol 277:F195-F203, 1999
84. Weiss C, Passow H, Rothstein A: Autoregulation of flow in isolated rat kidney in the absence of red cells. Am J Physiol 196:1115-1118, 1959
85. Nishiitsutsuji-Uwo JM, Ross BD, Krebs HA: Metabolic activities of the isolated perfused rat kidney. Biochem J 103:852-62, 1967
86. Ross BD, Epstein FH, Leaf A: Sodium reabsorption in the perfused rat kidney. Am J Physiol 225:1165-1171, 1973
87. Epstein FH, Brosnan JT, Tange JD, Ross BD: Improved function with amino acids in the isolated perfused kidney. Am J Physiol 243:284-92, 1982
88. Baines AD, Shaikh N, Ho P: Mechanisms of perfused kidney cytoprotection by alanine and glycine. Am.J..Physiol. 259:F80-F87, 1990
89. Schurek HJ, Kriz W: Morphologic and functional evidence for oxygen deficiency in the isolated perfused rat kidney. Lab Invest 53:145-155, 1985
90. Lieberthal W, Stephens GW, Wolf EF, Rennke HG, Vasilevsky ML, Valeri CR, Levinsky NG: Effect of erythrocytes on the function and morphology of the isolated perfused rat kidney. Ren Physiol 10:14-24, 1987
91. Endre ZH, Ratcliffe PJ, Tange JD, Ferguson DJ, Radda GK, Ledingham JG: Erythrocytes alter the pattern of renal hypoxic injury: predominance of proximal tubular injury with moderate hypoxia. Clin.Sci. 76:19-29, 1989
92. Ratcliffe PJ, Endre ZH, Scheinman SJ, Tange JD, Ledingham JG, Radda GK: 31P nuclear magnetic resonance study of steady-state adenosine 5'- triphosphate levels during graded hypoxia in the isolated perfused rat kidney. Clin.Sci. 74:437-448, 1988
93. Lieberthal W, Sheridan AM, Valeri CR: Protective effect of atrial natriuretic factor and mannitol following renal ischemia. Am J Physiol 258:1266-72, 1990
94. Cross M, Endre ZH, Stewart-Richardson P, Cowin GJ, Westhuyzen J, Duggleby RG, Fleming SJ: 23Na-NMR detects hypoxic injury in intact kidney: increases in sodium inhibited by DMSO and DMTU. Magn Reson.Med. 30:465-475, 1993
95. Cowin GJ, Crozier S, Endre ZH, Leditschke IA, Brereton IM: Cortical and medullary βine-GPC modulated by osmolality independently of oxygen in the intact kidney. Am J Physiol 277:F338-F346, 1999
96. Steinhausen M, Snoei H, Parekh N, Baker R, Johnson PC: Hydronephrosis: a new method to visualize vas afferens, efferens, and glomerular network. Kidney Int 23:794-806, 1983
97. Loutzenhiser R, Epstein M, Horton C: Inhibition by diltiazem of pressure-induced afferent vasoconstriction in the isolated perfused rat kidney. Am J Cardiol 59:72-75, 1987
98. Carmines PK, Morrison TK, Navar LG: Angiotensin II effects on microvascular diameters of *in vitro* blood-perfused juxtamedullary nephrons. Am J Physiol 251:610-618, 1986
99. Carmines PK, Navar LG: Disparate effects of Ca channel blockade on afferent and efferent arteriolar responses to angiotensin II. Am J Physiol 256:1015-1020, 1989
100. Moore LC, Casellas D: Tubuloglomerular feedback dependence of autoregulation in rat juxtamedullary afferent arterioles. Kidney Int 37:1402-1408, 1990

101. Ichihara A, Imig JD, Navar LG: Cyclooxygenase-2 modulates afferent arteriolar responses to increases in pressure. Hypertension 34:843-847, 1999
102. Schurek HJ, Alt JM: Effect of albumin on the function of perfused rat kidney. Am J Physiol 240:569-76, 1981
103. Bullivant M: Autoregulation of plasma flow in the isolated perfused rat kidney. J Physiol 280:141-53, 1978
104. Schurek HJ, Brecht JP, Lohfert H, Hierholzer K: The basic requirements for the function of the isolated cell free perfused rat kidney. Pflugers Arch 354:349-65, 1975
105. Lieberthal W, Vasilevsky ML, Valeri CR, Levinsky NG: Interactions between ADH and prostaglandins in isolated erythrocyte-perfused rat kidney. Am J Physiol 252:331-7, 1987
106. Silva P, Hallac R, Spokes K, Epstein FH: Relationship among gluconeogenesis, QO2, and Na^+ transport in the perfused rat kidney. Am J Physiol 11:508-513, 1982
107. Baumgartl H, Leichtweiss HP, Lubbers DW, Weiss C, Huland H: The oxygen supply of the dog kidney: measurements of intrarenal pO 2. Microvasc.Res. 4:247-257, 1972
108. Schurek HJ, Jost U, Baumgartl H, Bertram H, Heckmann U: Evidence for a preglomerular oxygen diffusion shunt in rat renal cortex. Am J Physiol 259:F910-F915, 1990
109. Brezis M, Heyman SN, Dinour D, Epstein FH, Rosen S: Role of nitric oxide in renal medullary oxygenation. Studies in isolated and intact rat kidneys. J Clin Invest 88:390-5, 1991
110. Heyman SN, Karmeli F, Rachmilewitz D, Haj-Yehia A, Brezis M: Intrarenal nitric oxide monitoring with a Clark-type electrode: potential pitfalls. Kidney Int 51:1619-23, 1997
111. Dowd T, Barac-Nieto M, Gupta RK, Spitzer A: 31P nuclear magnetic resonance and saturation transfer studies of the isolated perfused rat kidney. Ren Physiol Biochem 12:161-70, 1989
112. Gupta RK, Dowd TL, Spitzer A, Barac-Nieto M: 23Na, 19F, 35Cl and 31P multinuclear nuclear magnetic resonance studies of perfused rat kidney. Ren Physiol Biochem. 12:144-160, 1989
113. Barac-Nieto M, Gupta RK, Spitzer A: NMR studies of phosphate metabolism in the isolated perfused kidney of developing rats. Pediatr Nephrol 4:392-8, 1990
114. Barac-Nieto M, Dowd TL, Gupta RK, Spitzer A: Changes in NMR-visible kidney cell phosphate with age and diet: relationship to phosphate transport. Am J Physiol 261:153-62, 1991
115. Dowd TL, Gupta RK: Multinuclear NMR studies of intracellular cations in perfused hypertensive rat kidney. J.Biol.Chem. 267:3637-3643, 1992
116. Dowd TL, Gupta RK: NMR studies of the effect of hyperglycemia on intracellular cations in rat kidney. J Biol Chem 268:991-6, 1993
117. Dowd TL, Gupta RK: Multinuclear NMR studies of intracellular cations in the prehypertensive rat kidney. Biochim Biophys Acta 1226:83-8, 1994
118. Dowd TL, Gupta RK: NMR studies of the effect of Mg2+ on post-ischemic recovery of ATP and intracellular sodium in perfused kidney. Biochim Biophys Acta 1272:133-9, 1995
119. Endre ZH, Allis JL, Ratcliffe PJ, Radd GK: 87-rubidium NMR: a novel method of measuring cation flux in intact kidney. Kidney Int 35:1249-1256, 1989
120. Endre ZH, Solez K: Anatomical and functional imaging of transplant acute renal failure. Transplantation Review 9:147-158, 1995
121. Cowin GJ, Leditschke IA, Crozier S, Brereton IM, Endre ZH: Regional proton nuclear magnetic resonance spectroscopy differentiates cortex and medulla in the isolated perfused rat kidney. MAGMA 5:151-8, 1997
122. Endre ZH, Hull WE, Fichtner K-P, Werner U, Kriz W, Ritz E: Development of MR Microscopy for the functioning isolated perfused kidney, in Renal Magnetic Resonance: Experimental and Clinical Applications, edited by Endre ZH, New York, Marcel Dekker, 2002,
123. Endre ZH: Magnetic resonance imaging and spectroscopy in critical care nephrology, in Critical care nephrology, edited by C. R, Bellomo R, Dordrecht, Kluwer, 1998, pp 1517-1533
124. Alcorn D, Emslie KR, Ross BD, Ryan GB, Tange JD: Selective distal nephron damage during isolated kidney perfusion. Kidney Int 19:638-647, 1981
125. Brezis M, Rosen S, Silva P, Epstein FH: Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. J Clin.Invest 73:182-190, 1984
126. Lieberthal W, Wolf EF, Merrill EW, Levinsky NG, Valeri CR: Hemodynamic effects of different preparations of stroma free hemolysates in the isolated perfused rat kidney. Life Sci 41:2525-33, 1987
127. Lieberthal W, Vogel WM, Apstein CS, Valeri CR: Studies of the mechanism of the vasoconstrictor activity of stroma-free hemoglobin in the isolated perfused rat kidney and rabbit heart. Prog Clin Biol Res 319:407-20, 1989
128. Baines AD, Christoff B, Wicks D, Wiffen D, Pliura D: Cross-linked hemoglobin increases fractional reabsorption and GFR in hypoxic isolated perfused rat kidneys. Am J Physiol 269:628-36, 1995

129. Baines AD, Adamson G, Wojciechowski P, Pliura D, Ho P, Kluger R: Effect of modifying O2 diffusivity and delivery on glomerular and tubular function in hypoxic perfused kidney. Am J Physiol 274:744-52, 1998
130. Hayakawa H, Hirata Y, Suzuki E, Sugimoto T, Matsuoka H, Kikuchi K, Nagano T, Hirobe M: Mechanisms for altered endothelium-dependent vasorelaxation in isolated kidneys from experimental hypertensive rats. Am J Physiol 264:1535-41, 1993
131. Hirata Y, Hayakawa H, Kakoki M, Tojo A, Suzuki E, Nagata D, Kimura K, Goto A, Kikuchi K, Nagano T, Hirobe M, Omata M: Receptor subtype for vasopressin-induced release of nitric oxide from rat kidney. Hypertension 29:58-64, 1997
132. Hirata Y, Hayakawa H, Suzuki E, Omata M: Does endothelin work as an intrarenal mechanism to alter pressure natriuresis in spontaneously hypertensive rats? J Hypertens 12:251-7, 1994
133. Hirata Y, Hayakawa H, Suzuki Y, Suzuki E, Ikenouchi H, Kohmoto O, Kimura K, Kitamura K, Eto T, Kangawa K, et al.: Mechanisms of adrenomedullin-induced vasodilation in the rat kidney. Hypertension 25:790-5, 1995
134. Hayakawa H, Raij L: Relationship between hypercholesterolaemia, endothelial dysfunction and hypertension. J Hypertens 17:611-9, 1999
135. Kakoki M, Hirata Y, Hayakawa H, Tojo A, Nagata D, Suzuki E, Kimura K, Goto A, Kikuchi K, Nagano T, Omata M: Effects of hypertension, diabetes mellitus, and hypercholesterolemia on endothelin type B receptor-mediated nitric oxide release from rat kidney. Circulation 99:1242-8, 1999
136. Kakoki M, Hirata Y, Hayakawa H, Suzuki E, Nagata D, Tojo A, Nishimatsu H, Nakanishi N, Hattori Y, Kikuchi K, Nagano T, Omata M: Effects of tetrahydrobiopterin on endothelial dysfunction in rats with ischemic acute renal failure. J Am Soc Nephrol 11:301-9, 2000
137. Kakoki M, Hirata Y, Hayakawa H, Suzuki E, Nagata D, Nishimatsu H, Kimura K, Goto A, Omata M: Effects of vasodilatory antihypertensive agents on endothelial dysfunction in rats with ischemic acute renal failure. Hypertens Res 23:527-33, 2000
138. Galat JA, Robinson AV, Rhodes RS: A model of hypoxic renal failure. J Surg Res 44:764-71, 1988
139. Kadkhodaee M, Endre ZH, Towner RA, Cross M: Hydroxyl radical generation following ischemia-reperfusion in cell- free perfused rat kidney. Biochim.Biophys.Acta 1243:169-174, 1995
140. Kadkhodaee M, Hanson GR, Towner RA, Endre ZH: Detection of hydroxyl and carbon-centred radicals by EPR spectroscopy after ischemia and reperfusion of the rat kidney. Free Radic.Res. 25:31-42, 1996
141. Kadkhodaee M, Gobe G, Wilgoss DA, Endre ZH: DNA fragmentation reduced by anti-oxidants following ischemia-reperfusionin the isolated perfused rat kidney. Nephrology 4:163-175, 1998
142. Brezis M, Shanley P, Silva P, Spokes K, Lear S, Epstein FH, Rosen S: Disparate mechanisms for hypoxic cell injury in different nephron segments. Studies in the isolated perfused rat kidney. J Clin Invest 76:1796-806, 1985
143. Lieberthal W, Menza SA, Levine JS: Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. Am.J.Physiol. 274:F315-F327, 1998
144. Allis JL, Endre ZH, Radda GK: 87Rb, 23Na and 31P nuclear magnetic resonance spectroscopy of the perfused rat kidney. Ren Physiol Biochem. 12:171-180, 1989
145. Endre ZH, Cowin GJ, Stewart-Richardson P, Cross M, Willgoss DA, Duggleby RG: 23Na NMR detects protection by glycine and alanine against hypoxic injury in the isolated perfused rat kidney. Biochem.Biophys.Res.Commun. 202:1639-1644, 1994
146. Paller MS, Hoidal JR, Ferris TF: Oxygen free radicals in ischemic acute renal failure in the rat. J.Clin.Invest 74:1156-1162, 1984
147. Paller MS, Hedlund BE: Role of iron in postischemic renal injury in the rat. Kidney Int 34:474-480, 1988
148. Linas SL, Whittenburg D, Repine JE: Role of neutrophil derived oxidants and elastase in lipopolysaccharide- mediated renal injury. Kidney Int 39:618-623, 1991
149. Linas SL, Shanley PF, Whittenburg D, et al.: Neutrophils accentuate ischemia/reperfusion injury in isolated perfused rat kidneys. Am J Physiol 255:F725-F733, 1988
150. Ghielli M, Verstrepen W, Nouwen E, De Broe ME: Regeneration processes in the kidney after acute injury: role of infiltrating cells. Exp Nephrol 6:502-7, 1998
151. Ghielli M, Verstrepen WA, De Greef KE, Helbert MH, Ysebaert DK, Nouwen EJ, De Broe ME: Antibodies to both ICAM-1 and LFA-1 do not protect the kidney against toxic (HgCl2) injury. Kidney Int 58:1121-34, 2000
152. Paller MS, Neumann TV: Reactive oxygen species and rat renal epithelial cells during hypoxia and reoxygenation. Kidney Int 40:1041-1049, 1991
153. Brezis M, Rosen S, Stoff JS, Spokes K, Silva P, Epstein FH: Inhibition of prostaglandin synthesis in rat kidney perfused with and without erythrocytes: implication for analgesic nephropathy. Miner Electrolyte Metab 12:326-32, 1986
154. Brezis M, Rosen S: Hypoxia of the renal medulla—its implications for disease. N.Engl.J Med. 332:647-655, 1995
155. Heyman SN, Rosen S, Darmon D, Goldfarb M, Bitz H, Shina A, Brezis M: Endotoxin-induced renal failure. II. A role for tubular hypoxic damage. Exp Nephrol 8:275-82, 2000
156. Lieberthal W, Rennke HG, Sandock KM, Valeri CR, Levinsky NG: Ischemia in the isolated erythrocyte-perfused rat kidney. Protective effect of hypothermia. Ren Physiol Biochem 11:60-9, 1988
157. Endre ZH, Ratcliffe PJ: Patterns of ischaemic renal cell injury, in Acute Renal Failure, edited by Solez K, Racusen LC, New York, Marcel Dekker, 1990, pp 173-185

158. Mason J, Welsch J, Torhorst J: The contribution of vascular obstruction to the functional defect that follows renal ischemia. Kidney Int 31:65-71, 1987
159. Wolgast M, Bayati A, Hellberg O, Kallskog O, Nygren K: Osmotic diuretics and hemodilution in postischemic renal failure. Ren Fail 14:297-302, 1992
160. Beeri R, Symon Z, Brezis M, Ben-Sasson SA, Baehr PH, Rosen S, Zager RA: Rapid DNA fragmentation from hypoxia along the thick ascending limb of rat kidneys. Kidney Int. 47:1806-1810, 1995
161. Gobe G, Zhang XJ, Cuttle L, Pat B, Willgoss D, Hancock J, Barnard R, Endre RB: Bcl-2 genes and growth factors in the pathology of ischaemic acute renal failure. Immunol.Cell Biol. 77:279-286, 1999
162. Jaffe R, Ariel I, Beeri R, Paltiel O, Hiss Y, Rosen S, Brezis M: Frequent apoptosis in human kidneys after acute renal hypoperfusion. Exp Nephrol 5:399-403, 1997
163. Ueda N, Walker PD, Hsu S, Shah SV: Activation of a 15-kDa endonuclease in hypoxia/reoxygenation injury without morphologic features of apoptosis. Proc Natl Acad Sci USA 92:7202-7206, 1995
164. De Broe ME: Apoptosis in acute renal failure. Nephrol Dial Transplant 16:23-6, 2001
165. Gobe G, Willgoss D, Hogg N, Schoch E, Endre Z: Cell survival or death in renal tubular epithelium after ischemia- reperfusion injury. Kidney Int 56:1299-1304, 1999
166. Cuttle L, Zhang XJ, Endre ZH, Winterford C, Gobe GC: Bcl-X(L) translocation in renal tubular epithelial cells *in vitro* protects distal cells from oxidative stress. Kidney Int 59:1779-88, 2001
167. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479-489, 1997
168. Safirstein R: Renal stress response and acute renal failure. Adv.Ren Replace.Ther. 4:38-42, 1997
169. Ratcliffe PJ, Endre ZH, Nicholls LG, Tange JD, Ledingham JGG: The isolated perfused rat kidney: filtering and non-filtering models in the assessment of altered renal vascular resistance in nephrotoxicity, in Nephrotoxicity: Extrapolation from *in vitro* to *in vivo* and from animals to man., edited by Bach PH, Lock EA, New York, Plenum, 1989, pp 107-110
170. Mason J, Kain H, Welsch J, Schnermann J: The early phase of experimental acute renal failure. VI. The influence of furosemide. Pflugers Arch 392:125-33, 1981
171. Firth JD, Ratcliffe PJ, Raine AE, Ledingham JG: Endothelin: an important factor in acute renal failure? Lancet 2:1179-82, 1988
172. Firth JD, Ratcliffe PJ: Organ distribution of the three rat endothelin messenger RNAs and the effects of ischemia on renal gene expression. J Clin Invest 90:1023-31, 1992
173. Ritthaler T, Gopfert T, Firth JD, Ratcliffe PJ, Kramer BK, Kurtz A: Influence of hypoxia on hepatic and renal endothelin gene expression. Pflugers Arch 431:587-93, 1996
174. Kramer BK, Bucher M, Sandner P, Ittner KP, Riegger GA, Ritthaler T, Kurtz A: Effects of hypoxia on growth factor expression in the rat kidney *in vivo*. Kidney Int 51:444-7, 1997
175. Wilhelm SM, Simonson MS, Robinson AV, Stowe NT, Schulak JA: Endothelin up-regulation and localization following renal ischemia and reperfusion. Kidney Int 55:1011-8, 1999
176. Forbes JM, Hewitson TD, Becker GJ, Jones CL: Ischemic acute renal failure: long-term histology of cell and matrix changes in the rat. Kidney Int 57:2375-85, 2000
177. Forbes JM, Jandeleit-Dahm K, Allen TJ, Hewitson TD, Becker GJ, Jones CL: Endothelin and endothelin a/b receptors are increased after ischaemic acute renal failure. Exp Nephrol 9:309-16, 2001
178. Chan L, Chittinandana A, Shapiro JI, Shanley PF, Schrier RW: Effect of endothelin-receptor antagonist on ischemic acute renal failure. Am.J.Physiol. 266:F135-F138, 1994
179. Forbes JM, Hewitson TD, Becker GJ, Jones CL: Simultaneous blockade of endothelin A and B receptors in ischemic acute renal failure is detrimental to long-term kidney function. Kidney Int 59:1333-41, 2001
180. Lieberthal W, Wolf EF, Rennke HG, Valeri CR, Levinsky NG: Renal ischemia and reperfusion impair endothelium-dependent vascular relaxation. Am J Physiol 256:F894-F900, 1989
181. Allgren RL, Marbury TC, Rahman SN, Weisberg LS, Fenves AZ, Lafayette RA, Sweet RM, Genter FC, Kurnik BRC, Conger JD, Sayegh MH: Anaritide in acute tubular necrosis. N Engl J Med 336:828-834, 1997
182. Perico N, Dadan J, Remuzzi G: Endothelin mediates the renal vasoconstriction induced by cyclosporine in the rat. J Am Soc Nephrol 1:76-83, 1990
183. Bertelli A, Giovannini L, Palla R, Migliori M, Panichi V, Andreini B: Protective effect of L-propionylcarnitine on cyclosporine-induced nephrotoxicity. Drugs Exp Clin Res 21:221-8, 1995
184. Hirata Y, Hayakawa H, Suzuki E, Kimura K, Kikuchi K, Nagano T, Hirobe M, Omata M: Direct measurements of endothelium-derived nitric oxide release by stimulation of endothelin receptors in rat kidney and its alteration in salt-induced hypertension. Circulation 91:1229-35, 1995
185. Oldroyd S, Slee SJ, Haylor J, Morcos SK, Wilson C: Role for endothelin in the renal responses to radiocontrast media in the rat. Clin Sci (Colch) 87:427-34, 1994

186. Oldroyd SD, Haylor JL, Morcos SK: Bosentan, an orally active endothelin antagonist: effect on the renal response to contrast media. Radiology 196:661-5, 1995
187. Morcos SK, Oldroyd S, Haylor J: Effect of radiographic contrast media on endothelium derived nitric oxide-dependent renal vasodilatation. Br J Radiol 70:154-9, 1997
188. Wang A, Holcslaw T, Bashore TM, Freed MI, Miller D, Rudnick MR, Szerlip H, Thames MD, Davidson CJ, Shusterman N, Schwab SJ: Exacerbation of radiocontrast nephrotoxicity by endothelin receptor antagonism. Kidney Int 57:1675-80, 2000
189. Cantley LG, Spokes K, Clark B, McMahon EG, Carter J, Epstein FH: Role of endothelin and prostaglandins in radiocontrast-induced renal artery constriction. Kidney Int 44:1217-23, 1993
190. Erley CM, Duda SH, Schlepckow S, Koehler J, Huppert PE, Strohmaier WL, Bohle A, Risler T, Osswald H: Adenosine antagonist theophylline prevents the reduction of glomerular filtration rate after contrast media application. Kidney Int 45:1425-31, 1994
191. Katholi RE, Taylor GJ, McCann WP, Woods WT, Jr., Womack KA, McCoy CD, Katholi CR, Moses HW, Mishkel GJ, Lucore CL, et al.: Nephrotoxicity from contrast media: attenuation with theophylline. Radiology 195:17-22, 1995
192. Yoshioka T, Fogo A, Beckman JK: Reduced activity of antioxidant enzymes underlies contrast media- induced renal injury in volume depletion. Kidney Int 41:1008-15, 1992
193. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W: Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med 343:180-4, 2000
194. Endre ZH, Nicholls LG, Ratcliffe PJ, Ledingham JGG: Prevention and reversal of mercuric chloride-induced increase in renal vascular resistance by captopril, in Nephrotoxicity: Extrapolation from *in vitro* to *in vivo* and from animals to man., edited by Bach PH, Lock EA, New York, Plenum, 1989, pp 103-106
195. Rossi N, Churchill P, Ellis V, Amore B: Mechanism of adenosine receptor-induced renal vasoconstriction in rats. Am J Physiol 255:885-90, 1988
196. Rossi N, Ellis V, Kontry T, Gunther S, Churchill P, Bidani A: The role of adenosine in HgCl2-induced acute renal failure in rats. Am J Physiol 258:1554-60, 1990
197. Yanagisawa H, Nodera M, Kurihara N, Wada O: Altered expression of endothelin-1 and endothelial nitric oxide synthase in the juxtaglomerular apparatus of rats with HgCl2-induced acute renal failure. Toxicol Lett 98:181-8, 1998
198. Bhardwaj R, Moore PK: Endothelium-derived relaxing factor and the effects of acetylcholine and histamine on resistance blood vessels. Br J Pharmacol 95:835-43, 1988
199. Radermacher J, Forstermann U, Frolich JC: Endothelium-derived relaxing factor influences renal vascular resistance. Am J Physiol 259:9-17, 1990
200. Radermacher J, Klanke B, Kastner S, Haake G, Schurek HJ, Stolte HF, Frolich JC: Effect of arginine depletion on glomerular and tubular kidney function: studies in isolated perfused rat kidneys. Am J Physiol 261:779-86, 1991
201. Radermacher J, Klanke B, Schurek HJ, Stolte HF, Frolich JC: Importance of NO/EDRF for glomerular and tubular function: studies in the isolated perfused rat kidney. Kidney Int. 41:1549-1559, 1992
202. Garcia-Estan J, Atucha NM, Sabio JM, Vargas F, Quesada T, Romero JC: Increased endothelium-dependent renal vasodilation in cirrhotic rats. Am J Physiol 267:549-53, 1994
203. Garcia-Estan J, Atucha NM, Groszmann RJ: Renal response to methoxamine in portal hypertensive rats: role of prostaglandins and nitric oxide. J Hepatol 25:206-11, 1996
204. Vargas F, Sabio JM, Luna JD: Contribution of endothelium derived relaxing factors to acetylcholine induced vasodilatation in the rat kidney. Cardiovasc Res 28:1373-7, 1994
205. Vargas F, Osuna A, Fernandez-Rivas A: Vascular reactivity and flow-pressure curve in isolated kidneys from rats with N-nitro-L-arginine methyl ester-induced hypertension. J Hypertens 14:373-9, 1996
206. Rapacon M, Mieyal P, McGiff JC, Fulton D, Quilley J: Contribution of calcium-activated potassium channels to the vasodilator effect of bradykinin in the isolated, perfused kidney of the rat. Br J Pharmacol 118:1504-8, 1996
207. Bagate K, Grima M, Imbs JL, Jong WD, Helwig JJ, Barthelmebs M: Signal transduction pathways involved in kinin B(2) receptor-mediated vasodilation in the rat isolated perfused kidney. Br J Pharmacol 132:1735-42, 2001
208. Kaufmann MA, Castelli I, Pargger H, Drop LJ: Nitric oxide dose-response study in the isolated perfused rat kidney after inhibition of endothelium-derived relaxing factor synthesis: the role of serum albumin. J Pharmacol Exp Ther 273:855-62, 1995
209. Ziyyat A, Zhang BL, Benzoni D: Interactions between nitric oxide and prostanoids in isolated perfused kidneys of the rat. Br J Pharmacol 119:388-92, 1996
210. Boric MP, Bravo JA, Corbalan M, Vergara C, Roblero JS: Interactions between bradykinin and ANP in rat kidney *in vitro*: inhibition of natriuresis and modulation of medullary cyclic GMP. Biol Res 31:281-9, 1998
211. Rahimian R, Van Breemen C, Karkan D, Dube G, Laher I: Estrogen augments cyclopiazonic acid-mediated, endothelium-dependent vasodilation. Eur J Pharmacol 327:143-9, 1997

212. Muller C, Endlich K, Helwig JJ: AT2 antagonist-sensitive potentiation of angiotensin II-induced constriction by NO blockade and its dependence on endothelium and P450 eicosanoids in rat renal vasculature. Br J Pharmacol 124:946-52, 1998
213. Hayakawa H, Hirata Y, Kakoki M, Suzuki Y, Nishimatsu H, Nagata D, Suzuki E, Kikuchi K, Nagano T, Kangawa K, Matsuo H, Sugimoto T, Omata M: Role of nitric oxide-cGMP pathway in adrenomedullin-induced vasodilation in the rat. Hypertension 33:689-93, 1999
214. Miric G, Dallemagne C, Endre Z, Margolin S, Taylor SM, Brown L: Reversal of cardiac and renal fibrosis by pirfenidone and spironolactone in streptozotocin-diabetic rats. Br J Pharmacol 133:687-94, 2001
215. Raz E, Kaminski N, Brezis M: Effects of perfusion pressure and renal flow upon albumin excretion in isolated perfused kidneys [see comments]. Nephron 56:396-8, 1990
216. Lapinski R, Perico N, Remuzzi A, Sangalli F, Benigni A, Remuzzi G: Angiotensin II modulates glomerular capillary permselectivity in rat isolated perfused kidney. J Am Soc Nephrol 7:653-60, 1996
217. Perico N, Lapinski R, Konopka K, Aiello S, Noris M, Remuzzi G: Platelet-activating factor mediates angiotensin II-induced proteinuria in isolated perfused rat kidney. J Am Soc Nephrol 8:1391-8, 1997
218. Burne MJ, Adal Y, Cohen N, Panagiotopoulos S, Jerums G, Comper WD: Anomalous decrease in dextran sulfate clearance in the diabetic rat kidney. Am J Physiol 274:700-8, 1998
219. Eppel GA, Osicka TM, Pratt LM, Jablonski P, Howden BO, Glasgow EF, Comper WD: The return of glomerular-filtered albumin to the rat renal vein. Kidney Int 55:1861-70, 1999
220. Boyce NW, Holdsworth SR: Evidence for direct renal injury as a consequence of glomerular complement activation. J Immunol 136:2421-5, 1986
221. Fauler J, Wiemeyer A, Yoshizawa M, Schurek HJ, Frolich JC: Metabolism of cysteinyl leukotrienes by the isolated perfused rat kidney. Prostaglandins 42:239-49, 1991
222. Yared A, Albrightson-Winslow C, Griswold D, Takahashi K, Fogo A, Badr KF: Functional significance of leukotriene B4 in normal and glomerulonephritic kidneys. J Am Soc Nephrol 2:45-56, 1991
223. Jocks T, Zahner G, Helmchen U, Kneissler U, Stahl RA: Antibody and complement reduce renal hemodynamic function in isolated perfused rat kidney. Am J Physiol 270:179-85, 1996
224. Jocks T, Zahner G, Freudenberg J, Wolf G, Thaiss F, Helmchen U, Stahl RA: Prostaglandin E1 reduces the glomerular mRNA expression of monocyte- chemoattractant protein 1 in anti-thymocyte antibody-induced glomerular injury. J Am Soc Nephrol 7:897-905, 1996
225. Saunders JR, Aminian A, McRae JL, O'Farrell KA, Adam WR, Murphy BF: Clusterin depletion enhances immune glomerular injury in the isolated perfused kidney. Kidney Int 45:817-27, 1994
226. Gabbai FB, Peterson OW, Khang S, Wilson CB, Blantz RC: Glomerular hemodynamics in cell-free and erythrocyte-perfused isolated rat kidney. Am J Physiol 267:423-7, 1994
227. Edelstein CL, Schrier RW: ARF: Pathogenesis, diagnosis and management. In Schrier RW (ed), Renal and Electrolyte Disorders, 6 th Edition, Lippincott Williams and Wilkins, Philadelphia, 2003, pp 401-455
228. Kribben A, Edelstein CL, Schrier RW: Pathophysiology of acute renal failure. J.Nephrol. 12 Suppl 2:S142-S151, 1999
229. Edelstein CL, Ling H, Wangsiripaisan A, Schrier RW: Emerging therapies for acute renal failure. Am.J.Kid.Dis. 30:S89-S95, 1997
230. Lieberthal W, Nigam SK: Acute renal failure. II. Experimental models of acute renal failure: imperfect but indispensable. Am J Physiol Renal Physiol 278:F1-F12, 2000
231. Conger JD: Abnormal vascular function following ischemia-reperfusion injury. J.Invest.Med. 43:431-442, 1995
232. Harris RJ, Branston NM, Symon L, Bayhan M, Watson A: The effects of a calcium antagonist, nimodipine, upon physiological responses of the cerebral vasculature and its possible influence upon focal cerebral ischemia. Stroke 13:759-766, 1982
233. Bevan JA, Laher I: Pressure and flow-dependent vascular tone. FASEB J. 5:2267-2273, 1991
234. Tolins JP, Palmer RM, Moncada S, Raij L: Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. Am.J.Physiol 258:H655-H662, 1990
235. Pohl U, Holtz J, Busse R, Bassenge E: Crucial role of endothelium in the vasodilator response to increased flow *in vivo*. Hypertension 8:37-44, 1986
236. Cases A, Haas J, Burnett JC, Romero JC: Hemodynamic and renal effects of acute and progressive nitric oxide synthesis inhibition in anesthetized dogs. Am.J.Physiol Regul.Integr.Comp Physiol 280:R143-R148, 2001
237. Goetz KL, Wang BC, Madwed JB, Zhu JL, Leadley RJ, Jr.: Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. Am.J.Physiol 255:R1064-R1068, 1988
238. Blantz RC: Dynamics of glomerular ultrafiltration in the rat. Fed.Proc. 36:2602-2608, 1977
239. Casellas D, Navar LG: *In vitro* perfusion of juxtamedullary nephrons in rats. Am.J.Physiol 246:F349-F358, 1984
240. Casellas D, Carmines PK, Navar LG: Microvascular reactivity of *in vitro* blood perfused juxtamedullary nephrons from rats. Kidney Int 28:752-759, 1985

241. Ichihara A, Inscho EW, Imig JD, Navar LG: Neuronal nitric oxide synthase modulates rat renal microvascular function. Am.J.Physiol 274:F516-F524, 1998
242. Ichihara A, Hayashi M, Hirota N, Saruta T: Superoxide inhibits neuronal nitric oxide synthase influences on afferent arterioles in spontaneously hypertensive rats. Hypertension 37:630-634, 2001
243. Ichihara A, Navar LG: Neuronal NOS contributes to biphasic autoregulatory response during enhanced TGF activity. Am.J.Physiol 277:F113-F120, 1999
244. Ichihara A, Imig JD, Navar LG: Neuronal nitric oxide synthase-dependent afferent arteriolar function in angiotensin II-induced hypertension. Hypertension 33:462-466, 1999
245. Ikenaga H, Ishii N, Didion SP, Zhang K, Cornish KG, Patel KP, Mayhan WG, Carmines PK: Suppressed impact of nitric oxide on renal arteriolar function in rats with chronic heart failure. Am.J.Physiol 276:F79-F87, 1999
246. Inscho EW, Cook AK, Mui V, Miller J: Direct assessment of renal microvascular responses to P2-purinoceptor agonists. Am.J.Physiol 274:F718-F727, 1998
247. Nishiyama A, Inscho EW, Navar LG: Interactions of adenosine A1 and A2a receptors on renal microvascular reactivity. Am.J.Physiol Renal Physiol 280:F406-F414, 2001
248. Ichihara A, Imig JD, Inscho EW, Navar LG: Cyclooxygenase-2 participates in tubular flow-dependent afferent arteriolar tone: interaction with neuronal NOS. Am.J.Physiol 275:F605-F612, 1998
249. Imig JD, Deichmann PC: Afferent arteriolar responses to ANG II involve activation of PLA2 and modulation by lipoxygenase and P-450 pathways. Am.J.Physiol 273:F274-F282, 1997
250. Carmines PK, Fallet RW, Che Q, Fujiwara K: Tyrosine kinase involvement in renal arteriolar constrictor responses to angiotensin II. Hypertension 37:569-573, 2001
251. Inscho EW, Cook AK, Mui V, Imig JD: Calcium mobilization contributes to pressure-mediated afferent arteriolar vasoconstriction. Hypertension 31:421-428, 1998
252. Tonshoff B, Kaskel FJ, Moore LC: Effects of insulin-like growth factor I on the renal juxtamedullary microvasculature. Am.J.Physiol 274:F120-F128, 1998
253. De Vriese AS, Verbeuren TJ, Vallez MO, Lameire NH, De Buyzere M, Vanhoutte PM: Off-line analysis of red blood cell velocity in renal arterioles. J.Vasc.Res. 37:26-31, 2000
254. Edwards RM: Response of isolated renal arterioles to acetylcholine, dopamine, and bradykinin. Am.J.Physiol 248:F183-F189, 1985
255. Edwards RM: Response of isolated renal arterioles to acetylcholine, dopamine, and bradykinin. Am.J.Physiol 248:F183-F189, 1985
256. Yuan BH, Robinette JB, Conger JD: Effect of angiotensin II and norepinephrine on isolated rat afferent and efferent arterioles. Am.J.Physiol 258:F741-F750, 1990
257. Conger JD, Falk SA, Robinette JB: Angiotensin II-induced changes in smooth muscle calcium in rat renal arterioles. J.Am.Soc.Nephrol. 3:1792-1803, 1993
258. Loutzenhiser K, Loutzenhiser R: Angiotensin II-induced Ca(2+) influx in renal afferent and efferent arterioles: differing roles of voltage-gated and store-operated Ca(2+) entry. Circ.Res. 87:551-557, 2000
259. Li N, Teggatz EG, Li PL, Allaire R, Zou AP: Formation and actions of cyclic ADP-ribose in renal microvessels. Microvasc.Res. 60:149-159, 2000
260. Purdy KE, Arendshorst WJ: EP(1) and EP(4) receptors mediate prostaglandin E(2) actions in the microcirculation of rat kidney. Am.J.Physiol Renal Physiol 279:F755-F764, 2000
261. Edwards RM, Weidley EF: Lack of effect of atriopeptin II on rabbit glomerular arterioles in vitro. Am.J.Physiol 252:F317-F321, 1987
262. Lanese DM, Yuan BH, Falk SA, Conger JD: Effects of atriopeptin III on isolated rat afferent and efferent arterioles. Am.J.Physiol 261:F1102-F1109, 1991
263. Edwards RM, Trizna W: Characterization of alpha-adrenoceptors on isolated rabbit renal arterioles. Am.J.Physiol 254:F178-F183, 1988
264. Munger KA, Takahashi K, Awazu M, Frazer M, Falk SA, Conger JD, Badr KF: Maintenance of endothelin-induced renal arteriolar constriction in rats is cyclooxygenase dependent. Am.J.Physiol 264:F637-F644, 1993
265. Lanese DM, Conger JD: Effects of endothelin receptor antagonist on cyclosporine-induced vasoconstriction in isolated rat renal arterioles. J Clin.Invest 91:2144-2149, 1993
266. Chatziantoniou C, Dussaule JC, Arendshorst WJ, Ardaillou R: Angiotensin II receptors and renin release in rat glomerular afferent arterioles. Kidney Int 46:1570-1573, 1994
267. De Leon H, Garcia R: Characterization of endothelin receptor subtypes in isolated rat renal preglomerular microvessels. Regul.Pept. 60:1-8, 1995
268. Park F, Mattson DL, Skelton MM, Cowley AW, Jr.: Localization of the vasopressin V1a and V2 receptors within the renal cortical and medullary circulation. Am.J.Physiol 273:R243-R251, 1997

269. Yoshida H, Tamaki T, Aki Y, Kimura S, Takenaka I, Abe Y: Effects of angiotensin II on isolated rabbit afferent arterioles. Jpn.J.Pharmacol. 66:457-464, 1994
270. Kornfeld M, Gutierrez AM, Persson AE, Salomonsson M: Angiotensin II induces a tachyphylactic calcium response in the rabbit afferent arteriole. Acta Physiol Scand. 160:165-173, 1997
271. Ito S, Amin J, Ren Y, Arima S, Abe K, Carretero OA: Heterogeneity of angiotensin action in renal circulation. Kidney Int Suppl 63:S128-S131, 1997
272. Croft KD, McGiff JC, Sanchez-Mendoza A, Carroll MA: Angiotensin II releases 20-HETE from rat renal microvessels. Am.J.Physiol Renal Physiol 279:F544-F551, 2000
273. Vyas SJ, Blaschak CM, Chinoy MR, Jackson EK: Angiotensin II-induced changes in G-protein expression and resistance of renal microvessels in young genetically hypertensive rats. Mol.Cell Biochem. 212:121-129, 2000
274. Imig JD, Pham BT, LeBlanc EA, Reddy KM, Falck JR, Inscho EW: Cytochrome P450 and cyclooxygenase metabolites contribute to the endothelin-1 afferent arteriolar vasoconstrictor and calcium responses. Hypertension 35:307-312, 2000
275. Yu H, Carretero OA, Juncos LA, Garvin JL: Biphasic effect of bradykinin on rabbit afferent arterioles. Hypertension 32:287-292, 1998
276. Ito S: Characteristics of isolated perfused juxtaglomerular apparatus. Kidney Int Suppl 67:S46-S48, 1998
277. Ito S, Ren Y: Evidence for the role of nitric oxide in macula densa control of glomerular hemodynamics. J.Clin.Invest 92:1093-1098, 1993
278. Ito S, Carretero OA: An in vitro approach to the study of macula densa-mediated glomerular hemodynamics. Kidney Int 38:1206-1210, 1990
279. Melnikov VY, Ecder T, Fantuzzi G, Siegmund B, Lucia MS, Dinarello CA, Schrier RW, Edelstein CL: Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. J.Clin.Invest 107:1145-1152, 2001
280. Daemen MA, Denecker G, Van't Veer C, Wolfs TG, Vandenabeele P, Buurman WA: Activated caspase-1 is not a central mediator of inflammation in the course of ischemia-reperfusion. Transplantation 71:778-784, 2001
281. Ling H, Edelstein CL, Gengaro P, Meng X, Lucia S, Knotek M, Wangsiripaisan A, Shi Y, Schrier RW: Attenuation of renal ischemia-reperfusion injury in inducible nitric oxide synthase knockout mice. Am.J.Physiol. 277:F383-F390, 1999
282. Haq M, Norman J, Saba SR, Ramirez G, Rabb H: Role of IL-1 in renal ischemic reperfusion injury. J Am Soc Nephrol 9:614-619, 1998
283. Kelly KJ, Williams WWJr, Colvin RB, Bonventre JV: Antibody to intracellular adhesion molecule-1 protects the kidney against ischemic injury. Proc Natl Acad Sci USA 91:812-816, 1994
284. Rabb H, Daniels F, O'Donnell M, Haq M, Saba SR, Keane W, Tang WW: Pathophysiological role of T lymphocytes in renal ischemia-reperfusion injury in mice. Am.J.Physiol Renal Physiol 279:F525-F531, 2000
285. Noiri E, Dickman K, Miller F, Romanov G, Romanov VI, Shaw R, Chambers AF, Rittling SR, Denhardt DT, Goligorsky MS: Reduced tolerance to acute renal ischemia in mice with a targeted disruption of the osteopontin gene. Kidney Int 56:74-82, 1999
286. Stein JH, Gottschall J, Osgood RW, Ferris TF: Pathophysiology of a nephrotoxic model of acute renal failure. Kidney Int 8:27-41, 1975
287. Mauk RH, Patak RV, Fadem SZ, Lifschitz MD, Stein JH: Effect of prostaglandin E administration in a nephrotoxic and a vasoconstrictor model of acute renal failure. Kidney Int 12:122-130, 1977
288. Solez K, Marel-Maroger L, Sraer J: The morphology of acute tubular necrosis in man. Analysis of 57 renal biopsies and comparison with glycerol model. Medicine (Baltimore) 58:362-376, 1979
289. Baylis C, Rennke HR, Brenner BM: Mechanisms of the defect in glomerular ultrafiltration associated with gentamicin administration. Kidney Int 12:344-353, 1977
290. Neugarten J, Aynedjian HS, Bank N: Role of tubular obstruction in acute renal failure due to gentamicin. Kidney Int 24:330-335, 1983
291. Verstrepen WA, Persy VP, Verhulst A, Dauwe S, De Broe ME: Renal osteopontin protein and mRNA upregulation during acute nephrotoxicity in the rat. Nephrol.Dial.Transplant. 16:712-724, 2001
292. Wolfert AI, Laveri LA, Reilly KM, Oken KR, Oken DE: Glomerular hemodynamics in mercury-induced acute renal failure. Kidney Int 32:246-255, 1987
293. Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW: Mechanism of cis-platinum nephrotoxicity: II. Morphologic observations. J.Pharmacol.Exp.Ther. 213:551-556, 1980
294. Stein JH, Fried TA: Experimental models of nephrotoxic acute renal failure. Transplant.Proc. 17:72-80, 1985
295. Knotek M, Rogachev B, Gengaro P, Esson M, Edelstein CL, Dinarello CA, Schrier RW: Endotoxemic renal failure in mice:Role of tumor necrosis factor indepenent of inducible nitric oxide synthase. Kidney Int 59:2243-2249, 2001
296. Schwartz D, Mendoca M, Schwartz Y, Xia Y, Satriano J, Wilson CB, Blantz R: Inhibition of constitutive nitric oxide synthase (NOS) by nitric oxide generated by inducible NOS after lipopolysaccharide administration provokes renal dysfunction in rats. J.Clin.Invest. 100:439-448., 1997

297. Satriano J, Schwartz D, Ishizuka S, Lortie MJ, Thomson SC, Gabbai F, Kelly CJ, Blantz RC: Suppression of inducible nitric oxide generation by agmatine aldehyde: Beneficial effects in sepsis. J Cell Physiol 188:313-320, 2001
298. Carcillo JA, Herzer WA, Mi Z, Thomas NJ, Jackson EK: Treatment with the type IV phosphodiesterase inhibitor Ro 20-1724 protects renal and mesenteric blood flow in endotoxemic rats treated with norepinephrine. J Pharmacol.Exp.Ther. 279:1197-1204, 1996
299. Zurovsky Y, Gispaan I: Antioxidants attenuate endotoxin-induced acute renal failure in rats. Am J Kidney Dis. 25:51-57, 1995
300. Ruetten H, Thiemermann C: Effect of selective blockade of endothelin ETB receptors on the liver dysfunction and injury caused by endotoxaemia in the rat. Br.J Pharmacol. 119:479-486, 1996
301. Mitaka C, Hirata Y, Yokoyama K, Nagura T, Tsunoda Y, Amaha K: Improvement of renal dysfunction in dogs with endotoxemia by a nonselective endothelin receptor antagonist. Crit Care Med. 27:146-153, 1999
302. Steinhausen M, Kucherer H, Parekh N, Weis S, Wiegman DL, Wilhelm KR: Angiotensin II control of the renal microcirculation: effect of blockade by saralasin. Kidney Int 30:56-61, 1986
303. Steinhausen M, Weis S, Fleming J, Dussel R, Parekh N: Responses of in vivo renal microvessels to dopamine. Kidney Int 30:361-370, 1986
304. Loutzenhiser R, Hayashi K, Epstein M: Atrial natriuretic peptide reverses afferent arteriolar vasoconstriction and potentiates efferent arteriolar vasoconstriction in the isolated perfused rat kidney. J.Pharmacol.Exp.Ther. 246:522-528, 1988
305. Endlich K, Steinhausen M: Natriuretic peptide receptors mediate different responses in rat renal microvessels. Kidney Int 52:202-207, 1997
306. Hayashi K, Epstein M, Loutzenhiser R: Pressure-induced vasoconstriction of renal microvessels in normotensive and hypertensive rats. Studies in the isolated perfused hydronephrotic kidney. Circ.Res. 65:1475-1484, 1989
307. Steinhausen M, Ballantyne D, Fretschner M, Parekh N: Sex differences in autoregulation of juxtamedullary glomerular blood flow in hydronephrotic rats. Am.J.Physiol 258:F863-F869, 1990
308. Bloom IT, Bentley FR, Wilson MA, Garrison RN: In vivo effects of endothelin on the renal microcirculation. J.Surg.Res. 54:274-280, 1993
309. Fretschner M, Endlich K, Gulbins E, Lang RE, Schlottmann K, Steinhausen M: Effects of endothelin on the renal microcirculation of the split hydronephrotic rat kidney. Ren Physiol Biochem. 14:112-127, 1991
310. Dietrich MS, Endlich K, Parekh N, Steinhausen M: Interaction between adenosine and angiotensin II in renal microcirculation. Microvasc.Res. 41:275-288, 1991
311. Chen J, Fleming JT: Juxtamedullary afferent and efferent arterioles constrict to renal nerve stimulation. Kidney Int 44:684-691, 1993
312. Takenaka T, Kanno Y, Kitamura Y, Hayashi K, Suzuki H, Saruta T: Role of chloride channels in afferent arteriolar constriction. Kidney Int 50:864-872, 1996
313. Hayashi K, Fujiwara K, Oka K, Nagahama T, Matsuda H, Saruta T: Effects of insulin on rat renal microvessels: studies in the isolated perfused hydronephrotic kidney. Kidney Int 51:1507-1513, 1997
314. Tang L, Loutzenhiser K, Loutzenhiser R: Biphasic actions of prostaglandin E(2) on the renal afferent arteriole: role of EP(3) and EP(4) receptors. Circ.Res. 86:663-670, 2000
315. Hayashi K, Suzuki H, Saruta T: Nitric oxide modulates but does not impair myogenic vasoconstriction of the afferent arteriole in spontaneously hypertensive rats. Studies in the isolated perfused hydronephrotic kidney. Hypertension 25:1212-1219, 1995
316. Zimmerhackl LB, Fretschner M, Steinhausen M: Cyclosporin reduces renal blood flow through vasoconstriction of arcuate arteries in the hydronephrotic rat model. Klin.Wochenschr. 68:166-174, 1990
317. Bloom IT, Bentley FR, Spain DA, Garrison RN: An experimental study of altered nitric oxide metabolism as a mechanism of cyclosporin-induced renal vasoconstriction. Br.J.Surg. 82:195-198, 1995
318. Nakamura A, Hayashi K, Fujiwara K, Ozawa Y, Honda M, Saruta T: Distinct action of aranidipine and its active metabolite on renal arterioles, with special reference to renal protection. J.Cardiovasc.Pharmacol. 35:942-948, 2000
319. Glazer AA, Inman SR, Stowe NT, Novick AC: Renal microcirculatory effects of lovastatin in a rat model of reduced renal mass. Urology 50:812-817, 1997
320. Krysztopik RJ, Bentley FR, Spain DA, Wilson MA, Garrison RN: Lazaroids prevent acute cyclosporine-induced renal vasoconstriction. Transplantation 63:1215-1220, 1997
321. Krysztopik RJ, Bentley FR, Spain DA, Wilson MA, Garrison RN: Free radical scavenging by lazaroids improves renal blood flow during sepsis. Surgery 120:657-662, 1996
322. Schlottmann K, Gulbins E, Rauterberg EW, Steinhausen M: Effects of systemic complement activation on renal circulation of rats. Eur.J.Clin.Invest 24:320-330, 1994
323. Kimura K, Tojo A, Hirata Y, Hayakawa H, Goto A, Omata M: Effects of a calcium antagonist and an angiotensin II receptor antagonist on rat renal arterioles. Blood Press Suppl 5:71-74, 1994

07

Renal cell culture models:

Contribution to the understanding of nephrotoxic mechanisms

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Introduction

The kidney represents a major target for toxic xenobiotics due to its role in the control of body fluid and electrolyte homeostasis. The high blood perfusion rate (20% of cardiac output) and the capability to extract, metabolize, secrete and concentrate toxic compounds make the kidneys extremely vulnerable to a wide variety of toxins, many of which are yet to be identified. It is estimated that therapeutic agents cause 20% of all diagnosed end stage renal disease (ESRD) and that chemicals and drugs may play a significant role in at least 50% of ESRD cases of unknown etiology [1].

Because of the functional and biochemical heterogeneity of the nephron, the susceptibility to a particu-

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 115-147

lar nephrotoxic insult will vary among nephron segments. Epithelial cells of the proximal nephron are target sites for a wide variety of nephrotoxic chemicals due to a large number of transport systems [2] and the presence of xenobiotic metabolizing enzymes such as cytochrome P-450, NADPH-cytochrome c reductase, glucuronyl transferase, sulfotransferases, glutathione S-transferases (including cysteine conjugate β-lyase), monooxygenases and prostaglandin H synthase [3]. Another factor is the intracellular concentration of glutathione (GSH) and GSH dependent enzymes activity, which is highest in proximal tubule and decreases progressively down the nephron [4, 5]. Injuries, which alter cellular REDOX state such as the oxidative stress during ischemia-reperfusion injury, will also vary along the nephron. Early proximal nephron segments (S2 segments) appear to be more resistant to oxidative injury than their outer medullary portions (S3 segments) and the thick ascending limbs of Henle´s loop (MTAL) [4].

Primary means of identifying the potential renal effects of chemicals involved testing in laboratory animals. However, the understanding of biochemical and cellular mechanisms together with advances in cell and tissue culture now permit the development and use of *in vitro* toxicity assays. The aim of the development of such *in vitro* tests is not only to refine, reduce and replace *in vivo* animal testing, but also to improve the relevance of data obtained for the safety evaluation in humans. The reasons for the current drive for *in vitro* assay development can be attributed to three main points: 1) Scientific experiments involving live animals are receiving bad press in recent years. Public opinion of such experiments is progressively more disapproving; and governments are listening. In 1986 the European Union issued a directive which states that animal experimentation shall not be performed if non-animal procedures are reasonable and practically available [6]; 2) *In vivo* test methods are expensive, time consuming and require the sacrifice of many animals. As an example, the rodent bioassay for assessing carcinogenicity costs $1-3 million and requires at least 3 years to complete [7]. The development of reliable *in vitro* models offers potential reduction of time and cost during new product development; 3) There is often doubt concerning the relevance to humans of toxicity or lack of toxicity, of compounds tested in animals. A compound toxic in one animal is not necessarily toxic in another animal and vice versa. For example thalidomide only causes birth defects in humans and rabbits and not in rats or mice [8]. Another example is the herbicide acetochlor, which caused the induction of nasal adenomas in rats in 2-year feeding studies. However after investigations including human tissue experiments it was concluded that the effects in rats were not relevant to humans [9]. Some of the species differences can be clarified; a specific isoenzyme of cytochrome P-450 found in the nasal epithelium of rat but not in humans, is thought to be responsible for the species dependent effect of acetochlor [10]. Further species variations in the expression of many Phase I and Phase II metabolizing enzymes are known [11-13]. There are, therefore, compelling ethical, financial and scientific reasons for developing *in vitro* alternatives to animal testing. The most important issue in the development of such tests is that the data produced by the test system is relevant to human risk.

Current status of established renal cell culture models

Renal cell cultures, which retain adequate renal cellular functions known to interfere with xenobiotics or drugs, have the advantage of providing an experimental model that is not influenced by higher-order regulatory systems. Alternative *in vitro* nephrotoxicity systems have been reviewed elsewhere [14]. For successful application of renal cell cultures, certain requirements must be met. The kidney is populated by more than 15 different cell-types, all with their own specific antigenic, biochemical and physiologic and pathophysiologic properties and sensitivity to chemical compounds [15]. The nephron and collecting duct system alone are populated by at least 12 different cell types (Figure 1, Table 1) [16]. By bringing kidney tissue into culture without applying any cell purification strategies, aforementioned *in vivo* heterogeneity will be reflected in a comparable heterogeneity *in vitro*. Such heterogeneity will inevitably interfere with the *in vitro* study of molecular and cellular biology at the level of a single cell-type. For culture systems to be valuable in studying the effects of selected mediators on various cell properties in a cell-type dependent way, it is critical to have access to cultures containing homogeneous cell populations of defined cell type/nephron segment origin [17].

Cells isolated from the kidney and successfully cultured should retain a phenotype, which preserves the key properties of the *in vivo et situ* components relevant for nephrotoxicity studies.

For example, *glomerular microvascular endothelial cells* in culture should maintain the characteristic fenestration, the presence of Weibel-Palade bodies (both observed by transmission electron microscopy), the basal expression of von Willebrand factor and CD 31 (platelet-endothelial cell adhesion molecule-1) and the binding of *lectin Ulex Europaeus* (for human cells) [18, 19]. Microvascular endothelial cells (regardless of their organ of origin) should respond to cytokines such as tumor necrosis factor α (TNF-α), by the increased expression of cell adhesion molecules for example E-selectin and intercellular adhesion molecule-1 (ICAM-1) [19, 20]. It is advisable to not only demonstrate the presence of specific characteristics, but also the absence of non-endothelial markers such as cytokeratin-8 (epithelial cells) [21], smooth muscle α actin (smooth muscle cells) and the intermediate filament protein desmin (pericytes) [22, 23]. The morphological characterization

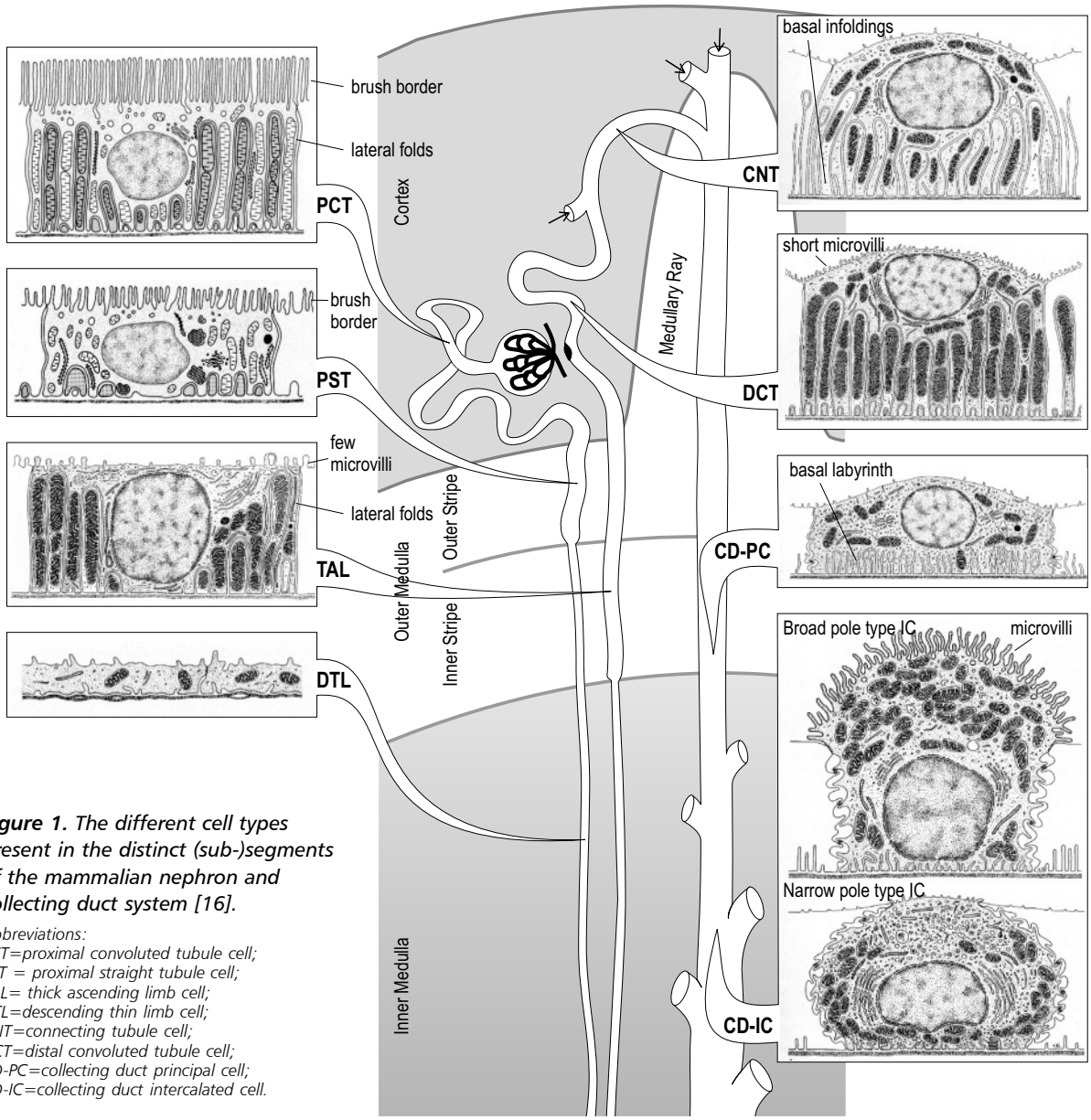

Figure 1. *The different cell types present in the distinct (sub-)segments of the mammalian nephron and collecting duct system [16].*

Abbreviations:
PCT=proximal convoluted tubule cell;
PST = proximal straight tubule cell;
TAL= thick ascending limb cell;
DTL=descending thin limb cell;
CNT=connecting tubule cell;
DCT=distal convoluted tubule cell;
CD-PC=collecting duct principal cell;
CD-IC=collecting duct intercalated cell.

Table 1. Anatomical structure of the mammalian nephron [16].

Micro-anatomical terms	Main divisions		Segmentation (preferred terms)		Cell types	Other frequently used descriptions
Proximal convolution	Proximal tubule	Pars convoluta	Proximal convoluted tubule (PCT)	S1 segment	S1 cells	P1 segment
				S2 segment	S2 cells	P2 segment
		Pars recta	Proximal straight tubule (PST)	S3 segment	S3 cells	P3 segment
Loop of Henle	Intermediate tubule	Pars descendans	Descending thin limb (DTL)		DTL cells	
		Pars ascendans	Ascending thin limb (ATL)		ATL cells	
	Distal tubule	Pars recta	Thick ascending limb (TAL) (or distal straight tubule)	Medullary straight part	TAL cells	
				Cortical straight part		
				Macula densa (MD)	MD cells	
				Post macular segment		
Distal convolution		Pars convoluta	Distal convoluted tubule (DCT)	DCT1	DCT cells (+ IC cells)	Early distal tubule — Distal tubule
				DCT2		
	Collecting system		Connecting tubule (CNT)		CNT cells (+ IC cells)	Late distal tubule — Distal tubule
Collecting duct		Collecting duct	Cortical collecting duct (CCD)		Principal cells (+IC cells)	Cortical collecting tubule (CCT)
			Outer medullary collecting duct (OMCD)			
			Inner medullary collecting duct (IMCD)		Principal cells	Inner medullary collecting tubule Papillary collecting duct (PCD) (Ducts of Bellini)

of endothelial cells by light microscopy must be used with caution due to the marked plasticity of these cells in culture [24]. However, glomerular endothelial cells can usually be distinguished at a light microscopy level from common contaminating cells such as fibroblasts, mesangial cells and epithelial cells [19].

Glomerular visceral epithelial cells (podocytes) in culture should at least have retained the potency to produce basement membrane constituents (collagen IV and glycosaminoglycans) and maintain the expression of cytokeratin, the membrane proteins megalin and podocalyxin [25], complement C3b and angiotensin II receptors, as well as the synthetic machinery for the synthesis of prostaglandins (Prostaglandin E2 and thromboxane) [26].

Glomerular mesangial cells in culture should display the basic properties of pericytes such as the expression of the cytoskeletal filaments smooth muscle actin [27], myosin, vimentin and desmin [28] and also functional properties of their *in vivo* counterparts such as, the capability to produce extracellular matrix molecules contributing to the formation of the glomerular basement membrane [29] and response to vasoconstrictive signals [30], growth factors and mitogenic signals and display phagocytic properties [31]. Morphologically they are recognized by the formation of multilayers and hillock structure as assessed by phase contrast microscopy and bundles of microfilaments orientated parallel to the plasma membrane at an electron microscopic level [32]. The absence of expression of non-mesangial mark-

ers such as cytokeratin and von Willebrand factor will exclude the possibility of contaminants from other cell types.

Renal tubular epithelial cells in culture represent an adequate *in vitro* model for nephrotoxicity studies if they have retained: (1) polar architecture and junctional assembly of epithelia, and proper polar distribution of membrane enzymes and transport systems, (2) vectorial transport of solutes and water, manifested by the formation of domes [33], the generation of transepithelial electrophysiological properties [34, 35], and cellular uptake of xenobiotics from either the apical or basolateral side, as observed *in vivo* [2] and (4) expression of nephron segment-specific characteristics, i.e. distinct antigen/enzyme differentiation markers, metabolic and transport properties, and hormone responsiveness [3, 17, 36]. Precise information concerning enzyme and antigen distribution along the course of the nephron has become available with the advent of microdissection techniques, (quantitative) histochemistry and immunohistochemistry. Figure 2 illustrates the *in vivo* expression patterns of a number of nephron (sub-)segment specific epitopes in the human nephron.

Whether these requirements are better met by primary cultures or renal cell lines is still subject of debate. To date, none of the primary cultures or continuous renal cell lines, at least for tubular epithelial cells, fully express all the differentiated functions found in their *in vivo* counterparts [37].

Methods of obtaining human primary renal tubular epithelial cell cultures

The use of primary cell cultures has certain advantages over perpetuated cell lines as the origin of the cell is defined and inherently represent a better level of differentiation.

Primary cultures containing more than one cell-type have up to now generally been considered less useful as one cannot ascribe the observed effects to a single defined cell-type. Consequently, over the past few years considerable effort has been expended to develop purified primary cultures containing only cells from an identifiable nephron segment and, whenever possible, containing but one single cell-type (reviewed in [42]). A more recent approach consists of developing ancillary techniques, which allow retrieval of cell specific responses in mixed cultures (containing cells from different nephron segments) [43].

The methods that have been described for obtaining human primary tubular epithelial cultures can be

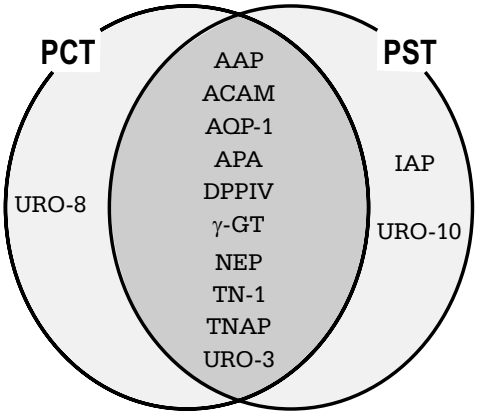

Figure 2A. *Diagram representing epitopes limited to the proximal convoluted tubule (PCT, left), epitopes limited to the proximal straight tubule (PST, right), and epitopes shared by proximal convoluted and proximal straight tubule (middle) [16].*

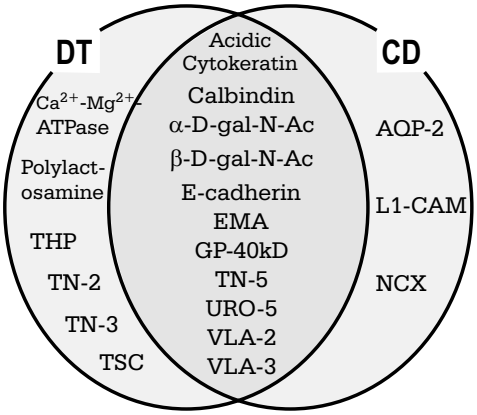

Figure 2B. *Diagram shows epitopes limited to the distal tubule cell (DT, left) epitopes limited to the collecting duct (principal) cell (CD, right), and epitopes shared by distal tubule and collecting duct cells (middle).*

Abbreviations: AAP: Alanine aminopeptidas (aminopeptidas N/M); ACAM: N-Cadherin; APA: Aminopeptidase 1 (angiotensinase); AQP-1: Aquaporin-1; AQP-2: Aquaporin 2; DPPIV: Dipeptidyl peptidase IV; EMA: Epithelial membrane antigen; GP-40kD: Glycoprotein 40kD [38]; IAP: intestinal alkaline phosphatase; L1-CAM: L1 Cell adhesion molecule; NCX: Na^+-Ca^{++} exchanger; NEP: Neutral endopeptidase; β-N-Ac: Dolichus biflorus; THP: Tamm-Horsfall protein; TN-1: TN-1 antigen [39]; TN-2: TN-2 antigen [39]; TN-3: TN-3 antigen [39]; TN-5: TN-5 antigen [39]; TNAP: Tissue non-specific alkaline phosphatase; TSC: Thiazide sensitive Na^+-Cl^- cotransporter; URO-10: Uro-10-antigen [39]; URO-3: Uro-3-antigen [41]; URO-5: Uro-5-antigen [41]; URO-8: Uro-8-antigen [40]; VLA-2: Very late antigen 2; VLA-3: Very late antigen 3; a-N-Ac: Arachis hypogea; γ-GT: γ-glutamyl transferase

classified into *preparative techniques,* in which the cultures are started off by seeding a 'bulk-mixture' of cells of variable nephron segment origin, and *pre-purification techniques,* in which cultures are started off by seeding one particular cell subset selected from the initial bulk-mixture. Preparative techniques have been widely applied for starting *mixed-type tubular epithelial cultures* and *homogeneous proximal tubular epithelial cultures* of both animal and human origin. Preparative techniques have also been described for obtaining rat and rabbit *distal tubular epithelial cultures*. To date no preparative techniques have been described for obtaining *human* distal tubule/collecting (duct) system cultures [44-45]. The following discussion will be limited to the techniques that have been published for obtaining the diverse types of *human* tubular epithelial cultures.

Preparative techniques (human cultures)

An overview of the preparative techniques that have been published for obtaining *homogeneous proximal tubular epithelial cultures* and *mixed-type tubular epi-*

Table 2. Overview of the published macro-separation techniques described to result in pure human proximal tubular epithelial cultures [52].

Author (et al), **year**	**Preparative Procedure**	**Growth Medium**	**Passage**	**Characterization**	**Application**
Detrisac 1984 [46]	Diss → Expl	DMEM/HAMF12 + HITS + T3 + EGF	P_6-P_{12}	**CC**: Glc-6-P$^+$, AcP$^+$, AP$^+$, **ICC**: CA$^+$, VWF$^-$, THP$^-$, keratin$^-$, **Morph**: Domes$^+$, Microvill$^+$	[53-55]
Triffilis 1985 [50]	Perf Coll → Diss → Coll → Siev → Seed	EMEM		**CC**: gamma-GT$^+$, **ICC**: cytokeratin$^+$, THP$^-$, vWF$^-$	[56, 57]
Miltenburg 1989 [58]	Diss → Expl	DMEM/HAMF12 + HITS + T3 + EGF	P_2-P_6	**ICC**: EMA^{++}, DPPIV$^+$, cytokeratin$^-$ **Morph**: Epith$^+$, Domes$^+$, Microvilli$^+$	[59-63]
McLaren 1990 [49]	Diss → Coll → Siev→ DGC → Seed	DMEM/HAMF12	P_0	**ICC**: AcP$^+$, NEP$^+$, LAP$^+$, vWF$^-$, EMA$^-$, Glc-3-P-DHG$^-$ **cAMP**: PTH$^+$, AVP$^-$, Calc$^-$ **T**: Na$^+$-dep. glc, anion and cation transport **Morph**: Epith$^+$, Domes$^+$, Microvill$^+$	[64]
Racusen 1991 [65]	Diss → Coll → Diss→ Siev → Seed	DMEM/HAMF12 + HITS + 10%FCS + T3	?	**ICC**: gamma-GT$^+$	[65]
Phillips 1995 [66]	Diss → Serial Siev → Coll → Seed	DMEM/HAMF12 + HITS + 10%FCS	P_3	**ICC**: AP$^+$, AcP$^+$, NSE$^+$, Glc-6-Pase$^+$, THP$^-$ **Morph**: Microvill$^+$	[67, 68]
Courjault-Gautier 1995 [69]	Diss → Coll → Siev → Coll → Siev → Seed	DMEM/HAMF12 + HI*TS *:t1: insulin$^-$, glc$^-$ t2:insulin$^+$,glc^{low}	P_1	**BC** (homog): AP$^+$, gamma-GT$^+$ **Morph**.: Epith$^+$, Microvilli, Domes$^+$, **cAMP**: PTH$^+$, AVP$^-$, Calc$^-$ **T**: Na$^+$-dep. Glc and P uptake	

AcP: acid phosphate
AGCU: adenosine, guanosine, cytosine and uridine supplemented
AP: alkaline phosphatase
APN: aminopeptidase N
AVP: arginine vasopressin
BC (homog): biochemical determination on culture homogenate
CA: carbonic anhydrase
Calc: calcitonin
cAMP: cyclic adenosine monophosphate
CC: cytochemistry
Coll: treatment with collagenase
CG: density gradient centrifugation
Dexa: dexamethasone
DHG: dehydrogenase
diss: dissection
DMEM: Dulbecco's modified Eagle medium
DPPIV: dipeptidyl peptidase IV
EGF: epidermal growth factor
EMA: epithelial membrane antigen
Epith: epithelial
Expl: explantation on culture substrate
FACS: fluorescence activated cell sorting/ analysis
FCS: fetal calf serum
glc: glucose
HITS: hydrocortisone/insulin/transferrin/ selenium supplemented
Homog: tissue discruption in sterile tissue homogenizer
ICC: immunocytochemistry
IT: insulin/transferring supplemented
LAP: leucine aminopeptidase
MACS: magnetic cell separation
Morph: morphologically
NEP: neutral endopeptidase
NSA: neuron specific enolase
P: phosphate
P_x: passage x
Pase: phosphatase
Perf coll: organ perfusion with collagenase
PTH: parathyroid hormone
Seed: seeding on culture substrate
Siev: sieving through steel sieve
T3: triiodothyronin
THP: Tamm-Horsfall protein
vWF: von Willebrand factor

thelial cultures of human origin is shown in Tables 2 and 3. Two basic culture methods can be distinguished: *explantation* of tissue fragments versus *seeding* of single cells/small tubular fragments. In the explantation method [46], renal cortical tissue is cut up into small fragments, approximately 1 mm^3 in size. These fragments are then explanted directly into a culture flask pre-coated with serum. The fragments are then immersed in cell culture medium, which results in outgrowth of cells from these tissue fragments. The outgrowing cells ultimately form confluent monolayers.

In the *seeding techniques*, the dissected cortex is additionally subjected to sieving through a steel mesh sieve [47], proteolytic digestion (using collagenase, trypsin, DNAse, or an enzyme mixture) [48] or both [49]. Triffilis et al. [50] perfuse the entire kidney with collagenase *before* dissecting it. In the method of McLaren et al. [49], the digested fragments are additionally centrifuged on a discontinuous Percoll®-gradient for separation of viable cells from small debris. The resulting suspension of cells/small tubular fragments is then seeded, cultured and passaged several times. Cells are usually grown on tissue-culture-treated plastic surfaces. In order to improve adherence and differentiation, several authors pre-coat the growth surface with serum and/or several extracellular matrix components.

Since the original description of Detrisac et al. [46] a 1:1 mixture of DMEM and HAMF12, supplemented with hydrocortisone, triiodothyronine, insulin, transferrin and selenate (currently known as K1 medium) [51], has been the most widely used medium in human (proximal) tubular epithelial cell culture. Several authors add serum because of its growth promoting effect.

Problems & pitfalls in determining cell composition in cultures obtained by macro-separation techniques

In all macro-separation techniques, the starting material is from multiple nephron segments. Hence, assessment of unwanted elements such as non-epithelial contaminants, non-tubular contaminants and/or presence of cells originating from 'undesired' nephron segments, is imperative. Culture composition has generally been studied by checking for presence of features characteristic of the cell-types of interest *in vivo*, and vice-versa, for absence of features characteristic of the contaminating cell-types. As far as *morphology* is concerned, most authors checked presence/absence of epithelial growth pattern, that is confluent monolayer

Table 3. Overview of the published macro-separation techniques described to result in heterogeneous human tubular epithelial cultures [52].

Author (et al), **year**	**Preparative Procedure**	**Growth Medium**	**Passage**	**Characterization**
Hang Yang 1987 [70]	Homog → Siev → Coll → Siev → Seed	Waymouths + 10%FCS	→P_5	**BC** (homog): LAP^+,AP^+, gamma-GT^+
Kempson 1989 [48]	Diss → Coll → Seed	DMEM/HAMF12 + 10%FCS	P_{3-} P_8	**BC** (homog): LAP^+, gamma-GT^+, $Maltase^+$ **T**: Na^+-dep. glc, P & alanine transport **cAMP**: PTH^{+++}, AVP^+ **Morph**: heterogeneous, $Epith^+$, $Domes^+$, $Microvilli^+$
Burton 1996 [71]	Diss → Coll → Siev → Seed	DMEM/HAMF12 + 5%FCS + HITS + T3	P_2	**ICC**: NSE^+, vWF^-, EMA^+, $keratin^+$ **cAMP**: PTH^{++}, AVP^+ **Morph**: $Epith^+$, $Domes^+$, $Microvilli^+$
Combe 1997 [72]	Diss → Coll → Siev → Seed	DMEM/HAMF12 + 5%FCS (P_3: + HITS + T3)	P_2-P_5	**ICC**: EMA^+, $DPPIV^+$, NSE^+, VWF^-, $cytokeratin^+$ **cAMP**: PTH^{++}, AVP^+ **Morph**: $Epith^+$, $Domes^+$
Orphanides 1997 [47]	Diss → Siev → Seed	RPMI1640 + 10%FCS + AGCU + Dexa	P_1-P_6	**CC**: AP^+, **ICC**: $desmin^-$, $vimentin^-$, α-SMA^-, $keratin^+$ Morph: $Epith^+$

Abbreviations: see table 2

growth, and presence of microvilli and dome formation, the latter indicating vectorial transport-activity and presence of tight junctions. Culture composition has also been studied by checking for presence/absence of expression of several membrane & intracellular constituents (brush-border ecto-enzymes, hormone receptors, intermediate filaments such as cytokeratins). Finally, some authors studied synthesis and release of specific products (such as cAMP-production following stimulation with diverse hormones) and presence of segment specific transport activities indicative of differentiated cell function (Tables 2 and 3).

However, despite using similar isolation procedures, culture techniques and culture media, different authors often draw different conclusions as to 'homogeneous proximal' versus 'heterogeneous tubular epithelial' composition of their resulting cultures (Table 2 versus Table 3). The different conclusions regarding the (in-)homogeneity of the resulting cell populations may not follow from imprecise cell characterization, but rather from a different interpretation of the cell characterization.

In several studies multiple passages of cultures precede characterization. Repeated subculturing of cells results in large cell numbers, often necessary for toxicological & mechanistic studies, but multiple passages promote loss of antigenic, biochemical and functional characteristics of cells [51, 73-75]. This loss of cell-type specific characteristics is likely to interfere with proper characterization of cultures by promoting a false homogeneous image of culture composition (*phenotype homogenization*). The finding that all cells in initially heterogeneous cultures show the same phenotype following several passages has generally been interpreted as reflecting purification caused by some form of selection pressure. It should be noted however that except in the study of Courjault-Gautier et al. [69], such selection pressure has never been confirmed. Consequently, it would seem more prudent to consider the possibility of latent cell-types persisting in culture, remaining undetected due to widespread loss of antigenic, biochemical and functional characteristics.

It is evident that differentiating true purification from phenotype homogenization will be facilitated by increasing the number of cell characteristics studied. In this regard it should be noted that in a number of studies, homogeneous proximal tubular cell composition was indeed based on a very limited amount of data [65, 76]. Differentiation between purification and phenotype homogenization will be facilitated by characterizing the cells early after isolation, to prevent loss of cell differentiation with increasing culture time [77, 78]. In this regard it is of note that cells were characterized very late in the explant method of Detrisac et al. [46] (passage 6-12), because cell (out-)growth proceeds very slow in the absence of serum.

Secondly, conclusions on cell composition were often inferred from characterization studies performed at the population level, rather than at the individual cell level. Examples are the studies in which proximal tubular brush-border enzyme activities were determined on culture homogenates [50]; studies in which proximal tubule transport characteristics (sodium-dependent glucose, amino-acid and/or phosphate uptake) were determined [49]; and studies in which adenylate cyclase activation patterns following hormonal stimulation were assayed. Such characterization studies indeed provide valuable information on differentiated state and function of cells present in the cultures under study; however, they do not provide information on homogeneity/purity of the culture and can not be used for this purpose. As an example, one approach to exclude the presence of non-proximal tubular cells in cultures is by demonstrating absence of a significant increase in cAMP production following addition of AVP and/or calcitonin [49]. Unfortunately, negative results of such population-based characterization studies do not exclude the presence of cell subsets if not accompanied by strong experimental evidence that the 'unwanted' cell subset actually continue to respond in the presumed way (evidence that was never provided, as the unwanted cell subsets were not available as pure populations).

Similarly, histochemical stainings of monolayers *in situ* provide more information on culture composition than enzyme determinations on culture homogenates. Nevertheless, small-unstained subsets may easily remain undetected and very few authors have actually counted the percentages of cells that stain positively (for example by staining cytospin-preparations). Often the information is merely qualitative. In the studies of Detrisac et al. [46], for example, expression of glucose-6-phosphatase is the only strong argument for proximal origin of the resulting cells; however, the authors provide only qualitative histochemical information on its expression in the resulting cultures. If a

subset of cells does not stain due to antigen/enzyme expression below detection limit, a contaminating cell subset may remain undetected.

Even cells grown in optimized culture conditions never reproduce the whole phenotype of their *in vivo* counterparts: alteration in phenotype may result from genetic drift, cell selection and/or environmental factors [51]. For example, certain markers that are cell-type specific *in vivo*, have been shown *in vitro* to be expressed on cell-types not expressing them *in vivo* (*off → on concept*, first described by Bander et al.) [79-84]. Other markers are known to disappear on cells once grown *in vitro* (*on → off concept*). *Pre-purified culture systems* are the ideal tool for studying the extent to which such phenotype alterations occur in distinct cell-types. In view of the lack of pre-purified cultures used to characterize cultures derived by macro-separation techniques, very little information on phenotype alterations is available. Hence, deduction of cell origin is largely based on the assumption that no such changes take place, contributing to misinterpretation of cell origin and culture composition.

With the availability of pre-purified TAL cultures (microdissection studies, see below), it is now known that THP is rapidly lost by TAL cells, once in culture. Nevertheless, many authors have inferred homogeneous proximal composition of cultures by demonstrating absence of THP-immunoreactivity on cultured cells [46, 50, 66]. Homogeneous epithelial composition was similarly often inferred simply from absence of the endothelial marker von Willebrand Factor (vWF)-immunoreactivity, without proof that vWF remains expressed on endothelial cells under their culture conditions [46, 49, 50].

DPPIV and AP are other examples of markers that are used improperly in cell culture for characterization purposes [46, 58, 66]. *In vivo*, DPPIV and AP are expressed only by proximal tubular cells. With the availability of pre-purified DT and CD system cultures, it is now clear that *in vitro* DPPIV and AP are also expressed on DT/CD system cells and CD cells, respectively. Thus any characterization study that uses markers of which the lineage-specificity was not previously confirmed *in vitro* should be viewed with utmost caution.

Finally, a number of conclusions with respect to cell origin were simply based on incorrect use of markers. As an example, some authors have inferred absence of non-epithelial cells from absence of vWF expression in their cultures [66]; whereas, vWF expression is limited to a subset of human renal endothelial cells. Similarly THP has regularly been used for excluding presence of non-proximal tubular epithelial cells [46, 50, 66, 58], whereas it expression is again limited to a subset of distal tubule cells, namely TAL cells, with none on collecting duct cells. Some authors [49] inferred homogeneous proximal origin based on strong upregulation of adenylate cyclase activity in their cultures which is PTH specific, and absent with AVP [46, 53, 54, 64]. However, microdissection studies in human have revealed that PTH stimulates adenylate cyclase in both proximal and distal tubule cells (see below) [85, 86]. Thus, the aforementioned hormone responsiveness pattern would be perfectly compatible with homogeneous distal tubule culture composition.

Comparison of the characterization results of the studies summarized in table 2 (supposedly homogeneous proximal tubular epithelial origin) with those in table 3 (supposedly heterogeneous tubular epithelial origin), yields relatively few convincing differences. Those authors who concluded that their cultures were not of homogeneous proximal origin seem to provide evidence of a more prudent and scientifically more correct conclusion. In our opinion a meticulous and detailed re-characterization of the cultures obtained by aforementioned macro-separation techniques (that is performed early, on the single cell level rather than on the population level, using only validated markers, and using them in a correct way) would yield a more heterogeneous antigenic/biochemical profile, and necessitate reconsideration of their initially reported homogeneous composition.

Pre-purification techniques

Manual microdissection

The most direct way to establish cultures from specific portions of the nephron is to initiate them from well-identified microdissected tubule segments. In the microdissection procedure, tubules are identified by their orientation within the kidney slice, their consistency, and their distinct morphological characteristics under the microscope.

Several groups have used this *manual microdissection* approach to isolate individual sub-segments. This resulted in very pure cultures of PT cells, TAL cells,

DCT cells and CD system cells from rabbit [85, 87-93]. Wilson and co-authors showed that *human* PCT (S1S2), PST (S3), TAL and CD can similarly be obtained by manual microdissection and can be grown in primary culture in defined media [94].

The major advantage of the microdissection technique is the quasi-absolute certainty concerning the origin of the cells. Hence, cultures obtained by microdissection provide the ideal tool for studying cell responses. Microdissection does not, however, allow separation of multiple cell-types present in the same segment *in vivo*. Wilson et al. indeed report that their microdissected CD cultures contain at least two distinct cell populations (principal cells and intercalated cells) [94]. The main disadvantage of the microdissection technique is the limited number of cells derived from a single isolate, particularly true primary (that is passage 0) cells.

Affinity purification (immunoselection) techniques

To date, monoclonal antibodies or lectins specific for almost every cell-type of the nephron and collecting (duct) system are available (Figure 2). This array of monoclonals/lectins represents a powerful tool for the immunoselection of individual cell-types. However, the applicability of antibodies and lectins for immunoselection is limited by the membrane expression density of the antigens/oligosaccharide moieties sought, their stability and affinity.

In the *panning technique,* originally developed by Wysocki and Sato [95], cell selection is achieved by incubating a mixture of cells (obtained by macro-separation techniques) on a culture dish pre-coated with a monoclonal antibody, which allows the adsorption of the cell-type bearing the corresponding antigen. The remaining cells are removed by repeated washings. Panning has been used for isolating rat PT cells, rabbit TAL cells and rabbit CD cells [96-100]. To date, panning has not been used for purification of human renal cell-types.

In *magnetic cell sorting* (MACS), cells labeled with primary antibody are incubated with a ferrous conjugated second antibody. Labeled cells are separated by decanting the cell suspension in a magnetic field. Pizzonia et al. [101] and Bacskai and co-authors [102] introduced MACS for immunoselection of murine distal tubule cells. Baer et al. [103] used MACS for immunodissection of human proximal and distal tubule cells, based on expression of the brush-border enzyme aminopeptidase M (CD13) and THP, respectively.

In *fluorescence activated cell sorting* (FACS), cells are sorted following labeling with fluorochrome-tagged primary or secondary antibodies. Cells are exposed to a laser beam and selected on the basis of presence or absence of cellular fluorescence. FACS was used for affinity purification of rabbit CD principal and intercalated cells using fluorochrome-tagged monoclonal antibodies and lectins [104-107]. It was first used to purify human cells by Van der Biest et al. [107]. FACS has been expanded since its original use and now allows isolation of a wide variety of cell types present in the human nephron: proximal tubule cells (antigen: leucine aminopeptidase), proximal convoluted tubule cells (antigen: TN20 antigen), proximal straight tubule cells (antigen: intestinal type alkaline phosphatase), distal tubule/collecting duct system cells (antigen: epithelial membrane antigen), thick ascending limb cells (antigen: epithelial membrane antigen), and principal cells (antigen: L1 cell adhesion molecule) [43].

Characterization of pre-purified cells: gold standard cultures

Prepurified cultures can be considered a 'golden standard' for the *in vitro* characteristics and/or for the *in vitro* 'behavior' of defined cell-types. Thus, antigen characteristics, transport processes and hormone profiles of proximal tubule, distal tubule and collecting duct cells have been characterized by Wilson et al. [87] (microdissection), Baer et al. [103] (MACS) and Van Der Biest et al. [107] (FACS) (Table 2).

As far as *morphologic characteristics* are concerned, *dome formation* was described in confluent PT, DT and CD system cultures, indicating vectorial solute and water transport in all these cell-types [94, 103, 107]. Van Der Biest et al. [107] using phase contrast microscopy, described proximal tubular monolayers to consist of closely packed polygonal cells, which display high granularity, indicative of high endocytic activity. On the other hand, DT/CD system cells are clearly less granular and present with a more curved outline [107]. At the ultrastructural level, microvilli have been described on both proximal and distal tubular cells [94, 103, 107]. On both cell-types, their density is substantially lower than on the corresponding cells *in vivo*. It is noteworthy to mention that in characterization studies of cultures obtained by macro-preparative tech-

niques, both dome formation and presence of microvilli has, until recently, been interpreted as proving proximal phenotype (thus origin) [46, 64, 66, 69].

The expression of several *in vivo* proximal tubule and distal tubule antigens remains present on *in vitro* preparations of proximal tubule and distal tubule cells (*proximal:* AP, γ-GT, DPPIV, APN; *distal:* EMA). Studies on these defined cell-types also revealed that some *in vivo* cell-type specific markers (such as THP) were no longer expressed on the corresponding cells *in vitro* [103]. Antigen expression studies on purified cultures similarly revealed that a number of *in vitro* nephron segment/cell-type specific markers are no longer confined to these cell-types once *in vitro*. In this way, the brush-border enzymes γ-GT and AP were found to be expressed weakly on DT cells and moderately on CD system cells, respectively [94, 103, 107].

Pre-purified cultures can be used to study hormone response patterns of defined renal epithelial cell-types. All authors agree on PTH responsiveness and AVP unresponsiveness by proximal cells in terms of adenylate cyclase activation [94, 103, 107]. Heterogeneous DT/CD system cultures were found to respond to both PTH and AVP stimulation [107]. Baer et al. [103] and Helbert et al. [52] found DT cells to respond strongly to PTH stimulation but very weakly to AVP. CD system cells on the other hand produced cAMP following PTH stimulation but not following stimulation with AVP. These results are in accordance with the data from Chabardes and co-authors [86], who studied the action sites for PTH and AVP along the human nephron by measuring adenylate cyclase activity using an *in vitro* single tubule micro-assay (tubules isolated using microdissection). In their hands, PTH increased adenylate cyclase activity in the PT and the DCT. AVP increased adenylate cyclase activity in the 'late distal convoluted tubule', that is the CNT, and the CD system [86]. The aforementioned hormone response patterns are in accordance with hormone receptor localization studies in rat by Yang et al. [108], Lee et al. [109], Terada et al. [110], Mimura et al. [111], Philips et al. [112] and Nonoguchi et al. [113].

Determination of responses in a cell-type dependent way in unpurified cultures

Because antigenic shifts (*on* → *off, off* → *on*) seem to be enhanced by multiple passaging of cells, it seems prudent to limit the use of renal (tubular epithelial) cell cultures as an *in vitro* model for the study of *in vivo* phenomena to low-passage-number subcultures, preferentially to 'true primary' (i.e. passage-0) cultures. At a first glance, the use of pre-purified passage-0 cultures may seem the solution to this problem. However, low cell yield of the primary isolate is the price of high purity. Consequently, their use for experiments requiring large cell numbers is impractical. Another approach consists of introducing ancillary techniques that allow evaluation of events/responses at the nephron segment level in cultures containing different cell types, as is the case when cultures are started off from a mixture of cells originating from different nephron segments. By studying the expression of a panel of cell surface markers in pre-purified proximal and distal tubule/collecting duct cultures, it is possible to identify a number of markers that retain their lineage specificity *in vitro*. Using these appropriate stable markers in FACS thus allows distinction of proximal and distal tubule/collecting duct subsets in previously not pre-purified cultures (Figure 3). When applying these markers in multi-parameter FACS analysis, assessment of cell events/responses becomes possible in a cell-type dependent way in mixed cultures [43].

Multi-parameter-FACS-analysis applied on mixed-type cultures combines two major advantages: first, it allows obtaining cell type specific information in true primary cells (passage 0), implying optimal differentiation. Second, it allows obtaining cell-type-specific information despite using a culture strategy that implies less workload and results in considerably higher cell yield (as compared to culture techniques requiring pre-purification).

Non-human primary cultures

Primary cultures of renal tubular, glomerular mesangial and endothelial cells from various species have been developed, including: mouse [114], rat [115, 116], rabbit [117] and pig [118]. Although cells in primary culture tend to dedifferentiate, the characteristics of those cells are usually closer to the *in vivo* situation than are animal cell lines, at least for a limited culture period. Primary cultures have been used successfully to study the short-term *in vitro* effects of cisplatin, gentamicin, cephalosporins, cysteine conjugates, butyl hydroperoxide, mercuric chloride, and cadmium chloride

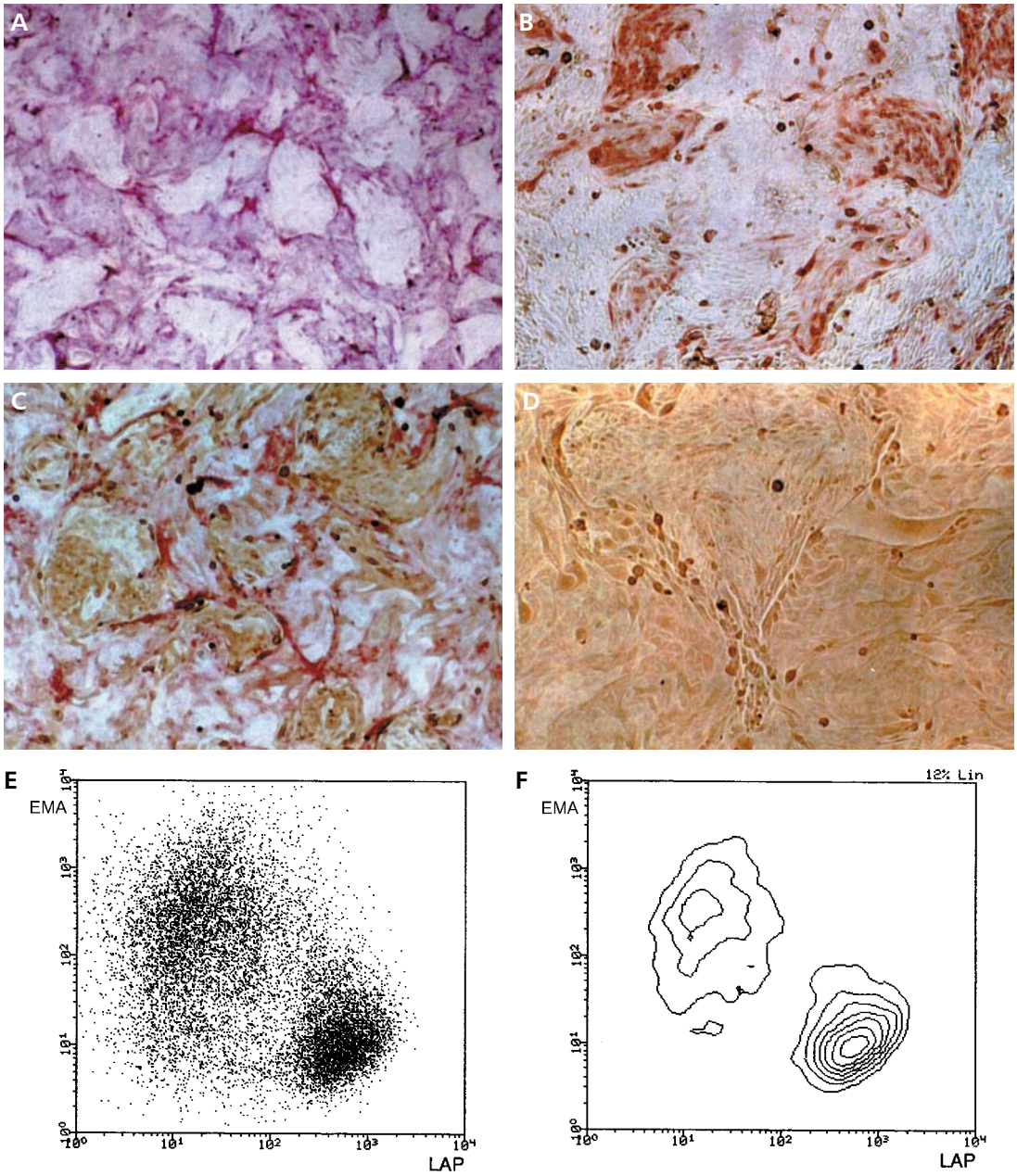

Figure 3. (Immuno-) cytochemical and flow cytometric staining of confluent not pre-purified human renal tubular epithelial cell cultures. *Cultures were stained/labeled for expression of validated markers: the proximal tubular marker* leucine aminopeptidase *(LAP), the distal tubular/collecting duct marker* epithelial membrane antigen *(EMA) and the epithelial marker* cytokeratin type 18. *LAP cytochemical staining shows that proximal tubular cells (LAP positive, pink) are organized into groups surrounding islands of cells not expressing LAP* ***(A)***. *Immunocytochemical staining for expression of EMA (brown) illustrates how EMA positive cells grow in similar island-like configurations* ***(B)***. *EMA (immunocytochemistry, brown)/LAP (cytochemistry, red) dual staining of these cultures demonstrates that the LAP negative islands match with the EMA positive islands and vice versa* ***(C)***. *The epithelial nature of these cultures is shown by their homogeneous staining for cytokeratin (immunocytochemistry, brown)* ***(D)***. *Flow cytometric analysis of these not pre-purified tubular epithelial cultures following EMA /LAP dual labeling similarly reveals the co-existence of proximal cells (LAP positive cluster) and distal tubular/collecting duct cells (EMA positive cluster). Dot plot representation* ***(E)*** *and contour plot representation of 10.000 dual labeled cells* ***(F)***. *Orig. magn.: A: x45; B-C: x55; D x130. Contour plots drawn in 10% linear-density. Adapted from [16], with permission.*

[119-122]. Nevertheless, the majority of *in vitro* nephrotoxicity studies have been performed on permanent or continuous renal epithelial cell lines.

Permanent and immortalized cell lines (human and non human origin)

Widely used permanent cell lines

Cell lines offer several advantages over primary cell cultures, such as an unlimited life-span and the lack of time-consuming isolation procedures. For this reason, the majority of *in vitro* nephrotoxicity studies have been performed on permanent or continuous renal epithelial cell lines. The most widely used permanent renal epithelial cell lines of animal origin are the LLC-PK_1 (Hampshire pig) [124,125], JTC-12 (cynomilgus monkey) [126] and OK (American opossum) [128] cell lines, which exhibit biochemical and antigenic characteristics suggestive of proximal origin, and the MDCK (Cocker Spaniel) [125,127], and A6 (clawed toad, Xenopus laevis) cell lines which exhibit properties suggestive of distal tubule/collecting duct origin. It should be noted that neither the cell-type of origin, nor the agent responsible for transformation are known for the aforementioned cell lines since no attempts were made to characterize their 'parent cells'. Their 'origin' was merely deduced from their morphologic and functional properties, which were usually studied years after emergence resulting in an ambiguous phenotype, leaving their true origin uncertain.

The MDCK cell line, for example, is one of the most widely employed cell lines in basic renal epithelial research. These MDCK cells show a hormonal profile consistent with collecting duct origin, express the furosemide-sensitive $Na^+/K^+/2Cl^-$ symporter, consistent with TAL origin, and express significant amounts of brush-border hydrolases (tnAP and LAP), consistent with PT origin [127, 129, 130].

Two cell lines that are often used as a model for the proximal nephron are the porcine cell line, LLC-PK_1 and the OK cell line from the opossum kidney. Both of these cell lines lack expression of the enzyme fructose-1, 6-bisphosphatase, rendering them incapable of gluconeogenesis, a key metabolic pathway in proximal nephron cells [4]. In addition, LLC-PK_1 cells are not responsive to parathyroid hormone, and lack a probenecid-sensitive organic anion transporter [131]. On the other hand they express vasopressin receptors [132, 133], which are not expressed in the *in vivo* correspondents. OK cells display very little γ-GT, and lack alkaline phosphatase, both considered to be markers for the proximal nephron [134].

Genetically engineered ('immortalized') cell lines

A combination of the advantages of a continuous cell line with improved differentiation of primary cultures can be obtained by immortalization of the latter. Immortalization of primary proximal tubular cell cultures has been achieved in several ways [135]. Proximal nephron cell lines have been produced by targeted oncogenesis in transgenic mice by using a pyruvate kinase-SV-40(T) antigen hybrid gene [136]. Transduction of the SV40 large T antigen has also been used to establish cell lines from rat primary proximal nephron cell cultures of the Wistar Kyoto rat [137] and the rabbit [138, 139]. Using a hybrid adeno 12-SV40 vector, successful immortalization of rabbit proximal primary cultures has been achieved [139, 140].

In theory, immortalized cell lines represent ideal cell culture models, provided that they derive from a single well-defined cell-type and maintain many characteristics of the parental cell. In practice, substantial loss of cell differentiation characteristics (including growth pattern, morphologic features, membrane properties and secretory patterns) are common drawbacks of cell immortalization, limiting their value as a model system for studying biology and (patho-)physiology of renal cells *in vitro*. This is illustrated in the rabbit-proximal tubule cell line RC.SV1 by down-regulation of transport systems and blunted or aberrant hormone response patterns [51, 139, 141].

Moreover, a number of immortalized cell lines were found to acquire atypical properties such as ion channel activities not present in the corresponding primary cells [142-145]. Certain cell lines were also shown to contain more than one morphologically/biochemically distinct cell-type, despite repeated subcloning [146, 147].

Cell lines of human origin

Many investigators are interested mainly in human disease but lack access to fresh human kidney tissue suitable for harvesting primary cultures. In this regard,

optimally differentiated *human* renal epithelial cell lines with extended *in vitro* growth potential would seem an ideal alternative. Nevertheless, to date there have been no reports on the use of continuous human renal cell lines to investigate site-specific renal toxicity and/or other site-specific renal processes, although there are several reports of primary human cells being used for such studies [144]. This is likely due the low number of more-or-less well-differentiated human immortalized cell lines and/or renal carcinoma cell lines that have been developed to date [148, 149]: several human renal adenocarcinoma cell lines were found to show very restricted expression of proximal tubular, even epithelial, tubular differentiation antigens [148, 149]. Human immortalized renal cell lines expressing a number of differentiated features of the proximal tubule were developed by Ryan et al. [150] (HK-2), Racusen et al. [64] (HKC 5/8/11) and Nanus and co-workers [151] (PT-KI, PT-HA]). HK-2 and HKC cell lines were shown to maintain epithelial morphology, monolayer growth pattern and Na^+-dependent glucose transport. HK-2 and HKC cells show moderate expression of the proximal brush-border enzymes γ-GT and AP (although substantially weaker when compared to primary proximal tubular cell cultures following several passages). HK-2 cells retained weak responsiveness to stimulation with parathyroid hormone (PTH); hormonal responsiveness was not studied in HKC cells. The only information available on the cell lines developed by Nanus et al. [151] is that they express a limited number of brush-border enzymes/antigens. To date no immortalized human distal tubule or collecting duct cell lines have been developed.

In summary, at present there is still a lack of well characterized and well differentiated cell lines of human origin representative of the different cell-types present in the nephron and the collecting duct system. However, once available, human immortalized cell lines of defined nephron segment origin will likely provide a welcome alternative to primary cultures for studying cell properties *in vitro* in a cell-type dependent way.

In vitro nephrotoxicity study variables

Culture medium composition

Since cell cultures were originally developed for the propagation of viruses needed for vaccine development, almost all of the established cell-culture methodologies, especially with respect to culture nutrients, are designed primarily for the selection of proliferating cells. This remains true for cell cultures used to study membrane traffic, gene expression, transport, membrane electrophysiological properties, and intracellular signal cascades. The efforts undertaken to create culture media for the generation of non-proliferating, but differentiated, cells have been limited to the development of serum-free media supplemented with certain hormones, growth factors or chemicals [152]; in most of the trials, no changes with respect to the basal composition of the media have been made.

Very few investigations define the physical conditions of medium application, culture medium volumes, growth support for cells, etc. It has recently been shown for renal epithelial cells in conventional static cultures that medium volumes markedly affect cell metabolism [153]. Distinct changes in medium composition have been shown to produce new cell phenotypes. For example, the transient omission of glucose, but maintenance of carbon sources for nucleoside synthesis (for example, uridine or pyruvate) led to a selection of gluconeogenic phenotypes in the renal LLC-PK_1 and OK cell lines [154, 155]. Regrowth in the presence of glucose demonstrated constitutive expression of the formerly missing gluconeogenic key enzymes, fructose-1, 6-biphosphatase and phosphenol pyruvate carboxykinase (PEPCK) [156].

Static versus non static cultures

Static cultures

Static cell cultures represent the classical approach in cell-culture technology. Primary cells or cell lines are seeded onto solid surfaces or growth supports made of a variety of plastic materials (polystyrene, polycarbonate, PTFE (polymeric tetrafluoroethylene - TEFLON®), PTX (polyester) or glass. The surfaces are usually hydrophobic and can be manipulated with regard to their surface charges by specific pretreatment. The surfaces can also be coated with extracellular matrix materials such as collagen, fibronectin or laminin or the matrix material, Matrigel™. It should be noted that complex matrix materials, such as Matrigel, could contain varying amounts of impurities in the form of growth factors or undefined autocrine or paracrine sig-

nal substances, which could affect the functional differentiation [159]. Epithelial cells can also be grown on microporous substrates, with nutrients supplied from both the apical and basolateral sides.

Growth substrate characteristics influence epithelial cell orientation and phenotypic expression due to the interaction of receptor sites on the cell surface with specific sites in the substrate/matrix. For example, growth of the Xenopus laevis kidney cell line A6 on microporous substrates could induce the expression of vasopressin receptors absent in solid support grown monolayers [157]. Similar observations have been reported when rabbit primary cultures of renal cortical collecting ducts were maintained on microporous supports under constant medium perfusion [158].

The major disadvantage of static cultures is that the medium composition is continuously modified by the cells, and needs replenishment at defined intervals. Furthermore, the access of medium to the growth-support side of the cell is not the same as that *in vivo,* and may therefore affect the cell phenotype [116]. In addition, gaseous exchange (CO_2, O_2) is affected. The latter can be improved by using oxygen-permeable and/or carbon dioxide-permeable thin plastic supports (such as Permanox®). Nutrient access to cells is improved by using microporous growth supports [160]. Microscopic examination of cultures may sometimes be problematic, since not all growth supports are readily transparent. Appropriate, but not always easy to perform, fluorescent microscopy techniques must be applied in these situations.

Non-static cultures

The most widely used non-static culture systems are roller and micro-carrier cultures. In roller cultures, if cells are adhesive, they will gradually attach to the inner surfaces of culture bottles and grow to form a monolayer. This system has three major advantages over static monolayer culture: a greater surface area; a constant, but gentle agitation of the medium; and an increased ratio of medium surface area to volume, allowing enhanced gaseous exchange to take place through the thin film of medium over the cells not actually submerged. The disadvantages are the difficulty of examining cultures microscopically, and the access of the medium to the cells from only one surface. In rocking or shaking cultures, the culture dishes are tilted slightly at a defined frequency. This exerts some mechanical stress on the monolayers and improves the gaseous exchange in a way similar to that which occurs with roller cultures [161]. In micro-carrier culture, monolayer cells are grown on plastic microbeads of approximately 100µm diameter and made of polystyrene, sephadex, polyacrylamide, collagen, or gelatin [162]. This culture system increases the maximum ratio of surface area to medium volume, and has the additional advantage that cells can be treated in suspension. An advantage of this technique would be the possibility of renewal of the medium at a constant rate.

In the perfusion culture technique, because the supply of medium and gaseous exchange become limiting at higher cell densities [163], a combination of microporous growth support and continuous replenishment of the medium was developed, originally based on the use of hollow microporous plastic fibers, maintained in a container permanently perfused with culture medium [164]. For this reason, several modifications have been developed, [158, 163-165]. The Minucell™ system, for example, combines the advantage of filter inserts with constant replenishment of the medium. Multiple filter inserts can be placed in a perfusion chamber. The inserts can even be supplied with different cell types (co-cultures), if the cultures can be maintained in the same culture medium. A modification of this system allowed, for the first time, the use of "gradient cultures" [158]. Here, barrier-forming, confluent monolayers, grown on a special filter insert sealed against the perfusion chamber by O-rings, are perfused with media of various compositions on the apical and basolateral side of the epithelial monolayers. Disadvantages of this system include an imperfect gas exchange within the chamber, as well as the fairly small growth surface area for cells, which makes the assessment of biochemical and molecular biology parameters difficult. The increased technical complexity of culturing cells under continuous perfusion precludes the possibility of this technique becoming routine at present.

Endpoints for *in vitro* nephrotoxicity

The precise knowledge of the physiological and biochemical properties of the *in vitro* models used and their monitoring during culture is mandatory for the selection of biologically relevant endpoints for *in vitro* nephrotoxicity studies. Marked alterations of these prede-

termined properties are indicative of toxicity. Examples of endpoints used to monitor the toxic effect on specific cellular functions are given in table 4.

Contribution of established renal cell culture models to the understanding of nephrotoxic mechanisms

In this section we provide a short review on the contribution of renal cell culture systems to the discovery of the mechanisms of the most well known nephrotoxins.

Cyclosporine A

The immunosuppressive drug cyclosporine A (CSA) has revolutionized transplant medicine. However, CSA induced-nephrotoxicity still represents a major therapeutic challenge. Chronic CSA nephropathy is characterized by a decrease in glomerular filtration rate (GFR), tubular atrophy, interstitial fibrosis and progressive renal dysfunction. It is difficult to delineate the mechanisms of CSA toxicity from clinical data since the majority of clinical experiences with CSA have been in renal transplant recipients. Animal models of CSA nephropathy have brought some insights, how-

Table 4. Endpoints for assessment of nephrotoxicity on renal cell culture systems.

Cell activity modulated	Type of modulation	Detectable changes via
Gene expression	Switching on of new genes	mRNA patterns
	DNA synthesis	^{3}H-thymidine incorporation
	Modulation of transcription	e.g. Kinases, NF Kappa B and various other regulators of transcription
	mRNA stability	Gel-electrophoresis (ethidium bromide)
Protein expression	Production	Rates of protein synthesis (^{14}C-leucine incorporation), specific protein expression and/or release (western blot, enzyme immuno assays)
	Degradation	Ubiquitinylation, myristilation, hydrolysis
Membrane activity	Ion pumps	Membrane potential, Na^+-K^+-ATPase activity, intracellular ion concentrations
	Membrane integrity	Leaking of cellular constituents (e.g. LDH) and trypan blue exclusion. Brush border integrity by loss of brush border enzyme (AP, γ-GT etc.).
	Transport processes	Transport of solutes (e.g. glucose) and water, volume regulatory properties, endo- and exocytosis
	Integrity of cell - cell interaction (junctional complexes)	Dome formation (only for solid supports), transepithelial resistance and paracellular permeability and modulation of junctional proteins (occludin, cadherin and catenin)
Energy production	Respiration rate	Oxygen consumption linked to ATP production, glucose and amino acid consumption, succinate dehydrogenase levels, mitochondrial membrane potential (fluorescent probes).
	Metabolism	Lactate/pyruvate ratio.
	REDOX potential	MTT assay
	Oxidative stress	GSH-GSSG ratio, generation of free radical species, lipid peroxidation products and DNA strand breaks
Cell cycle	Rate of Apoptosis / cell and population turn over	Caspase 3 upregulation, cyclin A levels, P53 level, Fas ligand expression, cell size and granularity (flow cytometric analysis), annexin V binding, DNA fragmentation, nuclei condensation
Morphological changes / Motility changes	Cytoskeleton, motor proteins	Light and electron microscopy, cell size and shape, vacuolization, actin, microtubule and microfilament levels and organization, cell contraction (plane cell surface area).

ever *in vitro* cell culture techniques allow the direct determination of toxicity at a cellular and molecular level, thus allowing dissection of the effects of CSA.

CSA causes a dose and time dependent increase in contractility of *cultured mesangial cells* [166, 167], as measured by changes in planar cell surface area (PCSA). Since mesangial contractility, (contributing to the ultrafiltration coefficient (K_f)) is a major effector in decreased glomerular filtration rate, mesangial cell contraction is a particularly useful endpoint in the elucidation of mediators involved in this response. A number of factors have been shown *in vitro* to attenuate CSA induced increase in mesangial cell contraction including, platelet activating factor antagonists [168], the calcium channel blocker verapamil and anti-endothelin antibodies [167]. CsA, also inhibited both basal and induced PGE2 synthesis in cultured rat mesangial cells [169] and increased expression of TGF β and its receptors (Type I and II) [169a]. Interestingly, CSA causes increased matrix accumulation in cultured mesangial cells isolated from mice susceptible to glomerulosclerosis, whereas in cultured mesangial cells from mice resistant to glomerulosclerosis CSA had no effect [170]. This observation suggests that genetical background may play a role in CSA-induced glomerular lesions.

The porcine *proximal tubule-like cell line LLC-PK_1*, has been the most widely utilized cell type in the study of the direct tubular effects of CSA. CSA induces direct toxicity to LLC-PK_1 cells, manifested by an increase in cell vacuolization, a decrease in cell proliferation [171] and a dose dependent decrease in overall cellular viability as measured by MTT assay and loss of membrane integrity. Such gross cell damage, with loss of membrane integrity (increased LDH release and decreases in trypan blue exclusion), is indicative of necrosis [172].

Low doses of CSA (nM concentrations) have been shown to induce apoptotic cell death in LLC-PK_1 cells as evidenced by a decrease in cell size and an increase in cell granularity (flow cytometric analysis), externalization of phosphatidylserine (FITC-annexin V binding), DNA fragmentation (TUNEL assay) and increases in Fas ligand (APO-1/CD95) expression [172]. CSA has also been shown to increase the tumor suppressor p53 in LLC-PK_1 cells with associated cell cycle arrest [173]. p53 is also a transcriptional modulator of the *bax* gene, which promotes apoptosis [174]. Apoptotic cell death of renal tubular epithelial cells (including DNA fragmentation, caspase 3 induction, increased p53 and bax expression, Fas ligand upregulation and decreases in Bcl-2 expression) has been associated with interstitial fibrosis in animal models of chronic CSA toxicity [175, 176]. However, in primary human proximal tubular cells no evidence of CsA induced apoptotic cell death could be demonstrated [176a].

CSA causes an inhibition of glucose uptake by LLC-PK_1 cells, which may be representative of glycosuria observed clinically in CSA treated renal transplant recipients [179, 180].

LLC-PK_1 cells also respond to CSA by increasing the synthesis and release of endothelin [181], and by increasing the activity of the basolateral Na-K ATPase [182]. These observations may implicate renal tubular cells themselves in the further contribution to CSA-induced alterations in renal and systemic hemodynamics.

CSA has also been shown to cause toxico-tolerance *in vitro*. CsA, which is transported via the P-glycoprotein, increases the expression of P-glycoprotein in rat proximal tubular cells [183] and in the human proximal tubular cell line HK-2 [183a]. CSA also induces the expression of HSP 70 in LLC-PK_1 cells, which increases tolerance to subsequent exposure to CSA [184].

Walker et al. demonstrated that streptomycin (a usual antibiotic additive to cell culture media) has synergistic effects on CSA toxicity to LLC-PK_1 cells [185]. Since that publication most investigators have conducted CSA experiments without antibiotics. This represents a good example that the utmost of care must be taken to exclude the possibility of interfering substances such as growth hormones, serum supplements and antibiotics, when conducting toxicity studies *in vitro*.

Johnson et al. used *human proximal tubular cells* (HPT) and *renal cortical fibroblasts* as a model of the tubulointerstitium to investigate CSA-induced fibrosis. CSA resulted in a suppression of matrix metalloproteinase activity and an increase in fibroblast collagen synthesis. CSA also induced the secretion of insulin like growth factor from the fibroblast cells and transforming growth factor β-1 (TGFβ-1) from the HPT cells, which preceded fibroblast collagen synthesis [177]. In a more recent study they could demonstrate that the angiotensin-converting enzyme (ACE) inhibitor, enalaprilat (the active form of the ACE inhibitor enalapril), could prevent CSA-induced collagen pro-

duction from fibroblast cells and CSA-induced TGFβ-1 production from HPT cells. However, enalaprilat failed to protect HPT from direct CSA cytotoxicity (decreased cell viability, decreased cell proliferation and inhibition of the sodium hydrogen exchanger) [178].

Cisplatin

Cisplatin is a widely used drug in the effective treatment of a number of human carcinomas [186]. However, cisplatin is also a nephrotoxic agent primarily damaging the epithelial cells of the S3 segment of the proximal tubule [187]. In primary *rabbit proximal tubular* (RPT) *cells* cisplatin exposure resulted in an inhibition of DNA synthesis, which is related to the primary anti-tumorigenic mechanism of this compound i.e. DNA inter and intra strand cross linking [188, 189]. RNA and protein synthesis were decreased in RPT cells and quiescent LLC-PK_1 cells upon cisplatin exposure [188, 190]. Other effects of cisplatin on cultured RPT include a decrease in glucose uptake, an inhibition of Na^+-K^+ ATPase and alterations in total glutathione content [189].

In the normal *rat kidney (NRK) cell line* cisplatin (1μM for 48h) induced a marked increase in the level of lipid peroxides [191].

Cisplatin is taken up preferentially by the basolateral membrane of microporous grown *LLC-PK_1 cells* and *opossum kidney (OK) cells* [192, 193]. Furthermore, cisplatin applied basolaterally was more toxic than apical exposure in LLC-PK_1 cells [192]. These studies suggest that tubular excretion of cisplatin is dominant over tubular reabsorption.

Cisplatin is a potent inducer of apoptosis in various proximal tubular cell models. In primary mouse proximal tubular cell cultures [193a] and LLC-PK_1 cells [194], high doses of cisplatin (mM) resulted in necrosis whereas low doses (μM) caused apoptotic cell death. In mouse proximal tubular cells and normal rat kidney epithelial cells (NRK52E), cisplatin induced an increase in Fas, Fas ligand and TNF α mRNAs [194a]. Cisplatin induced apoptosis in LLC-PK1 cells is brought about via activation and mitochondrial translocation of the pro-apoptopic molecule Bax, which leads to release of cytochrome C into the cytosol and activation of caspase 9 [194b]. The caspase 9 inhibitor LEHD-CHO could prevent cisplatin induced apoptosis in LLC-PK_1 cells whereas the caspase 8 inhibitor IETD-fmk did not [194b]. Cisplatin induced apoptosis could also be inhibited by overexpresssion of crm A (a suppressor of the interleukin-1β converting enzyme family) and by over expression of bcl-2 in immortalized mouse S3 cells [195].

Van de Water et al. studied the effect of focal adhesion kinase (FAK) on chemically induced apoptosis in *LLC-PK_1 cells*. LLC-PK_1 were stably transfected with deletion mutations of FAK (including, focal adhesion targeting domain (FAT)), which compete with endogenous FAK for localization to focal adhesions. FAT overexpression significantly increased apoptosis in dichlorovinylcysteine (DCVC) treated cells but not in cisplatin treated cells [196]. In other words, FAK protected against DCVC (also a potent acute nephrotoxin), but failed to protect against cisplatin-induced apoptosis, suggesting that FAK is not involved in cisplatin-induced apoptosis.

Iron chelators (including desferrioxamine) and hydroxyl radical scavengers (dimethyl sulfoxide, mannitol and benzoic acid) reduce the cytotoxic effects of cisplatin in LLC-PK_1 cells and reduce acute renal failure in rats [197]. It was therefore postulated that iron has a critical role in hydroxyl radical mediated cisplatin-induced nephrotoxicity. In a follow up study these investigators could show that inhibition of cytochrome P-450 (with cimetidine or piperonyl butoxide) prevented the cisplatin-induced increase in catalytic iron content and nephrotoxicity (both in LLC-PK_1 cells and *in vivo* in rats) [198]. They conclude that cytochrome P-450 is a significant source of catalytic iron capable of catalyzing free radical reactions in cisplatin induced injury, and thus cytochrome P-450 inhibitors may have clinical benefits in the prevention of cisplatin-induced nephrotoxicity. In a different study green tea tannin was shown to dose dependently protect against cisplatin-induced damage to LLC-PK_1 cells and nephrotoxicity to rats. This protective effect of green tea tannin was thought to be due to decreasing oxidative stress [199].

It should be noted that cisplatin appears to inhibit the activity of LDH [200]. Thus, the use of this enzyme as a marker of cisplatin-induced toxicity should be controlled appropriately. The possible interference of test compounds with toxicity assays is not always carefully controlled, but is a prerequisite for accurate scientific evaluation.

Aminoglycosides

Aminoglycosides are antibiotics particularly active against aerobic gram-negative bacteria and certain gram-positive organisms. Aminoglycosides are used in therapy of severe infections of abdominal organs, endocarditis or sepsis. However, the clinical use is limited by severe toxic effects to the kidney and inner ear. The mechanism of action of these antibiotics was thought to be the blockade of bacterial ribosomal protein biosynthesis. Recent studies, however, show that cationic antibiotic molecules create fissures in the outer cell membrane, resulting in the leakage of intracellular contents [201].

Gentamicin was found to induce an activation of *cultured mesangial cells*, as measured by contraction (PCSA) and proliferation. Since gentamicin increases the expression of inducible nitric oxide (iNOS) in these cells [202] and has been shown to elevate intracellular Ca^{2+} (via influx, and release from internal stores) [203], it is postulated that nitric oxide-induced Ca^{2+} elevation might be responsible for the observed effect. These results are in support of a mesangial cell role in the reduction of glomerular filtration rate after aminoglycoside intoxication [204, 205].

In *LLC-PK_1 cells* gentamicin induces membrane damage as shown by the loss of specific membrane enzymes (γ-glutamyl transpeptidase, alkaline phosphatase and aminopeptidase), a decrease of the lysosomal enzyme N-acetyl-β-D-glucosaminidase, an inhibition of apical Na^+-dependent glucose transporter and the basolateral Na-K-ATPase pump as well as a decrease in dome formation [206, 207]. Furthermore gentamicin results in a dose dependent decrease in intracellular ATP and cAMP [207, 208].

Chronic gentamicin exposure of LLC-PK_1 cells (10 mM gentamicin for 15 days) resulted in an increase in cell granularity and a decrease in cell size (flow cytometric analysis), implicating enhanced rates of apoptotic cell death. Interestingly, this effect was not paralleled by an increase in Fas ligand expression [209]. LLC-PK_1 cells over expressing the anti-apoptotic protein bcl-2 were protected from gentamicin-induced apoptosis [210].

LLC-PK_1 cells appear to be an acceptable model for the study of aminoglycoside toxicity due to the following observations: (1) A recent study confirmed *in vivo* observations that gentamicin was more toxic to LLC-PK_1 monolayers when exposed at the apical side, which was due to increased apical uptake [211]. (2) Furosemide when given in combination with gentamicin resulted in an amplification of cytotoxicity to these cells [212]. This phenomenon has also been observed clinically [213]. (3) Hori et al. [207] could show that LLC-PK_1 cells have the following sensitivities for aminoglycosides with respect to decreases in frequency of dome formation and an increased number of floating cells, neomycin > gentamicin > amikacin, tobramycin. Thus using these parameters LLC-PK_1 sensitivities to aminoglycosides reflects the *in vivo* situation.

Cephalosporins

Cephalosporins are a family of β-lactam-antibiotics, which are effective bactericidal therapeutics for infections of the bloodstream, skin, respiratory and urinary tract.

LLC-PK_1 cells lack an organic anion transporter, which is thought to be responsible for cephalosporin uptake by proximal tubular cells *in vivo*, however cephalosporin toxicity in these cells mimics closely *in vivo* observations. Kiyomiya et al. have demonstrated that in LLC-PK_1 cells, the cytotoxic effect of cephalosporins is due to a decreased activity of cytochrome C oxidase in the mitochondria causing decreases in intracellular ATP content and consequent increases in hydrogen peroxide and lipid peroxide levels [214, 214a]. Cephaloridine, ceftazidime and cefotaxime, were shown to cause direct toxicity to LLC-PK_1 monolayers, evaluated by enzyme release (brush border enzymes AP and γ-GT, cytosolic enzyme LDH and the mitochondrial enzyme glutamate dehydrogenase (GLDH)). In addition marked morphological damage was observed at both light and electron microscopic levels [215]. A decrease in transepithelial resistance (TER) was observed previous to other changes, demonstrating that TER is a highly sensitive endpoint for LLC-PK_1 toxicity [215].

Amphotericin B

Amphotericin B (AmB) a polyene macrolide antibiotic with strong activity against a broad spectrum of fungal infections has long been identified as nephrotoxic. The toxic mechanism is assumed to be that binding of AmB to sterols in the cell membrane results in the formation of aqueous pores, which leads to a disregulation of volume and ion concentrations within the cells [216]. However, Hsu et al. postulated by using

the patch-clamp technique that AmB, at least in *MDCK cells*, disturbs the normal ion channel function rather than forming pores in the cell membrane [217].

Amp B results in *mesangial cell* contraction *in vitro*. This effect was related to a Ca^{2+} entry from the extracellular space via calcium channels [218]. In LLC-PK_1 and renal medullary interstitial cells AmB, in therapeutic doses, induced apoptosis, which could be prevented by recombinant human insulin-like-growth-factor-1 (IGF-1). Similar results were observed in rats [219]. In a mechanistic study it could be shown that Amp B bound to high-density lipoprotein (HDL) was less toxic to LLC-PK_1 cells than AmB alone or AmB bound to low-density lipoprotein (LDL). This result was thought to be related to the absence of HDL receptors in LLC-PK_1 cells [220, 221]. The antifungal activity of AmB was not altered when associated to HDL or LDL. These results are of relevance for therapeutic applications [220].

Cadmium

Cadmium (Cd), an extremely toxic metal used in industry as an anti-corrosive agent, is found in food and cigarettes. The most serious consequence of chronic Cd poisoning is lung- and prostate cancer but the first effect during chronic intake is kidney damage, manifested by marked proteinuria. Under chronic exposure, cadmium is primarily taken up by the liver, where it induces synthesis of metallothionein (MT) and induces formation of cadmium-metallothionein (CdMT) complexes. Hepatic CdMT reaching the kidneys causes proximal tubular damage, which has been shown in animal studies to be due to apoptotic cell death [222].

Cd has been shown to be a specific inducer of c-fos in *mesangial cells* through activation of Erk kinase, protein kinase C and stress-activated protein kinase (SAPK) pathways [223, 224] and may therefore also play a carcinogenic role in the kidney.

Although Cd can also induce apoptosis in *LLC-PK_1 cells*, it appears that CdMT does not [225]. It is speculated that there is a down regulation of CdMT transport systems in both LLC-PK_1 cells and primary rat proximal tubular cells [226]. Thus, LLC-PK_1 cells are not a representative model for *in vivo* CdMT exposure.

However, inorganic Cd has numerous deleterious effects on renal epithelial cells. $CdCl_2$ exposure caused a disruption in the cadherin-catenin complex resulting in reduced trans-epithelial resistance and produced alterations in the actin cytoskeleton in LLC-PK_1 cells and MDCK cells [227, 227a]. Both LLC-PK_1 cells and MDCK cells take up $CdCl_2$ preferentially from the basolateral side and thus $CdCl_2$ is more toxic to these cultures when applied basolaterally [228, 227]. $CdCl_2$ also resulted in an early decrease in mitochondrial membrane potential and an increase in cytoplasmic Ca^{2+} in LLC-PK_1 cells, MDCK cells and OK cells [228a].

Mercury

Mercury is an industrial pollutant, which can contaminate food (e.g. fish and grain) and water sources [229]. The nephrotoxic potential of mercury is related to its accumulation in the proximal tubule region and the intracellular binding to several functional groups, which results in inactivation of different enzymes and inhibition of protein synthesis [230]. Inorganic mercury *in vivo* accumulates to a greater extent within the kidney than methyl mercury [231]. However, in cell culture experiments on rabbit renal proximal tubule, LLC-PK_1, MDCK and human proximal tubule cells (HPT) the opposite was shown, possibly due to the binding of inorganic mercury to synthesized metallothionein and the resulting protection of the cell [232]. Another protective mechanism appeared to be glutathione conjugation [232]. Of all cell types utilized LLC-PK_1 cells react most sensitive to exposure [231]. Additionally, in LLC-PK_1 cells, mercury chloride exposure caused elevated c-fos mRNA levels [233] and increased apoptosis [234]. In a study using normal rat kidney epithelial cells (NRK52E) it is postulated that Hg^{2+} enhances the sensitivity of kidney cells to apoptotic stimuli as a consequence of inhibition of NF-kappaB activity [234a].

Mycotoxins

Mycotoxins are defined as mould derived secondary metabolites, Ochratoxin A and aflatoxin B being the most widely studied. Ochratoxin A (OTA), produced by *Aspergillus ochraeus* and *Penicillium verrucosum*, can be found as a contaminant in grain, beer, coffee and meat. OTA is nephrotoxic, carcinogenic and genotoxic [235].

Nanomolar concentrations of OTA resulted in the dedifferentiation of an MDCK clone (MDCK-C7), i.e. a distinct morphology from the parent cell line (spindle-shape, pleiomorphic, narrow intercellular spaces, increased cell size) and a reduced proliferation rate and numerical chromosomal aberrations [236]. Further

studies could demonstrate that OTA exposure resulted in apoptosis, which was associated with induced c-jun amino-terminal-kinase (JNK) activation. These effects occurred at dose levels where no inducing signs of acute toxicity were seen (increase in LDH release, decrease in total protein) [237].

By utilizing horseradish-peroxidase-labeled OTA in western blots Schwerdt et al. [238] could show that in different renal cell lines (MDCK-C11, OK, LLC-PK_1 and immortalized human kidney epithelial cells (IHKE)) OTA binds directly to certain proteins. Thus OTA-protein binding damages normal protein function. Moreover, such binding results in a further accumulation of OTA [237].

Aflatoxin B1 (AFB1) is produced by such fungi as *Aspergillus flavus, niger* and *parasiticus.* AFB1 is known to induce liver cancer by causing a point mutation on the p53 gene [239, 240]. AFB1 is also a nephrotoxic agent. It is found mostly on stored nuts, sweet corn and bacon. It is worth mentioning that these mycotoxins are not eradicated by cooking.

Aflatoxin B leads to condensation of nuclei, separation of nuclei from the cytoplasm, cytoplasmic vacuolization, and loss of the brush border in MDBK (Madin-Darby bovine kidney) and PFBK cells (primary fetal bovine kidney) evidenced by electron microscopic examination [241]. In addition exposure of OK cells to Aflatoxin B resulted in an inhibition of inorganic phosphate uptake, which could not be abolished by application of parathyroid hormone (PTH) and insulin [221].

Hemoglobin and myoglobin

Hemoglobin and myoglobin are good examples of endogenous nephrotoxic substances. Hemoglobin (Hb), responsible for the transport and delivery of oxygen within the body, is composed of the prosthetic iron containing heme group and four globin molecules. Myoglobin (consisting of one globin molecule with a prosthetic group), is an oxygen store for the muscle. When hemoglobin as well as myoglobin are released into the extracellular compartment in large amounts due to pathological events (hemolysis, rhabdomyolysis) both will severely injure the kidney and may even lead to acute renal failure [242]. Several potential mechanisms of toxicity have been proposed for these compounds such as renal epithelial cell damage by iron-induced free oxidant injury [243, 244] and ischemic injury due to heme pigment-induced vasoconstriction [245].

In a rat model of hemolysis (by glycerol injection) iron released from hemoglobin resulted in hydroxyl radical formation, lipid peroxidation and renal dysfunction. Desferrioxamine (DFO), which binds free iron could protect against injury [243]. LLC-PK_1, OK and NHK-C cells respond to injury mediated by reactive oxygen molecules with an early decline in ATP levels and a late response consisting of cell detachment and cell lytic injury. Scavengers of hydroxyl radicals and iron chelators prevented these alterations [246].

A 24-hour exposure of the HK-2 cell line to myoglobin suppressed cell proliferation and resulted in DNA strand breaks and suppression of protein synthesis. DFO reduced myoglobin-induced cell death and also induced a growth suppressive effect [247]. In rats, as well as in OK cells, polymerized Hb solution increased heme oxygenase (HO) activity. Inhibition of HO enzyme activity by cimetidine did not change the grade of renal injury seen with Hb infusion alone, which indicates that Hb-evoked renal injury is not HO-dependent [248]. Mitochondrial and nuclear damage induced by myolysis in rats and verified by electron microscopy and TUNEL technique, could not be observed in MDCK and LLC-PK_1 cells [249]. Exogenous glutathione (GSH) resulted in increased myoglobin toxicity in HK-2 cells. Intracellular GSH depletion prevented this action [250]. Although oxygen-free-radical-induced renal damage remains a controversial topic, such studies provide further insight into the understanding of hemo/myoglobin induced renal damage.

In summary

It is clear from the above studies that *in vitro* renal cell culture can be used successfully to study the mechanisms of cell modulation by toxic compounds. Such systems allow a simple but sophisticated approach to the development of strategies to overcome nephrotoxicity of many important drugs. Once strategies are developed however they still need to be studied in relevant animal models.

Future requirements to study nephrotoxicity *in vitro*

Renal cell cultures have an unexploited potential in the screening and evaluation of possible nephrotox-

ins. These systems are theoretically suited not only to short term studies but also to long term exposures and thus may be useful in the screening of compounds on a chronic basis. Predictive models of chronic renal toxicity would be a major development in the assessment of human risk to a whole range of environmental, therapeutic and industrial compounds. However, if this is to be achieved successfully a number of requirements must be sought.

There is general agreement that all culture systems used for clinical risk assessment, especially when testing for long-term effects, should preferably be of human origin. Although this could be achieved by the use of primary cultures, their establishment is constantly hampered by the restriction of the availability of samples (usually from surgical sources), by the fact that life-span of cultures is limited, and because such cultures have a limited capability to be passaged. Furthermore, it has to be kept in mind that cell phenotypes can change rapidly, depending on the culture conditions. For this reason, more surrogate cell lines of human origin are needed, and those that are currently available need further characterization (e.g. the human proximal tubule, HK-2 cell line).

New cell culture techniques, which may improve the applicability of renal epithelial cultures, are also required. Currently there exist two commercially available cell culture perfusion systems, which allow the continuous perfusion of culture media and optimized oxygenation [165]. These systems allow stable long-term culture of quiescent adherent cells [158]. Continuous medium perfusion furthermore may lead to the re-expression of lost functions in continuous cell lines and the maintenance of differentiated properties in primary cells. Recently our laboratory has demonstrated that LLC-PK_1 cells maintained in a newly developed perfusion system (EpiFlow®) changed from a glycolytic to a more oxidative phenotype [251].

Evidence is also available from pilot experiments in our laboratory that this mode of cultivation helps to prolong the lifetime of primary cultures of proximal tubular cells. Combining perfusion culture with co-culture of a cell type that is an anatomical neighbor *in vivo* (e.g. epithelial with endothelial interstitial or immune cells) may improve the state of differentiation of both partner cells and increase the complexity of autacoid interaction.

The establishment of more organotypic types of renal cell cultures (perfusion and co-cultures) will in the future hopefully allow for long term testing of nephrotoxins. Co-culturing renal epithelial or mesangial cells with either endothelial and/or immune cells under perfusion conditions would allow testing of important signal molecules [252] such as therapeutic cytokines (interferons, interleukins, immune cell growth factors) and biotechnologically produced by industry. Due to the high species specificity of these latter compounds *in vitro* test systems based on human cells (primary cultures or cell lines) represent the only possibility to judge toxic side effects and therapeutic risks.

Additionally, efforts need to be invested to establish culture medium formulations, which are designed to maintain differentiated, quiescent cultures. At present this criteria are best, although still insufficiently, met by serum free hormonally defined media.

The elegant and sophisticated methodology introduced by Helbert [43, 69, 253], the combination of immunoselection of renal cells, with improved culture technologies regarding media-composition and media-application may help to establish long living highly differentiated "homogeneous" primary cultures of the various nephron cell types.

The utilization of renal cell culture techniques will gain added importance in the future for screening newly synthesized drugs or environmental contaminants for adverse effects to the kidney, or to investigate mechanistic aspects leading to renal cell injury. Especially with respect to the latter, renal cultures offer the possibility of easy access to the object of interest. Cell lines can be provided in nearly unlimited amounts, and they match reasonably well their site of nephron origin. In this context continuous renal cell lines represent the current experimental system of choice. They are easy to grow, maintain and handle, they are commercially available (e.g. from the American Type Culture Collection) and retain most of the basic functions of their ancestor cell, at least in case of permanent proximal and collecting duct cells (LLC-PK_1, OK, JTC-12, HK-2, MDCK, A6). Another advantage is the enormous amount of information about culture conditions and differentiated functions, metabolism, transport, and hormone responsiveness, available from the literature [37, 134]. The disadvantage is the fact that they may suffer from loss of the one or more *in vivo et situ* functions as a result of prolonged cultivation. Under these circumstances, if the lost function

is the predominant target for a nephrotoxic xenobiotic under investigation, a more laborious and difficult primary cultures must be selected. In addition one must keep in mind that continuous cell lines are heterogeneous, and multiple cell types might be present in an uncloned wild type culture.

It is desirable that methods should be developed to re-express the "lost functions" or to tailor new cell lines more closely matching the cell type of origin in continuous cell lines. Such an enterprise may include several already available cell biology techniques. The simplest approach could be adaptation to culture conditions that more closely resemble the *in vivo* environment of the respective cell type. As already mentioned, omittance or drastic reduction of glucose and replacement against pyruvate in the media used for cultivation of LLC-PK_1 cells enables re-expression of gluconeogenesis [155]. Fusion of cells of continuous cell lines [254] stemming from the same *in vivo* ancestor cell of different species and with different retention of cellular functions, i.e. metabolic pathways, transport systems or hormone receptors, may be used to establish new continuous lines more closely resembling the characteristics of the cell type of origin. Fusion of cells from primary cultures with cells from continuous lines delineated from the same nephron cell or nephron segment may help to either immortalize the respective primary culture or help to re-express lost functions within the continuous cell type. Genetic approaches [135] may deliver another route to tailor new, more "natural" permanent cell lines. Transfection with different but defined genes will be one of the important tools [255]. These strategies should even offer the possibility to establish cell lines expressing most or all of the functions of human renal cells. However, all these trials demand careful selection procedures to isolate the fused hybridoma, the mutated or transfected cells.

Last but not least there is urgent need to "harmonize" or "standardize" all these procedures so that an interlaboratory comparisons can be achieved. Such procedures include, cell isolation, growth substrates, cell culture media including the mode of medium application. A first initiative in this direction has been taken by ECVAM (European Center for the Validation of Alternative Methods, a section of the European Commission Institute for Health an Consumer Protection) by founding a task force dealing wit the creation of guidelines for "Good Cell Culture Practice".

References

1. Commission of the European Communities/International Programme on Chemical Safety. Health signficiance and early detection of nephrotoxicity. In: Proc. of the joint CEC/IPCS Workshop. Luxembourg, 26- 29 April 1988. Toxicol Lett 1989; 46: 1-306.
2. Ullrich KJ, Rumrich G, Gemborys MW, Dekant W. Renal transport and nephrotoxicity. In: Nephrotoxicity. Mechanisms, early diagnosis and therapeutic management. Bach PH, Gregg NJ, Wilks MF, Delacruz L (editor). Marcel Decker, Inc., New York, Basel, Hong Kong 1991; p. 1-8.
3. Morel F, Doucet A. Hormonal control of kidney functions at the cell level. Physiol Rev 1986; 66: 377-468.
4. Guder WG, Ross BD. Enzyme distribution along the nephron. Kidney Int 1984; 26: 101-111.
5. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. Biochem Pharmacol 1984; 33: 1801-1807.
6. European Economic Community: Council Directive 86/609. On the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other purposes. Off J Eur Commun 1986; L352: 1-29.
7. Stokes WS, Marafante E. Introduction and summary of the 13th meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): alternative testing methodologies. Environ Health Perspect 1998; 106 (Suppl 2): 405-412.
8. Webster WS, Brown-Woodman PD, Ritchie HE. A review of the contribution of whole embryo culture to the determination of hazard and risk in teratogenicity testing. Int J Dev Biol 1997; 41: 329-335.
9. Ashby J, Kier L, Wilson AG, Green T, Lefevre PA, Tinwell H, Willis GA, Heydens WF, Clapp MJ. Evaluation of the potential carcinogenicity and genetic toxicity to humans of the herbicide acetochlor. Hum Exp Toxicol 1996; 15: 702-735.
10. Green T, Lee R, Moore RB, Ashby J, Willis GA, Lund VJ, Clapp MJ. Acetochlor-induced rat nasal tumors: further studies on the mode of action and relevance to humans. Regul Toxicol Pharmacol 2000; 32: 127-133.
11. Hengstler JG, Van der Burg B, Steinberg P, Oesch F. Interspecies differences in cancer susceptibility and toxicity. Drug Metab Rev 1999; 31: 917-970.

12. Thier R, Wiebel FA, Hinkel A, Burger A, Bruning T, Morgenroth K, Senge T, Wilhelm M, Schulz TG. Species differences in the glutathione transferase GSTT1-1 activity towards the model substrates methyl chloride and dichloromethane in liver and kidney. Arch Toxicol 1998; 72: 622-629.
13. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995; 30: 445-600.
14. Pfaller W, Gstraunthaler G. Nephrotoxicity testing *in vitro*—what we know and what we need to know. Environ Health Perspect 1998a; 106 (Suppl 2): 559-569.
15. Kriz W, Bankir L. A standard nomenclature for structure of the kidney. The Renal Commission of the International Union of Physiological Sciences (IUPS). Anat Embryol (Berl) 1988; 178: N1-8.
16. Helbert MJF. Flow cytometric immunodissection of the human nephron and collecting duct system. Ph.D.-thesis, University of Antwerp, Belgium 2001.
17. Horster MF, Sone M. Primary culture of isolated tubule cells of defined segmental origin. Methods Enzymol 1990; 191: 409-426.
18. Green DF, Hwang KH, Ryan US, Bourgoignie JJ. Culture of endothelial cells from baboon and human glomeruli. Kidney Int 1992; 41: 1506-1516.
19. van Setten PA, van Hinsbergh VW, van der Velden TJ, van de Kar NC, Vermeer M, Mahan JD, Assmann KJ, van den Heuvel LP, Monnens LA. Effects of TNF α on verocytotoxin cytotoxicity in purified human glomerular microvascular endothelial cells. Kidney Int 1997; 51: 1245-1256.
20. Haraldsen G, Kvale D, Lien B, Farstad IN, Brandtzaeg P. Cytokine-regulated expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in human microvascular endothelial cells. J Immunol 1996; 156: 2558-2565.
21. van Muijen GN, Ruiter DJ, Warnaar SO. Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 1987; 57: 359-369.
22. Herman IM, D'Amore PA. Microvascular pericytes contain muscle and nonmuscle actins. J Cell Biol 1985; 101: 43-52.
23. Diaz-Flores L, Gutierrez R, Varela H, Rancel N, Valladares F. Microvascular pericytes: a review of their morphological and functional characteristics. Histol Histopathol 1991; 6: 269-286.
24. Hewett PW, Murray JC. Human microvessel endothelial cells: isolation, culture and characterization. *In vitro* Cell Dev Biol Anim 1993; 29A: 823-830.
25. Kerjaschki D, Sharkey DJ, Farquhar MG. Identification and characterization of podocalyxin—the major sialoprotein of the renal glomerular epithelial cell. J Cell Biol 1984; 98: 1591-1596.
26. Kazatchkine MD, Fearon DT, Appay MD, Mandet C, Bariety J. Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in seventy-five cases of renal diseases: loss of C3b receptor antigen in focal hyalinosis and in proliferative nephritis of systemic lupus erythematosus. J Clin Invest 1982; 69: 900-912.
27. Johnson RJ, Iida H, Alpers CE, Majesky MW, Schwartz SM, Pritzi P, Gordon K, Gown AM. Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis. A-smooth muscle actin is a marker of mesangial cell proliferation. J Clin Invest 1991; 87, 847-858.
28. Stamenkovic I, Skalli O, Gabbiani G. Distribution of intermediate filament proteins in normal and diseased human glomeruli. Am J Pathol 1986; 125: 465-475.
29. Ishimura E, Sterzel RB, Morii H, Kashgarian M. Extracellular matrix protein: gene expression and synthesis in cultured rat mesangial cells. Nippon Jinzo Gakkai Shi 1992; 34: 9-17.
30. Potier M, L'Azou B, Cambar J. Isolated glomeruli and cultured mesangial cells as *in vitro* models to study immunosuppressive agents. Cell Biol Toxicol 1996; 12: 263-270.
31. Ardaillou R, Chansel D, Stefanovic V, Ardaillou N. Cell surface receptors and ectoenzymes in mesangial cells. J Am Soc Nephrol 1992; 2: S107-S115.
32. Kriz W, Elger M, Lemley K, Sakai T. Structure of the glomerular mesangium: a biomechanical interpretation. Kidney Int 1990; Suppl 30: S2-S9.
33. Gstraunthaler G, Pfaller W. Continuos renal cell lines as *in vitro* tools to study nephrotoxicity. *In vitro* Methods of Toxicology, CRC Press, Inc. 1992; 93-113.
34. Rabito CA. Occluding junctions in a renal cell line (LLC-PK1) with characteristics of proximal tubular cells. Am J Physiol 1986; 250: F734-F743.
35. Simmons NL. Tissue culture of established renal cell lines. Methods Enzymol 1990; 191: 426-436.
36. Morel F. Sites of hormone action in the mammalian nephron. Am J Physiol 1981; 240: F159-F164.
37. Pfaller W, Gstraunthaler G. Nephrotoxicity testing *in vitro* - what we know and what we need to know. Environ Health Perspect 1998b; 106 (Suppl 2): 559-569.
38. Bander NH, Cordon-Cardo C, Finstad CL, Whitmore WF, Darracott-Vaughan E, Oettgen HF, Melamed M, Old L. Immunohistologic dissection of the human kidney using monoclonal antibodies. J Urol 1985; 133: 502-505.

39. Müller GA, Müller C. Characterisation of renal antigens on distinct parts of the human nephron by monoclonal antibodies. Klin Wochenschrft 1983; 61:893-902.
40. Bander NH, Finstad CL, Cordon-Cardo C, Ramsawak RD, Vaughan ED, Whitmore WF, Oettgen HF, Melamed MR, Old LJ. Analysis of a mouse monoclonal antibody that reacts with a specific region of the human proximal tubule and subsets renal cell carcinomas. Cancer Res 1989; 49: 6774-6780.
41. Fradet Y, Cordon-Cardo C, Thomson T, Daly ME, Whitmore WF Jr, Lloyd KO, Melamed MR, Old LJ. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. Proc Natl Acad Sci USA 1984; 81:224-228.
42. Toutain H, Morin JP. Renal proximal tubule cell cultures for studying drug-induced nephrotoxicity and modulation of phenotype expression by medium components. Renal Failure 1992; 14: 371-383.
43. Helbert MJ, Dauwe S, De Broe ME. Flow cytometric immunodissection of the human nephron *in vivo* and *in vitro*. Exp Nephrol 1999; 7: 360-376.
44. Bello-Reus E, Weber MR. Electrophysiological studies of primary cultures of rabbit distal tubule cells. Am J Physiol 1987; 252: F899-F909.
45. Lajeunesse D, Regnier L, Thales S; Hebert RL. Simultaneous isolation and biochemical characterization of connecting and collecting tubules by Ficoll Gradient. Kidney Int 1995; 47: 306-311.
46. Detrisac CJ, Sens MA, Garvin AJ, Spicer SS, Sens DA. Tissue culture of human kidney epithelial cells of proximal tubule origin. Kidney Int 1984; 25: 383-390.
47. Orphanidis C, Fine LG, Normal JT. Hypoxia stimulates proximal tubular cell matrix production via a TGF-a1-independent mechanism. Kidney Int 1997; 52: 637-647.
48. Kempson SA, McAteer JA, Al-Mahrouq HA, Dousa TP, Dougherty GS, Evan AP. Proximal tubule characteristics of cultured human renal proximal tubule epithelium. J Lab Clin Med 1989; 113: 285-296.
49. McLaren J, Whiting PH, Hawksworth GA. Maintenance of glucose uptake in suspensions and cultures of human renal proximal tubular cells. Toxicol Letters 1990; 53: 237-241.
50. Triffillis AL, Regec AL, Trump BF. Isolation, culture and characterization of human renal tubular cells. J Urol 1985; 133: 324-329.
51. Ardaillou R, Ronco P, Rondeau E. Biology of renal cells in culture. In: Brenner and Rector's The Kidney. Brenner BM (editor). WB Saunders 1996, Philadelphia; p. 99-192.
52. Helbert MJ, Dauwe SE, De Broe ME. Flow cytometric immunodissection of the human distal tubule and cortical collecting duct system. Kidney Int 2001; 59: 554-564.
53. Blackburn JG, Hazen-Martin DJ, Detrisac CJ, Sens DA. Electrophysiology and ultrastructure of cultured human proximal tubule cells. Kidney Int 1988; 33: 508-516.
54. Middleton JP, Dunham CB, Onorato JJ, Sens DA, Dennis VW. Protein kinase A, cytosolic calcium and phosphate uptake in human proximal renal cells. Am J Physiol 1989; 257: F631-F638.
55. Gansler T, Hsu W-C, Gramling TS, Robinson KA, Buse MG, Blocker N, Roy L, Green S, garvin AJ, Sens DA. Growth factor binding and bioactivity in human kidney epithelial cultures. *In vitro* Cell Dev Biol 1990; 26: 285-290.
56. Warren JW, Mobley HLT, Hebel JR, Trifillis AL. Cytolethality of hemolytic Escherichia Coli to primary human renal proximal tubular cell obtained from different donors. Urology 1995; 45: 706-710.
57. Regec AL, Trump BF, Trifillis AL. Effect of gentamycin on the lysosomal system of cultured human proximal tubular cells: endocytic activity, lysosomal pH, and membrane fragility. Biochem Pharmacol 1989; 38: 2527-2534.
58. Miltenburg AM, Meijer-Paape ME, Daha MR, Van Bockel JH, Weening JJ, Van Es LA, van der Woude FJ. Donor-specific lysis of human kidney proximal tubular epithelial cells by renal allograft infiltrating lymphocytes. Transplantation 1989; 48: 296-302.
59. Gerritsma JS, Hiemstra PS, gerritsen AF, Prodjosudjadi W, Verweij CL, van Es LA, Daha MR. Regulation and production of IL-8 by human proximal tubular epithelial cells *in vitro*. Clin Exp Immunol 1996; 103: 289-294.
60. Prodjosudjadi W, Gerritsma JS, Klar-Mohamad N, gerritsen AF, Bruijn JA, van Es LA. Production and cytokine mediated regulation of monocyte-chemoattractant protein 1 by human proximal tubular cells. Kidney Int 1995; 48: 1477-1486.
61. Boswell RN, Yard BA, Schrama E, van Es LA, Daha MR, van der Woude FJ. Interleukin-6 production by human proximal tubular epithelial cells *in vitro*: analysis of the effect of interleukin-1 α (IL-1 α) and other cytokines. Nephrol Dial Transplant 1994; 9: 599-606.
62. Seelen MA, Brooimans RA, van der Woude FJ, van Es LA, Daha MR. Ifn-γ mediates stimulation of complement C4 biosynthesis in human proximal tubular epithelial cells. Kidney Int 1993; 44: 50-57.
63. Brooimans RA, Stegmann AP, Van Dorp WT, Van der Ark AA, van der Woude FJ, van Es LA, Daha MR. Interleukine-2 mediates stimulation of complement C3 biosynthesis in human proximal tubular epithelial cells. J Clin Invest 1991; 88: 379-384.
64. McLay JS, Chatterjee P, Nicolson AG, Jardine AG, McKay NG, Ralston SH, Grabowski P, Haites NE, McLeod AM, Hawsksworth GM. Nitric oxide production by human proximal tubular cells: a novel immunomodulatory mechanism ? Kidney Int 1994; 46: 1043-1049.

65. Racusen LC, Monteil C, Sgrignoli A, Lucskay M, Marouillat S, Rhim JGS, Morin J-P. Cell lines with extended *in vitro* growth potential from human renal proximal tubule: characterization, response to inducers, and comparison with established cell lines. J Lab Clin Med 1997; 129: 318-329.
66. Phillips AO, Steadman R, Topley N, Williams JD. Elevated D-glucose concentrations modulate TGF-a1 synthesis by human cultured renal proximal tubular cells. Am J Pathol 1995; 147: 362-374.
67. Phillips AO, Topley AN, Morrisey K, Williams JD, Steadman R. Basic fibroblast growth factor stimulates the release of performed transforing growth factor a1 from human proximal tubular cells in the absence of de novo gene transcription or mRNA translation. Lab Invest 1997a; 76: 591-600.
68. Phillips AO, Steadman R, Morrisey K, Martin J, Eynstone L, Williams JD. Exposure of human renal tubular cells to glucose leads to accumulation of type IV collagen and fibronectin by decreased degradation. Kidney Int 1997b; 52: 973-984.
69. Courjault-Gautier F, Chevalier J, Abbou CC, Chopin DK, Toutain HJ. Consecutive use of hormonally defined serum-free media to establish highly differentiated human renal proximal tubule cells in primary culture. J Am Soc Nephrol 1995; 5: 1949-1963.
70. Hang Yang A, Gould-Kostka J, Oberley TD. *In vitro* growth and differentiation of human kidney tubular cells on a basement membrane substrate. *In vitro* Cell Dev 1987; 23: 34-46.
71. Burton CJ, Combe C, Walls J, Harris KPG. Fibronectin production by human tubular cells. Kidney Int 1996; 50: 760-767.
72. Combe C, Burton CJ, Dufourco P, Weston S, Horsburgh T, Walls J, Harris KP. Hypoxia induces ICAM-1 on cultured human tubular cells. Kidney Int 1997; 51: 1703-1709.
73. Maata A, Glumoff V, Paakkonen P, Liska D, Penttinen RP, Elima K. Nuclear factor binding to an AP-1 site is associated with the activation of pro-α-1(I)-collagen gene in dedifferentiating chondrocytes. Biochem J 1993; 294: 365-371.
74. Nobeling R, Buhrle CP. The mesangial cell culture: a tool for the study of the electrophysiological and pharmacological properties of the glomerular mesangial cell. Differentiation 1987; 36: 47-56.
75. Gloy J, Greger R, Schollmeyer P, Huber M, Pavenstadt H. Influence of cell culture conditions and passage number on the response of membrane voltage to ATP and angiotensin II in rat mesangial cells. Renal Physiol Biochem 1994; 17: 62-72.
76. Racusen LC. Alterations in human proximal tubule cell attachment in response to hypoxia: role of microfilaments. J Lab Clin Med 1994; 123: 357-364.
77. Koechlin N, Pisam M, Poujeol P, Tauc M, Rambourg A. Conversion of a rabbit proximal convoluted tubule (PCT) into a cell monolayer: ultrastructural study of cell dedifferentiation and redifferentiation. Eur J Cell Biol 1991; 54: 224-236.
78. Baer PC, Tunn UW, Nunez G, Scherberich JE, Geiger H. Transdifferentation of distal but not proximal tubular epithelial cells from human kidney in culture. Exp Nephrol 1999; 7: 306-313.
79. Bander NH. Comparison of antigen expression of human renal cancer *in vivo* and *in vitro*. Cancer 1984; 53: 1235-1239.
80. Rettig WJ, Real FX, Spengler BA, Biedler JL, Old LJ. Human melanoma proteoglycan: expression in hybrids controlled by intrinsic and extrinsic signals. Science 1986a; 231: 1281-1284.
81. Rettig WJ, Murty VV, Mattes MJ, Chaganti RS, Old LJ. Extracellular matrix-modulated expression of human cell surface glycoproteins A42 and J143. Intrinsic and extrinsic signals determine antigenic phenotype. J Exp Med 1986b; 164: 1581-1599.
82. Eguchi G, Kodama R. Transdifferentiation. Curr Opin Cell Biol 1993; 5: 1023-1028.
83. Hay ED. Extracellular matrix alters epithelial differentiation. Curr Opin Cell Biol 1993; 5: 1029-1035.
84. Helbert M, De Meester I, Scherberich J, Nouwen E, De Broe ME. Adenosine deaminase complex protein is expressed on both proximal and distal tubular epithelial cells *in vitro*. Nephrol Dial Transpl 1996; 11: A29 (Abstract).
85. Chabardes D, Imbert M, Clique A, Montegut M, Morel F. PTH sensitive adenyl cyclase activity in different segments of the rabbit nephron. Pflügers Arch 1975; 354: 229-239.
86. Chabardes D, Gagnan-Brunette M, Imbert-Teboul M, Gontcharevskaia O, Montegut M, Clique A, Morel F. Adenylate cyclase responsiveness to hormones in various portions of the human nephron. J Clin Invest 1980; 65: 439-448.
87. Wilson PD, Anderson RJ, Breckon RD, Nathrath W, Schrier RW. Retention of differentiated characteristics by cultres of defined rabbit kidney epithelia. J Cell Physiol 1987; 130: 245-254.
88. Chabardes D, Imbert-Teboul M, Montegut M, Clique A, Morel F. Distribution of calcitonin-sensitive adenylate cyclase activity along the rabbit kidney tubule. Proc Natl Acad Sci 1976b; 73: 3608-3612.
89. Aigner J, Kloth S, Kubitza M, Kashgarian M, Dermietzel R, Minuth WW. Maturation of renal collecting duct cells *in vivo* and under perfusion culture. Epithelial Cell Biol 1994; 3: 70-78.
90. Minuth WW, Gilbert P. The expression of specific proteins in cultured renal collecting duct cells. Histochemistry 1988; 88: 435-441.
91. Merot J, Bidet M, Gachot B, Le Maout S, Koechlin S, Tauc M, Poujeol P. Electrical properties of rabbit early distal convoluted tubule in primary culture. Am J Physiol 1989; 257: F288-F299.
92. White S, Reeve H. Primary culture of collecting duct cell epithelium from neonate rabbit kidney in monolayer. Exp Physiol 1992; 77: 129-139.
93. Chabardes D, Imbert-Teboul M, Montegut M, Clique A, Morel F. Catecholamine sensitive adenyl-cyclase activity in different segments of the rabbit nephron. Pflügers Arch 1975b; 361: 9-15.

94. Wilson PD, Dillingham DA, Breckon RD, Anderson RJ. Defined human renal tubular epithelia in culture: growth, characterization, and hormonal response; Am J Physiol 1985; 248: F436-F443.
95. Wysocki LJ, Sato VL. Planning for lymphocytes: a method for a cell selection. Proc Natl Acad Sci 1978; 75: 2844-2848.
96. Stanton RC, Mendrick DL, Rennke HG, Seifter JL. Use of monoclonal antibodies to culture rat proximal tubular cells. Am J Physiol 1986; 251: C780-C786.
97. Rose UM, Hartog A, Jansen JW, Van Os CH, Bindels RJ. Anoxia-induced increases in intracellular calcium concentration in primary cultures of rabbit thick ascending limb of Henle's loop. Bioch Biophys Acta 1994; 1226: 291-299.
98. Bindels RJ, hartog A, Timmermans J, Van Os CH. Active Ca^{2+} transport in primary cultures of rabbit kidney CCD: stimulation by 1, 25-dihydroxyvitamin D3 and PTH. Am J Physiol 1991; 261: F799-F807.
99. Allen ML, Nakao A, Sonnenburg WK, Burnatowska-Hledin M, Spielman WS, Smith WL. Immunodissection of cortical and mecullary thick ascending limb cells from rabbit kidney. Am J Physiol 1988; 255: F704-F710.
100. Spielman WS, Sonnenburg WK, Allen ML, Arend LJ, Gerozissis K, Smith WL. Immunodissection and culture of rabbit cortical collecting tubule cells. Am J Physiol 1986; 251: F348-F357.
101. Pizzonia JH, Gesek FA, Kennedy SM, Coutermarsh BA, Bacskai BJ, Friedman PA. Immunomagnetic separation, primary culture and characterization of cortical thick ascending limb plus distal convoluted tubule cells from mouse kidney. *In vitro* Cell Dev Biol 1991; 27A: 409-416.
102. Bacskai BJ, Friedman PA. Activation of latent Ca^{2+}-channels in renal epithelial cells by parathyroid hormone. Nature 1990; 347: 388-391.
103. Baer PC, Nockher WA, Haase W, Scherberich JE. Isolation of proximal and distal tubule cells from human kidney by immunomagnetic separation. Technical note. Kidney Int 1997; 52: 1321-1331.
104. Fejes-Toth G, Naray-Fejes-Toth A. Isolated principal and intercalated cell hormone responsiveness and Na-K-ATPase activity. Am J Physiol 1989; 256: F742-F750.
105. Fejes-Toth G, Chen WR, Rusvai E, Moser T, Naray-Fejes-Toth A. Differential expression of AE-1 in renal HCO_3-secreting and –reabsorbing interacalated cells. J Biol Chem 1994; 269: 26717-26721.
106. Fejes-Toth G, Naray-Fejes-Toth A. Effect of acid base balance on H^+-ATP-ase 31 kD subunit mRNA levels in collecting duct cells. Kidney Int 1995; 48: 1420-1426.
107. van der Biest I, Nouwen EJ, Van Dromme SA, De Broe ME. Characterization of pure proximal and heterogeneous distal human tubular cells in culture. Kidney Int 1994; 45: 85-94.
108. Yang T, Hassan S, Huang YG, Smart AM, Bruggs JP, Schnermann JB. Expression of PTHrP, PTH/PTHrP receptor, and Ca(2+)-sensing receptor mRNAs along the rat nephron. Am J Physiol 1997; 272: F751-F758.
109. Lee K, Brown D, Urena P, Ardaillou N, Ardaillou R, Deeds J, Segre GV. Localization of parathyroid hormone/parathyroid hormone-related peptide receptor mRNA in kidney. Am J Physiol 1996; 270: F186-F191.
110. Terada Y, Tomita K, Nonoguchi H, Yang T, Marumo F. Different localization and regulation of two types of vasopressin receptor messenger RNA in microdissected rat nephron segments using reverse transcription polymerase chain reaction. J Clin Invest 1993; 92: 2339-2345.
111. Mimura Y, Ogura T, Hayakawa N, Otsuka F, Hashimoto M, Yamauchi T, Makino H, Ogawa N. *In vitro* macro- and microautoradiographic localization of V1 and V2 receptors in the rat kidney using OPC-21268 and OPC-31260. Nephron 1997; 76: 331-336.
112. Phillips PA, Abrahams JM, Kelly JM, Mooser V, Trinder D, Johnston CI. Localization of vasopressin binding sites in rat tissues using specific V1 and V2 selective ligands. Endocrinology 1990; 126: 1478-1484.
113. Nonoguchi H, Owada A, Kobayashi N, Takayama M, Terada Y, Koike J, Ujiie K, Marumo F, Sakai T, Tmita K. Immunohistochemical localization of V2 Vasopressin receptor along the nephron and functional role of luminal V2 receptor in terminal inner medullary collecting ducts. J Clin Invest 1995; 96: 1768-1778.
114. Taub M, Sato G. Growth of functional primary cultures of kidney epithelial cells in defined medium. J Cell Physiol 1980; 105: 369-378.
115. Boogaard PJ, Nagelkerke JF, Mulder GJ. Renal proximal tubular cells in suspension or in primary culture as *in vitro* models to study nephrotoxisity. Chemico Biological Interactions 1990; 76: 251-292.
116. Chen TC, Curthoys NP, Langenaur CF, Puschett JB. Characterization of primary cell cultures derived from rat renal proximal tubules. *In vitro* Cell Dev Biol 1989; 25(8): 714-722.
117. Aleo MD, Taub ML, Nickerson PA, Kostyniak PJ. Primary cultures of rabbit renal proximal tubule cells: I. Growth and biochemical characteristics. *In vitro* Cell Dev Biol 1989; 25: 776-783.
118. Kruidering M, Maasdam DH, Prins FA, de Heer E, Mulder GJ, Nagelkerke JF. Evaluation of nephrotoxicity *in vitro* using a suspension of highly purified porcine proximal tubular cells and characterization of the cells in primary culture. Exp Nephrol 1994; 2: 324-344.
119. Blais A, Morvan-Baleynaud J, Friedlander G, Le Grimellec C. Primary culture of rabbit proximal tubules as a cellular model to study nephrotoxicity of xenobiotics. Kidney Int 1993; 44: 13-18.

120. Bogaard B, Clausen J. An *in vitro* system for evaluation of oxidative stress and the effects of antioxidants. ATLA 1997; 25: 279-287.
121. van de Water B, Zoeteweij JP, de Bont HGM, Mulder G, Nagelkerke JF. Role of mitochondrial Ca in the oxidative stress-induced dissipation of the mitochondrial membrane potential. J Biol Chem 1994; 269: 14564-14552.
122. Wallin A, Zhang G, Jones TW, Jaken S, Stevens JL. Mechanism of the nephrogenic repair response. Laboratory Investigation 1992; 66: 474-484.
124. Gstraunthaler G, Pfaller W, Kotanko P. Biochemical characterization of renal epithelial cell cultures (LLC-PK1 and MDCK). Am J Physiol 1985; 248: F536-F544.
125. Hull RN, Cherry WR, Weaver GW. The origin and characteristics of a pig kidney cell strain LLC-PK_1. *In vitro* 1976; 12: 670-677.
126. Takaoka T, Katsuta H, Endo M. et al. Establishement of a cell strain, JCT-12, from cynomolgus monkey kidney tissue. Jpn J Exp Med 1962; 32: 351.
127. Gaush CR, Hard WL, Smith TF. Characterization of an established cell line of canine kidney cells (MDCK). Proc Soc Exp Biol Med 1966; 122: 931-935.
128. Koyama H, Goodpasture C, Miller MM, Teplitz RL, Riggs AD. Establishment and characterization of a cell line from the American opossum (Didelphys virginiana). *In vitro*. 1978 Mar;14(3):239-46
129. Saier MH. Growth and differentiated properties of a kidney epithelial cell line (MDCK). Am J Physiol 1981; 240: C106-109.
130. Louvard D. Apical membrane aminopeptidase appears at site of cell-cell contact in cultured kidney epithelial cells. Proc Natl Acad Sci 1980; 77: 4132-4136.
131. Fouda AK, Fauth C, Roch-Ramel F. Transport of organic cations by kidney epithelial cell line LLC-PK1. J Pharmacol Exp Ther 1990; 252: 286-292.
132. Tang MJ, Weinberg JM. Vasopressin-induced increases of cytosolic calcium in LLC-PK1 cells. Am J Physiol 1986; 251: F1090-F1095.
133. Wohlwend A, Vassalli JD, Bertrand D, Orci L. Calcitonin and vasopressin affect epithelial properties in a renal cell line. J Cell Physiol 1986; 128: 71-75.
134. Gstraunthaler GJ. Epithelial cells in tissue culture. Ren Physiol Biochem 1988; 11(1-2): 1-42.
135. Hopfer U, Jacobberger JW, Grunert DC, Eckert RL, Jat PS, Whisett JA. Immortaliztion of epithelial cells. Am J Physiol 1996; 270: C1-C11.
136. Cartier N, Lacave R, Vallet V, Hagege J, Hellio R, Robine S, Pringault E, Cluzeaud F, Briand P, Kahn A, et al. Establishment of renal proximal tubule cell lines by targeted oncogenesis in transgenic mice using the L-pyruvate kinase-SV40 (T) antigen hybrid gene. J Cell Sci 1993; 104 (Pt 3): 695-704.
137. Woost PG, Orosz DE, Jin W, Frisa PS, Jacobberger JW, Douglas JG, Hopfer U. Immortalization and characterization of proximal tubule cells derived from kidneys of spontanous hypertensive and normotensive rats. Kidney Int 1996; 50: 125-134.
138. Romero MF, Douglas JG, Eckert RL, Hopfer U, Jacobberger JW. Development and characterization of rabbit proximal tubular epithelial cell lines. Kidney Int 1992; 42: 1130-1144.
139. Vandewalle A, Lelongt B, Geniteau LM, Baudouin B, Antoine M, Estrade S, Chatelet P, Cassingena R, Ronco P. Maintenance of proximal and distal cell functions in SV40-trandformed tubular cell lines derived from rabbit kidney cortex. Journal of Cellular Physiology 1989; 141: 203-221.
140. Rhim JS, Jay G, Arnstein P, Price FM, Sanford KK, Aaronson SA. Neoplastic transformation of human epidermal keratinocytes by AD12-SV40 and Kirsten sarcoma viruses. Science 1985; 227: 1250-1252.
141. Arend LJ, Handler JS, Thim JS, Gusovsky F, Spielman WS. Aadenosine-sensitive phospho-inositide turnover in a newly established renal cell line. Am J physiol 1989; 256: F1067-F1074.
142. Kastner Kastner S, Soose M, Stolte H. Human kidney cells in *in vitro* pharmatoxicology. In: Human cells in *in vitro* pharmatoxciology. Rogiers V, Sonck W, Shepard E, Vercruysse A (editors). VUBpress, Brussels 1993; p. 197-238.
143. Handler JS, Perkins FM, Johnson JP. Studies of renal cell function using cell culture techniques. Am J Physiol 1980; 238: F1-F9.
144. Charlton JA, Simmons NL. Established human renal cell lines: pnenotypic characteristics define suitability for use in *in vitro* models for predictive toxicology. Toxic *In vitro* 1993; 7: 129-136.
145. Teulon J, Ronco PM, Geniteau-Legendre M, Baudouin B, Estrade S, Cassingena R, Vandewalle A. transformation of renal tubule epithelial cells by Simian virus-40 is associated with emergence of Ca^{2+}-insensitive K^+ channels and altered mitogenic sensitivity to K^+ channel blockers. J Cell Physiol 1992; 151: 113-125.
146. Prie D, Dussaule JC, Lelongt B, Geniteau-Legendre M, Chatelet F, Cassingena R, Vandewalle A, Ronco PM. Principal cell-specific antigen and hormonal regulatory network in RC.SvtsA58 cell line. Am J Physiol 1994; 266: C1628-C1638.
147. Nakazato Y, Suzuki H, Saruta T. Characterizations of subclones of Madin Darby canine kidney renal epithelial cell line. Biochim Biophys Acta 1989; 1014: 57-65.
148. Ebert T, Bander NH, Finstad CL, Ramsawak RD, Old LJ. Establishment and characterisation of human renal cancer and normal kidney cell lines. Cancer Res 1990; 50: 5531-5536.

149. Matsuda M, Osafune M, Nakano E, Kotake T, Sonoda T, Watanabe S, Hada T, Okashi T, Higashino K, Yamamura Y, Abe T. Characterization of an established cell line from human renal carcinoma. Cancer Res 1979; 39: 4694-4699.
150. Ryan MJ, Johnson G, Kirk J, Fuerstenberg SM, Zager RA, Torok-Storb, B. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. Kidney Int 1994; 45: 48-57.
151. Nanus DM, Lynch SA, Rao PH, Anderson SM, Jhanwar SC, Albino AP. Transformation of human kidney proximal tubule cells by a src-containing retrovirus. Oncogene 1991; 6(11): 2105-2111.
152. Freshney RI. Culture of animal cells: a manual of basic technique. In: Anonymous. Wiley and Liss, New York 1994.
153. Gstraunthaler G, Seppi T, Pfaller W. Impact of culture conditions, culture media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? Cell Physiol Biochem 1999; 9: 150-172.
154. Gstraunthaler G, Thurner B, Weirich-Schweiger H, Pfaller W. A novel gluconeogenic strain of OK cells with metabolic properties different from gluconeogenic LLC-PK1 cells. Renal Physiol Biochem 1993; 3: 78-88.
155. Gstraunthaler G, Gersdorf E, Fischer WM, Joannidis M, Pfaller W. Morphological and biochemical changes of LLC-PK1 cells during adaptation to glucose-free culture conditions. Ren Physiol Biochem 1990; 13: 137-153.
156. Holcomb T, Curthoys NP, Gstraunthaler G. Subcellular localization of PEPCK and metabolism of gluconeogenic substrains of renal cell lines. Am J Physiol 1995; 268: C449-C457.
157. Lang MA, Muller J, Preston AS, Handler JS. Complete response to vasopressin requires epithelial organization in A6 cells in culture. Am J Physiol 1986; 250: C138-C145.
158. Minuth WW, Dermietzel R, Kloth S, Hennerkes B. A new method culturing renal cells under permanent superfusion and producing a luminal-basal medium gradient. Kidney Int 1992; 41: 215-219.
159. Schlechte W, Brattain M, Boyd D. Invasion of extracellular matrix by cultured colon cancer cells: dependence on urokinase receptor display. Cancer Commun 1990; 2: 173-179.
160. Steele RE, Preston AS, Johnson JP, Handler JS. Porous-bottom dishes for culture of polarized cells. Am J Physiol 1986; 251: C136-C139.
161. Sahai A, Cole LA, Clarke DL, Tannen RL. Rocking promotes differentiated properties in LLC-PK cells by improved oxygenation. Am J Physiol 1989; 256: C1064-C1069.
162. Griffith JB. Scaling up of animal cell cultures. In: Animal cell culture, a practical approach. Freshney RI (editor). Oxford IRL Press at Oxford University Press, Oxford 1992; p. 47-93.
163. Knatzek RA, Gullino P, Kohler PO, Dedrick R. Cell culture on artificial capillaries. Science 1972; 178: 65-67.
164. McBridge J, Oehlschlager SL, Hanley AB. The use of a flow cell bioreactor to measure chronic exposure to toxins. In: Biomarkers in Food Chemical Risk Assesment. Crews HM, Hanley AB (editors). Royal Society of Chemistry 1995; p. 64-66.
165. Pfaller W, Felder E, Koppelstaetter C, Abbot SE, Westwick J. Epiflow: a new cell culture system utilising continuous medium exchange and continuous supply with respiratory gases. ATLA 1999b; 27: 423 (Abstract).
166. Potier M, Wolf A, Cambar J. Comparative study of cyclosporin A, cyclosporin G, and the novel cyclosporin derivative IMM 125 in isolated glomeruli and cultured rat mesangial cells: a morphometric analysis. Nephrol Dial Transplant 1998; 13: 1406-1411.
167. L'Azou B, Medina J, Frieauff W, Cordier A, Cambar J, Wolf A. *In vitro* models to study mechanisms involved in cyclosporine A-mediated glomerular contraction. Arch Toxicol 1999; 73: 337-345.
168. Rodriguez-Puyol D, Lamas S, Olivera A, Lopez-Farre A, Ortega G, Hernando L, Lopez-Novoa JM. Actions of cyclosporin A on cultured rat mesangial cells. Kidney Int 1989; 35: 632-637.
169. Stahl RA, Adler S, Baker PJ, Johnson RJ, Chen YP, Pritzl P, Couser WG. Cyclosporin A inhibits prostaglandin E2 formation by rat mesangial cells in culture. Kidney Int 1989; 35: 1161-1157.
169a. Waiser, J.; Dell, K.; Bohler, T.; Dogu, E.; Gaedeke, J.; Budde, K., and Neumayer, H. H. Cyclosporine A up-regulates the expression of TGF-β1 and its receptors type I and type II in rat mesangial cells. Nephrol Dial Transplant. 2002 Sep; 17(9):1568-77.
170. Fornoni A, Lenz O, Tack I, Potier M, Elliot SJ, Striker LJ, Striker GE. Matrix accumulation in mesangial cells exposed to cyclosporine A requires a permissive genetic background. Transplantation 2000; 70, 587-593.
171. Becker GM, Gandolfi AJ, Nagle RB. Effects of cyclosporin A on a kidney epithelial cell line (LLC-PK1). Res Commun Chem Pathol Pharmacol 1987; 56: 277-280.
172. Healy E, Dempsey M, Lally C, Ryan MP. Apoptosis and necrosis: mechanisms of cell death induced by cyclosporine A in a renal proximal tubular cell line. Kidney Int 1998; 54: 1955-1966.
173. Lally C, Healy E, Ryan MP. Cyclosporine A-induced cell cycle arrest and cell death in renal epithelial cells. Kidney Int 1999; 56: 1254-1257.
174. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995; 80: 293-299.
175. Shihab FS, Andoh TF, Tanner AM, Yi H, Bennett WM. Expression of apoptosis regulatory genes in chronic cyclosporine nephrotoxicity favors apoptosis. Kidney Int 1999; 56: 2147-2159.
176. Thomas SE, Andoh TF, Pichler RH, Shankland SJ, Couser WG, Bennett WM, Johnson RJ. Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. Kidney Int 1998; 53: 897-908.

176a. Bakker, R. C. ; van Kooten, C.; van de Lagemaat-Paape, M. E.; Daha, M. R., and Paul, L. C. Renal tubular epithelial cell death and cyclosporin A. Nephrol Dial Transplant. 2002 Jul; 17(7):1181-8.
177. Johnson DW, Saunders HJ, Johnson FJ, Huq SO, Field MJ, Pollock CA. Fibrogenic effects of cyclosporin A on the tubulointerstitium: role of cytokines and growth factors. Exp Nephrol 1999; 7: 470-478.
178. Johnson DW, Saunders HJ, Vesey DA, Qi W, Field MJ, Pollock CA. Enalaprilat directly ameliorates *in vitro* cyclosporin nephrotoxicity in human tubulo-interstitial cells. Nephron 2000; 86: 473-481.
179. Scoble JE, Senior JC, Chan P, Varghese Z, Sweny P, Moorhead JF. *In vitro* cyclosporine toxicity. The effect of verapamil. Transplantation 1989; 47: 647-650.
180. Chan P, Chapman JR, Morris PJ. Glycosuria: an index of cyclosporine nephrotoxicity. Transplant Proc 1987; 19: 1780.
181. Nakahama H. Stimulatory effect of cyclosporine A on endothelin secretion by a cultured renal epithelial cell line, LLC-PK1 cells. Eur J Pharmacol 1990; 180: 191-192.
182. Ellis D, Gabriel ME, Ellis MP. Phospholipase-C and Na-K ATPase activation by cyclosporine and FK506 in LLC-PK1, cells. Possible implications in blood pressure regulation. Transplantation 1991; 52: 349-353.
183. Hauser IA, Koziolek M, Hopfer U, Thevenod F. Therapeutic concentrations of cyclosporine A, but not FK506, increase P-glycoprotein expression in endothelial and renal tubule cells. Kidney Int 1998; 54: 1139-1149.
183a. Romiti, N.; Tramonti, G., and Chieli, E. Influence of different chemicals on MDR-1 P-glycoprotein expression and activity in the HK-2 proximal tubular cell line. Toxicol Appl Pharmacol. 2002 Sep 1; 183(2):83-91.
184. Yuan CM, Bohen EM, Musio F, Carome MA. Sublethal heat shock and cyclosporine exposure produce tolerance against subsequent cyclosporine toxicity. Am J Physiol 1996; 271: F571-F578.
185. Walker RJ, Lazzaro VA, Duggin GG, Horvath JS, Tiller DJ. Synergistic toxicity of cyclosporin A and streptomycin in renal epithelial cell cultures. Res Commun Chem Pathol Pharmacol 1988; 62: 447-460.
186. Loehrer PJ, Einhorn LH. Drugs five years later. Cisplatin. Ann Intern Med 1984; 100: 704-713.
187. Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW. Mechanism of cis-platinum nephrotoxicity: II. Morphologic observations. J Pharmacol Exp Ther 1980; 213: 551-556.
188. Tay LK, Bregman CL, Masters BA, Williams PD. Effects of cis-diamminedichloroplatinum(II) on rabbit kidney *in vivo* and on rabbit renal proximal tubule cells in culture. Cancer Res 1988; 48: 2538-2543.
189. Courjault F, Leroy D, Coquery L, Toutain H. Platinum complex-induced dysfunction of cultured renal proximal tubule cells. A comparative study of carboplatin and transplatin with cisplatin. Arch Toxicol 1993b; 67: 338-346.
190. Montine TJ, Borch RF. Quiescent LLC-PK1 cells as a model for cis-diamminedichloroplatinum(II) nephrotoxicity and modulation by thiol rescue agents. Cancer Res 1988; 48: 6017-6024.
191. Fukuishi N, Gemba M. Use of cultured renal epithelial cells for the study of cisplatin toxicity. Jpn J Pharmacol 1989; 50: 247-249.
192. Okuda M, Tsuda K, Masaki K, Hashimoto Y, Inui K. Cisplatin-induced toxicity in LLC-PK1 kidney epithelial cells: role of basolateral membrane transport. Toxicol Lett 1999; 106: 229-235.
193. Endo T, Kimura O, Sakata M. Carrier-mediated uptake of cisplatin by the OK renal epithelial cell line. Toxicology 2000; 146: 187-195.
193a. Lieberthal, W.; Triaca, V., and Levine, J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. Am J Physiol. 1996 Apr; 270(4 Pt 2):F700-8.
194. Okuda M, Masaki K, Fukatsu S, Hashimoto Y, Inui K. Role of apoptosis in cisplatin-induced toxicity in the renal epithelial cell line LLC-PK1. Implication of the functions of apical membranes. Biochem Pharmacol 2000; 59: 195-201.
194a. Tsuruya, K.; Ninomiya, T.; Tokumoto, M.; Hirakawa, M.; Masutani, K.; Taniguchi, M.; Fukuda, K.; Kanai, H.; Kishihara, K.; Hirakata, H., and Iida, M. Direct involvement of the receptor-mediated apoptotic pathways in cisplatin-induced renal tubular cell death. Kidney Int. 2003 Jan; 63(1):72-82.
194b. Park, M. S.; De Leon, M., and Devarajan, P. Cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways. J Am Soc Nephrol. 2002 Apr; 13(4):858-65.
195. Takeda M, Kobayashi M, Shirato I, Osaki T, Endou H. Cisplatin-induced apoptosis of immortalized mouse proximal tubule cells is mediated by interleukin-1 β converting enzyme (ICE) family of proteases but inhibited by overexpression of Bcl-2. Arch Toxicol 1997; 71: 612-621.
196. van de Water B, Houtepen F, Huigsloot M, Tijdens IB. Suppression of chemically induced apoptosis but not necrosis of renal proximal tubular epithelial (LLC-PK1) cells by focal adhesion kinase (FAK). Role of FAK in maintaining focal adhesion organization after acute renal cell injury. J Biol Chem 2001; 276(39): 36183-36193.
197. Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV. *In vitro* and *in vivo* evidence suggesting a role for iron in cisplatin- induced nephrotoxicity. Kidney Int 1998a; 53: 394-401.
198. Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV. Role of cytochrome P-450 as a source of catalytic iron in cisplatin- induced nephrotoxicity. Kidney Int 1998b; 54: 1562-1569.

199. Yokozawa T, Nakagawa T, Lee KI, Cho EJ, Terasawa K, Takeuchi S. Effects of green tea tannin on cisplatin-induced nephropathy in LLC-PK1 cells and rats. J Pharm Pharmacol 1999; 51: 1325-1331.
200. Hannemann J, Baumann K. Inhibition of lactate-dehydrogenase by cisplatin and other platinum- compounds: enzyme leakage of LDH is not a suitable method to measure platinum-compound-induced kidney cell damage *in vitro*. Res Commun Chem Pathol Pharmacol 1988; 60: 371-379.
201. Montie T, Patamasucon P. Aminoglycosides: the complex problem of antibiotic mechanisms and clinical applications. Eur J Clin Microbiol Infect Dis 1995; 14: 85-87.
202. Rivas-Cabanero L, Rodriguez-Lopez AM, Martinez-Salgado C, Saura M, Lamas S, Lopez-Novoa JM. Gentamicin treatment increases mesangial cell nitric oxide production. Exp Nephrol 1997; 5: 23-30.
203. Martinez-Salgado C, Rodriguez-Barbero A, Tavares P, Eleno N, Lopez-Novoa JM. Role of calcium in gentamicin-induced mesangial cell activation. Cell Physiol Biochem 2000; 10, 65-72.
204. Klotman PE, Yarger WE. Reduction of renal blood flow and proximal bicarbonate reabsorption in rats by gentamicin. Kidney Int 1983; 24: 638-643.
205. Schor N, Ichikawa I, Rennke HG, Troy JL, Brenner BM. Pathophysiology of altered glomerular function in aminoglycoside-treated rats. Kidney Int 1981; 19: 288-296.
206. Inui K, Saito H, Iwata T, Hori R. Aminoglycoside-induced alterations in apical membranes of kidney epithelial cell line (LLC-PK1). Am J Physiol 1988; 254: C251-C257.
207. Hori R, Yamamoto K, Saito H, Kohno M, Inui K. Effect of aminoglycoside antibiotics on cellular functions of kidney epithelial cell line (LLC-PK1): a model system for aminoglycoside nephrotoxicity. J Pharmacol Exp Ther 1984; 230: 724-728.
208. Shehata M, Cope GH, Johnson TS, Raftery AT, El Nahas AM. Cyclosporine enhances the expression of TGF-β in the juxtaglomerular cells of the rat kidney. Kidney Int 1995; 48: 1487-1496.
209. Choi KH, Kim TI, Chong DL, Lee HY, Han DS. Gentamicin induced apoptosis of renal tubular epithelial (LLC-PK1) cells. Korean J Intern Med 2000; 15: 218-223.
210. El Mouedden M, Laurent G, Mingeot-Leclercq MP, Tulkens PM. Gentamicin-induced apoptosis in renal cell lines and embryonic rat fibroblasts. Toxicol Sci 2000; 56, 229-239.
211. Kiyomiya K, Matsushita N, Matsuo S, Kurebe M. Differential toxic effects of gentamicin on cultured renal epithelial cells (LLC-PK1) on application to the brush border membrane or the basolateral membrane. J Vet Med Sci 2000a; 62: 971-975.
212. Hensen J, Haenelt M, Gross P. Lithium induced polyuria and renal vasopressin receptor density. Nephrol Dial Transplant 1996; 11: 622-627.
213. Noel P, Levy VG. [Renal toxicity of the association: gentamicin/furosemide. One case (author's transl)]. Nouv Presse Med 1978; 7: 351-353.
214. Kiyomiya K, Matsushita N, Matsuo S, Kurebe M. Roles of oxygen radical production and lipid peroxidation in the cytotoxicity of cephaloridine on cultured renal epithelial cells (LLC- PK1). J Vet Med Sci 2000b; 62: 977-981.
214a. Kiyomiya, K.; Matsushita, N.; Kurebe, M.; Nakagawa, H., and Matsuo, S. Mitochondrial cytochrome c oxidase as a target site for cephalosporin antibiotics in renal epithelial cells (LLC-PK(1)) and renal cortex. Life Sci. 2002 Nov 22; 72(1):49-57.
215. Steinmassl D, Pfaller W, Gstraunthaler G, Hoffmann W. LLC-PK1 epithelia as a model for *in vitro* assessment of proximal tubular nephrotoxicity. *In vitro* Cell Dev Biol Anim 1995; 31: 94-106.
216. Warnock DW. Amphotericin B: an introduction. J Antimicrob Chemother 1991; 28 (Suppl B): 27-38.
217. Hsu S, Burnette RR. Characterization of the effects of amphotericin B on ion channels in MDCK cells using the patch-clamp technique. Biochim Biophys Acta 1997; 1329: 26-38.
218. Sabra R, Branch RA. Effect of amphotericin B on intracellular calcium levels in cultured glomerular mesangial cells. Eur J Pharmacol 1992; 226: 79-85.
219. Varlam DE, Siddiq MM, Parton LA, Russmann H. Apoptosis contributes to amphotericin B-induced nephrotoxicity. Antimicrob Agents Chemother 2001; 45: 679-685.
220. Wasan KM, Lopez-Berestein G. Modification of amphotericin B's therapeutic index by increasing its association with serum high-density lipoproteins. Ann N Y Acad Sci 1994; 730: 93-106.
221. Glahn RP, Van Campen D, Dousa TP. Aflatoxin B1 reduces Na(+)-P(i) co-transport in proximal renal epithelium: studies in opossum kidney (OK) cells. Toxicology 1994; 92: 91-100.
222. Hamada T, Nakano S, Iwai S, Tanimoto A, Ariyoshi K, Koide O. Pathological study on beagles after long-term oral administration of cadmium. Toxicol Pathol 1991; 19: 138-147.
223. Ding W, Templeton DM. Activation of parallel mitogen-activated protein kinase cascades and induction of c-fos by cadmium. Toxicol Appl Pharmacol 2000; 162: 93-99.
224. Templeton DM, Wang Z, Miralem T. Cadmium and calcium-dependent c-fos expression in mesangial cells. Toxicol Lett 1998; 95: 1-8.
225. Ishido M, Tohyama C, Suzuki T. Cadmium-bound metallothionein induces apoptosis in rat kidneys, but not in cultured kidney LLC-PK1 cells. Life Sci 1999; 64: 797-804.

226. Liu J, Liu Y, Klaassen CD. Nephrotoxicity of CdCl2 and Cd-metallothionein in cultured rat kidney proximal tubules and LLC-PK1 cells. Toxicol Appl Pharmacol 1994; 128: 264-270.
227. Zimmerhackl LB, Momm F, Wiegele G, Brandis M. Cadmium is more toxic to LLC-PK1 cells than to MDCK cells acting on the cadherin-catenin complex. Am J Physiol 1998; 275: F143-F153.
227a. Gennari, A.; Cortese, E.; Boveri, M.; Casado, J., and Prieto, P. Sensitive endpoints for evaluating cadmium-induced acute toxicity in LLC-PK1 cells. Toxicology. 2003 Feb 1; 183(1-3):211-20.
228. Prozialeck WC, Niewenhuis RJ. Cadmium (Cd2+) disrupts intercellular junctions and actin filaments in LLC-PK1 cells. Toxicol Appl Pharmacol 1991; 107: 81-97.
228a. Alvarez-Barrientos, A.; O'Connor, J. E.; Nieto Castillo, R.; Moreno Moreno, A. B., and Prieto, P. Use of flow cytometry and confocal microscopy techniques to investigate early CdCl(2)-induced nephrotoxicity in vitro. Toxicol In Vitro. 2001 Aug-2001 Oct 31; 15(4-5):407-12.
229. Nicholson JK, Kendall MD, Osborn D. Cadmium and mercury nephrotoxicity. Nature 1983; 304: 633-635.
230. Zalups RK. Molecular interactions with mercury in the kidney. Pharmacol Rev 200; 52: 113-143.
231. Aleo MD, Taub ML, Kostyniak PJ. Primary cultures of rabbit renal proximal tubule cells. III. Comparative cytotoxicity of inorganic and organic mercury. Toxicol Appl Pharmacol 1992; 112: 310-317.
232. Bohets HH, Van Thielen MN, Van der Biest I, Van Landeghem GF, D'Haese PC, Nouwen EJ, De Broe ME, Dierickx PJ. Cytotoxicity of mercury compounds in LLC-PK1, MDCK and human proximal tubular cells. Kidney Int 1995; 47: 395-403.
233. Matsuoka M, Wispriyono B, Igisu H. Induction of c-fos gene by mercury chloride in LLC-PK1 cells. Chem Biol Interact 1997; 108: 95-106.
234. Duncan-Achanzar KB, Jones JT, Burke MF, Carter DE, Laird HE 2nd. Inorganic mercury chloride-induced apoptosis in the cultured porcine renal cell line LLC-PK1. J Pharmacol Exp Ther 1996; 277: 1726-1732.
234a. Woods, J. S. ; Dieguez-Acuna, F. J.; Ellis, M. E.; Kushleika, J., and Simmonds, P. L. Attenuation of nuclear factor kappa B (NF-kappaB) promotes apoptosis of kidney epithelial cells: a potential mechanism of mercury-induced nephrotoxicity. Environ Health Perspect. 2002 Oct; 110 Suppl 5:819-22.
235. Baudrimont I, Sostaric B, Yenot C, Betbeder AM, Dano-Djedje S, Sanni A, Steyn PS, Creppy EE. Aspartame prevents the karyomegaly induced by ochratoxin A in rat kidney. Arch Toxicol 2001; 75: 176-183.
236. Gekle M, Gassner B, Freudinger R, Mildenberger S, Silbernagl S, Pfaller W, Schramek H. Characterization of an ochratoxin-A-dedifferentiated and cloned renal epithelial cell line. Toxicol Appl Pharmacol 1998; 152: 282-291.
237. Meyer-Lehnert H, Schrier RW. Cyclosporine A enhances vasopressin-induced Ca2+ mobilization and contraction in mesangial cells. Kidney Int 1988; 34: 89-97.
238. Schwerdt G, Freudinger R, Mildenberger S, Silbernagl S, Gekle M. The nephrotoxin ochratoxin A induces apoptosis in cultured human proximal tubule cells. Cell Biol Toxicol 1999; 15(6): 405-415.
239. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253: 49-53.
240. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 1991; 350, 427-428.
241. Yoneyama M, Sharma RP, Kleinschuster SJ. Cytotoxicity of citrinin in cultured kidney epithelial cell systems. Ecotoxicol Environ Saf 1986; 11: 100-111.
242. Sillix DH, McDonald FD. Acute renal failure. Crit Care Clin 1987; 3: 909-925.
243. Paller MS. Hemoglobin- and myoglobin-induced acute renal failure in rats: role of iron in nephrotoxicity. Am J Physiol 1988; 255: F539-F44.
244. Shah SV, Walker PD. Evidence suggesting a role for hydroxyl radical in glycerol-induced acute renal failure. Am J Physiol 1988; 255: F438-F443.
245. Savitsky JP, Doczi J, Black J, Arnold JD. A clinical safety trial of stroma-free hemoglobin. Clin Pharmacol Ther 1987; 23: 73-80.
246. Andreoli SP, McAteer JA. Reactive oxygen molecule-mediated injury in endothelial and renal tubular epithelial cells *in vitro*. Kidney Int 1990; 38: 785-794.
247. Iwata M, Zager RA. Myoglobin inhibits proliferation of cultured human proximal tubular (HK- 2) cells. Kidney Int 1996; 50: 796-804.
248. Somers M, Piqueras AI, Strange K, Zeidel ML, Pfaller W, Gawryl M, Harris HW. Interactions of ultrapure bovine hemoglobin with renal epithelial cells *in vivo* and *in vitro*. Am J Physiol 1997; 273: F38-F52.
249. Nath KA, Grande JP, Croatt AJ, Likely S, Hebbel RP, Enright H. Intracellular targets in heme protein-induced renal injury. Kidney Int 1998; 53: 100-111.
250. Zager RA, Burkhart KM. Differential effects of glutathione and cysteine on Fe2+, Fe3+, H2O2 and myoglobin-induced proximal tubular cell attack. Kidney Int 1998; 53: 1661-1672.
251. Felder, E., Jennings, P., Seppi, T., and Pfaller, W.. LLC-PK1 cells maintained in a new perfusion cell culture system exhibit an improved oxidative metabolism. Cell Physiol Biochem 2002; 12, 153-62.

252. Pfaller W, Felder E, Brandauer R, Ryan MP, Westwick J, Abbott SE, Morin J-P, Hartung T, van Gompel J, Manning F. Novel human renal and hepatic co-culture *in vitro* systems for the evaluation of biotechnology-derived cytokines. ATLA 1999; 27: 303 (Abstract).
253. Helbert MJ, Dauwe SE, Van der Biest I, Nouwen EJ, De Broe ME. Immunodissection of the human proximal nephron: flow sorting of S1S2S3, S1S2 and S3 proximal tubular cells. Kidney Int 1997; 52: 414-428.
254. Kersting U, Joha H, Steigner W, Gassner B, Gstraunthaler G, Pfaller W, Oberleithner H. Fusion of cultured dog kidney (MDCK) cells: I. Technique, fate of plasma membranes and of cell nuclei. J Membr Biol 1989; 111: 37-48.
255. Pouyssegur J, Franchi A, L'Allemain G, Magnaldo I, Paris S, Sardet C. Genetic approach to structure, function, and regulation of the Na^+/H^+ antiporter. Kidney Int 1987; Suppl 23: S144-S155.

08

Aminoglycosides and vancomycin

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Epidemiology and clinical importance of aminoglycoside nephrotoxicity

This chapter covers the aminoglycosides used in present clinical practice. Most of these molecules have been introduced between the mid-60's and the early 80's (gentamicin, tobramycin, dibekacin, amikacin, netilmicin). Since then, only isepamicin and arbekacin have been developed and brought to market in only a few countries. Aminoglycoside antibiotics have important antibacterial properties. They demonstrate concentration-dependent bactericidal activity against susceptible organisms. The addition of an aminoglycoside to a β-lactam has increased survival in patients with gram-negative bacteremia, particularly in those with hypotension and severe illness [1]. A general description of aminoglycosides with respect to structures, activities and mechanisms of bacterial resistance has been reviewed by Mingeot et al. [2]. Of particular interest is the fact that, in contrast to cephalosporins or fluoroquinolones, which are used for many of the same indications, emergence of bacterial resistance during ami-

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noglycoside therapy is distinctly rare.

All of the aminoglycoside family share a potential for nephrotoxicity, ototoxicity, and rarely, neuromuscular blockade. With the exception of the aminocyclitol, spectinomycin, the aminoglycoside antibiotics all have the potential for causing injury to the renal proximal convoluted tubules, the cochlea and/or vestibular apparatus of the ear, and inducing neuromuscular blockade [3, 4]. The inherent toxicity and relative toxic potential of the aminoglycosides is partly related to their positive electrical charge at physiologic pH. At intracellular sites, the pH is even lower and the drugs are more cationic.

The reported incidence of nephrotoxicity varies from 0-50% with most in the 5-25% range [5-7]. The variability results from differences in definition of nephrotoxicity, frequency of, and tests used to measure renal function and the clinical setting in which the drugs were given. The incidence in a small group of healthy young volunteers dosed over 11 days with a single daily infusion was zero; the incidence in infected older patients with multisystem disease and exposure to other potential nephrotoxins ranged as high as 35-50% [5, 6, 8]. In prospective randomized studies with definitions of nephrotoxicity that reflect a substantive decrement of glomerular filtration rate (GFR) in seriously ill patients, the reported incidence of nephrotoxicity varies between 5-10% of patient courses [5, 9]. In a routinely applied once-daily dosing program, the incidence of nephrotoxicity may even be lower. The Hartford hospital reported an incidence of nephrotoxicity of 1.2% in a series of 2, 184 adult patients [10].

In studies of the etiology of acute renal failure, medication-induced renal injury is reported as a major cause. In an analysis of over 2000 hospitalized patients, almost 100 experienced renal insufficiency and seven episodes were attributed solely to aminoglycoside therapy [11]. A high percentage of neonatal patients are treated with aminoglycosides and in the involvement of drugs in neonatal acute renal failure has increased up to 8-fold in the last 10 years [12].

In general, the aminoglycoside-induced toxicity is manifested as a non-oliguric decline in creatinine clearance. Progression to dialysis-dependent oliguric-anuric renal failure is unusual unless other risk factors are present [8]. As in animal models, the renal failure is generally reversible. In a few patients there has been documented recovery of renal function despite continued administration of the aminoglycoside [13].

A study from 1987 has measured the economic impact of aminoglycoside nephrotoxicity. This study had an incidence of nephrotoxicity of 7.3%. There were 2.74 additional regular hospital days and 1.50 intensive care unit days. The average additional cost of this renal complication calculated over each course of prescribed therapy was US$ 2501 in 1987 [14]. With the inflation in medical costs this impact was already increased to US$ 4583 per case in 1997 [15] and can be calculated to US$ 6133 in 2002.

Risk factors of aminoglycoside nephrotoxicity

Studies on the pathogenesis of aminoglycosides are largely carried out in otherwise normal animals while clinically these drugs are administered to patients with serious infections and associated co-morbidities. Based on clinical observations, there appear to be a variety of factors that predispose to the development of renal dysfunction with aminoglycoside therapy [8] (Table 1).

Age

The incidence of aminoglycoside nephrotoxicity rises with advancing age from 7% in patients under age 30 to 15% in patients over 70 years of age [16]. It is likely that dosage may be excessive in older patients based on overestimates of drug excretory capacity by insensitive renal function tests such as the serum urea nitrogen or serum creatinine. This age effect has been confirmed in a retrospective stepwise discriminant analysis of 214 patients in randomized prospective trials [17]. The mechanism of this age effect is unclear since experimental studies show a decrease in drug uptake in older animals compared to similarly dosed younger animals [18].

Pre-existing renal disease

Moore et al. [17] studied patients with pre-existing renal disease, as estimated by serum creatinine greater than 2 mg/dl and found no increased risk of toxicity if the dose was carefully adjusted. Yet, pre-existing renal failure clearly exposes the patient to unsuspected overdosing. In addition, the kidney from patients with

Table 1. Risk factors for aminoglycoside nephrotoxicity [17, 42, 46].

RISK OF TOXICITY INCREASE	RISK OF TOXICITY DECREASE
Patient factors:	
Older patients* [16]	Younger patients*
Pre-existing renal disease	Normal renal function
Female gender [17]; male gender [46]*	
Magnesium potassium, calcium deficiency*	
Intravascular volume depletion*, hypotension* [27]	Normotensive*
Hepatic syndrome	No hepatic dysfunction
Sepsis syndrome*	
Aminoglycoside factors:	
Recent aminoglycoside therapy	No recent aminoglycoside therapy
Larger doses*	Smaller doses*
Treatment of 3 or more days* [16]	Treatment of less than 3 days*
Drug choice: e.g. gentamicin [50]*, amikacin [9]*	Drug choice: e.g. tobramycin [50]*, netilmicin [51]*
Frequent dosing interval*	Once-daily dosing*
Concomitant drugs:	
Amphotericin B	Extended spectrum penicillins [47]*[1]
Cephalosporins [45, 52]	
Cisplatin	
Clindamycin	
Cyclosporine	
Foscarnet	
Furosemide	
IV radiocontrast agents	
Piperacillin [49]	
Vancomycin [53, 54]	

**In concurrent with experimental nephrotoxicity data*

[1]Extended spectrum penicillins: e.g. carbenicillin, ticarcillin

pre-existing renal disease may have a decreased ability to recover from ischemic and/or toxic insults [19, 20].

Other demographic and patient factors

Retrospective analyses of clinical studies suggest that females are more susceptible to aminoglycoside nephrotoxicity than males [17]. This is in contradiction to the increased susceptibility of male rodents to aminoglycosides [21]. In experimental animals with streptozotocin diabetes, nephrotoxicity and renal drug uptake are markedly reduced [22, 23]. There seems to be no clinical counterpart to this experimental observation.

Depletion of intravascular volume is an important risk factor for aminoglycoside-induced nephrotoxicity whether induced by sodium depletion, hypoalbuminemia, diuretics, systemic acid-base disturbances or sepsis [24-28]. These co-morbid conditions per se are not associated with increased risk if acid-base and electrolyte/volume status are maintained [29]. Hypokalemia and hypomagnesemia may be both predisposing risk factors or the consequences of aminoglycoside-induced damage [30, 31].

Accumulated evidence suggests that liver disease is an important clinical risk factor for aminoglycoside nephrotoxicity [32]. This is particularly true of patients with biliary obstruction or cholangitis as distinct from other causes of liver disease such as alcoholic cirrhosis [33]. When liver disease is defined as any three of six criteria consisting of, AST > 2 times normal, total bilirubin > 2.5 µg/dl, albumin < 3 g/dl, elevated alkaline phosphatase, prothrombin time > 15 seconds or

ascites, the relative risk of developing tobramycin-induced renal dysfunction was 31.8 (95% confidence interval: 19.7-51.4) based on an analysis of 179 patients [34].

Sepsis

Because of the unique importance of aminoglycosides in treating patients with difficult gram-negative sepsis, the hemodynamic and metabolic perturbations of the sepsis syndrome often coalesce with the drug to produce synergistic nephrotoxicity. Acute or chronic endotoxemia amplifies the nephrotoxic potential and renal uptake of gentamicin in rats [35-37]. Endotoxin and other bacterial toxins or virulence factors trigger increased reactive oxygen intermediates in renal tubular cells which may be additive to the membrane damage produced by the aminoglycosides themselves [38, 39]. The increased renal metabolic demands due to fever along with shock, ischemia and foci of tissue necrosis enhance aminoglycoside nephrotoxicity by accelerating the course and severity of the toxic insult [40, 41]. In any critically ill patient with acute renal dysfunction, it is often difficult to discern the precise etiologic role of aminoglycosides.

In order to identify individuals at high risk for aminoglycoside nephrotoxicity, a predictive model was developed based on retrospective analysis of risk factors in 338 patients receiving aminoglycosides. Nephrotoxicity developed in 17.5% of the sample population with duration of therapy the strongest associated risk factor [42]. Initial one hour post-dose blood level - which directly correlated with the dose of aminoglycoside administered-, liver disease, age, and female sex were other risk factors of significance based on multivariate analysis. Using their new model, it prospectively predicted 14 of 15 patients with nephrotoxicity (93% sensitivity) and 106 of 160 without toxicity (66% specificity) [42]. However, a later study using the same model found only a 42% sensitivity and 54% specificity [43].

Drug-related risk factors

Toxicity is obviously related to techniques used to administer the aminoglycosides (choice of drug, dosage, schedule,...), however, before discussing the practical applications, the reader must appreciate the mechanism of renal aminoglycoside drug handling and the pathogenesis of nephrotoxicity.

Toxicity of aminoglycosides is enhanced by co-administration of other drugs. Some concomitant drugs having additive effect do so because of potential kidney toxicity: e.g., amphotericin B, vancomycin, foscarnet, cyclosporine, contrast dye and cisplatin. Others with statistically identified risk factors defy explanation at present: e.g., clindamycin. All these drugs are commonly given to patients who receive or will receive aminoglycosides.

On the other hand, certain drugs have been reported to decrease aminoglycoside toxicity, such as antipseudomonal penicillins. Thus, when gentamicin or tobramycin plus carbenicillin or ticarcillin are administered to a febrile neutropenic patient, the reported incidence of nephrotoxicity is between 2-6% as compared to a 10-15% incidence when the aminoglycoside is combined with other β-lactam drugs [44, 45]. In part this protection may be due to the increased sodium content of these two penicillins when administered in large doses [46, 47]. Experimentally, piperacillin seems to protect against the early gentamicin-induced alterations in animals [48], but and unambiguous clinical counterpart has not been demonstrated. A recent analysis of risk factors actually reported an increased risk of aminoglycoside nephrotoxicity with concomitant piperacillin but not carbenicillin or ticarcillin [49]. The authors speculated that the lower sodium content of piperacillin might explain the difference. It is possible that the difference between the incidence of nephrotoxicity when penicillins are given with aminoglycosides versus when cephalosporins are the co-administered drug, relates to relative protection by the penicillins, not enhancement by the cephalosporins.

Physiopathology

Renal handling

Because of their polar nature, the distribution of aminoglycosides is largely restricted to the extracellular space. Consequently, their distribution volume is small both in animals and humans. It equals the volume of the extracellular space or approximately 0.25 L/kg body weight, in normal subjects.

The plasma pharmacokinetics of aminoglycosides have been described by a three interrelated compart-

ment model [55, 56]. The first phase (α or distributive) corresponds to the distribution of the drug from the vascular to extracellular spaces, and occurs with a half-life of 15 to 30 minutes following intravenous administration. The second phase (β or eliminative) involves excretion of drug from the plasma and the extravascular spaces, and is essentially determined by the GFR. This phase is most important for the adjustment of the dose. The third (or γ) phase corresponds to the prolonged, slow elimination of drug that has accumulated in the 'so-called' deep compartment, which essentially corresponds to the kidney. For most dose-adjustment decisions, plasma kinetics can be simplified to a one-compartment model because the distribution phase is short and because the γ phase influences serum levels that are significantly lower than therapeutic concentrations (the activity of aminoglycosides is primarily related to the serum peak concentration). Moreover, the influence of the third compartment on plasma kinetics is minimal because release of the drug from this compartment is only detected in the urine.

The plasma half-life of different aminoglycosides is very similar [1.5 to 3.5 hours in humans with normal renal function [57] and correlates with the GFR [58, 59]. Aminoglycosides are excreted unchanged from the body, primarily by glomerular filtration. The renal clearance of aminoglycosides is, however, about 10 to 30 % lower than GFR [56]. Theoretically this difference between aminoglycoside clearance and glomerular filtration could be due to a decreased glomerular ultrafiltrability of aminoglycoside. Pastoriza-Munoz et al. [60] found in Munich-Wistar rats a Bowman's space plasma gentamicin/inulin ratio of 0.86. The authors attributed this restriction in gentamicin ultrafiltrability primarily to the influence of the Donnan effect on the passive distribution of these polycations across a semipermeable membrane, rather than to binding of drug to plasma proteins. However, the main reason for the observed difference between aminoglycoside clearance and GFR is partial reabsorption of aminoglycosides along the tubules (see below). Because of the dominant role of glomerular filtration in aminoglycoside clearance, half-life is prolonged in all cases of decrease in renal function, such as in the elderly [61] or in very young and premature infants (up to 8 hours) whose kidneys are immature. Aminoglycoside half-life is considerably shorter in small rodents (approximately 30 min) because of the characteristically larger renal clearance in these animals as compared to man.

Cortical uptake

Initial understanding of the physiopathology of aminoglycosides nephrotoxicity occurred in the seventies with the demonstration of aminoglycoside accumulation in the renal cortex [62, 63]. This finding was first documented in animals, but later confirmed in the human kidney [64-67]. Autoradiographic studies have consistently shown that cortical accumulation is the result of aminoglycoside uptake by the proximal tubular cell [68-71]. Autoradiographic, micropuncture and immunocytochemical studies have localized aminoglycosides to S1/S2 proximal tubule cells [69, 71-74]. Much attention had been focused on the identification of pathway(s) responsible for aminoglycosides uptake in proximal tubular cells, but the proposed mechanism remained a matter of controversy. On one hand a common transport system for gentamicin and polyamines (e.g. polylysine or spermine) at the brush border membrane of the renal proximal tubular cells was proposed [75-77]. In parallel, it was suggested that gentamicin was transported across the brush border membrane through a cation/H^+ exchange [78]. However, adsorptive endocytosis soon became the most likely mechanism, but its molecular basis, and the reason why uptake takes place only in proximal tubular cells remained to be resolved. Thus, early studies showed that gentamicin binds to anionic phosphatidyl inositides in the luminal membrane before being ingested into the renal cortical cells by endocytosis [79-81]. Soon evidence was presented that gentamicin binds to the brush-border membrane of renal epithelial cells through electrostatic interactions [82, 83]. A key role for megalin, as an endocytotic receptor expressed on the apical surface of the proximal tubular epithelium, was proposed in the mid 90's. Megalin binds low molecular weight plasma proteins and is critical for their reabsorption from the glomerular filtrate [84]. Megalin is also responsible for the re-uptake of many xenobiotics from the luminal urine, and particular polybasic substances which interact with the abundant negatives charges present on the extracellular receptor domain [85]. Several studies in the rat have addressed the role of megalin in renal aminoglycosides uptake: Moestrup et al. [85] used the specific antagonist receptor-associated protein (RAP), to block the activity of

megalin in perfused rat proximal tubules, causing a 20% reduction in gentamicin clearance. Nagai [86] demonstrated similar results in rats treated with maleate (which impairs the receptor-mediated uptake of megalin ligands). Finally, the demonstration that mice with genetic or functional megalin deficiency do not accumulate aminoglycosides in their proximal tubular cells and are protected against aminoglycoside-induced nephrotoxicity has provided compelling evidence for the role of this protein as the principle *in vivo* drug target [87].

Intracellular handling

Following binding to megalin in the brush border, aminoglycosides traffic via the endocytotic system to lysosomes, where they accumulate in large amounts (reaching concentration 10-100 time the serum concentration). This process probably relates to the capacity of lysosomes to retain non-diffusible solutes, while water and other diffusible solutes can leave these organelles [88]. Furthermore, aminoglycosides are resistant to degradation by lysosomal hydrolases (lysosomal glycosidases act only on neutral or acidic sugars). Recent information (Figure 1), also indicates that a measurable (5-10%) amount of internalized gentamicin traffics directly and rapidly from the surface membrane to the Golgi apparatus [89-92]. This novel finding for gentamicin is consistent with the movements of other surface ligands such as the Shiga or Ricin toxins [93-95]. Potentially injurious situations, such as ischemia, may increase the shunting to the Golgi apparatus [96].

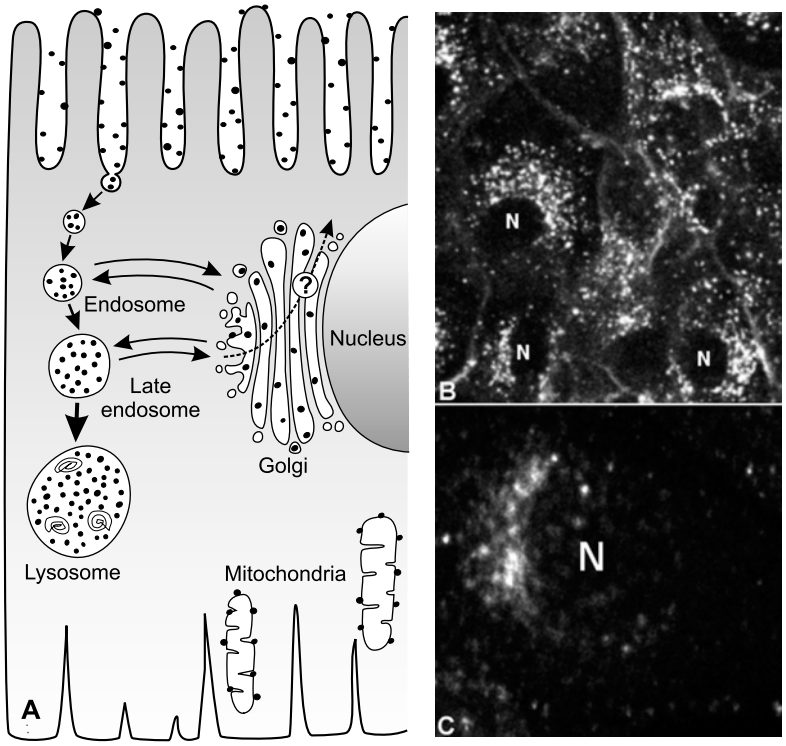

Figure 1. *Binding uptake and intracellular trafficking of gentamicin in renal proximal tubular cells.* ***A.*** *Gentamicin (•) is shown binding to the surface membrane and being internalized by a receptor (megalin) – mediated endocytic process. Gentamicin also enters the cell through fluid phase endocytosis. It moves through the endocytic system into late endosomes and from there into lysosomal structures. A small but quantifiable fraction (5-10%) of gentamicin directly traffics from the surface membrane into the trans-Golgi network and from there throughout the Golgi Apparatus. Panel* ***B*** *shows total cellular Texas Red Gentamicin fluorescence following two hours of uptake in LLCPK1 cells. Panel* ***C*** *shows cellular uptake with quenching of lysosomal and endosomal fluorescence utilizing an HRP method. The paranuclear fluorescence has been shown, using co-localization studies, to be consistent with Golgi uptake. In both inserts N stands for nucleus. Mitochondria have also been shown to contain aminoglycoside following treatment.*

Cellular toxicity

High dose

Pathologic alterations reported vary from minor changes to frank, extended necrosis depending on the duration of the treatment and the amount of drug used. Initial rodent studies used large doses (40 mg/kg gentamicin and above), because of the belief that these animals had reduced sensitivity to aminoglycosides because of a faster drug elimination rate. Under these circumstances, acute tubular necrosis is readily observed, but it is difficult to ascertain the sequence of events leading to this stage of tissue injury. High dose studies were instrumental in demonstrating the relationship between injury of proximal tubules and kidney dysfunction, the onset of an intense regeneration leading to the repopulation by immature cells which resulted in a state of apparent refractiveness to further aminoglycoside insult. The successive events of alteration and recovery have been partially dissected and characterized by using infused animal models, which allow transient, acute exposures [97]. High dose studies have also allowed delineation of the potential involvement of alterations in distal tubules [98] and in the glomerulus [99] as contributing to kidney dysfunction. Major changes in these anatomic locations have been described, but may not be pertinent to the situation in humans.

Low dose

An intriguing aspect of aminoglycoside nephrotoxicity is that very large amount of drugs (usually 10 times the therapeutic doses) must be administered to animals in order to have active acute tubular necrosis and concomitant alteration of the renal function [100, 101], while rats given low, therapeutically relevant doses, show neither extensive tubular necrosis nor gross kidney dysfunction. Low doses induce an array of alterations involving the apical and basolateral membranes, the lysosomes and various other subcellular components of proximal tubular cells, none of which correlate with organ dysfunction [102]. The recent observation that aminoglycosides induce apoptosis both *in vivo* and *in vitro* [2, 71] at therapeutically relevant doses has shed new lights on the early aspects of toxic mechanisms. Apoptosis, or programmed cell death, was first described by Kerr in 1972 [103], and is characterized by specific features of cell shrinkage, increased cytoplasmic density, condensation of chromatin and fragmentation of the DNA. While apoptosis is an important, physiological event in many normal processes such as embryogenesis, remodeling of tissue, or maturation of the immune system, it can also be triggered by a large array of toxic agents [104]. Rats treated with low doses of aminoglycosides show a marked apoptotic reaction in proximal tubules (Figure 2) which is (i) detectable after 4 days of treatment, and conspicuous after 10 days, (ii) dose-dependent and (iii) occurring in absence of necrosis [2]. Gentamicin-induced apoptosis can be also demonstrated on cultured cells of renal (LLC-PK1; MDCK) and non-renal (embryonic fibroblasts) origin [71]. Current work suggest that lysosome destabilization is a key triggering event in the onset of apoptosis in LLC-PK1 cells incubated with gentamicin, with other pathways, such as the mitochondrial also are involved.

In addition to apoptosis and necrosis, aminoglycosides also cause multiple morphological and functional evidence of organelle toxicity. In apical membranes there is inhibition of alkaline phosphatase activity and decreased transport of D-glucose [105], while

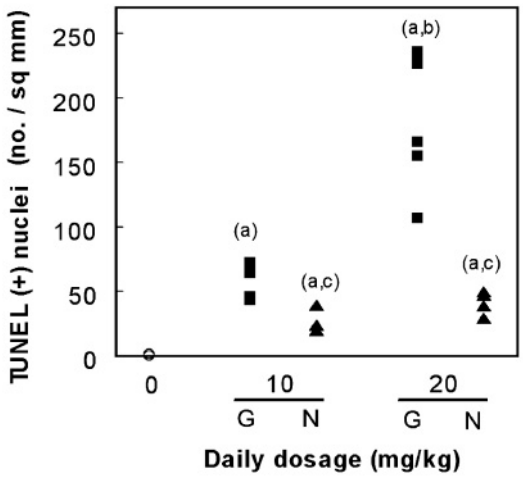

Figure 2. *Enumeration of apoptotic nuclei observed in cortex sections after application of TUNEL. Open circles, control animals; filled squares, animal treated for 10 days with gentamicin (G); filled triangles, animals treated with netilmicin (N). Each point refers to the pooled counts of one individual animal (n=6 in each group). Statistical analysis (ANOVA followed by Scheffe post hoc test with P<0.05): a, significantly higher than control; b, significantly higher than the lower dose; c, significantly lower than the other aminoglycoside at the same dosage [116].*

basolateral membranes show a marked inhibition of Na-K-ATPase activity [75, 106, 107] when the drugs are presented in the cytoplasmic but not external surface of the membrane [75]. Aminoglycosides inhibit the phosphatidylinositol cascade in proximal tubule cells in culture stimulated by parathyroid hormone [108]. Most conspicuously, aminoglycosides induce an intense phospholipidosis that has been demonstrated in cell culture [109], experimental animals [97, 110-112] and man [65]. This phospholipidosis occurs rapidly involving all major phospholipids, with the predominant increase being phosphatidylinositol [110, 111]. Accumulation of phospholipids within the lysosomes is responsible for the formation of the so-called 'myeloid bodies' [109, 113, 114]. Subcellular fractionation studies, however, have also showed an increased in the phosphatidylinositol content in other organelles such as brush-border and basolateral membranes, mitochondria, and endoplasmic reticulum [92, 110-112], with a significant increase in total phospholipids limited to basolateral membrane [112]. The cellular contents of neutral lipids and cholesterol are not increased. The phospholipidosis induced by aminoglycosides has been proposed to result primarily from an impaired degradation due to the inhibition of phospholipases A and C and sphingomyelinase [97, 109, 115]. The activity of the other lysosomal enzymes are unaffected [109]. A recent study has suggested that phospholipidosis could be due to the increased synthesis of phosphatidylinositol and phosphatidylethanolamine [115]. At present, it is unknown whether phospholipidosis is linked to apoptosis and necrosis. Some aminoglycosides induce a marked phospholipidosis with little apoptosis or necrosis (netilmicin, e.g.; [116, 117]). Moreover, gentamicin induces apoptosis in LLC-PK1 cells without associated phospholipidosis [71]. Besides lysosomes, aminoglycoside-induced alterations of mitochondria have also been described. Bendirdjian et al. [118] showed a competitive interaction between gentamicin and Mg^{2+} resulting in reduced mitochondrial respiration. Simmons et al. [119] showed a reduced respiration of mitochondria isolated from rats treated with gentamicin, and additional studies have revealed competitive displacement of Mg^{2+} at the inner mitochondrial membrane sites by gentamicin [120]. In this context, Sundin et al. reported that gentamicin might traffic to the Golgi complex and mitochondria *in vivo* [92]. This observation may have particular significance since polyamines are known to activate mitochondria and cause the release of cytochrome c, an important precursor to apoptosis [121]. More recently, proteomic analysis following gentamicin administration indicate energy production impairment and a mitochondrial dysfunction occurring in parallel to the onset of nephrotoxicity [122]. Involvement of oxygen radicals species at the level of the mitochondria are also proposed as an potentially important mechanism [123, 124].

Aminoglycosides are known to inhibit prokaryotic protein synthesis by binding to ribosomes, blocking initiation and causing mistranslation [125, 126]. Studies indicate gentamicin that administered *in vivo* rapidly reduces renal cortical endoplasmic reticulum protein synthesis *in vitro* [127, 128]. Data indicates that a major of the *in vivo* inhibitory effect of gentamicin on protein occurs during the first two days after antibiotic administration [92]. The mechanism by which this occurs is unknown, but could involve inhibition of nuclear transcription or alteration of endoplasmic reticulum or Golgi-mediated post-translational modifications.

In summary, while aminoglycoside antibiotics are known to induce cellular toxicity by affecting numerous intracellular organelles and processes, the mechanisms by which this occurs remain poorly understood. At present, critical questions regarding the understanding of aminoglycoside nephrotoxicity center around the compartmentalization of aminoglycoside within endosomal/lysosomal structures and subsequent movement throughout the cell. Do aminoglycosides diffuse out of the endocytotic compartment into the cytosol where they can induce direct subcellular organelle damage, or do they remain sequestered within these compartments and traffic to other subcellular organelles via retrograde vesicular trafficking (Figure 1). How does co-existing ischemia alter the intracellular compartmentalization of aminoglycosides, and why does oxygen deprivation result in markedly enhanced cellular toxicity ?

Secondary cellular and tissue alterations

Two major observations made during the low dose studies and post-treatment recovery, are that aminoglycosides induce (a) a dramatic increase in tubular cell turnover, and, (b) a marked proliferation in the cortical interstitial compartment [98, 129]. The first phe-

nomenon most likely results from the necrosis/apoptosis process and is certainly essential for the recovery or maintenance of a normal kidney structure. Conflicting results involving the role of growth factors in the regenerative response in the kidney after toxic injury have been reported. Administration of a high doses of gentamicin to rats provoke an early and dramatic decline in amounts of mRNA coding for epidermal growth factor, resulting in undetectable amounts after four days and below normal levels for at least ten days. Thus, this decline persists throughout the complete regeneration phase [129]. Subcutaneous infusion of rats with epidermal growth factor during gentamicin nephrotoxicity and repair shortens the period of tubular necrosis, but changes in serum creatinine, BUN values, or cell proliferation do not different significantly from rats given only gentamicin [130]. The proliferative response in the kidney of gentamicin-treated rats starts in the interstitial compartment [98, 129]. An interstitial infiltrate of leukocytes, including macrophages and T lymphocytes is observed. For the moment, the role of this infiltration in the initiation of tubular damage and subsequential recovery is unclear.

Prevention of aminoglycoside nephrotoxicity

The above descriptions lead to a number of potential and useful applications at the level of drug development and evaluation, as well as that of the direct care of the patient (Table 2).

Experimental studies

In vitro and *in vivo* models, which explore the above considerations have been useful in rationally analyzing the potential differences among aminoglycosides with respect to renal tolerance. Thus, aminoglycoside congeners appear to have quite different nephrotoxicity profiles when examined in experimental set-ups. There is obviously a hierarchy among the drugs used in clinical practice, and this hierarchy is based on the combination of pharmacokinetic parameters (essentially the level of cortical accumulation) and the intrinsic toxicity (i.e. its ability to cause tubular insult for a given cortical concentration). Ranking by these two criteria is not identical, because the molecular parameters involved differ. The two types of ranking in experimental animals are presented in Table 3. One is cautioned against using a direct animal to human transformation. First, it will only apply to patients who are, otherwise, strictly identical in terms of risk factors and underlying conditions. This is rarely the case and explains why conflicting clinical reports have been published concerning the relative nephrotoxicity of different aminoglycosides. There has been little dispute about such a ranking in experimental animals. Thus, for instance, amikacin which is active against many gentamicin- or tobramycin-resistant strains, has long been considered as an aminoglycoside to be used only in patients at high risk (and therefore most often more severely ill or in more complex situations) or in patients who experienced failures with the other aminoglycosides because of resistance problems but who were already partly intoxicated by the first course. Second, the difference between drugs may be sufficiently narrow (see for instance the difference between gentamicin and netilmicin in animals treated with low, clinically-relevant doses [131]) so as to almost vanish when examined in the context of a clinical study where

Table 2. Possible measures to prevent aminoglycoside-induced nephrotoxicity.

1. Limit the duration of treatment to maximal 7 days
2. Choose the least toxic aminoglycoside
3. Adapt dose to renal function
4. Avoid concomitant administration of potentially nephrotoxic drugs
5. Determine clinical risk factors

Table 3. High to low nephrotoxicity of aminoglycosides in rats.

According to renal cortical accumulation (% of administered dose)	According to intrinsic toxicity
Neomycin	Neomycin
Gentamicin	Gentamicin
Netilmicin	Tobramycin
Tobramycin	Netilmicin
Amikacin	Amikacin
Streptomycin	Streptomycin

variations among patients and other causes of nephrotoxic insult cannot be avoided and cause 'background noise' to an extent equal to that of the aminoglycoside-induced toxicity itself. Given these methodological caveats, it is nevertheless interesting to note that there is a clinical consensus that gentamicin, at the one end of the spectrum, tends to be more nephrotoxic than amikacin, with tobramycin and netilmicin somewhere 'in between', as suggested from the experimental studies. There are so far no direct clinical data comparing the nephrotoxic potentials of dibekacin and sisomicin with other aminoglycosides in comparable patients. Data with isepamicin are still scanty in Caucasian populations, but the Japanese experience seems very positive (note however that aminoglycosides are given at considerably lower doses in Japan compared to other countries, which may obscure the picture). Neomycin, was recognized early on as a very nephrotoxic aminoglycoside so that its parenteral use was discontinued. Thus, there is only very limited clinical information available beyond anecdotes or 'case reports'. Streptomycin is accepted as being virtually non-nephrotoxic, but one has to remember that it was never included in any large clinical trial for comparison with other aminoglycosides.

Another development in experimental studies has been the search for agents or approaches that would protect against aminoglycoside nephrotoxicity. Among them, the use of polyaspartic acid has been most successful since the co-administration of this acidic polymer with gentamicin or amikacin provides almost complete protection against the development of all measurable histological or functional signs related to aminoglycoside treatment in the experimental animal, using a wide variety of conditions (low and high doses, acute and chronic treatment...). Discovery of polyaspartic acid as a protectant against aminoglycoside-induced nephrotoxicity actually arises from the erroneous assumption that it would prevent or compete with the aminoglycoside uptake by kidney tubular cells. This proved to be wrong, since co-administration of polyaspartic acid actually increases the amount of aminoglycoside recovered from the kidney cortex, even though it eventually, and quite paradoxically, achieves protection [132]. Available data suggest that polyaspartic acid actually complexes aminoglycosides in the lysosomes of proximal tubular cells (binding of the acidic polymer to the polycationic drug is fostered by the acid pH of lysosomes) and thereby prevents drug binding to lysosomal acidic phospholipids and/or other anionic intracellular targets. Interestingly enough, this observation provides further evidence that the intralysosomal aminoglycoside acts as a key factor in the onset and/or development of aminoglycoside-induced nephrotoxic insult.

Clinical studies: the once-a-day schedule

Paradoxically, early experimental studies had conclusively demonstrated that daily doses of aminoglycosides given according to conventional clinical schedule of "thrice-a-day" (TID, i.e. the daily dose split into 3 administrations at 8-hour intervals) over a one-week period would invariably cause more intense toxicity than the same daily dose given in only one administration per 24 hours [133]. A high peak of aminoglycoside has long been noted, and accepted, as a statistically significant risk factor in clinical situations. But this was based on retrospective studies in which the schedule of administration was kept unchanged (TID) so that high peaks were manifestation of overdosing. The bulk of the evidence now shows that aminoglycoside nephrotoxicity can be dissociated from the height of the peak of the aminoglycoside blood level. Over the years, it became obvious that for a given total daily dose of any specific aminoglycoside, the magnitude of the toxicity could be manipulated by changing the schedule of administration and that toxicity was greatest when the daily dose was being divided into multiple small administrations (in experimental toxicological evaluations, a practical approach to detect toxicity with minimal amounts of drug has been the use of implanted osmotic pumps which can give almost continuous infusions for a couple of days). The reason for this apparent paradox is that the drug uptake is saturable, as seen above, so that maintaining a low serum level maximizes the relative drug uptake. Quite importantly, the apparent saturation constant lies close to values round which the serum concentration will vary when changing the schedule of administration from thrice a day (TID) to once-day (QD) in patients. Moreover, that concentration (15 mg/L for gentamicin) is consistent with the dose needed to achieve effective therapy. Indeed, at the same time, bacteriological evidence became available that high concentrations of aminoglycosides (above 10 mg/L for gentamicin)

(1) enhance bacterial killing, (2) prolong the so-called post-antibiotic effect of aminoglycosides (which is the period during which no bacterial regrowth will be observed following drops in the drug concentration below the *in vitro* minimum inhibitory concentration for the bacteria under study), and (3) could also be useful in preventing the selection of bacteria with intermediate sensitivity to aminoglycosides (reviewed in [2]). This prompted the launching of several clinical trials comparing conventional dosing (TID; BID) of aminoglycosides with a QD mode of administration. When small groups of patients were combined, no difference in efficacy or increase in toxicity was evident, rather the magnitude of the subclinical signs of aminoglycoside-induced tubular insult, like phospholipiduria was reduced [134, 135].

Several meta-analyses pooled the data of individual RCT (Table 4) [136-145], including a meta-analysis specifically of the studies in immunocompromised patients [145]. It is apparent that only the meta-analyses that combined the results of the individual RCT by means of a fixed-effects model yielded significant results in favor of less nephrotoxicity in the single daily dose regimens. However, given the inhomogeneity of the study designs and the different aminoglycosides used, it seems prudent to use the random-effects model to combine the individual studies. The meta-analyses that used this technique did not show a significant difference in the two dosing regimens. Nevertheless, in all analyses the single daily dose regimen was associated with an increase in nephrotoxicity.

In recent years three new prospective studies have been published. The study of Rybak [146] is remarkable since it was not only randomized but also performed in a double-blinded fashion. Once-daily versus twice-daily administration of aminoglycosides was evaluated in 123 patients with suspected or proven gram-negative bacterial infections. Once-daily dosing of aminoglycosides had a predictably lower probability of causing nephrotoxicity than twice-daily dosing. Uijtendaal et al. (147) evaluated the efficacy and nephrotoxicity of once-daily administration of gentamicin versus multiple-daily administration in 52 children and infants. Nephrotoxicity occurred in 6 patients, 3 in each group. Both regimens were equally effective.

Karachalios et al. [148] investigated the efficacy and safety of amikacin administered once-daily versus twice-daily in adult patients with systemic infections. There was no difference in clinical response, bacterial cure rate, or in the incidence of nephrotoxicity, which was unusually low in this series (5 patients out of 136).

In conclusion, although a decrease in nephrotoxicity rates in once-daily dose regimens has not been established, extended-interval dosing strategies have never been associated with an increased risk of nephrotoxicity. The main reason why the majority of acute care hospitals [149] have adopted this strategy is that once-daily dosing provides a cost-effective method for administration of aminoglycosides by reducing work load among service personnel and by reducing or even

Table 4. Meta-analyses of the incidence of nephrotoxicity in single daily dosing versus multiple dosing of aminoglycosides.

Author	N of RCT	Method	Result (95% CI)
Blaser & König, 1995 [136]	24	Summation	RR 0.82
Galloe et al., 1995 [137]	16	Not given	RR 1.00 (0.98-1.02)
Barza et al., 1996 [138]	21	Random-effects model	RR 0.78 (0.57-1.07)
Munckhof et al., 1996 [139]	15	Random-effects model	RD –1.3 % (-5% – 3.1%)
Ferriols-Lisart & Alos-Aliminana, 1996 [140]	18	Fixed-effects model	OR 0.60 (0.40-0.86)
Freeman & Strayer, 1996 [141]	15	Fixed-effects Peto	OR 0.70 (0.51-0.94)
Hatala et al., 1996 [142]	13	Random-effects model	RR 0.87 (0.60-1.26)
Ali & Goetz, 1997 [143]	26	Random-effects model	RD –0.18% (-0.99% - 3.75%)
Bailey et al., 1997 [144]	22	Random-effects model	RD –0.6% (-2.4% - 1.1%)
Hatala et al., 1997 [145]	4	Random-effects model	RR 0.78 (0.31 – 1.94)

OR: odds ratio; RR: risk ratio; RD: risk difference

eliminating the need for therapeutic drug monitoring [150, 151].

Clinical studies: pharmacokinetic dosing

To reduce the toxicity burden of aminoglycosides, monitoring of drug serum levels and applications of pharmacokinetic principles to achieve predefined levels in patients is common practice. Eight prospective, randomized controlled trials specifically designed to investigate the effect of pharmacokinetic dosing [152] on aminoglycoside expression of nephrotoxicity could be identified from the literature [153-160]. These individual studies have been unable to detect any change in the incidence of this adverse event. We combined the results of the studies using a 'random effect model' [161] (Figure 3). The overall relative risk for nephrotoxicity in the pharmacokinetically dosed group was found to be 0.90 (95% confidence interval 0.61-1.31), thus strengthening the findings of the individual studies. The reason may be that those 'predefined levels' were not those above which toxicity rates would be importantly modified. But, more basically, it must be emphasized that an underlying and indispensable assumption in the concept of drug monitoring to prevent drug toxicity is, that serum levels are the main determinant of toxicity. This assumption may be flawed for aminoglycosides [162, 151]. First, the development of the once-a-day concept has made clear that high peak levels per se are not necessarily associated with toxicity (what a high peak level, for a given scheme of administration mean can be either a high total dosage or a small volume of distribution, and these two conditions may already have opposite meanings in terms of toxicity). Peak levels, nowadays, are probably more useful to ascertain aminoglycoside effectiveness in situations where they could be abnormally low such as in patients with a larger volume of distribution or when infection with organisms with intermediate sensitivity is feared. Trough levels undoubtedly are of toxicological significance; because their elevation would cause the kidney to be exposed for prolonged periods of time to drug concentrations below saturation of binding to the brush border, i.e. conditions which maximize the uptake and the toxicity. Yet, an elevated trough level will most often mean a decrease in the glomerular filtration of the drug and an actual reduction of its availability to the proximal tubular cells (remember that an increased creatinine clearance - and therefore an increased filtration rate of the aminoglycoside may be an independent risk factor for toxicity, whereas a decreased creatinine clearance *per se* is not). Thus, the interpretation of an elevated trough level is that there has been a delay in drug elimination. Consequently, less drug needs to be given or more prolonged intervals can be applied between drug administrations. Universal acceptance is difficult because (1) the lack of normative objectively defined values and (2) that trough levels are often difficult to accurately measure, certainly in extended interval dosing schemes [151].

Another assumption needed to accept serum level determinations as useful, is that the degree of toxicity is linearly related to the trough level. Current evidence does not support this correlation, since the early data of Schentag and coworkers [163] clearly demonstrated that the extent of renal uptake - which in turns will trigger toxicity - may vary widely among patients. What we actually need is a direct estimation of the amount of drug retained in the kidney, but that information is not easy to obtain by non-invasive techniques.

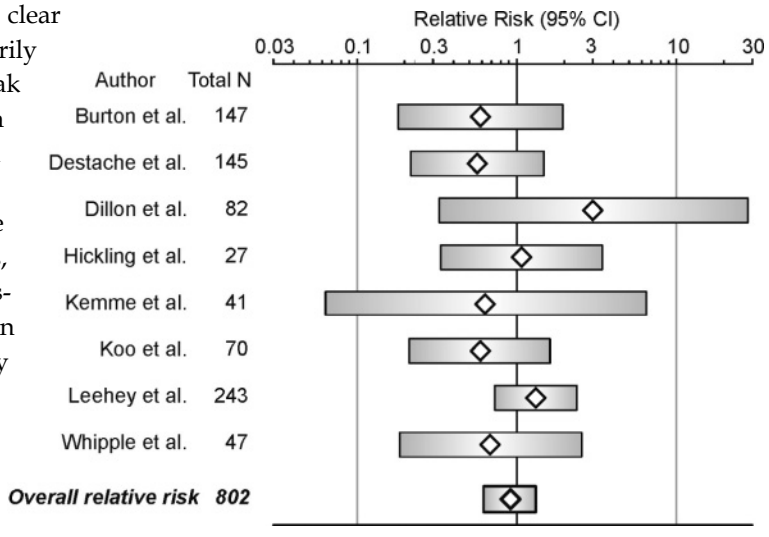

Figure 3. *Forest plot of the relative risk of nephrotoxicity (value < 1 favors pharmacokinetic dosing) of the 8 individual randomized controlled trials. The overall relative risk was 0.90 (95% CI 0.63-1.31).*

Vancomycin

Vancomycin is a glycopeptide antibiotic with a molecular weight of 1500. The drug is poorly absorbed from the gastrointestinal tract. Vancomycin cannot be given by the intramuscular route and should be administered as a slow 60-minute infusion, when parenteral therapy is necessary. Approximately 30% is bound to serum protein [164, 165]. Renal vancomycin elimination is predominantly by glomerular filtration [166]. The renal clearance of vancomycin amounts to 90% of inulin clearance and 80% of creatinine clearance. In healthy subjects, 30% of the systemic clearance is by non-renal mechanisms. The vancomycin clearance is decreased in the elderly and dosage should be adjusted in this population [167]. A reduction of dosage is also necessary in patients with renal insufficiency. Dosing nomograms for use in patients with renal insufficiency are available [168, 169]. Since vancomycin is not eliminated by hemodialysis or peritoneal dialysis, there is no need for additional dosing after dialysis [170].

The worldwide increase in the incidence of resistant gram-positive infections has renewed the interest in the T glycopeptide antibiotics [171].

Vancomycin is the drug of choice against serious infections caused by methicillin-resistant strains of *Staphylococcus aureus* and coagulase-negative staphylococci [172]. It may also be used for treatment of infections by gram-positive organisms in penicillin-intolerant patients. Vancomycin has been extensively used to treat endocarditis caused by streptococci, enterococci and staphylococci. The empiric treatment of intravenous catheter sepsis and hemodialysis vascular access infection by vancomycin has led to a linear increase in its use in the last decade [173]. Oral vancomycin is efficacious in the treatment of *Corynebacterium difficile*-mediated diarrhea. Of major concern is the recent emergence of vancomycin-resistant enterococcus strains [174, 175].

In the early years of its use, vancomycin developed a reputation as being a relatively toxic drug. The most important side effects associated with vancomycin therapy are an anaphylactoid reaction referred to as the 'red man' syndrome or the 'red neck' syndrome, ototoxicity, and nephrotoxicity. Typically, the 'red man' syndrome develops upon rapid infusion of vancomycin and consists of pruritus, a rash involving the face, neck, and upper torso, and exceptionally hypotension. It is believed to be mediated by non-immunological release of histamine and other mediators. Pretreatment with H_1-antihistamines may be protective for the syndrome [176]. Improved purification procedures, however, have diminished adverse reactions. In a recent study comparing once-daily versus twice-daily administration of vancomycin for infections in hospitalized patients, red man syndrome occurred in 13.7 % and 9.6 % in QD and BID groups, respectively [177].

The incidence of nephrotoxicity associated with vancomycin is low (5-15%) when the drug is used alone [54, 178, 179]. In a subset of patients who did not receive any other potentially nephrotoxic agents, Cohen et al. [177] found 4 of 37 patients with nephrotoxicity in the QD vancomycin group and 3 of 39 patients in the BID group. There is, however, clinical and experimental evidence that vancomycin can enhance the nephrotoxic potential of aminoglycosides. Wood et al. [180] reported that rats treated concomitantly with tobramycin and vancomycin showed more extensive tubular necrosis than rats treated with either tobramycin or vancomycin alone. In a prospective study, Rybak et al. found an incidence of nephrotoxicity of 5% in patients treated with vancomycin alone and of 11% in patients treated with an aminoglycoside alone [54]. In patients who received the combination of vancomycin with an aminoglycoside the incidence was increased to 22%. In a meta-analysis, Goetz and Sayers calculated that the incidence of nephrotoxicity associated with combination therapy was 13.3% greater than therapy with vancomycin alone and 4.3% greater than therapy with an aminoglycoside alone [178]. In a prospective study, evaluating the effect of aminoglycoside dosing regimens on rates of observed nephrotoxicity, concomitant use of vancomycin was found to be a significant predictor of nephrotoxicity by multivariate logistic regression analysis [146]. In a prospective study comparing continuous versus intermittent infusion of vancomycin in severely ill patients Wysocki et al. [181] found a significant rise in serum creatinine during treatment only in those patients who received vancomycin with other antibiotics including aminoglycosides. A prospective study of vancomycin toxicity in oncology patients [182] essentially came to the same conclusion that concomitant administration of nephrotoxic antibiotics increases the risk for nephrotoxicity.

Whether concomitant vancomycin therapy can en-

hance cyclosporine-induced nephrotoxicity remains controversial. In a retrospective analysis, Chandrasekar and Cronin did not find an additive nephrotoxic potential for vancomycin when used concomitantly in bone marrow transplant recipients with cyclosporine and aminoglycosides [183]. However, in a prospective, randomized, and double-blind study in febrile neutropenic patients, Kureishi et al. found a significant deterioration of renal function when vancomycin and cyclosporine, but not teicoplanin and cyclosporine, were used concurrently [184].

Monitoring vancomycin serum concentrations is not cost-effective in preventing vancomycin-induced nephrotoxicity in patients with normal renal function, since the correlation between serum levels and antibacterial efficacy or toxicity remains controversial [185, 186, 182]. Serum level determination may be helpful in patients with increased volume of distribution, in patients with decreased renal function, in children or neonates, and in the elderly [165].

Teicoplanin, a glycopeptide antibiotic similar to vancomycin, is devoid of nephrotoxicity. Used at lower doses (6 mg/kg/day) it is better tolerated than vancomycin. However, there is some concern about its efficacy in severe infections like endocarditis [171] and the possible selection of resistance in Staphylococcus aureus and in coagulase-negative staphylococci. At higher doses (12 mg/kg/day) it may be more effective in the treatment of various gram-positive infections [187]. For the time being, teicoplanin should be considered as an alternative for vancomycin in the treatment of gram-positive infections only in patients who are at risk for developing drug-induced nephrotoxicity. Recently, two new antibiotics – quinupristin/dalfopristin and linezolid – came available as treatment options for infections due to drug-resistant gram-positive cocci [188]. Although devoid of nephrotoxicity, both drugs have important adverse effects and they are costly. Nevertheless, quinupristin/dalfopristin and linozolid may be useful in selected patients who cannot tolerate vancomycin or in the case of infection with resistant organisms.

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References

1. Craig WA, Andes D. Aminoglycosides are useful for severe respiratory tract infections. Semin Respir Infect 1997; 12(4): 271-277.
2. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. Antimicrob Agents Chemother. 1999; 43: 727-737.
3. Pittinger CB, Adamson R. Antibiotic blockade of neuromuscular function. Ann Rev Pharmacol 1972; 12: 169-184.
4. Brummett RE, Fox KE. Aminoglycoside-induced hearing loss in humans. Antimicrob Agents Chemother 1989; 33: 797-800.
5. Lane AZ, Wright GE, Blair DC. Ototoxicity and nephrotoxicity of amikacin. Am J Med 1977; 62: 911-918.
6. Noone P. Sisomicin, netilmicin and dibekacin: a review of their antibacterial activity and therapeutic use. Drugs 1984; 27: 548-578.
7. Bertino JS, Booker LA, Franck PA, Jenkins PL, Franck KR, Natziger AN. Incidence of and significant risk factors for aminoglycoside-associated nephrotoxicity in patients dosed by using individualized pharmacokinetic monitoring. J Infect Dis 1993; 167: 173-179.
8. Appel GB. Aminoglycoside nephrotoxicity. Am J Med 1990; 88(suppl C): 16S-20S.
9. Smith CR, Baughman KL, Edwards CQ, Rogers JF, Lietman PS. Controlled comparison of amikacin and gentamicin. N Engl J Med 1977; 296: 349-353.
10. Nicolau DP, Freeman CD, Belliveau PP, Nightingale CH, Ross JW, Quintiliani R. Experience with a once-daily aminoglycoside program administered to 2, 184 adult patients. Antimicrob Agents Chemother 1995; 39(3): 650-655.
11. Hou SH, Bushinsky DA, Wish JB, Cohen JJ, Harrington JT. Hospital-acquired renal insufficiency: a prospective study. Am J Med 1983; 74: 243-248.
12. Fanos V, Cataldi L. Antibacterial-induced nephrotoxicity in the newborn. Drug Safety 1999; 20(3): 245-267.
13. Trollfors B. Gentamicin-associated changes in renal function reversible during continued treatment. Antimicrob Agents Chemother 1983; 12: 285-287.
14. Eisenberg JM, Koffer H, Glick HA, Connell ML, Loss LE, Talbot GH, Shusterman NH, Strom BL. What is the cost of nephrotoxicity associated with aminoglycosides ? Ann Intern Med 1987; 107: 900-909.

15. Slaughter RL, Cappelletty DM. Economic impact of aminoglycoside toxicity and its prevention through therapeutic drug monitoring. Pharmacoeconomics 1998; 14(4): 385-394.
16. Pateson DL, Robson JM, Wagener MM. Risk factors for toxicity in elderly patients given aminoglycosides once daily. J Gen Intern Med 1998; 13(11): 735-739.
17. Moore RD, Smith CR, Lipsky JJ, Mellits ED, Lietman PS. Risk factors for nephrotoxicity in patients treated with aminoglycosides. Ann Intern Med 1984; 100: 352-357.
18. Beauchamp D, Gourde P, Bergeron MG. Effect of age on the intracortical accumulation kinetics of gentamicin in rats. Antimicrob Agents Chemother 1989; 33(11): 2006-2008.
19. Manian FA, Stone WJ, Alford RH. Adverse antibiotic effects associated with renal insufficiency. Rev Infect Dis 1990; 12(2): 236-249.
20. Beauchamp D, Gourde P, Thereault G, Bergeron MG. Age-dependent gentamicin experimental nephrotoxicity. J Pharm Exp Ther 1992; 260: 444-449.
21. Bennett WM, Parker RA, Elliott WB, Gilbert DN, Houghton DC. Sex: a determinant of susceptibility to gentamicin nephrotoxicity in the rat. J Infect Dis 1982; 145: 370-373.
22. Teixeira RB, Kelley J, Alpert H, Pardo V, Vaamonde CA. Complete protection from gentamicin-induced acute renal failure in the diabetes mellitus rat. Kidney Int 1982; 21: 600-612.
23. Elliott WB, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM. Experimental gentamicin nephrotoxicity: effect of streptozotocin-induced diabetes. J Pharm Exp Ther 1985; 233: 264-270.
24. Bennett WM, Hartnett MN, Gilbert D, Houghton D, Porter GA. Effect of sodium intake on gentamicin nephrotoxicity in the rat. Proc Soc Exp Biol Med 1976; 151: 736-738.
25. Chiu PJS, Miller GH, Long JF, Waitz JA. Renal uptake and nephrotoxicity of gentamicin during urinary alkalinization in rats. Clin Exp Pharmacol Physiol 1979; 6: 317-326.
26. Thompson JR, Simonsen R, Spindler MA, Southern PM, Cronin RE. Protective effect of KCl loading in gentamicin nephrotoxicity. Am J Kidney Dis 1990; 15: 583-591.
27. Adelman RD, Spangler WL, Beasom F, Ishizaki G, Conzelman GM. Furosemide enhancement of experimental gentamicin nephrotoxicity: comparison of functional and morphological changes with activities of urinary enzymes. J Infect Dis 1979; 140: 342-352.
28. Gamba G, Contreras AM, Cortes J, Nares F, Santiago Y, Espinosa A, Bobadilla J, Sanchez GJ, Lopez G, Valadez A, Pena JC. Hypoalbuminemia as a risk factor for amikacin nephrotoxicity. La Revista de Investigacion Clinica 1990; 42(3): 204-209.
29. Luft FC, Bennett WM, Gilbert DN. Experimental aminoglycoside nephrotoxicity: accomplishments and future potential. Rev Infect Dis 1983; 5(suppl 2): S268-S293.
30. Nanji AA, Denegri JF. Hypomagnesemia associated with gentamicin therapy. Drug Intelligence Clin Pharmacy 1984; 18: 596-598.
31. Zaloga GP, Chernow B, Pock A, Wood B, Zaritsky A, Zucker A. Hypomagnesemia is a common complication of aminoglycoside therapy. Surg Gyn Obstet 1984; 158: 561-565.
32. Lietman PS. Liver disease, aminoglycoside antibiotics, and renal dysfunction. Hepatology 1988; 4: 966-968.
33. Desai TK, Tsang TK. Aminoglycoside nephrotoxicity in obstructive jaundice. Am J Med 1988; 85: 47-50.
34. Moore RD, Smith CR, Lietman PS. Increased risk of renal dysfunction due to interaction of liver disease and aminoglycosides. Am J Med 1986; 80: 1093-1097.
35. Ngeleka M, Beauchamp D, Tardif D, Auclair P, Gourde P, Bergeron MG. Endotoxin increases the nephrotoxic potential of gentamicin and vancomycin plus gentamicin. J Infect Dis 1990; 161: 721-727.
36. Auclair P, Tardif D, Beauchamp D, Gourde P, Bergeron MG. Prolonged endotoxemia enhances the renal injuries induced by gentamicin in rats. Antimicrob Agents Chemother 1990; 34: 889-895.
37. Tardif D, Beauchamp D, Bergeron MG. Influence of endotoxin on the intracortical accumulation kinetics of gentamicin in rats. Antimicrob Agents Chemother 1990; 34: 576-580.
38. Keane WF, Welch R, Gekker G, Peterson PK. Mechanism of Escherichia coli a-hemolysin-induced injury to isolated tubular cells. Am J Pathol 1987; 126: 350-357.
39. Joly V, Bergeron Y, Bergeron MG, Carbon C. Endotoxin-tobramycin additive toxicity on renal proximal tubular cells in culture. Antimicrob Agents Chemother 1991; 35: 351-357.
40. Zager RA. A focus of tissue necrosis increases renal susceptibility to gentamicin administration. Kidney Int 1988; 33: 84-90.
41. Spiegel DM, Shanley PF, Molitoris BA. Mild ischemia predisposes the S3 segment to gentamicin toxicity. Kidney Int 1990; 38: 459-464.
42. Sawyers CL, Moore RD, Lerner SA, Smith CR. A model for predicting nephrotoxicity in patients treated with aminoglycosides. J Infect Dis 1986; 153: 1062-1068.
43. Garrison MW, Rotschafer JC. Clinical assessment of a published model to predict aminoglycoside-induced nephrotoxicity. Ther Drug Monitoring 1989; 11: 171-175.

44. The EORTC International Antimicrobial Therapy Project Group. Three antibiotic regimens in the treatment of infection in febrile granulocytopenic patients with cancer. J Infect Dis 1978; 137: 14-29.
45. Klastersky J, Hensgens C, Debusscher I. Empiric therapy for cancer patients: comparative study of ticarcillin-tobramycin, ticarcillin-cephalothin, and cephalotin-tobramycin. Antimicrob Agents Chemother 1975; 7: 640-645.
46. Humes HD. Aminoglycoside nephrotoxicity. Kidney Int 1988; 33: 900-911.
47. Sabra R, Branch RA. Role of sodium in protection by extended spectrum penicillins against tobramycin-induced nephrotoxicity. Antimicrob Agents Chemother 1990; 340: 1020-1025.
48. Hayashi T, Watanabe Y, Kumano K, Kitayama R, Yasuda T, Saikawa I, Katahira J, Kumada T, Shimizu K. Protective effect of piperacillin against nephrotoxicity of cephaloridine and gentamicin in animals. Antimicrob Agents Chemother 1988; 32: 912-918.
49. Halstenson CE, Wong MO, Herman CS, Heim-Duthoy KL, Teal MA, Affrime MB, Kelloway JH, Keane WF, Awni WM. Effects of concomitant administration of piperacillin on the dispositions of isepamicin and gentamicin in patients with end-stage renal disease. Antimicrob Agents Chemother 1992; 36: 1832-1836.
50. Smith CR, Lipsky JJ, Laskin OL, Hellmann DB, Mellits ED, Langstreth J, Lietman PS. Double-blind comparison of the nephrotoxicity and auditory toxicity of gentamicin and tobramycin. N Engl J Med 1980; 302: 1106-1109.
51. Lerner AM, Cone LA, Jansen W, Reyes MP, Blair DC, Wright GE, Lorber RR. Randomized, controlled trial of the comparative efficacy, auditory toxicity, and nephrotoxicity of tobramycin and netilmicin. Lancet 1983;1123-1131.
52. Wade JC, Smith CR, Petty BG, Lipsky JJ, Conrad G, Ellner J, Lietman PS. Cephalothin plus an aminoglycoside is more nephrotoxic than methicillin plus and aminoglycoside. Lancet 1978; 3: 604-606.
53. Farber BF, Moellering RC. Retrospective study of the toxicity of preparations of vancomycin from 1974-1981. Antimicrob Agents Chemother 1983; 23: 138-141.
54. Rybak MJ, Albrecht LM, Boike SC, Chandrasekar PH. Nephrotoxicity of vancomycin alone and with an aminoglycoside. J Antimicrob Chemother 1990; 25: 679-687.
55. Laskin OL, Longstreth JA, Smith CR, Lietman PS. Netilmicin and gentamicin multidose kinetics in normal subjects. Clin Pharmacol Ther 1983; 34: 644-650.
56. Schentag JJ, Jusko WJ. Renal clearance and tissue accumulation of gentamicin. Clin.Pharmacol.Ther 1977; 22: 364-370.
57. Gyselynck AM, Forrey A, Cutler R. Pharmacokinetics of gentamicin: distribution and plasma and renal clearance. J Infect Dis 1971;124 (Suppl 124): 70-76.
58. Hull JH, Sarubbi FA, Jr. Gentamicin serum concentrations: pharmacokinetic predictions. Ann.Intern.Med 1976; 85: 183-189.
59. Reymann MT, Bradac JA, Cobbs CG, Dismukes WE. Correlation of aminoglycoside dosages with serum concentrations during therapy of serious gram-negative bacillary disease. Antimicrob.Agents Chemother 1979; 16: 353-361.
60. Pastoriza-Munoz E, Timmerman D, Feldman S, Kaloyanides GJ. Ultrafiltration of gentamicin and netilmicin *in vivo*. J Pharmacol Exp Ther 1982; 220: 604-608.
61. Welling PG, Baumueller A, Lau CC, Madsen PO. Netilmicin pharmacokinetics after single intravenous doses to elderly male patients. Antimicrob Agents Chemother 1977; 12: 328-334.
62. Luft FC, Kleit SA. Renal parenchymal accumulation of aminoglycoside antibiotics in rats. J Infect Dis 1974; 130: 656-659.
63. Fabre J, Rudhardt M, Blanchard P, Regamey C. Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. Kidney Int 1976; 10: 444-449.
64. Edwards CQ, Smith CR, Baughman KL, Rogers JF, Lietman PS. Concentrations of gentamicin and amikacin in human kidneys. Antimicrob.Agents Chemother 1976; 9: 925-927.
65. De Broe ME, Paulus GJ, Verpooten GA, Roels F, Buyssens N, Wedeen R, Van Hoof F, Tulkens PM. Early effects of gentamicin, tobramycin, and amikacin on the human kidney. Kidney Int 1984; 25: 643-652.
66. Verpooten GA, Giuliano RA, Verbist L, Eestermans G, De Broe ME. Once-daily dosing decreases renal accumulation of gentamicin and netilmicin. Clin Pharmacol Ther 1989; 45: 22-27.
67. De Broe ME, Verbist L, Verpooten GA. Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. J Antimicrob Chemother 1991; 27 Suppl C: 41-47.
68. Just M, Erdmann G, Habermann E. The renal handling of polybasic drugs. 1. Gentamicin and aprotinin in intact animals. Naunyn Schmiedebergs Arch Pharmacol 1977; 300: 57-66.
69. Silverblatt FJ, Kuehn C. Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kidney Int 1979; 15: 335-345.
70. Vandewalle A, Farman N, Morin JP, Fillastre JP, Hatt PY, Bonvalet JP. Gentamicin incorporation along the nephron: autoradiographic study on isolated tubules. Kidney Int 1981; 19: 529-539.
71. Wedeen RP, Batuman V, Cheeks C, Marquet E, Sobel H. Transport of gentamicin in rat proximal tubule. Lab Invest 1983; 48: 212-223.
72. Molitoris BA, Meyer C, Dahl R, Geerdes A. Mechanism of ischemia-enhanced aminoglycoside binding and uptake by proximal tubule cells. Am J Physiol 1993; 264: F907-F916.

73. Pastoriza-Munoz E, Timmerman D, Kaloyanides GJ. Renal transport of netilmicin in the rat. J Pharmacol Exp Ther 1984; 228: 65-72.
74. Senekjian HO, Knight TF, Weinman EJ. Micropuncture study of the handling of gentamicin by the rat kidney. Kidney Int 1981; 19: 416-423.
75. Williams PD, Trimble ME, Crespo L, Holohan PD, Freedman JC, Ross CR. Inhibition of renal Na^+, K^+-adenosine triphosphatase by gentamicin. J Pharmacol Exp Ther 1984; 231: 248-253.
76. Josepovitz C, Pastoriza-Munoz E, Timmerman D, Scott M, Feldman S, Kaloyanides GJ. Inhibition of gentamicin uptake in rat renal cortex *in vivo* by aminoglycosides and organic polycations. J Pharmacol Exp Ther 1982; 223: 314-321.
77. Lipsky JJ, Cheng L, Sacktor B, Lietman PS. Gentamicin uptake by renal tubule brush border membrane vesicles. J Pharmacol Exp Ther 1980; 215: 390-393.
78. Sokol PP, Huiatt KR, Holohan PD, Ross CR. Gentamicin and verapamil compete for a common transport mechanism in renal brush border membrane vesicles. J Pharmacol Exp Ther 1989; 251: 937-942.
79. Walker RJ, Duggin GG. Drug nephrotoxicity. Annu Rev Pharmacol Toxicol 1988; 28: 331-345.
80. Sastrasinh M, Knauss TC, Weinberg JM, Humes HD. Identification of the aminoglycoside binding site in rat renal brush border membranes. J Pharmacol Exp Ther 1982; 222: 350-358.
81. Schacht J. Isolation of an aminoglycoside receptor from guinea pig inner ear tissues and kidney. Arch Otorhinolaryngol 1979; 224: 129-134.
82. Hori R, Okuda M, Ohishi Y, Yasuhara M, Takano M. Surface binding and intracellular uptake of gentamicin in the cultured kidney epithelial cell line (LLC-PK1). J Pharmacol Exp Ther 1992; 261: 1200-1205.
83. Takano M, Ohishi Y, Okuda M, Yasuhara M, Hori R. Transport of gentamicin and fluid-phase endocytosis markers in the LLC-PK1 kidney epithelial cell line. J Pharmacol Exp Ther 1994; 268: 669-674.
84. Christensen EI, Gliemann J, Moestrup SK. Renal tubule gp330 is a calcium binding receptor for endocytic uptake of protein. J Histochem Cytochem 1992; 40: 1481-1490.
85. Moestrup SK, Cui S, Vorum H, Bregengard C, Bjorn SE, Norris K, Gliemann J, Christensen EI. Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. J Clin Invest 1995; 96: 1404-1413.
86. Nagai J, Tanaka H, Nakanishi N, Murakami T, Takano M. Role of megalin in renal handling of aminoglycosides. Am J Physiol Renal Physiol 2001; 281: F337-F344.
87. Schmitz C, Hilpert J, Jacobsen C, Boensch C, Christensen EI, Luft FC, Willnow TE. Megalin deficiency offers protection from renal aminoglycoside accumulation. J Biol Chem 2002; 277: 618-622.
88. Tulkens P, Trouet A. The uptake and intracellular accumulation of aminoglycoside antibiotics in lysosomes of cultured rat fibroblasts. Biochem Pharmacol 1978; 27: 415-424.
89. Molitoris BA. Cell biology of aminoglycoside nephrotoxicity: newer aspects. Curr Opin Nephrol Hypertens 1997; 6: 384-388.
90. Sandoval R, Leiser J, Molitoris BA. Aminoglycoside antibiotics traffic to the Golgi complex in LLC-PK1 cells. J Am Soc Nephrol 1998; 9: 167-174.
91. Sandoval RM, Dunn KW, Molitoris BA. Gentamicin traffics rapidly and directly to the Golgi complex in LLC- PK(1) cells. Am J Physiol Renal Physiol 2000; 279: F884-F890.
92. Sundin DP, Sandoval R, Molitoris BA. Gentamicin inhibits renal protein and phospholipid metabolism in rats: implications involving intracellular trafficking. J Am Soc Nephrol 2001; 12: 114-123.
93. Sandvig K, van Deurs B. Endocytosis, intracellular transport, and cytotoxic action of Shiga toxin and ricin. Physiol Rev 1996; 76: 949-966.
94. Lord JM, Roberts LM. Toxin entry: retrograde transport through the secretory pathway. J Cell Biol 1998; 140: 733-736.
95. Johannes L, Goud B. Surfing on a retrograde wave: how does Shiga toxin reach the endoplasmic reticulum ? Trends Cell Biol 1998; 8: 158-162.
96. Sandoval RM, Bacallao RL, Dunn KW, Leiser JD, Molitoris BA. Nucleotide depletion increases trafficking of gentamicin to the Golgi complex in LLC-PK1 cells. Am J Physiol Renal Physiol 2002; 283: F1422-F1429.
97. Giuliano RA, Paulus GJ, Verpooten GA, Pattyn VM, Pollet DE, Nouwen EJ, Laurent G, Carlier MB, Maldague P, Tulkens PM. Recovery of cortical phospholipidosis and necrosis after acute gentamicin loading in rats. Kidney Int 1984; 26: 838-847.
98. Nouwen EJ, Verstrepen WA, Buyssens N, Zhu MQ, De Broe ME. Hyperplasia, hypertrophy, and phenotypic alterations in the distal nephron after acute proximal tubular injury in the rat. Lab Invest 1994; 479-493.
99. Luft FC, Aronoff GR, Evan AP, Connors BA. The effect of aminoglycosides on glomerular endothelium: a comparative study. Res.Commun.Chem.Pathol.Pharmacol. 1981; 34: 89-95.
100. Gilbert DN. Aminoglycosides. In: Principles and practices of infectious diseases. Mandell GL, Bennett JE, Dolin R (editors). Churchill Livingstone, New York 1995, p. 279-306.
101. Parker RA. BWHPGA. Animal models in the study of aminoglycoside nephrotoxicity. In: The aminoglycosides: microbiology, clinical use and toxicology. Marcel Dekker, New York 1982: 235-267.
102. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother 1999; 43: 1003-1012.

103. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-257.
104. Vermes I, Haanen C. Apoptosis and programmed cell death in health and disease. Adv Clin Chem 1994; 31: 177-246.
105. Takahashi M, Aramaki Y, Inaba A, Tsuchiya S. Inhibition of alkaline phosphatase activity and D-glucose uptake in rat renal brush-border membrane vesicles by aminoglycosides. Biochim Biophys Acta 1987; 903: 31-36.
106. Williams PD, Bennett DB, Gleason CR, Hottendorf GH. Correlation between renal membrane binding and nephrotoxicity of aminoglycosides. Antimicrob Agents Chemother 1987; 31: 570-574.
107. Cronin RE, Nix KL, Ferguson ER, Southern PM, Henrich WL. Renal cortex ion composition and Na-K-ATPase activity in gentamicin nephrotoxicity. Am J Physiol 1982; 242: F477-F483.
108. Ramsammy LS, Josepovitz C, Kaloyanides GJ. Gentamicin inhibits agonist stimulation of the phosphatidylinositol cascade in primary cultures of rabbit proximal tubular cells and in rat renal cortex. J Pharmacol Exp Ther 1988; 247: 989-996.
109. Aubert-Tulkens G, Van Hoof F, Tulkens P. Gentamicin-induced lysosomal phospholipidosis in cultured rat fibroblasts. Quantitative ultrastructural and biochemical study. Lab Invest 1979; 40: 481-491.
110. Knauss TC, Weinberg JM, Humes HD. Alterations in renal cortical phospholipid content induced by gentamicin: time course, specificity, and subcellular localization. Am J Physiol 1983; 244: F535-F546.
111. Feldman S, Wang MY, Kaloyanides GJ. Aminoglycosides induce a phospholipidosis in the renal cortex of the rat: an early manifestation of nephrotoxicity. J Pharmacol Exp Ther 1982; 220: 514-520.
112. Josepovitz C, Farruggella T, Levine R, Lane B, Kaloyanides GJ. Effect of netilmicin on the phospholipid composition of subcellular fractions of rat renal cortex. J Pharmacol Exp Ther 1985; 235: 810-819.
113. Kosek JC, Mazze RI, Cousins MJ. Nephrotoxicity of gentamicin. Lab Invest 1974; 30: 48-57.
114. Ramsammy LS, Josepovitz C, Lane B, Kaloyanides GJ. Effect of gentamicin on phospholipid metabolism in cultured rabbit proximal tubular cells. Am J Physiol 1989; 256: C204-C213.
115. Laurent G, Kishore BK, Tulkens PM. Aminoglycoside-induced renal phospholipidosis and nephrotoxicity. Biochem Pharmacol 1990; 40: 2383-2392.
116. El Mouedden M, Laurent G, Mingeot-Leclercq MP, Taper HS, Cumps J, Tulkens PM. Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. Antimicrob Agents Chemother 2000; 44(3): 665-675.
117. Luft FC, Yum MN, Kleit SA. Comparative nephrotoxicities of netilmicin and gentamicin in rats. Antimicrob. Agents Chemother 1976; 10: 845-849.
118. Bendirdjian J, Gillaster J, Foucher B. Mitochondrial modifications with aminoglycosides. In: The aminoglycosides, microbiology, clinical use and toxicology. Marcel Dekker, New York 1982; p..
119. Simmons CF, Jr., Bogusky RT, Humes HD. Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation. J Pharmacol Exp Ther 1980; 214: 709-715.
120. Weinberg JM, Humes HD. Mechanisms of gentamicin-induced dysfunction of renal cortical mitochondria. I. Effects on mitochondrial respiration. Arch Biochem Biophys 1980; 205: 222-231.
121. Mather M, Rottenberg H. Polycations induce the release of soluble intermembrane mitochondrial proteins. Biochim Biophys Acta 2001; 1503: 357-368.
122. Charlwood J, Sekhel JM, King N, Camilleri P, Lord P, Bugelski P, Atif U. Proteomic analysis of rat kidney cortex following treatment with gentamicin. J Proteome Res 2002; 1(1): 73-82.
123. Walker PD, Shah SV. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. J Clin Invest 1988; 81: 334-341.
124. Ueda N, Guidet B, Shah SV. Gentamicin-induced mobilization of iron from renal cortical mitochondria. Am.J.Physiol 1993; 265: F435-F439.
125. Davies J, Gorini L, Davis BD. Misreading of RNA codewords induced by aminoglycoside antibiotics. Mol Pharmacol 1965; 1: 93-106.
126. Tai PC, Davis BD. Triphasic concentration effects of gentamicin on activity and misreading in protein synthesis. Biochemistry 1979; 18: 193-198.
127. Buss WC, Piatt MK. Gentamicin administered *in vivo* reduces protein synthesis in microsomes subsequently isolated from rat kidneys but not from rat brains. J Antimicrob Chemother 1985; 15: 715-721.
128. Bennett WM, Mela-Riker LM, Houghton DC, Gilbert DN, Buss WC. Microsomal protein synthesis inhibition: an early manifestation of gentamicin nephrotoxicity. Am J Physiol 1988; 255: F265-F269.
129. Verstrepen WA, Nouwen EJ, Yue XS, De Broe ME. Altered growth factor expression during toxic proximal tubular necrosis and regeneration. Kidney Int 1993; 43: 1267-1279.
130. Morin NJ, Laurent G, Nonclercq D, Toubeau G, Heuson-Stiennon JA, Bergeron MG, Beauchamp D. Epidermal growth factor accelerates renal tissue repair in a model of gentamicin nephrotoxicity in rats. Am J Physiol 1992; 263: F806-F811.
131. Porter GA, Bennett WM, Gilbert DN. Unraveling aminoglycoside nephrotoxicity using animal models. J Clin Pharmacol 1983; 23: 445-452.

132. Gilbert DN, Wood CA, Kohlhepp SJ, Kohnen PW, Houghton DC, Finkbeiner HC, Lindsley J, Bennett WM. Polyaspartic acid prevents experimental aminoglycoside nephrotoxicity. J Infect Dis 1989; 159: 945-953.
133. Bennett WM, Plamp CE, Gilbert DN, Parker RA, Porter GA. The influence of dose regimen on experimental gentamcicn nephrotoxicity: dissociation of peak serum levels from renal failure. J Infect Dis 1979; 140: 576-580.
134. Tulkens PM. Pharmacokinetic and toxicological evaluation of a once-daily regimen versus conventional schedules of netilmicin and amikacin. J Antimicrob Chemother 1991; 27 (suppl C): 49-61.
135. Van der Auwera P, Meunier F, Ibrahim S, Kaufman L, Derde MP, Tulkens PM. Pharmacodynamic parameters and toxicity of netilmicin (6 mg/kg/day) given once daily or in three divided doses to cancer patients with urinary tract infection. Antimicrob Agents Chemother 1991; 35: 640-647.
136. Blaser J, Konig C. Once-daily dosing of aminoglycosides. Eur J Clin Microbiol Infect Dis 1995; 14: 1029-1038.
137. Galloe AM, Graudal N, Christensen HR, Kampmann JP. Aminoglycosides: single or multiple daily dosing? A meta-analysis on efficacy and safety. Eur J Clin Pharmacol 1995; 48(1): 39-43.
138. Barza M, Ioannidis JP, Cappelleri JC, Lau J. Single or multiple daily doses of aminoglycosides: a meta-analysis. Brit Med J 1996; 312(7027): 338-345.
139. Munckhof WJ, Grayson ML, Turnidge JD. A meta-analysis of studies on the safety and efficacy of aminoglycosides given either once daily or as divided doses. J Antimicrob Chemother 1996; 37(4): 645-663.
140. Ferriols-Lisart R, Alos-Alminana M. Effectiveness and safety of once-daily aminoglycosides: a meta-analysis. Am J Health Syst Pharm 1996; 53(10): 1141-1150.
141. Freeman CD, Strayer AH. Mega-analysis of meta-analysis: an examination of meta-analysis with an emphasis on once-daily aminoglycoside comparative trials. Pharmacother 1996; 16(6): 1093-1102.
142. Hatala R, Dinh T, Cook DJ. Once-daily aminoglycoside dosing in immunocompetent adults: a meta-analysis. Ann Intern Med 1996;124(8): 717-725.
143. Ali MZ, Goetz MB. A meta-analysis of the relative efficacy and toxicity of single daily dosing versus multiple daily dosing of aminoglycosides. Clin Infect Dis 1997; 24(5): 796-809.
144. Bailey TC, Little JR, Littenberg B, Reichley RM, Dunagan WC. A meta-analysis of extended-interval dosing versus multiple daily dosing of aminoglycosides. Clin Infect Dis 1997; 24(5): 786-795.
145. Hatala R, Dinh TT, Cook DJ. Single daily dosing of aminoglycosides in mmunocompromised adults: a systematic review. Clin Infect Dis 1997; 24(5): 810-815.
146. Rybak MJ, Abate BJ, Kang SL, Ruffing MJ, Lerner SA, Drusano GL. Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. Antimicrob Agents Chemother 1999; 43(7): 1549-1555.
147. Uijtendaal EV, Rademaker CM, Schobben AF, Fleer A, Kramer WL, van Vught AJ, Kimpen JL, van Dijk A. Once-daily versus multiple-daily gentamicin in infants and children. Ther Drug Monit 2001; 23: 506-513.
148. Karachaliou I, Halkiadaki D. Prospective randomized study of once-daily versus twice-daily amikacin regimens in patients with systemic infections. Int J Clin Pharmacol Ther 1998; 36(10): 561-54.
149. Chuck SK, Raber SR, Rodvold KA, Areff D. National survey of extended-interval aminoglycoside dosing. Clin Infect Dis 2000; 30(3): 433-439.
150. Nicolau DP, Freeman CD, Belliveau PP, Nightingale CH, Ross JW, Quintiliani R. Experience with a once-daily aminoglycoside program administered to 2, 184 adult patients. Antimicrob Agents Chemother 1995; 39(3): 650-655.
151. McCormack JP. An emotional-based medicine approach to monitoring once-daily aminoglycosides. Pharmacother 2000; 20: 1524-1527.
152. Burton ME, Brater DC, Chen PS, Day RB, Huber PJ, Vasko MR. A bayesian feedback method of aminoglycoside dosing. Clin Pharmacol Ther 1985; 37: 349-357.
153. Dillon KR, Dougherty SH, Casner P, Polly S. Individualized pharmacokinetic versus standard dosing of amikacin: a comparison of therapeutic outcomes. J Antimicrob Chemother 1989; 24: 581-589.
154. Burton ME, Ash CL, Hill DP, Handy TR, Shepherd MD, Vasko MR. A controlled trial of the cost benefit of computerized bayesian aminoglycoside administration. Clin Pharmacol Ther 1991; 49: 685-694.
155. Whipple JK, Ausman RK, Franson T, Quebbeman EJ. Effect of individualized pharmacokinetic dosing on patient outcome. Crit Care Med 1991; 19: 1480-1485.
156. Leehey DJ, Braun BI, Tholl DA, Chung LS, Gross CA, Roback JA, Lentino JR. Can pharmacokinetic dosing decrease nephrotoxicity associated with aminoglycoside therapy ? J Am Soc Nephrol 1993; 4: 81-90.
157. Koo J, Tight R, Rajkumar V, Hawa Z. Comparison of once-daily versus pharmacokinetic dosing of aminoglycosides in elderly patients. Am J Med 1996; 101(2): 177-183.
158. Hickling K, Begg E, Moore ML. A prospective randomised trial comparing individualized pharmacokinetic dosage prediction for aminoglycosides with prediction based on estimated creatinine clearance in critically ill patients. Intensive Care Med 1989; 15: 233-237.
159. Kemme DJ, Daniel CI. Aminoglycoside dosing: a randomised prospective study. Southern Med J 1993; 86: 46-51.

160. Destache CJ, Meyer SK, Bittner MJ, Hermann KG. Impact of a clinical pharmacokinetic service on patients treated with aminoglycosides: a cost-benefit analysis. Ther Drug Monit 1990; 12(5): 419-426.
161. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clinical Trials 1986; 7: 177-188.
162. McCormack JP, Jewesson PJ. A critical reevaluation of the 'therapeutic range' of aminoglycosides. Clin Infect Dis 1992; 14: 302-339.
163. Edwards DJ, Mangione A, Cumbo TJ, Schentag JJ. Predicted tissue accumulation of netilmicin in patients. Antimicrob Agents Chemother 1981; 20: 714-717.
164. Sande MA, Mandell GL. Vancomycin. In: The pharmacological basis of therapeutics, 8th edition. Goodman LS, Gilman A (editors). McGraw Hill, New York 1991; p. 1138-1140.
165. Cunha BA. Vancomycin. Med Clin North Am 1995; 79: 817-831.
166. Golper TA, Noonan HM, Elzinga L, Gilbert D, Brummett R, Anderson JL, Bennett WM. Vancomycin pharmacokinetics, renal handling, and nonrenal clearances in normal human subjects. Clin Pharmacol Ther 1988; 43: 565-570.
167. Cutler NR, Narang PK, Lesko LJ, Ninos M, Power M. Vancomycin disposition: the importance of age. Clin Pharmacol Ther 1984; 36: 803-810.
168. Moellering RC, Krogstad DJ, Greenblatt DJ. Vancomycin therapy in patients with impaired renal function: a nomogram for dosage. Ann Int Med 1981; 94: 343-346.
169. Brown DL, Mauro LS. Vancomycin dosing chart for use in patients with renal impairment. Am J Kidney Dis 1988; 11: 15-19.
170. Magera BE, Arroyo JC, Rosansky SJ, Postic B. Vancomycin pharmacokinetics in patients with peritonitis on peritoneal dialysis. Antimicrob Agents Chemother 1983; 23: 710-714.
171. Zeckel ML. A closer look at vancomycin, teicoplanin, and antimicrobial resistance. J Chemother 1997; 9(5): 311-331; discussion 332-335.
172. Wilhelm MP. Vancomycin. Mayo Clin Proc 1991; 66: 1165-1170.
173. Ena J, Dick RW, Jones RN, Wenzel RP. The epidemiology of intravenous vancomycin usage in a university hospital. JAMA 1993; 269: 598-602.
174. Morris JG, Shay DK, Hebden JN. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establisment of endemicity in a university medical center. Ann Int Med 1995; 123: 250-259.
175. Spera RV, Farber BF. Multiply-resistant Enterococcus faecium. The nosocomial pathogen of the 1990s. JAMA 1992; 268: 2563-2564.
176. Editorial. Red men should go: vancomycin and histamine release. Lancet 1990; 335: 1006-1007.
177. Cohen E, Dadashev A, Drucker M, Samra Z, Rubinstein E, Garty M. Once-daily versus twice-daily intravenous administration of vancomycin for infections in hospitalized patients. J Antimicob Chemother 2002; 49: 155-160.
178. Goetz MB, Sayers J. Nephrotoxicity of vancomycin and aminoglycoside therapy seperately and in combination. J Antimicrob Chemother 1993; 32: 325-334.
179. Salama SE, Rotstein C. Prospective assessment of nephrotoxicity with concomitant aminoglycoside and vancomycin therapy. Can J Hosp Pharm 1993; 46: 53-59.
180. Wood CA, Kohlhepp SJ, Kohnen PW, Houghton DC, Gilbert DN. Vancomycin enhancement of experimental tobramycin nephrotoxicity. Antimicrob Agents Chemother 1986; 30: 20-24.
181. Wysocki M, Delatour F, Faurisson F, Rauss A, Pean Y, Misset B, Thomas F, Timsit JF, Similowski T, Mentec H, Mier L, Dreyfuss D. Continuous versus intermittent infusion of vancomycin in severe sthaphylococcal infections: prospective multicenter randomized study. Antimicob Agents Chemother 2001; 45: 2460-2467.
182. Elting LS, Rubenstein EB, Kurtin D, Rolston KV, Fangtang J, Martin CG, Raad II, Whimbey EE, Manzullo E, Bodey GP. Mississippi mud in the 1990s: risks and outcomes of vancomycin-associated toxicity in general oncology practice. Cancer 1998; 83(12): 2597-2607.
183. Chandrasekar PH Cronin SM. Nephrotoxicity in bone marrow recipients receiving aminoglycoside plus cyclosporine or aminoglycoside alone. J Antimicrob Chemother 1991; 27: 845-849.
184. Kureishi A, Jewesson PJ, Rubinger M, Cole CD, Reece DE, Phillips GL, Smith JA, Chow AW. Double-blind comparison of teicoplanin versus vancomycin in febrile neutropenic patients receiving concomitant tobramycin and piperacillin: effect on cyclosporin A-associated nephrotoxicity. Antimicrob Agents Chemother 1991; 35: 2246-2252.
185. Cantu TG, Yamanaka-Yuen NA, Lietman PS. Serum vancomycin concentrations: reappraisal of their clinical value. Clin Infect Dis 1994; 19(6): 1180-1182.
186. Freeman CD, Quintiliani R, Nightingale CH. Vancomycin therapeutic drug monitoring: is it necessary ? Ann Pharmacother 1993; 27: 594-598.
187. Shea KW, Cuhna BA. Teicoplanin. Med Clin North Am 1995; 79: 833-844.
188. Rehm SJ. Two new treatment options for infections due to drug-resistant gram-positive cocci. Cleve Clin J Med 2002; 69(5): 397-401, 405-413.

09

Beta-lactam antibiotics

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Introduction

The large family of β-lactams comprises penicillins, cephalosporins, cephamycins, monobactams, carbacephems and carbapenems and are so named since they all containing the β-lactam moiety.

Penicillin was the first β-lactam antibiotic and was discovered in 1928 by Sir Alexander Fleming at St. Mary's Hospital, London [1]. The β-lactam chemical structure for penicillin was first proposed by Abraham and Chain in 1943 and finally established in 1945 by X-ray crystallographic analysis. In the same year, Giuseppe Brotzu, a Sardinian professor of bacteriology, isolated *Cephalosporium acremonium* from the sea near a sewage outfall at Cagliari, which produced antibiotic material with a broad spectrum of activity. It was almost eight years later in 1953 when Newton and Abraham, while studying the production of antibiotics by Brotzu's Cephalosporium, that they discovered a penicillin-like substance providing resistance to hydrolysis by penicillinases which was named cephalosporin C.

By 1959, Rolinson and coworkers completed the isolation of the penicillin nucleus, 6-aminopenicillanic acid, (Figure 1) in quantity. At about the same time the β-lactam-dihydrothiazine structure for the cephalosporin C was proposed [2] and confirmed subsequently by X-ray crystallographic analysis. In 1962, Morin and coworkers established a chemical method for the production of 7-aminocephalosporanic acid (Figure 1) from cephalosporin C in quantity. These developments opened the way to the preparation of a large number of semi-synthetic *cephalosporins* with hopes of being used as therapeutic agents. Cephalothin was prepared in 1962 and was the first semi-synthetic cephalosporin to find extensive clinical use in the 1960s. Cephalothin was followed by cephaloridine, in which the acetoxy group at C-3' of cephalothin was replaced by a pyridinium group (Figure 2). These cephalosporins were followed by four generation of cephalosporins that are now categorized based on their spectrum of activity.

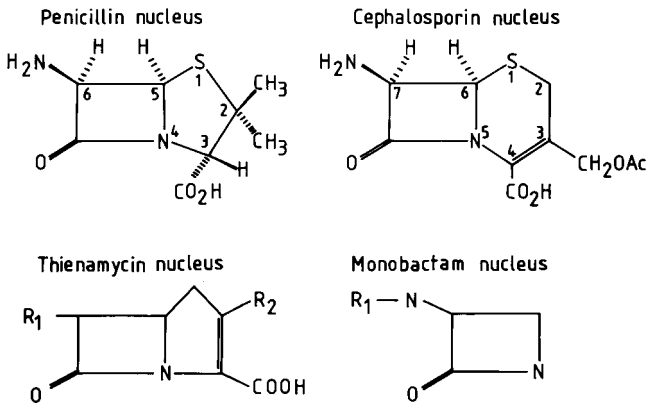

Figure 1. Core structure of penicillins, cephalosporins, carbapenems and monobactams.

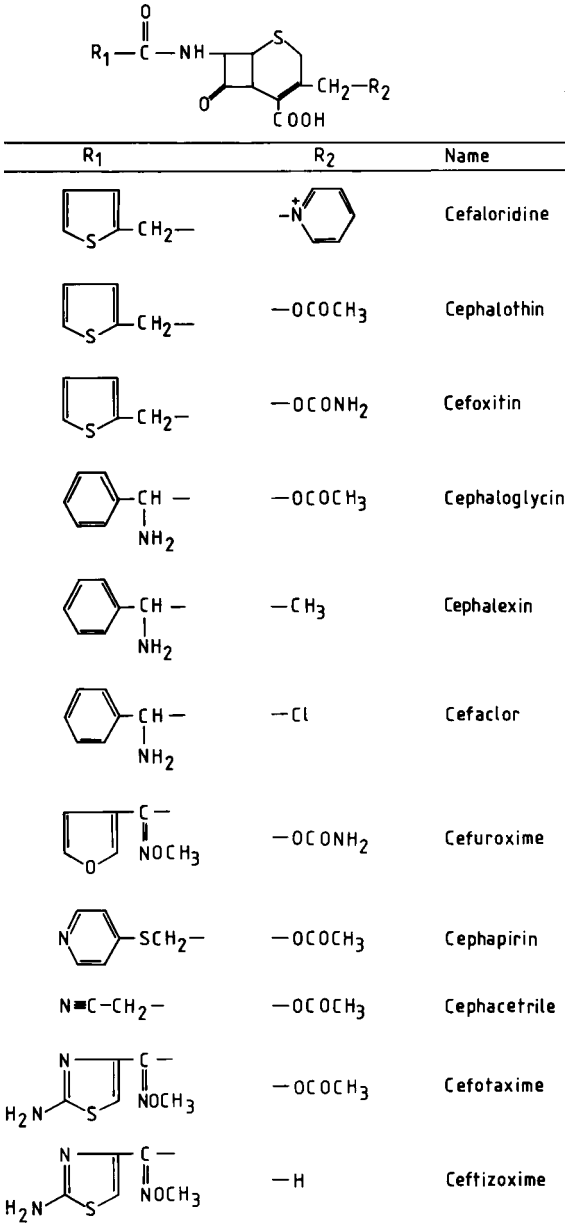

Figure 2. Various side chains attached to the β-lactam nucleus, which are involved in renal toxicity.

Cephamycins, including cefoxitin, cefotetan, cefmetazole and moxalactam (latamoxef), are related to cephalosporins but contain a metoxy group rather than a hydrogen at the 7-position on the β-lactam ring of the cephalosporin nucleus [3]. Cefoxitin (Figure 2) is the best known semi-synthetic cephamycin antibiotic derived from cephamycin C, a substance produced by *Streptomyces lactamdurans*. Molecular alterations such as an exchange of oxygen for sulfur at the S1 position in the dihydrothiazine ring resulted in the development of moxalactam, which is an oxa-β-lactam rather than a cephalosporin. Moxalactam is stable to β-lactamases due to the presence of a 7-methoxy group in its chemical structure. It is highly active and has a broad spectrum of activity except against *Pseudomona aeruginosa*.

Clavulanic acid was discovered in 1976 and is a β-lactam antibiotic with low antibiotic activity, but does protect β-lactamase-sensitive compounds of high intrinsic activity such as benzylpenicillin, ampicillin, and amoxicillin from β-lactamase destruction. Subsequently other β-lactamase inhibitors such as sulbactam and tazobactam were developed [4].

Carbacephems are structurally related to cephalosporins [5]. Loracarbef, the first this new class of β-lactam antibiotics, is a carbacephem analog of cefaclor (Figure 2) in which the sulfur atom in the dihydrothiazine ring has been replaced by a methylene group. Carbacephems have greater chemical stability than cephalosporins. Loracarbef has *in vitro* activity against most pathogenes responsible for upper respiratory tract infections. It is active *in vitro* against *Streptococcus pneumoniae* and has activity against most strains of *Staphylococcus aureus*.

Carbapenems, which are β-lactam antibiotics (penems) that are neither penicillins (penams) nor cephalosporins (cephems), proved to be of clinical significance and scientific interest. The first compound of this new type of β-lactam class was thienamycin [6]. Replacing the sulfur atom with a carbon atom altered the penem ring of thienamycin. All biologically active members of the class contain the unsaturated carbapen-2-em carboxylic acid nucleus (Figure 1). The carbapenem derivatives of thienamycin such as imipenem and panipenem showed exceptional activity against a wide range of bacteria including strains of *Pseudomona aeruginosa* and are high resistance to hydrolysis by β-lactamases. Imipenem is a semi-synthetic β-lactam antibiotic and is the N-formidoyl derivative of thienamycin, a carbapenem antibiotic produced by *Streptomyces cattleya*. The derivative imipenem is formulated in combination with cilastatin, which prolongs the half-life of imipenem by preventing its inactivation by dehydropeptidases in the kidney. Meropenem and biapenem are newer carbapenems, which show stability to renal hydrolysis and do not need to be combined with cilastatin [7].

From the *monobactam* group, aztreonam is a monocyclic β-lactam antibiotic (Figure 1), which is produced by *Chromobacterium violaceum*. Aztreonam has a high activity against gram-negative aerobic bacteria including *Pseudomona aeruginosa*, but it is virtually inactive against gram-positive bacteria and anaerobes. Aztreonam shows a high degree of stability to a wide range of both plasmid- and chromosomally-mediated β-lactamases comparable to the third-generation cephalosporins [8].

Nephrotoxic beta-lactams

Beta-lactams such as cephaloridine, cephalothin, cefotiam and imipenem have been associated with nephrotoxicity in humans and experimental animals [9]. An understanding of their nephrotoxicity mechanisms may provide valuable information for elucidation of the biochemical mechanisms of newer β-lactam nephrotoxicity. Similarly to cephaloridine, third- generation cephalosporins such as ceftazidime and cefsulodin and fourth-generation cephalosporins such as cefpirome and cefepime possess a quaternary nitrogen attached to the dihydrothiazine ring which may impart nephrotoxic potential [10]. Clinical and animal studies carried out with β-lactams, such as cephaloglycin, cephaloridine, cephalothin or imipenem, indicated that they show a differential accumulation at the site of their toxicity, the renal cortex [11]. Elucidation of the mechanism of toxic action of these model β-lactams has become the focus of several research efforts [12-16].

Penicillins

Penicillins are β-lactam antimicrobials, which have a 4-membered β-lactam ring that is fused to a 5-membered thiazolidine ring, thus forming the penam nucleus (Figure 1). Modifications of the parent compound can alter the bacterial spectrum of these β-

lactams. The natural penicillins, penicillins G and V, remain the drugs of choice for infections caused by S. pyrogens, Peptococcus, Treponema and other organisms. The penicillinase resistant drugs such as methicillin and oxacillin are primarily used for staphylococcal infections. Whereas aminopenicillins such as amoxicillin and ampicillin are effective against E. coli, Proteus, Salmonella and Shigella, the extended spectrum penicillins such as tircarcillin and carbecillins are active against Enterobacteriaceae and Pseudomonas.

When 1500 mg/kg ampicillin was administered to female rabbits as a single dose, there was no evidence of nephrotoxicity judged by the absence of tubular necrosis 48 hours after administration [17]. In the other hand, carbenicillin, methicillin and ampicillin have been associated with acute interstitial nephritis (AIN) [18-20].

AIN is characterized by fever, eosinophilia, hematuria, mild proteinuria and skin rash occur an average of 15 days after exposure (range 2-40). Rising serum creatinine concentration and acute renal failure with oliguria develop in about 50% of AIN cases, especially in older patients. Histologically, interstitial granulomas and variable degrees of tubular necrosis may be seen on renal biopsy.

Benzylpenicillin is a β-lactam with low or no renal toxicity [21]. However, when administered in large doses, benzylpenicillin or amoxicillin [22] have the potential to induced nephrotoxicity. Acute interstitial nephritis and disturbances of blood electrolytes have also been reported [23]. By comparison, dicloxacillin induced a pathological increase of creatinine, while cloaxicillin had only a marginal effect on the renal function [24].

The peroxidative potential of mezlocillin was determined by measuring both the generation of superoxide and malondialdehyde (MDA). The results showed that the amount of generated superoxide was almost equal to that produced by cefsulodin, under the same experimental conditions, while the amount of MDA was about 50% of that generated by cefsulodin [10]. After incubation of renal cortical slices with mezlocillin there was no change in the accumulation of the organic anion para-aminohippurate (PAH) in slices when compared to control whereas a significant decrease in the accumulation of the cation tetraethylammonium (TEA) occurred [10], suggesting a preferential sensitivity of organic cation transporter.

Cephalosporins and cephamycins

Therapeutic demands for new antimicrobial antibiotics arise from the emergence and dissemination of new opportunistic pathogens, especially in a expanding immune system-debilitated host population. As the number of β-lactams, and especially of cephalosporins, continues to expand, the need for classification increases. Changing the molecular environment of the β-lactam ring resulted in the development of β-lactam antibiotics possessing a penam or cephem nucleus known as "classical β-lactams" and of those with an unusual nucleus as "non-classical β-lactams" such as carbapenems (Figure 1). The introduction of specific side chains to the penam ring or cephem ring has resulted in a variety of changes in biological properties of these drugs: expansion of the antibacterial spectrum, increase in stability against β-lactamase and improved pharmacokinetic properties, slower elimination allowing longer dosage intervals and lower toxicity, especially nephrotoxicity [25, 26].

Cephalosporins are β-lactam antibiotics in which the β-lactam ring is fused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus. To differentiate between the successive waves of cephalosporins that have appeared since 1975, the introduction of the term "generations" served for separate one cephalosporin group from another. Cephalosporins have been classified as belonging to a first-, second-, third- or fourth-generation on the basis of their biological characteristics and clinical use for management of specific infections [25].

First-generation cephalosporins are mainly active against gram-positive cocci (except enterococci) and numerous Enterobacteriaceae. First generation cephalosporins (cephalothin, cephaloridine, cefazolin, cephalexin, cephapirin) have limited activity against gram-negative bacteria although some strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and Shigella may be inhibited *in vitro* by these drugs. However, gram-negative bacteria, which possess β-lactamases, are able to hydrolyze these cephalosporins partially or totally. Cefazolin has a substantially longer half-life and reaches much higher serum concentrations than other members of this group.

The nephrotoxicity of β-lactams, such as cephaloridine, is characterized by decreased glomerular filtration rate, proteinuria, enzymuria, urinary granular

casts, impaired ability of renal cortical slices to accumulate organic ions and to synthesize glucose [27].

Ultrastructural changes of proximal tubular cells occur as early as 1 hour after cephaloridine administration to rabbits and are characterized by loss of brush border, less elongated mitochondria and disappearance of structures associated with endocytosis. Later ultrastructural changes include disorganization of lateral interdigitations of plasma cell membrane and mitochondrial swelling [28,29].

Treatment of rabbits [30] or rats [31] with inhibitors of renal organic anion transport, such as probenecid, decreases renal cortical accumulation of cephaloridine and its nephrotoxicity. This correlates with the results of more recent studies, which indicate that cephaloridine, is actively transported into proximal tubular cells by a renal organic anion transporter 1 [32, 33]. Results from numerous *in vitro* and *in vivo* animal studies using real cortical slices, isolated tubule fragments and renal cortical microsomes [26, 31, 34-41] as well as *in vitro* studies using human renal cortical slices and human renal microsomes [9] revealed marked cephaloridine-induced lipid peroxidation. Cephaloridine-induced oxidative stress caused a significant decrease in renal accumulation of organic anions and cations, plus significantly impairing the ability of the renal cortical tissue to synthesize glucose [26, 31, 36, 37, 39, 42]. Similar results were obtained with cephalothin, which was less nephrotoxic than cephaloridine; cefazolin-induced lipid peroxidation was substantially less than that caused by cephalothin and did not affect the renal cortical accumulation of the cation tetraethylammonium (TEA) or gluconeogenesis [26]. It appears that cephaloridine-induced lipid peroxidation antedates the inhibition of organic ion accumulation [37]. Furthermore, species differences in cephaloridine- [9, 42] or cephalothin-induced nephrotoxicity have been reported [43]. Selenium deficiency potentiated cephaloridine nephrotoxicity [35, 44]. In contrast to selenium deficiency, copper deficiency did not increase cephaloridine-induced nephrotoxicity [44].

Second-generation cephalosporins (cefuroxime, cefotiam, cefonicid, cefaclor, cefamandole) differ from the first generation in that they are generally less active against staphylococci and streptococci, may have more resistance to β-lactamases and have greater *in vitro* activity against gram-negative bacteria than the first generation cephalosporins. Cefuroxime and cefamandole have enhanced activity against most strains of *Haemophilus influenzae* and some Enterobacteriaceae. Second generation cephalosporins are inactive against methicillin-resistant Staphylococci and enterococci. The N-methyl-tetrazole-thiol (NMTT) side chain in position 3 of the cephem nucleus confers epileptogenic activity, disulfiram-like activity and hypoprothrombinemia to cephamandole and cefotiam [26, 45].

Histochemistry and electron microscopy of rabbit kidneys treated with large doses of cefotiam revealed both loss of microvilli and the presence of degenerative processes in the proximal tubule [46]. When compared to the first-generation cephalosporin cephazolin, cefotiam has a comparable peroxidative potential but greater nephrotoxicity [26].

Third-generation cephalosporins (cefotaxime, cefodizime, ceftizoxime, cefixime, ceftriaxone, ceftazidime, cefsulodin, cefoperazone) have an expanded spectrum of activity against gram-negative bacteria compared with the first- and second-generation compounds. They are very resistent to β-lactamases, high potency against Enterobacteriaceae and some activity against *Pseudomona aeruginosa* and *Bacteroides fragilis*. However, they are usually less active *in vitro* against susceptible staphylococci than the first generation cephalosporins.

Cefoperazone, like cefamandole, has the ability to induce epileptogenic activity, disulfiram-like activity, and hypoprothrombinemia. It appears that hypothrombinemia occurs more frequently with cefoperazone than with other cephalosporins [45].

Two clinical studies indicated a small but significant decrease in glomerular filtration rate during ceftazidime therapy [47, 48]. A significant elevation in the excretion of alanine aminopeptidase was also observed [48]. Although cefotaxime and cefoperazone have peroxidative potential, they do not affect TEA accumulation and glucose synthesis by renal cortical slices [26]. Results of studies conducted with renal cortical microsomes showed that cefsulodin, when compared with ceftazidime and cefotaxime, was the most potent cephalosporin, causing superoxide production and induction of lipid peroxidation [10]. In studies conducted with renal cortical slices, ceftazidime induced the greatest decrease in PAH accumulation when compared to cefotaxime and cefsulodin but all three decreased TEA accumulation to a similar extent [10].

Fourth-generation cephalosporins such as cefpirome, cefepime, cefoselis, cefluprenam and cefclidin

are zwitterionic 7-methoxyimino cephalosporins, which are active *in vitro* and *in vivo* against both gram-negative and gram-positive bacteria. These zwitterionic β-lactams remain active against some, but not all, ceftazidime-resistant Enterobacteriaceae. Antipseudomonas activities are generally similar to that of ceftazidime except for cefclidin which is more active. However, these cephalosporins are not active against *Bacteroides fragilis*.

The nephrotoxic potential of the fourth-generation cefpirome and of two third-generation cephalosporins, cefotaxime and ceftazidime was compared using both *in vitro* and *in vivo* studies with renal cortical slices [49]. While cefpirome and cefotaxime did not have an effect on gluconeogenesis, ceftazidime caused a significant decrease. Furthermore cefpirome and ceftazidime decreased TEA accumulation whereas cefotaxime showed no effect [49]. These differences may be explained, at least in part, by the zwitterionic structure of cefpirome and ceftazidime as opposed to cefotaxime which lacks a pyridinium ring. Other factors besides peroxidative injury may play a role in the decrease of TEA accumulation caused by ceftazidime and cefpirome. Little or no evidence is yet available concerning the nephrotoxic potential of other fourth-generation cephalosporins.

Cephamycins include β-lactam such as cefoxitin, cefotetan, cefmetazole and latamoxef (moxalactam). Cephamycins are active against anaerobic bacteria, are less active against gram-positive cocci and, have no activity against methicillin-resistant staphylococci and enterococci. Cephamycin antibiotics such as cefotetan and latamoxef have a side chain called the methylthiotetrazole group (MTT), which predisposes to hypothrombinemia and bleeding, and alcohol intolerance by causing a disulfiram reaction.

The nephrotoxicity of cefotetan in rabbits was considerably less than that of cefazolin [50]. When compared to cefotiam, cefoxitin appears to be better tolerated by the kidney since the cefotiam-induced decrease of TEA accumulation and the decrease of gluconeogenesis in renal cortical slices was 2-3 times greater than with cefoxitin [26].

Carbacephems and carbapenems

Chemically, *carbacephems* differ from cephalosporin antibiotics in the dihydrothiazine ring where a methylene group has been substituted for the sulfur group (Figure 1). Loracarbef is the carbacephem analog of cefaclor. Loracarbef has been shown to be active against gram-positive aerobes such as *Staphylococcus pneumoniae* and gram-negative aerobes such as *Escherichia coli* and *Haemophilus influenzae*. When administered to female rabbits (1500 mg/kg) cefaclor and its carbacephem analog loracarbef differentiate in their nephrotoxicity with loracarbef showing a greater potential to cause tubular necrosis than cefaclor [17]. A case of acute interstitial nephritis associated with loracarbef resulting in end-stage renal failure has been described [51].

Carbapenems are a relatively new class of β-lactam antibiotics (penems) with a remarkably broad spectrum. These antibiotics have potent activity against gram-positive cocci including enterococci, and potent activity against gram-negative organisms, including *Pseudomonas aeruginosa*. Carbapenems also display high activity against gut anaerobes.

When given as a large single dose, imipenem can produce acute proximal tubular necrosis in experimental animals [52]. Imipenem has an unsaturated ring adjacent to the β-lactam ring, which is normally hydrolyzed by dehydropeptidase-1, a renal tubular brush border enzyme [53]. Cilastatin, a specific inhibitor of dehydropeptidase-1, blocks the inactivation of imipenem, resulting in high imipenem urinary concentrations and reduced nephrotoxicity. The nephroprotective effect of cilastatin is due to the inhibition of the contraluminal imipenem transport reducing the intracellular accumulation and preventing high tissue concentrations and nephrotoxicity [52]. Newer carbapenems such as meropenem are stable to the hydrolytic action of dehydropeptidase-1, without combination with cilastatin, and are well tolerated in elderly and renal impaired patients [54,55].

In an earlier study of the effects of imipenem in the rabbit kidney it was shown that imipenem caused a significant decrease of mitochondrial respiration, depletion of reduced glutathione, increased production of oxidized glutathione and lipid peroxidation [56]. However, these effects were less than those produced by a comparable nephrotoxic dose of cephaloridine [56]. Panipenem induced nephrotoxicity at a dose of 200 mg/kg, i.v., but this was less severe than that caused by a single dose of imipenem [57]. Simultaneous administration of βmipron (N-benzoyl-3-propionic

acid) with imipenem and panipenem reduced the nephrotoxicity of these carbapenems by inhibiting the active transport of carbapenems in the renal cortex [57].

In a more recent study, peroxidative and nephrotoxic injuries induced by meropenem and imipenem/cilastatin in rat and human cortical slices and microsomes were compared to those induced by cephaloridine [9]. While meropenem and imipenem/cilastatin did produce lipid peroxidation and depressed PAH accumulation and gluconeogenesis in rat and human renal cortex, the effect was substantially less than with cephaloridine [9]. The human renal cortical tissue appears to be less susceptible to β-lactam induced lipid peroxidation than the rat renal cortical tissue; with meropenem showed lower renal toxicity then imipenem/cilastatin [9].

Monocyclic beta-lactams

Aztreonam, a *monobactam*, is a useful alternative for patients with aerobic gram-negative infections who are allergic to penicillins, but has no activity against anaerobes. Aztreonam appears to be the only β-lactam antibiotic that can be safely administered to penicillin-allergic patients [58]. Aztreonam has a spectrum of activity that is comparable to the aminoglycosides but it is less nephrotoxic in patients [59] and it appears to be well tolerated in infants and children [60].

Results of *in vitro* experiments carried out with rat renal microsomes and renal cortical slices showed that aztreonam has a low potential to induce reactive oxygen species and lipid peroxidation [10]. However, aztreonam caused a decrease in renal cortical accumulation of PAH comparable to that of paraquat without a significant decrease in TEA accumulation [10]. Therefore, it appears that the nephrotoxic activity of aztreonam may be not directly related to the superoxide generation and lipid peroxidation.

Relationship between beta-lactam structure and renal toxicity

A consequence of the development of the large number of cephalosporins is that the molecular structures have become more and more complex. Alterations in the cephalosporin molecule have resulted in differences between cephalosporins in spectrum of activity, protein binding, peak serum level, serum half-life, route of excretion, cerebrospinal fluid levels and toxicity. Cephalosporins are semi-synthetic antibiotics derived from 7-aminocephalosporanic acid, which is also called the cephalosporin nucleus. The cephem ring ("nucleus") is composed of a β-lactam ring fused with a dihydrothiazine ring (Figure 1).

Cephalosporins differ in the substituents attached to the 3 and/or 7 positions of the cephem ring. Usually ***modifications at position 7*** influences the antibacterial spectrum and resistance against β-lactamases (Figure 2). For example, the presence of a methoxyimino group at the position 7 as found in cefuroxime, cefotaxime, ceftizoxime and ceftriaxone, confers enhanced β-lactamase stability with some loss of gram-positive activity. Addition of an aminothiazolyl side chain, as found in all the above except cefuroxime, provides unusually high affinity for the penicillin binding proteins found in gram-negative bacteria. Ceftazidime has a propylcarboxyl group at this location, which produces superior Pseudomonas activity but markedly reduces effectiveness against gram-positive organisms. The presence of a methoxy group at position 7 of the cephamycins cefoxitin and cefotetan, by steric hindrance, confers resistance to gram-negative β-lactamases, although it also reduces affinity for penicillin binding proteins [61].

Substitutions at position 3 of the dihydrothiazine ring play a major role in the overall pharmacokinetic properties and toxicity. For example, the unusually long half-life of ceftriaxone appears to be caused by the presence of a triazine substituted at this position [62]. Cephalosporins such as cephalothin, cephaloglycin, cephapirin, cephacetrile and cefotaxime share an acetoxymethyl group at the position 3 (Figure 2) and are all metabolically converted to desacetyl derivatives and to the antibacterially inactive lactone of these substances.

With increasing complexity of the molecular structure it seems inevitable that the ***toxic profile will be altered***. The most striking example of the effect of chemical alterations on the safety profile of β-lactams is the 3-methylthiotetrazole (MTT) side ring attached in position 3 of the cephem nucleus (Figure 3) The MTT side ring is present in many cefamycins (cefotetan, moxalactam) and cephalosporins (cefmenoxime, cefoperazone and cefamandole) and confers epileptogenic activity, disulfiram-like activity and reduced synthesis of prothrombin. Hypoprothrombinemia and bleed-

ing complications during therapy with these drugs occurred in geriatric, debilitated, or other patients with vitamin K deficiency or in patients with severe renal failure or following radical gastrointestinal surgery [63]. The substitution of the MTT side chain and the presence of the 2-methyl-1,3,4-thiodiazole-5-thiole (MTD) side ring in position 3 of the cephem nucleus of cefotiam, cefonicid and cefazolin (Figure 3) induces weaker but similar effects to those caused by the MTT side ring. It appears that the ionization of the N-dimethylaminoethyl group attached to the N-methyltetrazole-thiol (NMTT) side chain of cefotiam enhances its secretory transport in kidney epithelial cells [64].

Cephalosporins such as cephaloglycin, cephalexin and cefaclor have in common a ***D-phenylglycyl side chain at C-7'*** but they differ in the side chain on the C-3' of the cephem nucleus (Figure 2). While cephaloglycin possess an acetoxymethyl group at C-3 and a high intrinsic nephrotoxic potential, cephalosporins such as cephalexin and cefaclor, which in place of the acetoxymethyl group contain a methyl group or a chloride, respectively, are basically not nephrotoxic [11].

While the D-phenylglycyl side chain is not totally responsible for nephrotoxic potential of the β-lactam molecule, it does increase the nephrotoxic potential of the β-lactam if other molecular components are not metabolically detoxified or if renal metabolism occurs at a slow rate, as in the case of cephaloglycin [65].

However, the ***acetoxymethyl side chain in position 3*** of the cephem ring may confer nephrotoxic potential as in the case of cephaloglycin [11] and cephalothin [43] but not with cefotaxime (Figure 2) [26]. It is likely that the presence of D-phenylglycyl side chain in the cephaloglycin molecule and its global molecular configuration insures that the acetoxymethyl side chain will be metabolized at a slower rate by the renal enzymes. This leads to an intracellular accumulation of the intact cephaloglycin sufficient to reach threshold nephrotoxic concentration [64]. Thus, these results suggest that the presence of the acetoxymethyl group on the position 3 of the cephem ring does not lead to inevitable renal damage and thus cannot be solely responsible for the occurrence of nephrotoxicity. The difference between cephaloglycin and cefotaxime is due to the presence on the position 7 of the cephem nucleus of the D-phenylglycyl side chain for cephaloglycin and of the ***aminothiazolyloximino side ring*** for cefotaxime. The lack of the nephrotoxic potential of the aminothiazolyloximino side chain is proven by the molecular structure of ceftizoxime, which has only a hydrogen atom on the position 3 of cephem nucleus (Figure 2).

More interesting, the presence of the ***thiophene ring in position 7*** of the cephem nucleus (Figure 2) has been associated with nephrotoxic effects in the case of cephaloridine and cephalothin and to a lesser extent in the case of the cephamycin cefoxitin [26, 66]. When compared to cephalothin, small alterations of the cefoxitin

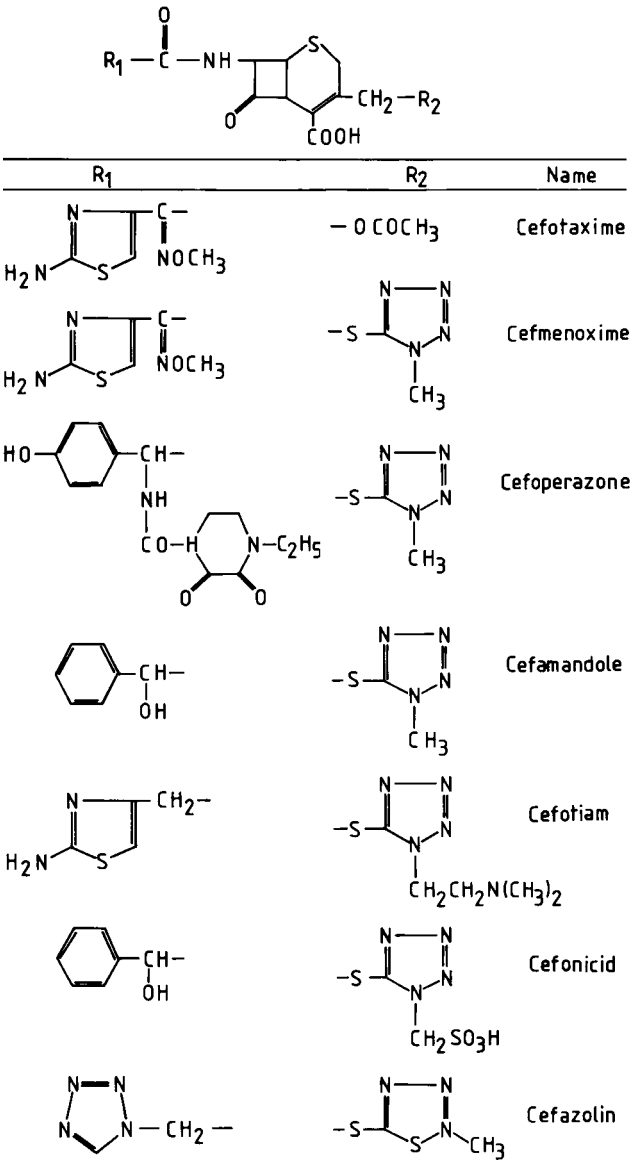

Figure 3. Side chains attached to the cephem nucleus, which have a toxic potential.

molecule in positions 3 and 7 of the cephem nucleus, such as replacement of the methyl group with an amino group and addition of the methoxy group respectively, reduced its nephrotoxic potential [66]. The aminoacetoxy side chain of cefoxitin that is also present in the molecule of cefuroxime (Figure 2), did not, by itself, confer a nephrotoxic potential to these two β-lactams.

The presence of other structures in position 7 of the cephem nucleus, such as the aminothiazolyloximino side ring of cefotaxime, ceftizoxime and cepirome (Figure 4) or the corresponding complex structure of cefoperazone (Figure 3), does not confer a nephrotoxic potential to these β-lactams [26, 49]. Furthermore, the results of various studies suggested that the ***pyridinium ring when attached at position 3*** to the cephem nucleus could be implicated in the nephrotoxic effects seen with cephaloridine, cefsulodin and ceftazidime [10, 26, 34]. The concomitant presence of the thiophene ring and pyridinium ring in the molecule (Figure 4) creates the most nephrotoxic cephalosporin to date, cephaloridine. The presence of an acetoxymethyl side chain instead of the pyridinium ring resulted in the reduction of the nephrotoxic potential of cephalothin by about 30-50% when compared to cephaloridine [9, 26].

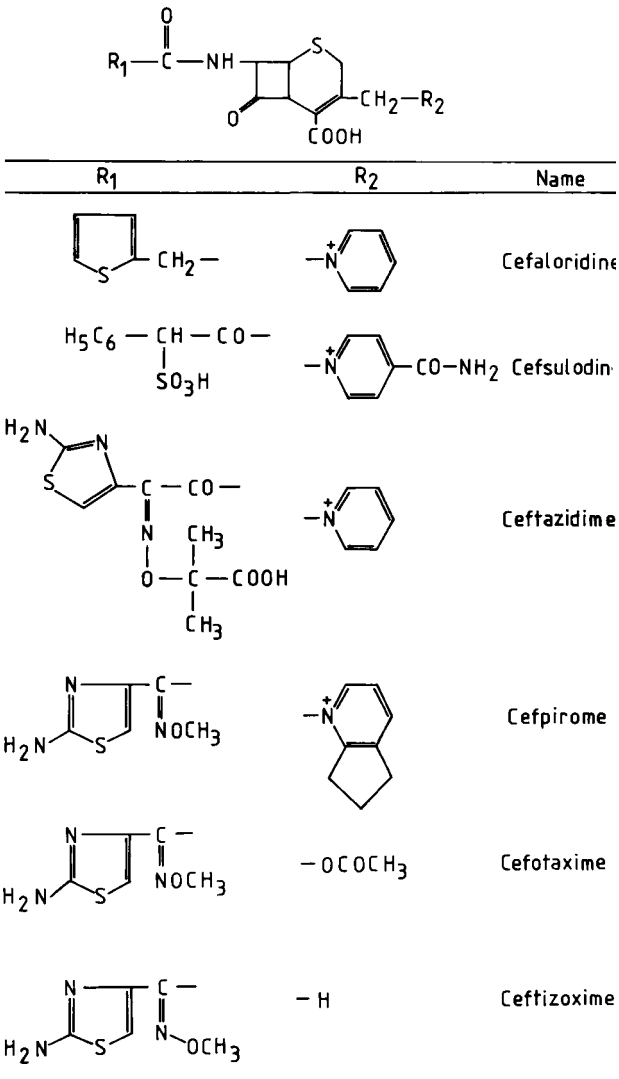

Figure 4. Cephalosporins containing the pyridinium ring attached to the cephem nucleus.

However, β-lactams such as cefotiam or imipenem which contain neither a D-phenylglycyl nor a pyridinium ring in their molecule act as nephrotoxicants [9, 26, 67]. Imipenem induces acute proximal tubular necrosis in monkeys [68] and rabbits [56] similar to that produced by cephaloridine. Both impairment of mitochondrial respiration and oxidative injury appear to be involved in the nephrotoxic action of imipenem [35, 69]. Nephrotoxic cephalosporins have an active leaving group in their C-3 side chain and cause mitochondrial injury by acylating and inactivating the mitochondrial receptors [11]. Although imipenem does not have any leaving group, it also causes similar nephrotoxicity [56].

The differences in nephrotoxicity of carbapenems are due to the different structural features, especially the physicochemical properties. The structure of meropenem differs from the structure of imipenem and panipenem due to the presence of a 1β-methyl group and the lesser basicity of the amino group in the C-2 side chain. The basicity of meropenem is much lower than that of imipenem and panipenem [70]. The reduced meropenem nephrotoxicity is not related to the presence of the 1β-methyl group. However, the basicity of the C-2 side chain of carbapenems is important for the occurrence of nephrotoxicity [70].

The strained structure of carbapenems confers a higher reactivity to the carbapenem skeleton than that of cephalosporin skeleton. It has been suggested that both β-lactam ring and the basicity of C-2 side play a major role in carbapenem-induced nephrotoxicity [70].

To summarize, the nephrotoxic potential of β-lactams is not solely due to the presence of a specific

side chain in the molecule, but rather, the entire molecular structure determines the nephrotoxic action.

Effects on plasma cell membrane and subcellular organelles

Plasma cell membrane

The cell membrane serves as a protective barrier in renal cells. It is the initial site which β-lactams encounter in their journey to the cellular environment from the blood or tubular fluid. β-lactams may disrupt the functional organization of the membrane through peroxidation of membrane lipids, which, in turn, leads to the inability of membrane to serve as an osmotic barrier and causes the cytosol contents to leak. As a result of the cephalosporins disruptive effect on cell membrane, increased leakage of the cytosolic enzyme lactate dehydrogenase (LDH) occurs. The increased LDH concentration was from the cytosol of the renal cortex [49,71] or from isolated proximal and distal tubular cells [39] or in the urine of experimental animals [39]. The results of these studies indicate that plasma membrane became permeable to large molecules such as LDH. After cephalosporin treatment, cephaloridine caused the greatest decrease of LDH concentration in cytosol [49]. Whereas, cephaloridine induced a greater release of LDH from proximal tubular cells than cephalothin and cephalexin, distal cells were not affected by any of these cephalosporins [38,39].

Endoplasmatic reticulum

The major intracellular source of reactive oxygen species such as superoxide anion and hydrogen peroxide are the cytochrome P-450 system of the endoplasmatic reticulum and mitochondria.

The endoplasmic reticulum is a continuous anastomosing network of lipoprotein membranes extending from plasma membrane to nucleus and mitochondria. The microsomal fraction derived from endoplasmatic reticulum consists of membranous vesicles. Microsomal cytochromes P-450 are a superfamily of hemoproteins that play a central role in the metabolism of a large variety of xenobiotics plus synthesis and catabolism of endogenous compounds. Results of studies using nephron fragments have shown that cytochrome P-450 was localized exclusively in the proximal tubule [72] whereas NADPH-cytochrome c reductase was distributed along the entire nephron [73]. The average concentration of cytochrome P450 in unstimulated renal cortex microsomal membranes is about 0.150 nmol/mg protein in rats while in humans it amounts to about 0.050 nmol/mg protein [73, 74].

The renal cytochrome P-450 enzyme system is involved in oxidative reactions in which an atom of molecular oxygen is inserted in an organic molecule. The flavoprotein NADPH-cytochrome P-450 reductase is an essential component of the mixed-function oxidase systems (MFO). Microsomal membranes appear to be particularly subject to attack by reactive oxygen radicals due to their high content of unsaturated fatty acids and the presence of the cytochrome P-450 system [40]. Cephaloridine-induced peroxidation of membrane lipids is decreased by the cytochrome P-450 inhibitor cobalt chloride [31], suggesting a role for a cytochrome P-450 reductase in the β-lactam-induced generation of reactive oxygen species and subsequent peroxidation products.

Cephaloridine-induced reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radical could, in addition to inducing peroxidative damage of membrane lipids [40], destroy and/or inactivate renal cortical membrane proteins and enzymes [75,77]. Treatment of rats with cephaloridine (CPH) caused an *in vivo* depletion of the microsomal cytochrome P-450 and b5 as well as induction of a polypeptide of molecular weight 44,000. SDSD-gel electrophoresis and phenobarbital induction studies indicated that the two depleted polypeptides were cytochrome P-450 isoenzymes [74]. The *in vivo* depletion of renal cortical cytochrome P-450 by CPH was dose-dependent. It is worth noting that statistically significant depletion of cytochrome P-450 occurred at relatively low dosage. More interestingly, the time course of CPH-induced decrease in renal cortical content of cytochrome P-450 isoenzymes indicates that a significant depletion occurred as early as 3 h after a single dose of 1200 mg/kg CPH [74].

An almost complete depletion of cytochrome P450 was measured at 12 and 24 hours after CPH treatment followed by a slow recovery of the cytochrome P-450 content over 48 to 72 hours despite continuing CPH-treatment (1200 mg/kg/d). Measurement of MDA content in the same alloguate of renal cortex and microsomes showed that a significant increase in CPH-

induced lipid peroxidation occurred 24 hours after administration of 1200 mg/kg/d CPH [74]. The time course of these biochemical events indicates that the onset of CPH-induced cytochrome P-450 loss distinctly precedes the onset of CPH-induced lipid peroxidation.

After intravenous treatment of rats with 1200 mg/kg/d CPH for 3 days, homogenates of the renal cortex were separated into subcellular fractions and their protein composition analyzed. The results of the SDS-gel electrophoresis of the renal cortical subfractions showed significant alterations of the polypeptide pattern in the microsomal fraction. The analysis of the polypeptide composition of the microsomal fraction indicated that paralleling to the depletion of cytochrome P-450 isoenzymes in the molecular weight range 50-53,000 was the induction of a polypeptide of molecular weight 44,000 [74].

The question rose whether the CPH-induced 44,000 molecular weight polypeptide is a cytochrome P450-isoenzyme. Thus, induction experiments were carried out in which saline-treated rats were compared with phenobarbital- and CPH-treated rats. Analysis of the polypeptide composition of the microsomal fraction from the phenobarbital group indicated a significant increase of polypeptides in the 50-53,000 molecular weight (P-450 region), but no increase in 44,000 molecular weight polypeptides [75]. However, in the renal cortical microsomes from the CPH treated rats (1200 mg/kg/d for 3 days), there was a time- and dose-dependent increase in the amount of the 44,000 molecular weight polypeptide with a simultaneous depletion of the 50-53,000 molecular weight polypeptides from the cytochrome P-450 region. These results suggest that the 44,000 renal microsomal polypeptide induced by CPH-treatment is not a cytochrome P-450 isoenzyme nor is it the result of degradation of high molecular weight proteins by CPH [75].

Solubilization experiments revealed that the CPH-induced 44,000 molecular weight polypeptide is a peripheral rather than an integral membrane protein [75]. The precise function of the inducible 44,000 molecular weight microsomal protein is not known at the present time. However, data showing an increase in the enzymatic activities of drug metabolizing enzymes such as renal cortical microsomal GSH-S-transferase (3.5-fold) suggest that the CPH-induced 44,000 polypeptide is an enzyme of the endoplasmatic reticulum involved in the detoxification of the reactive species evolving from intracellular bioactivation of CPH [74, 75].

CPH-treatment of rats (1200 mg/kg/d for 2 d) induced the enzymatic activities of other renal cortical drug-metabolizing enzymes such as 7-ethoxycoumarine-O-deethylase and cytosolic GSH-S-transferase whereas the enzymatic activities of aniline hydroxylase and aminopyrine-N-demethylase were simultaneously decreased or remained unchanged, respectively (Table 1) [74]. Treatment of male and female rats with cephaloridine (750 mg/kg/d) for two weeks to three months resulted in a 2-fold increase of glutathione-S-transferase activity in the renal cortex [76]. These results suggest an adaptive response to cephaloridine subchronic treatment.

Table 1. Effects of cephaloridine on the activity of drug metabolizing enzymes from rat renal cortex microsomes.

Enzymes	Control rats	Treated rats	% of control
NADPH-cytochrome-c-reductase (nmoles/mg protein/min)	13.64 ± 2.81	11.65 ± 2.03	85.2
Aminopyrine-N-demethylase (nmoles/mg protein/min)	0.81 ± 0.12	0.82 ± 0.07	101.2
Anilline hydroxylase (nmoles/mg protein/min)	0.330 ± 0.02	0.018 ± 0.003*	5.5
7-Ethoxycoumarin-O-deethylase (nmoles/mg protein/min)	0.095 ± 0.02	0.130 ± 0.07*	136.8
Glutathione-S-transferase (nmoles/ mg protein/min):			
I. **Microsomal**	9.8 ± 1.6	35.0 ± 1.8*	357.1
II. **Cytosolic**	94.75 ± 7.4	298.62 ± 11.3*	315.2

*Cephaloridine was administered for 2 days (1200 mg/kg/d, i.v.). Results are mean ± SD from 5 different preparations. * Values are significant at P< 0.05.*

Renal brush border

The effects of CPH-treatment of rats (1200 mg/kg/d for 3d) on the polypeptide composition of renal brush border from the proximal tubule cells; enzymatic activities and transport systems of the brush border membrane vesicles (BBMV) were investigated [77]. The results of these studies showed that CPH-treatment induces a 20-30% decrease in the specific activities of renal brush border enzymes leucine aminopeptidase and γ-glutamyltransferase. SDS-gel electrophoresis showed that CPH-treatment induced a decrease of the intensity of 3 brush border polypeptides of molecular weights of 72,000, 58,000 and 39,000 [77].

Lysosomes

Cephaloridine has been shown to interact with lysosome phospholipids; this reaction is, in part, hydrophobic in nature [78]. High cephaloridine concentrations have a disruptive effect on lysosomes whereas at low concentrations cephaloridine has a stabilizing effect on the lysosomal membrane system [78,79]. The stabilizing effect is greater with cefazolin and cephaloridine than with ampicillin [80]. This membrane stabilizing effect could be due to the cephaloridine inhibition of the lysosomal membrane bound phospholipase 2 [78].

Treatment of rats with latamoxef (2000 mg/g day) for 5 days induced an insignificant increase in the release of N-acetyl-β-D-glucosaminidase from lysosomes, when compared to control rats [81]. After intravenous treatment of rats with 1200 mg/kg/d CPH for 3 days, homogenates of the renal cortex were separated into subcellular fractions and their protein composition was analyzed. The results of the SDS-gel electrophoresis of the renal cortical subfractions showed no relevant alterations of the polypeptide pattern in the lysosomal fraction [75].

Mitochondria

Administration of cephaloridine induces mitochondria elongation followed by mitochondria swelling [28, 29] which lead to mitochondrial dysfunction. Cephaloridine and other β-lactams decreased mitochondrial respiration significantly, suggesting a loss of mitochondrial integrity [35,56]. It has been suggested that mitochondrial damage may mediate, at least in part, the nephrotoxicity of some β-lactams. Nephrotoxic β-lactams (cephaloridine, cephaloglycin, imipenem) cause similar patterns of respiratory depression whereas non-nephrotoxic β-lactams do not alter mitochondrial function [67]. It is also possible that intracellular accumulation of the nephrotoxic β-lactams cause disruption of lysosomal membrane, release of lysosomal hydrolases which inflict mitochondrial membrane injuries and mitochondrial dysfunction. Structural damage, which can be observed by light microscopy usually, means that the β-lactam-induced toxicity is severe. Under this conditions it may be difficult to decide whether or not the mitochondrial effects are the cause of renal toxicity or are secondary to the death of the cell. Many studies have shown that the β-lactam-induced injuries are early indications of cell injuries. However, some caution must be observed in interpreting the data as mitochondria can undergo reversible changes in conformation, which may reflect changes in osmolality of the cell rather than a direct mitochondrial inhibition.

Mechanisms of action

High intracellular concentration

Contraluminal uptake of organic ions from blood along with the luminal secretion and/or uptake of organic ions plays a crucial role in the renal handling of organic ions, especially β-lactams [82, 83].

Cephalosporins such as cephalothin and cephaloridine interact with both the anionic (p-aminohippurate, PAH) and cationic (tetraethylammonium, TEA or N-methylnicotinamid, NMN) transport systems [31, 67, 85]. β-lactams have been shown to be secreted by the S2 segment of the proximal tubule via the PAH transport system [85]. Cephalothin inhibited the transport of PAH in rabbit basolateral and in brush border membrane vesicles [84] while cephaloridine inhibited the transport of PAH and TEA and in rat renal cortical slices [26, 77] and of PAH [77, 84] or NMN [84] in rat brush border membrane vesicles (BBMV).

Cephaloridine-induced nephrotoxicity is not restricted to the S2 segment but also involves the S3 segment of the proximal tubule [86]. More recent studies suggest that the rat renal organic anion transporter 1 (OAT1) located in the renal basolateral cell membrane,

is the major transporter responsible for the renal secretion of antibiotics, especially that of β-lactams [32, 33]. The luminal secretion of β-lactam across brush-border membrane into urine has also thought to be carrier-mediated. The multispecific organic anion transporter, multidrug resistance-associated protein 2 (MRP2), is localized to the luminal membrane of all proximal tubule segments [87] and mediates the efflux of anionic lipophilic compounds such as glucuronides and glutathione conjugates from the cell. MRP2 or MRP2 isoforms are possible carrier candidates for the luminal secretion of organic anions such as β-lactams.

Apart from the secretion mechanisms, brush border membrane also contains transport systems for reabsorption of compounds from the luminal urine. Treatment of rats with 1200 mg/kg/d cephaloridine greatly reduced the uptake of cephalexin and cefotiam into BBMV whereas the posttreatment uptake of cephaloridine by the BBMV remained unaffected [77]. The unaffected uptake of cephaloridine into BBMV from cephaloridine treated rats indicates that cephaloridine is transported by a transport system, which is different from the dipeptide transporter. OCTN2 is an organic cation/carnitine transporter, which can transport not only organic cations but also of the zwitterions cephaloridine, carnitine and acylcarnitins [88]. Cephaloridine and other β-lactams with quaternary nitrogen such as cefoselis and cefepime are recognized by OCTN2 as transportable substrates [88]. β-lactams that do not contain a quaternary nitrogen but possess an α-amino group are recognized as transportable substrates by the peptide transporters PEPT1 and PEPT2 [89]. PEPT2 has a much higher affinity for β-lactams such as cephadroxil and amoxicillin [89], which do not contain a quaternary nitrogen but posses an α-amino group in the penam or cephem nucleus. Available evidence indicates that PEPT2 mediates H^+-peptide cotransport from the luminal urine across brush-border membrane into the proximal tubule cells [90].

Available experimental data indicates that cephaloridine-induced nephrotoxicity is dependent upon its renal cortical concentration [67]. Experimental data showed that probenecid, 2-4-dinitrophenol, ouabain and anoxia decreased the renal cortex accumulation of cephaloridine and cephalexin [91]. Concomitant exposure of renal cortical slices to cephaloridine and probenecid decreased cephaloridine-induced nephrotoxicity as shown by TEA accumulation in renal cortical slices [35]. Since inhibitors of organic ion transport prevent both transport and nephrotoxicity of cephaloridine, it could be concluded that the nephrotoxicity of cephaloridine is related to high intracellular concentrations resulting from active transport [67, 91] and bioactivation within kidney cells [34, 40].

Cytochrome P-450 and renal bioactivation

Kidneys are able to carry out extensive oxidation, reduction hydrolysis and conjugation reactions. The attractive hypothesis that cephaloridine is metabolized prior to producing nephrotoxicity [92] was not substantiated by experimental data. However, pretreatment of rats with 60 mg/kg cobalt chloride decreased cephaloridine-induced lipid peroxidation in renal cortical slices [31]. These results suggest that prior to producing nephrotoxicity, cephaloridine is taken up into renal cells, where, with the involvement of cytochrome P-450, it induces peroxidation of cell membrane lipids.

Whereas many cephalosporins such as cefaclor, cefadroxil, cefonicid, ceforanide, ceftazidime, ceftizoxime, cefuroxime, cephalexin, and cephradine are not metabolized, cefamandole naftate is rapidly hydrolyzed in plasma to cefamandole, which has greater antibacterial activity than the parent compound. Ceftriaxone is metabolized to a small extent to microbiologically inactive metabolites in the intestines after biliary excretion. Cefuroxime axetil is rapidly hydrolyzed to cefuroxime, the microbiologically active form of the drug, by nonspecific esterases in the intestinal mucosa and blood following oral administration. The axetil moiety is further metabolized to acetaldehyde and acetic acid [93].

Desacetylation of cephalosporins occurs in liver and kidney via the activity of acetylesterases. Desacetylated cephalosporins all maintain some antibacterial activity. Desacetylcefotaxime penetrates well extravascular body sites, achieves high tissue concentrations and acts synergistically with cefotaxime [94, 95]. Desacetylation of cephaloglycin, cephalothin and cephapirin resulted in formation of less active desacetyl forms [94] and less toxicity [64]. About 50% of cephaloglycin is metabolized to desacetylcephloglycin, which is less nephrotoxic at equal dosage [17]. Thus, desacetylation appears to be a detoxification mechanism for toxic cephalosporins such as cephaloglycin and cephalothin [64, 96].

Reactivity of the beta-lactam nucleus

The central β-lactam nucleus is involved in the molecular events leading to renal toxicity. Among other factors reactivity of the central β-lactam core contributes to the antimicrobial potency of these compounds. In the bacterial cell wall β-lactams form covalent complexes with membrane bound proteins (acylation), thus blocking cell wall formation and bacterial proliferation. The ability of a variety of penicillins and cephalosporins to acylate bacterial cell wall proteins was ranked as following: ceftazidime > cefaclor > cephaloglycin > cephalothin > or = cephaloridine > or = cefazolin >> penicillins > cephalexin and other 3-methyl-cephalosporins [97]. It appears that there is a partial correlation between β-lactam acylation potency and their nephrotoxicity which ranked as following: cephaloglycin > cephaloridine > cephalothin > cefazolin > cefaclor > penicillins, cephalexin, ceftazidime and cefotaxime [26, 67]. Moreover, cefaclor which appears to have high acylation potential has low renal toxicity.

Further, cephaloridine with moderate acylating activity is one of the most nephrotoxic cephalosporins. It was speculated that this discrepancy may be due to the presence of the cationic nitrogen group near to the carboxyl group of cephaloridine; this could limit cephaloridine access to the anionic targets and thus requiring high intracellular concentration to induce nephrotoxicity [67]. Whereas experimental evidence supports the concept that some β-lactams may induce acylation of mitochondrial substrate carries, little is known about the functional consequences of acylation of other cellular proteins [67].

Mitochondrial dysfunction

It has been suggested that mitochondrial injury may mediate, at least in part, the nephrotoxicity of some β-lactams [67]. Mitochondrial respiration with and uptake of succinate after exposure to toxic doses of cephaloridine, cephaloglycin, or imipenem [98] showed significant reduction of both functions. Cephalexin did not affect either the mitochondrial uptake or respiration with succinate. Depressed mitochondrial respiration secondary to acylation of the mitochondrial transporter for succinate appears to be implicated in renal toxicity caused by cephalosporins and carbapenems [98]. The organic anion fluorescein accumulates in mitochondria of renal proximal tubular cells [99, 100]. Valproate, indometacin, and salicylate induced a significant inhibition of fluorescein [101]. However, cephaloglycin and cephaloridine did not inhibited the fluorescein uptake. This is contrast with the results of previous studies in which an activation of the mitochondrial transporter was described [56]. This discrepancy between the results of these studies may be explained by the involvement of other carrier systems and/or species differences.

Using t-butyl hydroperoxide as a model hydroperoxide, the temporal sequence of cellular events leading to renal proximal tubular cell death was determined [102]. The results of the *in vitro* studies using rabbit isolated tubule suspensions showed that lipid peroxidation and glutathione oxidation are the initial events in t-butyl hydroperoxide-induced toxicity followed by mitochondrial dysfunction and cell death [102]. The temporal sequence of cellular events causing functional impairment and cell death was determined after exposure of rat renal cortical slices or suspensions of rabbit renal cortical tubules to cephaloridine [37, 38]. The results of these studies indicate that GSH depletion and lipid peroxidation are initial events, which precede mitochondrial dysfunction, impairment of the cellular uptake of organic ions and cell death. Moreover, supplementation of GSH to the incubation medium containing renal cortical microsomes significantly reduced cephaloridine-induced lipid peroxidation within the first 3 minutes after onset of incubation [36].

Glutathione and glutathione transferases

Reduced glutathione is the most important nonprotein thiol present in animal cells [103]. Most of the intracellular GSH is found in the cytosol. However, a minor mitochondrial pool of GSH contributes to the total cellular pool of glutathione [102, 104, 105].

GSH transferases are inducible enzymes with overlapping substrate specificity [73]. They are also found in renal cells as cytosolic enzymes or as membrane-bound microsomal transferases. GSH conjugates are usually less toxic than their parent compounds and are readily excreted in the bile and in the urine as their corresponding mercapturic acids. However, evidence is accumulating that GSH conjugates and/or their corresponding cysteine conjugates are nephrotoxic [106, 107].

Moreover, intracellular accumulation and cytochrome P450 catalyzed bioactivation of β-lactams such as cephaloridine overwhelms of the GSH redox cycle by inhibiting glutathione reductase activity [35, 56], depletion of GSH and accumulation of GSSG [35, 42, 49, 56]. Most of GSSG formed is subsequently reduced by glutathione reductase and GSH is regenerated with concomitant oxidation (consumption) of NADPH to $NADP^+$ [104].

Depletion of GSH by cephaloridine [34, 35, 56], cephaloglycin and imipenem [56]) was accompanied by a significant rise of GSSG concentration of the renal cortex. GSH depletion in renal cortex was dose-dependent and was greatest in rabbits, intermediate in rats and least in mice [42]. This pattern is consistent with the species susceptibility to cephaloridine nephrotoxicity. Further *in vitro* studies [36-38] using kidney slices and renal proximal tubule suspension were aimed at establishing the temporal sequence of biochemical events leading to cell death. The results of these studies showed that GSH depletion and lipid peroxidation were the earliest measurable events (0.25 to 1.5 hours) occurring after exposure of the renal tissue to cephaloridine [36-38].

These results correlate with the *in vivo* studies where a significant GSH depletion was measured 1 h after treating animals with cephaloridine, cephaloglycin or imipenem [42, 56].

Modulation of GSH level in cells (inhibition or stimulation) prior to treatment with different compounds affects the cellular response and drug toxicity [42,104,109]. Pretreatment of mice with buthionine sulfoximine enhanced peroxidative injury and trichloroethylene-mediated nephrotoxicity [109]. Similarly, diethylmaleate significantly depleted GSH in the rat renal cortex and potentiated cephaloridine-induced nephrotoxicity [42]. GSH synthesis may be stimulated by the drug oxothiazolidine-4-carboxylate (OTZ). After uptake in the cell, OTZ is enzymatically decarboxylated to yield cysteine, which is then used to synthesize GSH and thus increasing cellular GSH levels [110]. Another way of increasing cellular and tissue GSH levels is by use of GSH esters. The ester group attached to GSH facilitates penetration through the cell membrane inside the cell, where esterases hydrolyze the ester group to yield free GSH.

Reactive oxygen species and lipid peroxidation

It has been shown that the renal bioactivation of xenobiotics such as the herbicides paraquat and diquat [10, 111, 112], and of β-lactams such as cephaloridine and cefsulodin [10, 40, 41] or the antitumor agent adriamycin [113, 114] can induce the generation of reactive oxygen species (oxidative stress) which can be involved in alterations of the structure and functions of cell membranes, cytoskeletal injury, mutagenicity, carcinogenicity, and cell necrosis [115-117].

Reactive oxygen species

Although the mechanism(s) of β-lactam-induced nephrotoxicity is not fully elucidated, there is growing evidence that for some of the β-lactams, oxidative stress plays a pivotal role in the chain of events leading to nephrotoxicity and cell death [10, 34, 40].

The univalent reduction during redox cycling of compounds such as paraquat or cephaloridine, after exposure to renal microsomes, leads to production of the superoxide anion radical (Figure 5) [10, 40, 112]. Recent *in vitro* studies utilizing renal microsomes demonstrate that cephaloridine-induced reactive oxygen species readily oxidized porphyrinogens to porphyrin [118]. Results of *in vivo* studies in rats show that treatment with cephaloridine (10-500 mg/kg) produced a dose-dependent increase in urine concentration of the total porphyrin levels [118]. These results support cephaloridine-induced production of reactive oxygen species, *in vivo*. Pyridinium ring containing cephalosporins such as cephaloridine, cefsulodine and ceftazidime as well as other β-lactams such as mezlocillin and aztreonam, which do not contain a pyridinium ring, also induce superoxide production in the presence of rat renal microsomes and NADPH [10].

The capacity to generate, and the amount of superoxide produced by a *in vitro* renal microsome system is dependent on the molecular structure of the specific β-lactam. Superoxide production is a function of exposure time and β-lactam concentration (Figure 5). The rank order of the magnitude of superoxide production by β-lactams *in vitro* is as follows: cephaloridine > cefsulodin > mezlocillin > aztreonam > ceftazidime > cefotaxime [10].

The magnitude of renal damage caused by oxygen reactive species will also be influenced by the presence or absence of transition metals. Addition of $FeCl_2$ to a

renal microsomes system increased cephaloridine-induced peroxidation of membrane lipids in a concentration-dependent manner [36]. These data are relevant to *in vivo* conditions where the availability of physiological concentrations of iron is critical. Ferritin, which is present at the subcellular level in the cytosol and endoplasmatic reticulum, appears to be the source for ferric iron *in vivo* [119].

Superoxide generated by xanthine oxidase or in the redox cycling of paraquat can cause the reductive release of $F3^+$ from ferritin, a process that is dependent on the activity of microsomal NADPH-cytochrome P-450 reductase [119]. Iron appears to be an essential component in the formation of reactive species such as superoxide and hydroxyl radical via redox cycling of cephaloridine. Addition of EDTA or of the specific iron chelator desferrioxamine to an incubation system containing renal cortex microsomes and cephaloridine depressed cephaloridine-induced peroxidation of microsomal lipids significantly; EDTA showed a weaker effect than desferrioxamine at equimolar concentrations. By chelating $F3^+$ preferentially [120], desferrioxamine reduced the availability of $F2^+$ produced by the iron redox cycle and decreased cephaloridine-stimulated peroxidation of membrane lipids [36, 37].

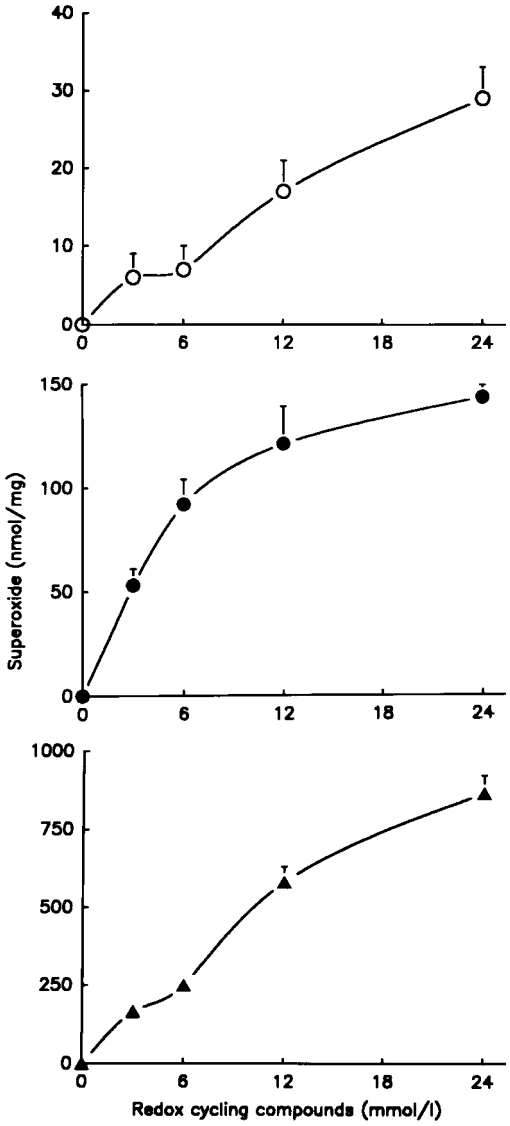

Figure 5. *Concentration-dependent production of superoxide induced by paraquat (▲), cephaloridine (●) and ceftazidime (○).*

Previous studies have shown that renal cortical microsomes are able to catalyze the reduction of cephaloridine in the presence of NADPH with subsequent formation of superoxide and hydrogen peroxide [40]. The divalent reduction of oxygen or the univalent reduction of superoxide yields non-radical species that are protonated at physiological pH to give hydrogen peroxide in a concentration-dependent manner (Figure 6). Hydrogen peroxide, which is a long-lived and membrane permeable species can diffuse and cause injury of cell macromolecules at considerable distances from its generation site.

Beta-lactam-induced generation of superoxide and hydrogen peroxide triggers formation of further highly reactive and cytotoxic oxygen species such as hydroxyl radical. Hydroxyl radical can further contribute in the presence of iron salts, to the decomposition of hydro-

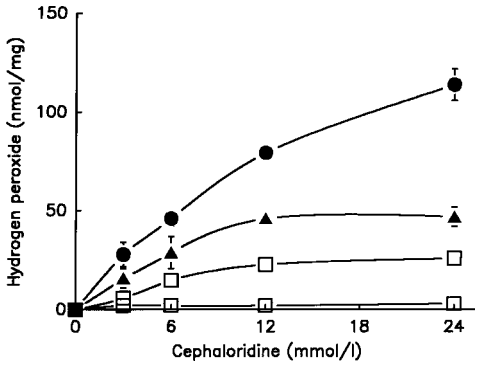

Figure 6. *Concentration-dependent hydrogen peroxide production induced by cephaloridine: 3-24 mmol/l (●), cephaloridine and catalase: 60 µg/ml (▲), cephaloridine and catalase: 120 µg/ml (❑), cefotaxime: 3-24 mmol/l (⧈).*

gen peroxide and formation of additional reactive oxygen species such as singlet oxygen [40].

Beta-lactam induced lipid peroxidation

Free radical chain reactions, which occur during lipid peroxidation, lead to formation of lipid hydroperoxides that decompose to several types of secondary free radicals and a large number of secondary reactive compounds, such as aldehydes, all resulting in the destruction of cellular membranes and other cytotoxic responses.

Under *in vivo* conditions, liver and kidney microsomal NADPH-cytochrome P-450 reductases are also able of initiating peroxidation reactions resulting in the breakdown of polyunsaturated fatty acids to short-chain products. Uncontrolled, these peroxidation reactions can cause disorganization of membrane structure, leading to the inactivation of membrane-associated enzymes, membrane leakage and cell death. Activated oxygen species resulting from bioactivation of paraquat and β-lactams react with polyunsaturated fatty acids to cause peroxidation of the cell membrane lipids and subsequent nephrotoxicity [10, 34, 37, 39, 40, 56]. Among the more stabile end products of lipid peroxidation are compound such as malondialdehyde (MDA), ethane, pentane, and hydroxy-trans-neonal. Generation of conjugated dienes, MDA and pentane have been frequently used to demonstrate *in vivo* induction of β-lactam peroxidative damage in the kidney [34, 37, 56, 109].

Because NADPH-cytochrome P-450 reductase activity is highest in the cortex [77,121] and medullary microsomes lack cytochrome P450 [121], renal cortical tissue was used to investigate peroxidative injury caused by β-lactam accumulation in the kidney. Renal cortical microsomes, slices, tubule and cell suspensions, primary cultured renal cells and established kidney cell lines were exposed to β-lactams with the aim to investigate the subcellular mechanism of the nephrotoxic injury.

Studies conducted with renal cortical slices from pig, rabbit and rat revealed that slices from rabbit and rat renal cortex are more susceptible to β-lactam induced peroxidative injury [43]. Comparing the peroxidative potential of cephalosporins of different generations revealed that not only first-generation cephalosporins, but also second-generation cephalosporins such as cefotiam and third- and fourth-generation cephalosporins (Table 2) can produce a significant increase of lipid peroxidation measured as MDA production [10,26,49].

Exposure of renal cortical microsomes or primary renal epithelial culture cells to different type of antibiotics led to a significant increase in production of superoxide and MDA after cephaloridine and mezlocillin [10, 40] but not after gentamicin [40, 122].

Significant increase in the cephaloridine-induced MDA generation was manifest in the proximal tubule suspensions while incubation of distal tubules with cephaloridine failed to increase MDA production tubule cell toxicity [36, 41, 50]. Exposure of rabbit and rat isolated proximal tubules or rat renal cortical slices to cephaloridine caused a time- and concentration-dependent generation of MDA [37, 38, 40, 41]. Inhibition of

Table 2. Malondialdehyde (MDA) content and gluconeogenesis as a function of the cephalosporinconcentration in the incubation medium.

	MDA (nmol/h/g tissue)					**Gluconeogenesis** (μmol/h/g tissue)				
Cephalosporin (mg/ml)	**0**	**1.25**	**2.5**	**5**	**10**	**0**	**1.25**	**2.5**	**5**	**10**
Cephaloridine	36.0 ± 6.2	48.4 ± 5.5	65.5* ± 3.1	96.4* ± 2.0	111.2* ± 2.5	26.7 ± 1.7	18.8* ± 2.1	16.2* ± 1.3	5.4* ± 1.9	4.4* ± 1.2
Ceftazidime	38.0 ± 3.6	41.5 ± 3.4	44.4* ± 1.4	48.6* ± 0.4	57.4* ± 2.3	26.9 ± 2.4	28.8 ± 1.3	8.2* ± 3.3	13.8* ± 2.8	2.8* ± 0.7
Cefpirome	41.2 ± 3.1	46.5 ± 4.1	45.2 ± 1.2	47.3 ± 4.0	58.3 * ± 3.1	25.6 ± 3.3	25.7 ± 1.9	21.2 ± 2.9	18.9 ± 3.9	23.0 ± 3.5
Cefotaxime	41.3 ± 2.9	42.8 ± 2.4	41.3 ± 1.9	46.6 ± 3.6	55.4 * ± 4.2	25.5 ± 1.6	25.3 ± 2.7	24.3 ± 4.1	25.7 ± 1.6	26.8 ± 1.3

Data represent mean ± SD from at least 4 rats. Values are significant at P< 0.05.*

cephaloridine uptake into kidney slices [40] or isolated proximal tubules by 1.0 and 2.0 mM probenecid reduced MDA production in a concentration-dependent manner [39]. These results provide indirect evidence that biochemical processes leading to MDA production do not occur in the incubation medium but within the cortical cells after an obligatory uptake process across the cell membrane. Furthermore, pretreatment of rats with 60 mg/kg cobalt chloride significantly decreased cephaloridine-induced lipid peroxidation in renal cortical slices [31]. Addition of $FeCl_2$ to the incubation medium of renal cortical microsomes caused a significant stimulation of the cephaloridine-induced lipid peroxidation [36, 37]. Collectively, these results are indicative of the cytochrome P450 involvement in the intracellular bioactivation of cephaloridine and its subsequent peroxidative and nephrotoxic action [123]. However, it appears that β-lactams are nephrotoxic through more than one molecular mechanism.

Protection by antioxidants and radical scavengers

Under normal physiological conditions the liver and the kidney cells appear to possess adequate defense mechanisms against lipid peroxidation. The most crucial intracellular components of the antiperoxidant defense system are glutathione and the glutathione-dependent enzymes.

The use of the detoxifying enzymes superoxide dismutase and catalase to suppress formation of superoxide and hydrogen peroxide, respectively, as well as specific radical scavengers for the hydroxyl radical and singlet oxygen such as mannitol, (+)-cyanidanol-3, thiourea, sodium benzoate, N-acetyl tryptophan and histidine, effectively decreased paraquat- or cephaloridine-induced peroxidation of microsomal lipids *in vitro* [15, 40, 41]. The chelation of iron should inhibit the production of hydroxyl radical and therefore mitigate the lipid peroxidation. Deferoxamine, a specific iron chelator, significantly inhibited peroxidation and protects against nephrotoxicity [36, 37]. Moreover, non-specific antioxidants such as vitamin E, N,N'-diphenyl-phenylenediamine, promethazine, probucol or reduced glutathione significantly depressed cephaloridine-induced peroxidation of lipids in renal cortical slices and microsomes [37, 40, 41, 124]. Intracellular signaling pathways of cAMP and protein kinase C (PKC) have been reported to modulate cephaloridine-induced free radicals and nephrotoxicity. [72, 125]. Phorbol myristate acetate (PMA) enhancement of cephaloridine-induced lipid peroxidation and cell injury was blocked by a PKC inhibitor [71].

Alterations of cellular biochemical processes

Various β-lactam antibiotics such as cephalosporins and guanylureido penicillins may cause nonimmunologic nephrotoxic effects. The elucidation of the precise biochemical mechanisms involved in nephrotoxicity of β-lactams is of obvious importance for their rational and efficient utilization in the clinical management of infectious disease and for development of future cephalosporins.

Renal transport systems

For the zwitterion cephaloridine (CPH) a quantitative correlation between CPH-concentration and the degree of nephrotoxicity has been found [126]. CPH is taken up from blood into the proximal tubule cells and it was assumed that CPH uptake across the basolateral membrane occurs by the transport systems for PAH [127, 128]. However, it was also shown that zwitterionic β-lactams such as CPH can interact with the cation transport systems [82].

Because rats treated with CPH had altered protein composition and enzymatic activities of membranes from endoplasmic reticulum membranes [74, 75] and since intracellular CPH accumulation and nephrotoxicity was ascribed to relative impermeability of the luminal membrane for CPH [129], the effects of CPH-treatment on transport systems located in the brush border membrane were investigated [77].

The uptake of *D-glucose* into renal brush border vesicles (BBMV) from control rats is a Na+-dependent transport process which demonstrates an overshoot phenomenon. After treatment of rats with CPH, the D-glucose transport into renal BBMV shows neither a Na^+-dependency nor an overshoot phenomenon (Figure 7). Furthermore, the equilibrium values for D-glucose uptake were reduced to 35% of controls in these studies. Similar results were obtained with BBMV from small intestine after treatment of animals with the anticancer drug mitomycin C [130, 131] but the equilibrium uptake values for D-glucose remained unchanged.

The effect of CPH-treatment upon the Na^+-dependent transport of the amino acid *L-alanine* was investigated [75]. The results of these studies showed that Na+-dependent transport of L-alanine was also reduced by the treatment with CPH and the overshoot phenomenon completely eliminated (Figure 7). In contrast to D-glucose, the equilibrium uptake values for L-alanine remained unchanged. Weinberg and colleagues [132] found that alanine and glycine can be protective against injury associated with increases in cytosolic free Ca^{2+}, reactive oxygen species, ATP depletion, and Na-K-ATPase inhibition in isolated kidney tubule cells in culture. Thus, the cephaloridine-induced decrease of alanine transport at the luminal cell membrane would diminish the cell defense ability against the toxic injuries caused by oxygen reactive species resulting from intracellular bioactivation of accumulated cephaloridine.

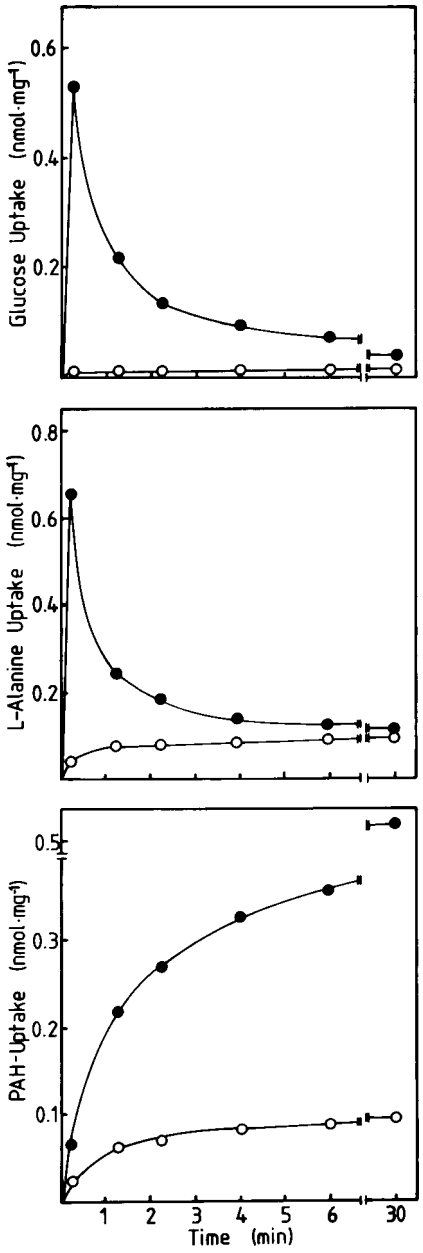

Figure 7. *Time-dependent uptake of PAH, L-alanine and glucose by renal brush border membrane vesicles. (●), control rats; (○), rats treated with cephaloridine (1200 mg/kg for 3 days).*

The carrier-mediated uptake of *p-aminohippuric acid* (PAH) into BBMV (Figure 7) and PAH accumulation by renal cortical slices [69,77] were also significantly reduced by CPH treatment (1200 mg/kg/d for 3d). Furthermore, the transport of other cephalosporins across the renal brush border membrane is also affected by CPH-treatment; the uptake of cephalexin and cefotiam into BBMV was greatly reduced whereas the uptake of CPH remained unaffected [77]. Secretion of cephalosporins across the brush border membrane is assumed to occur by the PAH-system as well as by the organic cation/H+-antiporter [127, 133]. Reabsorption of many cephalosporins is performed by the dipeptide transport system [69, 133]. The unaffected uptake of CPH into BBMV from CPH-treated rats indicates that CPH is transported by a system different from the dipeptide transporter. This is in agreement with results of other studies [82, 127] indicating that CPH interacts with transport systems for organic cations and anions in the brush border membrane. The similar uptake values for CPH in renal BBMV from untreated and CPH-treated rats do not support the previous hypothesis that the brush border membrane is impermeable for CPH [129].

Since cephalexin is transported by the dipeptide transport system [75,133], the question arose whether or not the reduction of cephalexin transport activity following CPH treatment could be caused by either reduction in the number of transport sites or an impairment of the transport system for β-lactam antibiotics and dipeptides [77]. Using photoaffinity labeling, two membrane polypeptides of brush border membrane of molecular weight of 130,000 and 95,000 were identified as constituents of the dipeptide transport system [77]. The results of this study demonstrated that CPH-treatment of rats greatly reduced the photoaffinity la-

beling of the binding protein for β-lactam antibiotics and dipeptides with apparent molecular weight 130000. The labeling of the polypeptide of 95000 molecular weight was almost completely depressed [77]. The decrease in labeling intensity of the putative dipeptide transporter is suggestive for a reduction in the number of transport sites following CPH treatment. These results provide further evidence to elucidate the biochemical mechanism by which cephaloridine-induced oxidative injuries alter cell membrane permeability.

Gluconeogenesis

Gluconeogenesis is an important metabolic function of the kidney [134]. Renal cortical slices from nive rats exposed to cephalosporins *in vitro* or renal cortical slices from animals treated with cephaloridine showed a time- and dose-dependent decrease of renal gluconeogenesis [26, 37, 49]. Glucose synthesis occurred in the proximal but not in the distal tubule suspensions [36]. Inhibition gluconeogenesis within 5 minutes of drug treatment may be an early event in cephaloridine-induced renal toxicity occurring prior to the onset of lipid peroxidation in renal cortical slices [135]. However, decreased gluconeogenesis should not cause cellular necrosis. Interestingly, antioxidants used to protect against cephaloridine-induced inhibition of organic ion accumulation do not block inhibition of gluconeogenesis by cephaloridine [135]. Cephaloridine-induced decrease in gluconeogenesis has been shown to be related to a simultaneous inhibition of the microsomal bound enzyme glucose-6-phosphatase activity in the renal cortex [135]. In contrast, the activity of another rate-limiting enzyme of gluconeogenesis, fructose-1,6-diphosphatase, was not inhibited by cephaloridine [12].

Renal lipid metabolism and protein degradation

Penicillin treatment of rabbit neonates (90,000 IU for 2d) altered *lipid metabolism in vivo* by significantly increasing serum concentration of non-esterified fatty acids and decreasing renal triglyceride content [136]. It appears that penicillin was either decreasing the utilization of non-esterified fatty acids or increasing release. The decrease of renal triglyceride content could be the result of the inhibition of the triglyceride synthesis or penicillin might have increased the utilization of this substrate.

Cephaloridine contains a quaternary nitrogen, exists as a zwitterion under physiological conditions and has structural similarities with carnitine. Proximal tubule cells are the internal sites of carnitine acylation [137]. Cephalosporin and carbapenem antibiotics inhibit carnitine tubular reabsorption [68, 138] and mitochondrial uptake of acylcarnitine leading to massive acylcarnitinuria [67]. Newer β-lactam such as cefepime and cefoselis, which possess a quaternary nitrogen as does carnitine, may also inhibit carnitine tubular reabsorption [88].

In order to be metabolized, long-chain fatty acids must first undergo conjugation to carnitine for transport by the acylcarnitine-carnitine carrier across the mitochondrial inner membrane [139]. Short-chain fatty acids enter the mitochondria through monocarboxylic acid transporters [139]. Studies were carried out to assess the effects cephaloridine, cephaloglycin and cephalexin on the mitochondrial oxidative metabolism of fatty acids such as butyrate and palmitate [67].

The results of these studies showed significant inhibition of palmitoylcarnitine-mediate respiration by cephaloridine *in vitro*, whereas cephaloglycin, which lacks structural homology with carnitine, caused a greater inhibition of the mitochondrial transport and oxidation of butyrate than cephaloridine. It is possible that the mitochondrial uptake of butyrate was not affected by cephaloridine maybe because the pyridinyl nitrogen hinders its attack on the monocarboxylate receptors. Cephalexin induced only mild *in vitro* toxicity to the mitochondrial uptake and oxidation of butyrate and palmitate [67].

Cephaloridine effect on the intracellular *renal protein degradation* was investigated using the labeled low molecular weight protein, ^{125}I-lysozyme. Treatment of rats with cephaloridine for 5 days was followed by administration of ^{125}I-lysozyme one hour prior sacrifice. Release of trichloroacetic acid (TCA) soluble radioactivity into incubation medium from renal cortical slices was used to quantify lysosomal degradation of lysozyme [141].

The results of these experiments showed that cephaloridine caused a dose-dependent decrease of intracellular protein degradation thus impairing the renal metabolism of endogenous and exogenous peptides and proteins taken up by the renal cells.

Clinical toxicity of beta-lactam antibiotics

Usually β-lactam induced adverse reactions are readily recognized by the clinician. On the other hand, the relationship between antimicrobial activity and the development of a drug-initiated adverse effect can be very subtle and elude the most astute clinician. If a β-lactam is uniquely advantageous for a patient, a carefully controlled rechallenge can be considered to more precisely identify a cause-effect relationship. With appropriate clinical management renal failure caused by β-lactams is often reversible. Identification and elimination of the risk factors associated with β-lactam nephrotoxicity is essential to the prevention of nephrotoxicity. Of these factors, correction of volume depletion and/or congestive heart failure and reversing diminished renal perfusion are of primary importance. While fluid resuscitation can limit the renal damage caused by nephrotoxic β-lactams, there is a risk of overhydration if renal failure develops. Monitoring of serum drug concentration should be helpful to confirm β-lactam-induced renal toxicity, especially when drug interactions are involved.

Interaction with other nephrotoxic drugs

Beta-lactam induced renal toxicity can results from their use in monotherapy or when used in combination with other nephrotoxic drugs such as aminoglycosides, amphotericin B, cisplatin, cyclosporine, furosemide, ifosfamide, vancomycin and nephrotoxic β-lactams. While the risk of nephrotoxic injury from monotherapy with β-lactams is relatively low, this risk is substantially increased when multiple drug combinations are required.

Benzylpenicillin and ureidopenicillins such as piperacillin and mezlocillin appear to have a little or no nephrotoxic potential when administered alone or in combination with other drugs.

Rats treated with piperacillin (1600 mg/kg) and furosemide (100 mg/kg) have elevation blood urea nitrogen (BUN) and creatinine concentration, and mild histologic degeneration of the proximal tubules. These alterations were similar to those observed in rats treated with furosemide alone [142].

The combination of cephalothin with an aminoglycoside was more nephrotoxic than methicillin plus aminoglycoside [143]. There is good evidence that concurrent administration of cephalothin and gentamicin are additive nephrotoxins in humans, especially in patients over 60 years of age as wells as in rabbits [144], and renal injuries are intensified in the presence of mild renal ischemia or endotoxemia [108]. The results of prospective randomized comparative studies of the combination mezlocillin/cefotaxime versus gentamicin/cefoxitin showed that the concurrent administration of mezlocillin/cefotaxime has low renal toxicity and can be recommended for the rational and empirical treatment of serious systemic infections [145].

Results from animal studies indicate that while furosemide enhanced cephaloridine nephrotoxicity no increased renal toxicity was observed by combining of piperacillin with furosemide [142]. Latamoxef and flomoxef may decrease nephrotoxicity of vancomycin by inhibiting its uptake into the kidney [146, 147]. The results of a retrospective study including renal transplant patients indicate that aztreonam can be safely administered with cyclosporine [148]. Combination therapy with ampicillin/aztreonam in neonates showed a lower renal toxicity than in the group with concurrent administration of oxacillin/amikacin [149].

New lactam antibiotics

There is a continuous need for new antibiotics to overcome resistance. However, in the case of β-lactams there is a need to inhibit β-lactamase enzymes, which hydrolyze, and thereby inactivate β-lactam antibiotics. Novel tricyclic carbapenems (trinems) and 2-naphtyl-carbapenems have broad spectrum and showed potent activities against gram-negative bacteria [150, 151] including methicillin-resistant *Staphylococcus aureus* (MRSA). The γ-lactams may be less susceptible to degradation by hydrolases. A number of compounds containing the γ-lactam (pyrrolidin-2-one) moiety show interesting biological and pharmaceutical activities. Some novel monocyclic thienyl γ-lactams are reported to show moderate to high antibacterial activity against gram-positive and gram-negative bacteria [152].

Prevention of clinical toxicity of beta-lactam antibiotics

Adverse drug effects represent a major source of morbidity and mortality and must be considered in the

differential diagnosis for patients who are experiencing new medical problems or whose clinical status is worsening. Familiarity with β-lactam induced adverse reactions can improve antibiotic selection and reduce adverse events. Before antibiotic therapy is started, the potential benefits and the possible adverse effects should be investigated in light of each patient's situation. Prevention should be considered in the first place, but if adverse events do occur, they must be recognized and corrected promptly.

The most important approach to decreasing β-lactam nephrotoxicity is judicious use of these drugs. If a β-lactam is uniquely advantageous for a patient, a carefully controlled rechallenge can be considered to more precisely identify a cause-effect relationship. When β-lactams are used in neonates, accurate determination of the dosage is required, especially for compounds with low therapeutic index and in patients with renal failure.

Occurrence of acute renal failure from β-lactam treatment may be prevented by early treatment of serious infections, together with maintenance of hemodynamic stability, renal perfusion, and urinary solute excretion. The β-lactam induced renal failure has a time course comparable to acute tubular necrosis of other origins. While there is no firm evidence that dialysis will speed up renal recovery, clinical stability and good nutrition are likely to improve recovery, as it is also the case with other types of renal failure.

Concomitant administration of piperacillin and cephaloridine to rabbits resulted in a significant protective effect against cephaloridine nephrotoxicity [153]. Cephaloridine nephrotoxicity can be prevented by administration of other cephalosporins or penicillins that produce little or no reduction of the cortical concentration of cephaloridine [154]. However, ceftriaxone protects against tobramycin nephrotoxicity by reducing the intracortical accumulation of tobramycin [155]. Combination of tobramycin with latamoxef protects the rat kidney from tobramycin nephrotoxicity, and the protective effect may be partially due to suppression of intrarenal accumulation of tobramycin by latamoxef. This suppression of nephrotoxicity is roughly dependent on the latamoxef dosage [81, 156].

Methimazole (1-methylimidazole-2-thiol) protects against cephaloridine-induced nephrotoxicity when was given 30 min prior cephaloridine administration to rats [157]. Furthermore, cephaloridine transport and accumulation in the kidney was not affected by methimazole [157].

Comparison of cephaloridine-induced nephrotoxicity in normoglycemic and diabetic rats showed lower renal toxicity in diabetic rats than normoglycemic rats. This is apparently due to the fact that the diabetic renal tissue accumulated less cephaloridine than the tissue from normoglycemic rats [158].

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References

1. Fleming A. On the antibacterial action of cultures of Penicillium with special reference to their use in the isolation of *B.influemzae*. Brit J Exper Pathol 1929; 10: 228-236.
2. Abraham EP, Newton GGF. The structure of cephalosporin C. Biochem J 1961; 79: 377-393.
3. Nagarajan R, Boeck LD, Gorman M, Hamil RC, Higgens CE, Hoehn MM, Stark WM, Whitney, JG. β-lactam antibiotics from Streptomyces. J Am Chem Soc 1971; 93: 2308-2310.
4. Page MG. B-Lactamase inhibitors. Drug Resist Updat 2000; 3(2): 109-125.
5. Cooper RD. The carbacephems: a new β-lactam antibiotic class. Am J Med 1992; 92(6A): 2S-6S.
6. Tally FP, Jacobus NV, Gorbach SL. *In vitro* activity of thienamycin. Antimicrob Agents Chemother 1978; 14 (3): 436-438.
7. Sader HS, Gales AC. Emerging strategies in infectious diseases: new carbapenem and trinem antibacterial agents. Drugs 2001; 61(5): 553-564.
8. Cimarusti CM, and Sykes RB. Monocyclic β-lactam antibiotics. Med Re. Rev 1984; 4(1): 1-24.
9. Yousif T, Pooyeh S, Hannemann J, Baumann J, Tauber R, Baumann K. Nephrotoxic and peroxidative potential of meropenem and imipenem/cilastatin in rat and human renal cortical slices. Inter. J Clin Pharmacol Therap 1999; 10: 475-486.

10. Duwe J, Cojocel C, Baumann K. Effect of paraquat-like substances and cephalosporins on accumulation of p-aminohippurate and tetraethylammonium in rat renal cortical slices, and on lipid peroxidation in rat renal microsomes and cortical alices. In: Nephrotoxicity. Mechanisms, early diagnosis, and therapeutic management. Bach PH, Gregg NJ, Wilks MF, Delacruz L (editors). Marcel Dekker Inc, New York 1991; p. 13-17.
11. Tune BM. The nephrotoxicity of cephalosporin antibiotics-structure-activity relationships. Comm Toxicol 1986; 1(2): 145-170.
12. Goldstein RS, Smith PF, Tarloff, JB, Contardi L, Rush GF, Hook JB. Biochemical mechanisms of cephaloridine nephrotoxicity. Life Sci 1988; 42(19): 1809-1916.
13. Kaloyanides GJ. Metabolic interactions between drugs and renal tubulo-interstitial cells. Role in nephrotoxicity. Kidney Int 1991; 39: 531-540.
14. Schnellmann RG, Griner RD. Mitochondrial mechanisms of tubular injury. In: Mechanisms of injury in renal disease and toxicity.Goldstein RS, Editor.CRC Press Inc, Boca Raton, Florida, USA 1994; p. 247-265.
15. Tomita M, Okuyama T, Watanabe S, Watanabe H. Quantitation of the hydroxyl radical adducts of salicylic acid by micellar electrokinetic capillary chromatography: oxidizing species formed by a Fenton reaction. Arch Toxicol 1994; 68: 428 - 433.
16. Tomita M., and Okuyama T. Effect of paraquat on the malondialdehyde level in rat liver microsomes (*in vitro*). Arch. Toxicol 1994; 68: 187-192.
17. Tune BM, Hsu C-H, Fravert D. Cephalothin and carbacephem nephrotoxicity. Role of tubular cell uptake and acylating potential. Biochem Pharmacol 1996; 51: 557-561.
18. Roselle GA, Clyne DH, Kaufman CA. Carbenicillin nephrotoxicity. South Med J 1978; 71(1): 84-86.
19. Elis D, Fried WA, Yunis EJ, Blau EB. Acute interstitial nephritis in children: a report of 13 cases and review of the literature. Pediatrics 1981; 67(6): 862-870.
20. Pommer W, Offerman G, Schultze G, Krause PH, Molzhan M. Acute interstitial nephritis caused by drugs. Dtsch Med Wochenschr 1983; 108(20): 783-788.
21. Browning MC, Tune BM. Reactivity and binding of β-lactam antibiotics in rabbit renal cortex. J Pharmacol. Exp Ther 1983; 226(3): 640-644.
22. Geller RJ, Chevalier RL, Spyker DA. Acute amoxicillin nephrotoxicity following an overdose. J. Toxicol Clin Toxicol 1986; 24(2): 175-182.
23. Schellie SF, Groshong T. Acute interstitial nephritis following amoxicillin overdose. Mo Med 1999; 96(6): 209-211.
24. Hedstrom SA, Hybbinette CH. Nephrotoxicity in isoxazolylpenicillin prophylaxis in hip surgery. Acta Orthop Scand 1988; 59(2): 144-147.
25. Dunn GL. Ceftizoxime and other third-generation cephalosporins: Structure activity relationships. J Antimicrob Chemother 1982; 10: 1-10.
26. Cojocel C, Goettsche U., Toelle K-L., Baumann, K. Nephrotoxic potential of first-, second-, and third-generation cephalosporins. Arch Toxicol 1988; 62: 458-464.
27. Fillastre JP, Kleinknecht D. Acute renal failure associated with cephalosporin therapy. Am Heart J 1975; 89(6): 809-810.
28. Silverblatt F, Turck M, Bulger R. Nephrotoxicitydue to cephaloridine: a light- and electron-microscopy study in rabbits. J Infect Dis 1970; 122(1): 33-44.
29. Yamada Y. Studies on the mechanisms of renal damage induced by nephrotoxic compounds. Nippon Hoigaku Zasshi 1995; 49(6): 447-457.
30. Tune BM. Effect of organic transport inhibitors on renal uptake and proximal tubular toxicity of cephaloridine. J Pharmacol Exper Therap 1972; 181: 250-256.
31. Cojocel C, Laeschke KH, Insellman G, Baumann K. Inhibition of cephaloridine-induced lipid peroxidation. Toxicology 1985; 35(4): 295-305.
32. Takeda M, Tojo A, Sekine T, Hosoyamada M, Kanai Y, Endou H. Role of organic anion transporter 1 (OAT1) in cephaloridin (CER)-induced nephrotoxicity. Kidney Int 1999; 56: 2128-2136.
33. Jariyawat S, Sekine T, Takeda M, Apiwattanakul N, Kanai Y, Sophasan S, Endou H. The interaction and transport of β-lactam antibiotics with the cloned rat renal organic anion transporter 1. J Pharmacol Exp Ther1999; 290 (2): 672-677.
34. Kuo C-H, Maita, K, Sleight SD, Hook JB. Lipid peroxidation: a possible mechanism of cephaloridine-induced nephrotoxicity. Toxicol Appl Pharmacol 1983; 67: 78-88.
35. Tune B, Fravert D, Hsu C-Y. Oxidative and mitochondrial toxic effects of cephalosporin antibiotics in the kidney. A comparative study of cephaloridine and cephloglycine. Biochem Pharmacol 1989; 38(3): 795-802.
36. Cojocel C, Mayer D. Protection by glutathione and antioxidants against cephaloridine-induced lipid peroxidation. Biochem. Pharmacol (Life Sci Adv) 1991; 10: 41-51.
37. Goldstein RS, Pasino DA, Hewit WR, Hook JB. Biochemical mechanisms of cephaloridine nephrotoxicity: time and concentration dependence of peroxidative injury. Toxicol Appl Pharmacol 1986; 83: 261-270.
38. Rush GF, Ponsler GD. Cephaloridine-induced biochemical changes and cytotoxicity in suspensions of rabbit isolated proximal tubules. Toxicol Appl Pharmacol 1991; 109: 314-326.

39. Rush GF, Heim RA, Ponsler GD, Engelhardt J. Cephaloridine-induced renal pathological and biochemical changes in female rabbits and isolated proximal tubules in suspension. Toxicol Pathol 1992; 20(2): 155-168.
40. Cojocel C, Hannemann J, Baumann K. Cephaloridine-induced lipid peroxidation initiated by reactive oxygen species as a possible mechanism of cephaloridine nephrotoxicity. Biochim Biophys Acta 1985; 834: 402–410.
41. Suzuki Y, Sudo J. Lipid peroxidation and generation of oxygen radicals induced by cephaloridine im renal cortical microsomes of rats. Jpn J Pharmacol 1990; 52(2): 233-243.
42. Kuo C-H, Hook JB. Depletion of renal gluthathione content and nephrotoxicity of cephaloridine in rabits, rats, and mice. Toxicol Appl Pharmacol 1982; 63: 292-302.
43. Cojocel C, Inselmann G, Laeschke KE, Baumann K. Species differences in cephalosporin-induced lipid peroxidation. Drugs Exptl Clin Res 1984; 10(11): 781-784.
44. Kays SE, Crowell WA, Johnson MA. Cephaloridine nephrotoxicity is potentiated by selenium deficiency but not by copper deficiency in rats. J Nutr 1992; 122: 1232-1241.
45. Alitalo R, Ruutu M, Valtonen V, Lehtonen T, Pentkäinen PJ. Hyoprothrombinaemia and bleeding during administration of cefamandole and cefoperazone. Ann Clin Res 1985; 17: 116-119.
46. Wachsmuth ED. Nephrotoxicity of cefotiam (CGP 14221/E) in rats and rabbits. Arch Toxicol 1996; 48: 135-156.
47. Norrby SR, Burman LA, Linderhol H, Trollfors B. Ceftazidime: pharmakokinetics in patients and effects on renal function. J Antimicrob Chemother 1982; 10(3): 199-206.
48. Alestig K, Trollfors B, Andersson R, Oolaison L, Suurkula M, Norrby SR. Ceftazidime and renal function. J Antimicrob Chemother 1984; 13(2): 177-181.
49. Cojocel C. Biochemical aspects of the renal tolerance for cefpirome and other cephalosporins. rzneimittelforschung 1990; 40(10):1140-1144.
50. Suzuki H, Imamura K, Yoshida T, Shibata M, Matsuzawa T, Ozaki H, Sakai T, Shiobara Y, Tachibana A, Yanko K. Animal pharmacokinetis and toxicology of cefotetan - a new cephamycin antibiotic. J Antimicrob Chemother 1983; 11(Suppl): 179-183.
51. Thieme RE, Caldwell SA, Lum GM. Acute interstitial nephritis associated with loracarbef therapy. J Pediatr 1995; 127(6): 997-1000.
52. Birnbaum J, Kahan FM, Kropp H, Macdonald JS. Carbapenems, a new class of β-lactam antibiotics: discovery and development of imipenem/cilastatin. Am J Med 1985; 78(Suppl 6A): 3-21.
53. Kropp H, Sundelof JG, Hajdu R, Kahan FM. Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase, dehydropeptidase-1. Antimicrob Ag Chemother 1982; 22: 62-70.
54. Cunha BA. Meropenem in elderly and renally impaired patients. Int J Antimicrob Agents 1988; 10: 107-117.
55. Asbel LE, Levison ME. Cephalosporins, carbapenems, and monobactams. Infect Dis Clin North Am 2000; 14(2): 435-437.
56. Tune BM., Fravert D, Hsu C-Y. Thienamycin nephrotoxicity. Mitochondrial injury and oxidative effects of imipenem in the rabbit kidney. Biochem Pharmacol 1989; 38(21): 3779-3783.
57. Hirouchi Y, Naganuma H, Kawahara Y, Okada R, Kammiya A, Inui K, Hori R. Preventive effect of ßmipron on nephrotoxicity and uptake of carbapenems in rabbit renal cortex. Jpn J Pharmacol 1994; 66(1): 1-6.
58. Cunha BA. Aztreonam. Urology 1993; 41(3): 249-258.
59. Moore RD, Lerener SA, Levine, DP. Nephrotoxicity and ototoxicity of aztreonam versus aminoglycoside therapy. J Infect Dis 1992; 165(4): 683-688.
60. Chartrand SA. Safety and toxicity profile of aztreonam. Pediatr Infect Dis J 1989; 8(Suppl 9): 120-123.
61. Ohya S, Yamazaki M, Sugawara S. Effect of 7 α substitution of cephems on their β-lactamase stability and affinity for penicillin-binding proteins in Morganella morganii. Antimicrob Agents Chemother 1983; 23(4): 522-525.
62. Patel IH, Kaplan SA. Phrmacokinetic profile of ceftriaxone in man. Am J Med 1984; 77(4C): 17-25
63. Sattler FR, Weitekamp MR, Ballard JO. Potential for bleeding with the new β-lactam antibiotics. Ann Intern Med 1986; 105(6): 924-931.
64. Goto K, Oda M, Saitoh H, Nishida M, Takada M. Effect of side chains including N-methyl-tetrazole-thiol group of β-lactam antibiotics on transport in cultured kidney epithelial cells LLC-PK1. Biol Pharm Bull 1988; 21(10): 113-116.
65. Williams PD, Laska DA, Tay LK, Hottendorf, GH. Comparative toxicities of cephalosporin antibiotics in a rabbit kidney cell line (LLC-RK1). Antimicrob Agents Chemother 1988; 32(3): 314-318.
66. Viotte G, Morin GP, Godin M, Fillastre JP. Chamges in the renal function of rats treated with cefoxitin and a comparison with other cephlosporins and gentamicin. J Antimicrob Chemother 1981; 7(5): 537-550
67. Tune BM. The renal toxicity of β-lactam antibiotics: mechanism and clinical impications. In: Clinical nephrotoxins – renal injury from drugs and chemicals.De Broe ME, Porter GA, Bennett WM, Verpooten GA (editors). Kluwer Academic Publ, Dordrecht, 1998; p. 121-134.
68. Topham JC, Murgatroyd LB, Johns DV, Goonetilleke UR, Wright J. Safety evaluation of meropenem in animals: studies on the kidney. J Antimicrob Chemother 1989; 24(Suppl A): 287-386.

69. Harrison MP, Jones DV, Pickford RJ, Wilson ID. ß-Hydroxybutyrate: a urinary marker of imipenem induced nephrotoxicity in the cynomolgus monkey detected by high field spectroscopy. Biochem Pharmacol 1991; 41(12): 2045-2049.
70. Nouda H, Matsumura H, Tanio T, Sunagawa M. Structural feature of carbapenem compounds or nephrotoxicity: effect of C-2 side chain. J Antibiotics 1996; 49(6): 603-606.
71. Kohda Y, Gemba M. Modulation by cyclic AMP and phorbol myristate acetate of cephaloridine-induced injury in rat renal cortical slices. Jpn J Pharmacol 2001; 85(1): 54-59.
72. Cojocel C, Maita K, Pasino DA, Kuo, C-H, Hook J B. Metabolic heterogeneity of proximal and distal tubules. Life Sci 1983; 33: 855-861.
73. Lock EA. Renal drug-metabolizing enzymes in experimental animals and humans. In: Mechanisms of injury in renal disease and toxicity. Goldstein R S (editor). CRC Press Inc, Boca Raton, Florida 1994; p. 173-206.
74. Cojocel C, Kramer W, Mayer D. Depletion of cytochrome P450 and alterations in activities of drug metabolizing enzymes induced by cephaloridine in rat kidney cortex. Biochemical Pharmacol 1988; 37(19): 3781-3785.
75. Kramer W, Cojocel C, Mayer D. Specific alterations of rat renal microsomal proteins induced by cephaloridine. Biochem Pharmacol 1988; 37 (21): 4135-4140.
76. Olivier MF, Dutertre-Catella H, Thevenin M, Martin C, Warnet JM, Claude JR. Increased reduced glutathione and glutathione S-transferase activity in chronic cephaloridine nephrotoxicity studies in the rat. Drug Chem Toxicol 1990; 13(1-2): 209-219.
77. Kramer W, Cojocel C, Mayer D. Effect of cephaloridine treatment on transport systems of the rat renal brush border membrane. Biochem Pharmacol (Life Sci Adv) 1990; 9: 127-133.
78. Fry M, Plummer DT. The interaction of cephaloridine with model membrane systems and rat kidney lysosomes. Chem Biol Interact 1979; 25(1): 113-124.
79. Ngaha EO. Further studies on the *in vivo* effect of cephaloridine on the stability of rat kidney lysosomes. Biochem Pharmacol 1982; 31(10): 1843-1847.
80. Yamagouchi A, Hiruma R, Sawai T. Phospholipid bilayer oermeability of β-lactam antibiotics. J Antibiot (Tokyo) 1982; 35(12): 1692-1696.
81. Kojima R, Ito M, Suzuki Y. Studies on the nephrotoxicity of aminoglycoside antibiotics and protection from these effects (4). Effects of tobramycin alone and in combination with latamoxef on the stability of rat kidney lysosomal membranes. Jpn J Pharmacol 1987; 43(1): 73-80.
82. Ullrich KJ, Rumrich G, Kloss S. Contraluminal organic anion and cation transport in the proximal renal tubule: V. Interaction with sulfamoyl- and phemoxy diuretics, and with β-lactam antibiotics. Kidney Int 1989; 36(1): 78-88.
83. Van Aubel RAMH, Masereeuw R, Russsel FGM. Molecular pharmacology of renal organic anion transporters. Am J Physiol Renal Physiol 2000; 279(2): F216-F232.
84. Williams PD, Hitchcock MJ, Hottendorf GH. Effect of cephalosporins on organic ion transport in renal membrane vesicles from the rat and rabbit kidney cortex. Res Commun Chem Pathol Pharmacol 1985; 47(3): 357-371.
85. Ullrich KJ, Rumrich G, Wieland T, Dekant W. Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. VI. Specificity: amino acids, their N-methyl-, Nacetyl- and N-benzoylderivatives; glutathione- and cysteine conjugates, di- and oligopeptides. Pflügers Arch 1989; 415(3): 342-350.
86. Atkinson RM, Curie LP, Prat PAH, Sharpe HM, Tomich EG. Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. Toxicol Appl Pharmacol 1966; 8(3): 398-406.
87. Schaub TP, Kartenbeck J, König J, Spring H, Dorsam J, Sthaeler G, Störkel S, Thon WF, Keppler D. Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubule and in renal carcinoma. J Am Soc Nephrol 1999; 10(6): 1159-1169.
88. Ganapathy ME, Huang W, Rajan DP, Carter AL, Sugawara M, Iseki K, Leibach FH, Ganapathy V. β-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. J Biol Chem 2000; 275(3): 1699-1707.
89. Terada T, Saito H, Mukai M, Inui K-I. Recognition of β-lactam antibiotics by rat peptide transportrers, PEPT1 and PEPT2 in LLC-Pk1 cells. Am J Physiol Renal Physiol 1997; 273(42): F706-F711.
90. Daniel H, Herget M. Cellular and molecular mechanisms of renal peptide transport. Am J Physiol Renal Physiol 1987; 273:F1-F8.
91. Hori R, Ishikawa Y, Takano M, Okano T, Kitazawa S, Inui K. The interaction of cephalosporin antibiotics with renal cortex of rats: accumulation to cortical slices and binding to purified plasma membranes. Biochem Pharmacol 1982; 31(13): 2267-2272.
92. McMurty RJ, Mitchell JR. Renal and hepatic necrosis after metabolic activation of 2-sustituted furans and thiophenes, including furosemide and cephaloridine. Toxicol Appl Pharmacol 1977; 42: 285-300.
93. Spurling NW, Harcourt RA, Hyde JJ. An evaluation of the safety of cefuroxime axetil during six months oral administration to beagle dogs. J. Toxicol Sci 1986; 11(4): 237-277.
94. Johns RN. A review of cephalosporin metabolism: a lesson to be learned for future chemotherapy. Diagn. Microbial Infect Dis 1989; 12(1): 25-32.

95. Marone P, Navarra A, Monzillo V, Traverso A. Antibacterial activity of combined cefotaxime and desacetyl-cefotaxime against aerobic gram-negative bacilli. Drugs Exp Clin Res 1990; 16(12): 629-633.
96. Hottendorf GH, Laska DA, Williams PD, Ford SM. Role of desacetylation in the detoxification of cephalothin in renal cells in culture. J Toxicol Environ Health 1987; 22(1): 101-111.
97. Indelicato JM, Dinner A, Peters LR, Wilham WL. Hydrolysis of 3-chloro-3-cephems. Intramolecular nucleophilic attack in cefaclor. J Med Chem 1977; 20: 961-963
98. Tune BM, Hsu C-Y. The renal mitochondrial toxicity of β-lactam antibiotics: *In vitro* effects of cephaloglycin and imipenem. J Amer Soc Nephrol 1990; 1(5): 815-821.
99. Masereeuw R, van den Bergh EJ, Bindels RJ, Russel Fg. Characterization of fluorescein transport in isolated proximal tubular cells of the rat: Evidence for mitochondrial accumulation. J Pharmacol Exp Ther 1994; 269: 1261-1267.
100. Miller DS, Stewart DE, Pitchard JB. Inracellular compartimentation of organic anions within renal cells. Am J Physiol 1993; 264: R882-R890.
101. Terlouw SA, Tanriseven O, Russel FGM, Masereeuw R. Metabolite anion carriers mediate the uptake of the anion drug fluorescein in renal cortical mitochondria. J Pharmacol Exp Ther 2000; 293(3): 968-973.
102. Schnellmann R G, Gilchrist S M, Mandel L J. Intracellular distribution and depletion of glutathione in rabbit renal proximal tubules. Kidney Int 1988; 34: 229-233.
103. Meister A, Anderson M A. Glutathione. Annu Rev Biochem 1985; 52: 711-760.
104. Reed D J. Regulation of reductive processes by glutathione. Biochem Pharcol 1986; 35(1): 7-13.
105. Moldeus P, Quanguang J. Importance of the glutathione cycle in drug metabolism. Pharmacol Ther 1987; 33: 37-40.
106. Elfarra AA, Anders MW. Renal processing of glutathione conjugates. Role in nephrotoxicity. Biochem Pharmacol 1984; 33(23): 3729-3732.
107. Beuter W, Cojocel C, Muller W, Donaubauer HH, Mayer D. Peroxidative damage and nephrotoxicity of dichlorovinylcysteine in mice. J Appl Toxicol. 1989; 9(3): 181-186.
108. Tune BM, Hsu C-Y, Fravert D. Mechanisms of bacterial endotoxin-cephaloridine toxic synergy and the protective effects of saline infusion in the rabbit kidney. J Pharmacol Exp Ther 1988; 244(2): 520-525.
109. Cojocel C, Beuter W, Müller W, Maye, D. Lipid peroxidation: a possible mechanism of trichloroethylene-induced nephrotoxicity. Toxicology 1989; 55: 131-141.
110. Mitchell JB, Biaglow JE, Russo A. Role of glutathione and other endogenous thiols in radiation protection. Pharmacol Ther 1988; 39(1-3): 269-274.
111. Bus JS, Gibson JE. Paraquat: model for oxidant-initiated toxicity. Environ Health Persp 1984; 55: 37-46.
112. Tomita M. Comparison of one-electron reduction activity against the bipyridylium herbicides, paraquat and diquat, in microsomal and mitochondrial fractions of liver, lung and kidney (*in vitro*). Biochemical Pharmacol 1991; 42(2): 303-309.
113. Gianni L, Zweier JL, Levy A, Mayers CE. Characterisation of the cycle of iron-mediated electron transfer from adriamycin to molecular oxygen. J Biol Chem 1985; 260(11): 6820- 6826.
114. Mimnaugh EG. Potentiation by reduced glutathione of adriamycin-stimulated lipid peroxidation in kidny microsomes. Biochem Pharmacol 1986; 35(23): 4337-4339.
115. Fridovich I. Biological effects of the superoxide radical. Arch Biochem Biophys 1986; 247: 1-11.
116. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys 1986; 246: 501-514.
117. Li W, Zhao Y, Chou IN. Paraquat-induced cytoskeletal injury in cultured cells. Toxicol Appl Pharmacol 1987; 91: 96-106.
118. Miller DM, Woods JS. Urinary porphyrins as biological indicators of oxidative stress in the kidney. Interaction of mercury and cephaloridine. Biochem Pharmacol 1993; 46 (12): 2235-2241.
119. Thomas CE, Aust SD. Reductive release of iron from ferritin by cation free radicals of paraquat and other bipyridils. J Biol Chem 1986; 261(28): 13064-13070.
120. Minotti G, Aust SD. The role of iron in the initiation of lipid peroxidation. Chem Phys Lipids 1987; 44(2-4): 191-208.
121. Zenser TV, Mattammal MB, Davis BB. Differential distribution of the mixed-function oxidase activities in rabbit kidney. J Pharmacol Exp Ther 1978; 207(3): 719-725.
122. Swann JD, Acosta D. Failure of gentamicin to elevate cellular malondialdehyde content or increase generation of intracellular reactive oxygen species in primary cultures of renal cortical epithelial cells. Biochem Pharmacol 1990; 40(7): 1523-1526.
123. Tune BM, Kuo CH, Hook JB, Hsu CY, Fravert D. Effects of piperonyl butoxide on cephalosporin nephrotoxicity in the rabbit. An effect on cephaloridine transport. J Pharmacol Exp Ther 1983; 224(3): 520-524.
124. Roy K, Saha A, De K, Sengupta C. Ceftriaxone induced lipid peroxidation and its inhibition with various antioxidants: Part II. Evaluation of glutathione and probucol as antioxidants. Acta Pol Pharm 2000; 57(5): 385-390.
125. Kawai Y, Gemba M. Amelioration by cAMP of cephaloridine-induced injury in the porcine kidney cell line LLC-PK1. Jpn J Pharmacol 1996; 72(1): 67-70.

126. Tune BM, Wu KY, Longerbeam DF, Kempson RL. Transport and toxicity of cephaloridine in the kidney. Effect of furosemide, p-aminohippurate and saline diuresis. J Pharmacol Exp Ther 1977; 202(2): 472-478.
127. Kasher JS, Holohan PD, Ross CR. Na+ gradient-dependent p-aminohippurate (PAH) transport in rat basolateral membrane vesicles. J Pharmacol Exp Ther 1983; 227(1): 122-129.
128. Tune BM, Fernholt M. Relationship between cephaloridine and p-aminohippurate transport in the kidney. Am J Physiol 1973; 225(5): 1114-1117.
129. Tune BM, Fernholt M, Schwartz A. Mechanism of cephaloridine transport in the kidney. J Pharmacol Exp Ther 1974; 191(2): 311-317.
130. Mizuno M, Hamaura T, Hashida M, Sezaki H. Changes in D-glucose uptake by brush-border vesicles from small intestine of rats treated with mitomycin C. Biochem Pharmacol 1986; 35(7): 1153-1158.
131. Mizuno M, Yoshino H, Hashida M, Sezaki H. Decreased transport of D-glucose and L-alanine across brush-border membrane vesicles from small intestine of rats treated with mitomycin C. Biochim Biophys Acta 1987; 902(1): 93-100.
132. Weinberg JM, Venkatachalam MA, Roeser NF, Davis JA, Varani J, Johnson KJ. Amino acid protection of cultured kidney tubule cells against calcium ionophore-induced lethal cell injury. Lab Invest 1991; 65: 671-678.
133. Inui K, Saito H, Takano M, Okano T, Kitazawa S, Hori R. Enzyme activities and sodium-dependent active D-glucose transport in apical membrane vesicles isolated from kidney epithelial cell line (LLC-PK1). Biochim Biophys Acta 1984; 769(2): 514-518.
134. Cersosimo E, Garlick P, Ferretti J. Renal substrate metabolism and gluconeogenesis during hypoglycemia in humans. Diabetes 2000; 49: 1186-1193.
135. Goldstein RS, Contardi LR, Pasino DA,Hook JB. Mechanisms mediating cephaloridine inhibition of renal gluconeogenesis. ToxicolApplPharmacol 1987; 87: 297-305.
136. Stroo WE, Hook JB. Dissociation of renal organic anion transport from renal lipid metabolism. I. Endogenous nonesterified fatty acids (NEFA) as determinants of transport. J Pharmacol Exp Ther 1983; 227(1): 55-59.
137. Wagner S, Deufel T, Guder WG. Carnitine metabolism in isolated rat kidney cortex tubules. Biol Chem Hoppe Seyler 1986; 367(1): 75-79.
138. Arrigoni-Martelli E, Caso V. Carnitine protects mitochondria and removes toxic acyls from xenobiotics. Drugs Exp Clin Res 2001; 27(10): 27-49.
139. Bieber LL, Farel SS. Carnitine acyl transferases. Enzymes 1983; 16: 624-644.
140. Ramsay RR, Gandour RD, van der Leij FR. Molecular enzymology of carnitine transfer and transport. Biochem Biophys Acta 2001; 1546(1): 21-43.
141. Cojocel C, Smith JH, Maita K, Sleight SD, Hook JB. Renal protein degradation: a biochemical target of specific nephrotoxicants. Fund Appl Toxicol 1983; 3: 278-284.
142. Hori R, Shimakura M, Aramata Y, Kizawa K, Nozawa I, Takahata M, Minami S. Nephrotoxicity of piperacillin combined with furosemide in rats. Jpn J Antibiot 2000; 53(8): 582-591.
143. Wade JC, Smith CR, Petty BG, Lipski JJ, Conrad G, Ellner J, Lietman PS. Nephrotoxicity of piperacillin combined with furosemide in rats. Jpn J Antibiot 2000; 53(8): 582-591.
144. Bendirdjian JP, Prime DJ, Browning MC, Hsu CY, Tune BM. Additive nephrotoxicity of cephalosporins and aminoglycosides in the rabbit. J Pharmacol Exp Ther 1981; 218(3): 681-685.
145. Kosmidis J, Daikos GK. Prospective randomized comparative studies of mezlocillin/cefotaxime vs. gentamicin/cefoxitin. J Antimicrob Chemother 1983; 11(Suppl C): 91-95.
146. Itoh F, Sato K, Harauchi T, Hirata M, Mizushima Y. Modification of vancomycin nephrotoxicity by other antibiotics in rats. Jpn J Antibiot 1995; 48(3): 380-388.
147. Toyoguchi T, Nakagawa Y. Nephrotoxicity and drug interaction of vancomycin (2). Nippon Yakurigaku Zasshi 1996; 107(5): 225-235.
148. Reitbroeck RC, Hoitsma AJ, Koene RA. Aztreonam can be safely used with cyclosporin without aggravating nephrotoxicity. Transpl Int 1989; 2(4): 232-234.
149. Fanos V, Musap M, Verlato G, Plebani M, Padovani EM. Evaluation of the antibiotic-induced nephrotoxicity in preterm neonates by determining urinary a1-microglobulin. Pediatr Nephrol 1996; 10: 645-647.
150. Greenlee ML, DiNinno F, Herrmann JJ, Jaworsky C, Muthard DA, Salzmann TN. 2-Naphthylcarbapenems: broad spectrum antibiotics with enhanced potency against MRSA. Bioorg Med Chem Lett 1999; 9(19): 2893-2896.
151. Kanno O, Shimoji Y, Ohya S, Kawamoto I. Synthesis and biological evaluation of novel tricyclic carbapenems (trinems). J Antib (Tokyo) 2000; 53(4): 404-414.
152. Kar GK, Roy BC, Adhikari SD, Ray JK, Brahama NK. Synthesis of some thieno γ lactam monocyclic acids with high antibacterial activity: a new look at an old molecular system. Bioorg Med Chem 1998; 6(12): 2397-2402.
153. Hayashi T, Watanabe Y, Kumano K, Kitayama R, Yasuda T, Saikawa I, Katahira J, Kumada T, Shimizu K. Protective effect of piperacillin against nephrotoxicity of cephaloridine and gentamicin in animals. Antimicrob Agents Chemother 1988; 32(6): 912-918.

154. Tune BM, Browning MC, Hsu CY, Fravert D. Prevention of cephalosporin nephrotoxicity by other cephalosporins and by penicillins without significant inhibition of renal cortical uptake. J Infect Dis 1982; 145(2): 174-180.
155. Beauchamp D, Theriault G, Grenier L, Gourde P, Perron S, Bergeron Y, Fontaine L, Bergeron MG. Ceftriaxone protects against tobramycin nephrotoxicity. Antimicrob Agents Chemother 1994; 38(4): 750-756.
156. Kojima R, Ito M, Suzuki Y. Studies on the nephrotoxicity of aminoglycoside antibiotics and protection from these effects (3). Protective effect of latamoxef against tobramycin nephrotoxicity and its protective mechanism. Jpn J Pharmacol 1986; 42(3): 397-404.
157. Sausen PJ, Elfarra AA, Cooley AJ. Methimazole protection of rats against chemically induced kidney damage *in vivo*. J Pharmacol Exp Ther 1992; 260(1): 393-401.
158. Valentovic M, Ball JG, Rogers BA, Meadows MK, Harmon RC, Moles J. Cephaloridine *in vitro* toxicity and accumulation in renal slices from normoglycemic and diabetic rats. Fundam Appl Toxicol 1997; 38(2): 184-190.

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Amphotericin B

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Introduction

In recent years, systemic mycoses have become a prominent cause of disease particularly in severely ill and immunocompromised patients. The factors contributing to the increased prevalence of fungal infections are related to larger number of patients with underlying immunosuppression, for example the acquired immunodeficiency syndrome (AIDS), more aggressive cancer chemotherapy, increase in transplantation, greater number of other immunocompromised patients, and more frequent use of prosthetic devices [1]. There have been a number of recent surveys, which illustrate the extent of this problem. The Center for Disease Control reported that among 51 USA hospitals, candidiasis was the eighth most common infection, accounting for 5% of the isolates [2]. This value can be considerably higher in certain specific patient groups. The National Cancer Institute estimated that 43% of patients dying with acute leukemia had systemic fungal infection at autopsy [3]. In patients with AIDS, the most common fungal infection is oropharyngeal candidiasis. However, in these patients, the fungal infection with the highest mortality rate is cryptococcosis. It is evident that systemic fungal infection is an important consideration in the treatment of a severely ill, immunosuppressed patients [4].

Amphotericin B (AmB) has remained the mainstay of therapy for serious fungal infections since its introduction in 1956, despite the availability of newer agents [5]. The usefulness of this agent, however, is limited by the frequent occurrence of several acute and chronic adverse effects that often necessitate changes in, or premature discontinuation of, therapy. These include fever, chills, nausea, vomiting, anorexia, headache, bronchospasm, hypotension, anaphylaxis, and bone marrow suppression. The most limiting adverse effect, however, is nephrotoxicity.

AmB is a member of the polyene macrolide class of antibiotics. The molecule consists of a large macrolide lactone ring of 37 carbon atoms, one side of which is composed of a rigid lipophilic chain of seven conjugated double bonds, and the opposite side of a similar number of hydrophilic hydroxylated carbon atoms (Figure 1). Thus, the molecule is amphipathic, and this feature of its structure is believed to be important in its mechanism of action [6]. The major action of AmB is believed to be on the cell membrane of fungal and mammalian cells. It is generally accepted that the drug binds to sterols in the cell membrane and induces formation of aqueous pores, which result in impairment of barrier function and loss of protons and cations from the cell. At low concentrations, the increased permeability is restricted to small molecules or cations such as sodium and potassium. At higher concentrations or after prolonged exposure, other cell constituents are lost and this leads to metabolic disruption and even cell death [6].

The cellular events that follow this membrane effect are complex and depend on a variety of factors, such as the growth phase of the cells, the dose, and the mode of AmB administration [7]. Some studies suggest that cell mortality is not simply a consequence of changes in permeability of membranes, and that formation of active oxygen species may play a role in the lytic or lethal actions of AmB [8, 9].

Clinical manifestations of nephrotoxicity

The most restrictive adverse effect associated with AmB therapy is its potential to induce nephrotoxicity, manifested as disturbances in both glomerular and tubular function. The clinical manifestations usually include azotemia, renal tubular acidosis, decreased concentrating ability of the kidney, and electrolyte disturbances such as urinary potassium wasting leading to hypokalemia, and magnesium wasting to result in hypomagnesemia [10].

Azotemia

The incidence of AmB-induced renal impairment is highly variable depending on the definition of nephrotoxicity and upon the presence of underlying risk factors. Following AmB introduction, a survey of 56

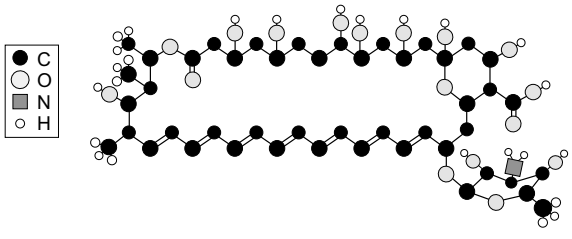

***Figure 1**. Chemical structure of amphotericin B.*

patients treated between 1956 and 1963 confirmed that 93% of patients developed values of BUN exceeding 200 mg/L, and 83% had serum creatinine levels greater than 15 mg/L [11]. A more recent report indicates that in almost every patient treated with AmB, the glomerular filtration rate (GFR) falls approximately 40% within the first 2 to 3 weeks of therapy, stabilizes at 20% to 60% of normal and remains at this level throughout the course of treatment [12]. Clements and Peacock [13] reported an incidence for azotemia of 60% in a retrospective analysis conducted between 1984 and 1987. In general, the incidence of azotemia due to AmB in the literature ranges between 50-90%. This variability may reflect various factors, among which are the definition used for nephrotoxicity, the dose of AmB, concomitant administration of other nephrotoxic agents, and the presence or absence of other proposed risk factors. In general, azotemia is transient and limited to the duration of therapy; renal function usually returns to pretreatment levels after discontinuation of the drug. In many cases, cessation of therapy for a few days allows renal function to recover enough to permit administration of the full course of therapy. In rare cases, however, permanent renal damage persists after cessation of therapy.

The relationship of the cumulative dose of AmB to the development of nephrotoxicity is controversial. Earlier studies suggested that greater cumulative doses of AmB (e.g. 3-4 g) were associated with a greater risk of nephrotoxicity [14]. This implies that the likelihood of a rise in the serum creatinine concentration increases in proportion to the length of therapy. However, we observed patients who developed azotemia at doses ranging from 100 mg to 1.5 g, with no significant increase in frequency as the cumulative dose increased. Our experience indicated that the frequency of nephrotoxicity did not increase with extended therapy over this dose range (Figure 2) [15]. With larger cumulative doses, renal impairment may be irreversible, as reported by Winn [16] who found persistent renal impairment in 88% of patients who had received cumulative doses exceeding 5 g.

In addition to cumulative dose, additional risk factors have been identified. The rate of infusion of AmB impacts toxicity. In a recent study involving patients with suspected or proven invasive fungal infection, AmB was less toxic when administered as a continuous infusion over 24 hours compared to the conventionally 4 hour infusion [17]. The frequency of dosing is an additional consideration, as administration of drug on alternate days reduces the incidence of nephrotoxicity [18, 19]. Additional risk factors include older age [14, 20] and diuretic use. A case-control study revealed that higher daily doses, concomitant diuretic use, and abnormal baseline renal function were each significant, independent, risk factors for AmB nephrotoxicity [21].

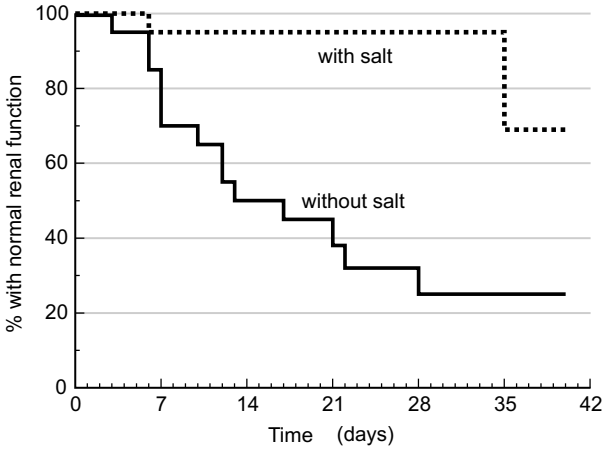

***Figure 2**. Estimated proportion of patients retaining normal renal function during therapy with amphotericin B. Patients received amphotericin B with (dotted line, n=17) or without (solid line, n=21) parenteral salt supplementation due to co-administration of ticarcillin. (Used with permission from [15])*

In a recent multivariate risk factor assessment, Luber et al. [22] demonstrated that the AmB nephrotoxicity incidence varied with the definition used for renal impairment. Among 178 patients a change in creatinine of >46 μmol/L over baseline occurred in 50%; a doubling of creatinine over baseline in 49%; a change in creatinine of >92 μmol/L in 29%; a doubling and/or a change in creatinine of >92 μmol/L in 49%; and an increase in creatinine to >230 μmol/L in 8%. In this study, nephrotoxicity was associated with a greater cumulative dose of AmB and concomitant nephrotoxic drugs for all definitions. In those patients who experienced severe nephrotoxicity (creatinine increased to >230 μmol/L), concomitant cyclosporine therapy was the most significant risk factor (odds ratio 18.8, P= 0.022).

Urinary concentration defects

Many studies have shown that AmB can induce a loss of concentrating ability of the kidney [11, 23, 24]. This abnormality is almost invariably present and occurs early (1-2 weeks) in the course of therapy. The impairment in concentrating ability probably reflects direct tubular toxicity since it occurs in the absence of a decrease in GFR, and is temporally unrelated to azotemia. Barbour et al. [25] reported a study of 3 patients whose inability to concentrate the urine was associated with a defect in free water reabsorption even under maximal stimuli, and concluded that a tubular functional abnormality was induced because of the failure of the vasopressin response in the medullary collecting tubule.

Electrolyte disturbances

Electrolyte disorders secondary to renal wasting of potassium and magnesium are commonly encountered adverse effects in patients receiving AmB [10, 21]. Although hypokalemia has been emphasized in prior studies, its impact on patient management and on the course of other manifestation of AmB nephrotoxicity has not been well examined. In addition to its known systemic effects (muscle weakness, fatigue, cramps, rhabdomyolysis and myoglobinuria), potassium depletion may alter renal function causing further impairment of concentrating ability, urinary acidification, renal insufficiency and abnormal sodium reabsorption [26]. It is conceivable that these effects may influence or contribute to the nephrotoxicity of AmB.

Approximately 75% of patients develop hypokalemia during the course of treatment with AmB [27]. However, if potassium supplementation, sufficient to maintain a normal plasma level of potassium, is regarded as an objective parameter of a potassium losing diathesis, the incidence is as high as 90% or more [13, 28]. The maintenance of normokalemia require up to 300 mEq of potassium chloride replacement a day. These patients are often severely ill and unable to tolerate oral supplementation, so prolonged (6-7 hours) of administration of large intravenous doses of potassium chloride with appropriate and careful monitoring may be necessary. The logistics of such continuous intravenous maintenance infusions can create problems in timely administration [13].

Some investigators consider hypokalemia a dose-dependent response, the mechanisms of urinary potassium wasting is unclear. A recent study has shown that AmB affects sodium flux in both the distal and transverse human colon, suggesting a change in sodium/potassium exchange to result in potassium loss [29]. Selective distal tubular epithelial toxicity seems to be, at least in part, responsible for the profound potassium wasting. The magnitude of urinary potassium loss increases in the presence of a high sodium chloride intake. Thus the potassium depletion and hypokalemia associated with AmB treatment can enhance the tubular toxicity due to AmB [30] and contribute to overall changes in renal function.

Magnesium wasting has also been reported as a consequence of AmB administration [31, 32]. A negative magnesium balance probably occurs in all patients, but clinically relevant magnesium depletion only occurs when the urinary loss is high and not replaced. In the study by Barton et al [31] the lowest serum level and the largest fractional excretion of magnesium were observed by the fourth week of AmB therapy, after a cumulative dose of approximately 500 mg. This abnormality was fully reversible, evidenced by the normal serum and urinary magnesium levels measured 1 year after discontinuation of therapy. As in the case of potassium depletion, if magnesium depletion is evaluated by measurement of magnesium balance rather than by the serum level, the incidence of magnesium depletion is high. In a recent study, a marked change in the urinary excretion of magnesium occurred after a cumulative AmB dose of only 150 mg, suggesting some degree of magnesium depletion, although serum magnesium levels remained in the low normal range [28].

The mechanisms for the observed AmB induced renal magnesium wasting remain unclear. Increased urinary excretion of magnesium, despite a reduced filtered load, suggests a tubular defect in magnesium reabsorption [31]. When magnesium and potassium wasting occur concomitantly, potassium replacement may not be successful unless magnesium deficiency is corrected first.

Renal tubular acidosis

Chronic features of renal tubular acidosis can be anticipated in patients receiving total doses of AmB of

0.5-1 g or more, and are generally reversible after therapy is discontinued [5]. In our experience this is one of the earlier manifestations of tubular toxicity, since all patients developed an acidification defect in response to an acid load after 2 weeks of therapy and a cumulative dose of 300 mg of AmB [28]. This defect appears to be a specific tubular effect of AmB, since impaired in acid secretion has been demonstrated in the isolated turtle bladder and attributed to increased passive permeability of the luminal membrane to hydrogen ions [33, 34], plus the impaired excretion of titratable acids is greater than can be accounted for by depression of GFR [35, 36]. It is also thought that distal renal tubular acidosis is a contributing pathogenic mechanism for urinary losses of potassium and magnesium [27, 35, 36].

Pathological findings

Despite the almost universal changes in renal function, histological changes associated with AmB therapy are minimal and occur in both glomerulus and renal tubule. Tubular damage primarily involves the distal convoluted tubule and the ascending limb of the loop of Henle [31]. Morphologic changes include fragmentation and thickening of basement membranes, necrosis and vacuolization of distal tubular epithelium and nephrocalcinosis [11, 23, 36, 37]. Glomerular changes include calcific foci, along with hypercellularity and vacuolization of smooth-muscle cells in small arteries and arterioles [24, 36]. In studies conducted in rats, cortical changes associated with AmB were restricted to the medullary ray, an area that is vulnerable to hypoxia, and consisted of focal rupture and calcification of the thick ascending limbs [38]. Calcification was also detected in the macula densa, an area rich in oxygen. Administration of AmB to salt depleted rats resulted in an extension of these changes to the area rich in vascular tissue between the medullary rays and to atrophic changes in the thick ascending limb in the inner stripe [38a] (Figure 3).

Mechanisms of nephrotoxicity

Before mechanisms can be proposed to account for renal cell injury, the possible sites of nephron involvement should be identified based upon structural and functional changes [39]. AmB is known to cause acute renal vasoconstriction and to preferentially damage the distal tubular epithelium, but the exact mechanisms mediating its nephrotoxicity have not been clearly defined. The initial event is thought to involve binding of AmB to membrane sterols in the renal vasculature and epithelial cells causing alter membrane permeability. This interaction may then trigger other cellular events that result in activation of second messenger systems, release of mediators or activation of renal homeostatic mechanisms. It is, therefore, possible that the membrane effect per se is not the sole factor that determines the extent of change in renal function.

Effects on cell membranes

It is generally accepted that AmB-induced injury to cells is due to its binding to sterols in the cell membrane: ergosterol in the case of fungal cells and cholesterol in mammalian cells [7]. This binding is more avid

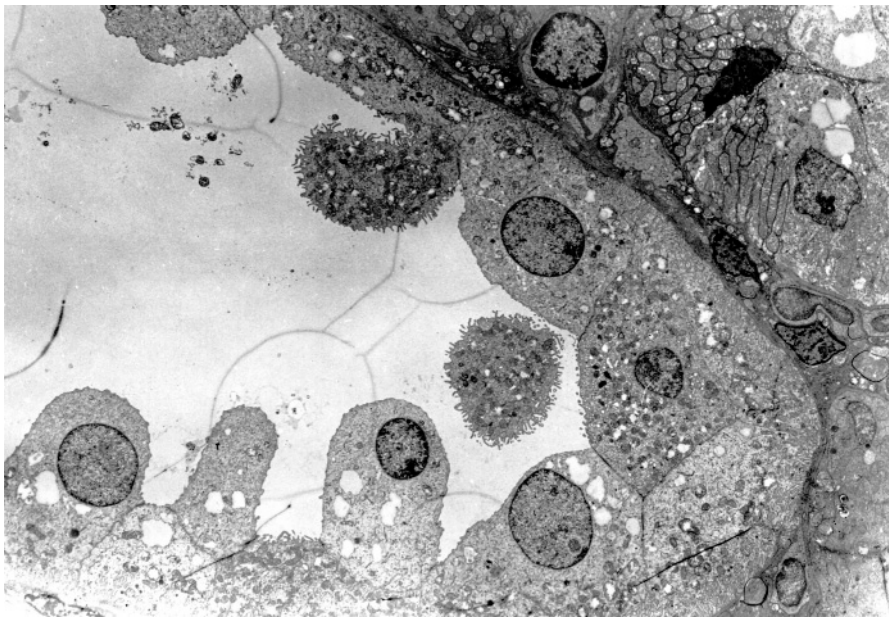

Figure 3. *Distal tubulus of rat kidney after 14 days of AmB administration. Electron microscopy shows intratubular casts and debris, loss of brush border, tubular cell vacuolization, and protrusion of cells into the tubulus lumen. Magnification 3600x. With permission from [38a].*

to ergosterol than to cholesterol, which explains AmB's relatively selective toxicity to fungal cells [40, 41].

In the early 1960s, studies showed that polyene antibiotics induced changes in cellular permeability that resulted in the leakage of important cellular constituents, followed by lysis and death [42-46]. It was also discovered that the toxic effect of the drugs on cells was dependent on the presence of sterols in the cell membranes, and that addition of sterols to the growth media of certain fungi prevented the polyene-induced inhibition of growth and permeability changes [42, 47, 48]. This increased permeability has been documented in both artificial and natural membranes [49]. It has been proposed that AmB, acting as a pseudophospholipid, interacts with sterol molecules to cause formation of aqueous pores, which consist of an annulus of polyene and sterol, in which the hydrophilic region of the drug molecule faces the interior of the pore (Figure 4) [49-51]. Among the documented effects of AmB on living tissues are increased permeability of the toad urinary bladder to urea, potassium and chloride ions [52-54], of erythrocytes and liposomes to potassium ions [55, 56], and of erythrocytes to sodium and chloride ions [57, 58]. It also alters the permeability of the turtle bladder and of purified renal brush border membrane vesicles to sodium and hydrogen ions [33, 59-61].

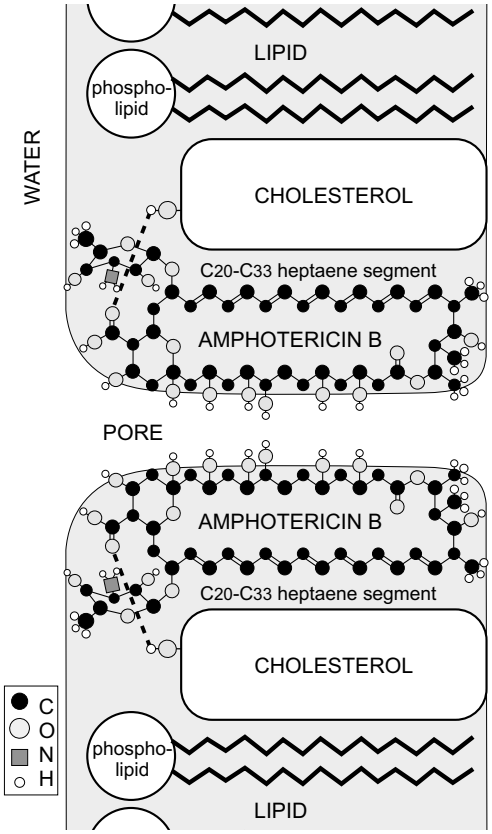

***Figure 4**. Proposed partial model for the AmB-induced pore in the cell membrane. The drug acts as a counterfeit phospholipid, with the C_{15} hydroxyl, C_{16} carboxyl, and C_{19} mycosamine groups situated at the membrane-water interface, and the C_1 to C_{14} and C_{20} to C_{33} chains aligned in parallel within the membrane. The heptaene chain will seek a hydrophobic environment while the hydroxyl groups will seek a hydrophilic environment. Thus, a cylindrical pore will be formed, the inner wall of which consists of the hydroxyl-substituted carbon chains of the AmB molecules, and outer wall of which is formed by the heptaene chains of the molecules and by sterol nuclei. (Used with permission from [49]).*

Considering the renal tubular effects of AmB observed in clinical practice, it is reasonable to suggest that part or all of these effects may be explained by a direct effect on tubular cell membranes. In support of this suggestion is the *in vivo* finding that while AmB binds to sterols in most tissues, the highest level documented is in the kidney [62]. Furthermore, binding of AmB to the cell membrane appears to be necessary for its toxic effect, since inhibition of sterol synthesis by ketoconazole reduces the binding of AmB as well as the permeability changes induced by AmB in a parallel fashion [60, 63]. In agreement with these suggestions is the finding of increased tubular permeability to inulin *in vivo* in rats following acute or chronic administration of AmB, resulting in back-leak of inulin [64].

Further evidence to support a direct toxic effect of AmB on renal cells is the demonstration of increased apoptosis in proximal tubular and medullary interstitial cell lines [65]. The occurrence of apoptosis has also been confirmed in an *in-vivo* model in rats in which AmB administration also caused hypokalemia, loss of concentrating ability and dehydration. Interestingly, prevention of apoptosis by recombinant human insulin growth factor-1 (rhIGF-1) ameliorated the tubular toxicity indicating the importance of apoptosis in AmB-induced renal tubular toxicity process. A possible mechanism for this action is suggested by a recent study that has demonstrated that AmB exposure increases cellular ceramide as well as sphingomyelin levels in proximal tubular cells [66]. It is noteworthy that

Table 1. Effect of amphotericin B infusions (0.05 mg/kg/min i.a.) on systemic glomerular hemodynamic parameters.

	Before	After	p-value
Mean arterial pressure (mmHg)	114 ± 5	117 ± 4	NS
Renal plasma flow (ml/min)	4.69 ± 0.35	2.82 ± 049	< 0.025
Glomerular filtration rate (ml/min)	1.03 ± 0.65	0.70 ± 0.09	< 0.005
Single nephron glomerular filtration rate (ml/min)	35.5 ± 2.2	22.8 ± 2.8	< 0.0005
Plasma flow (ml/min)	142 ± 12	89 ± 14	< 0.005
Single nephron filtration fraction	0.26 ± 0.03	0.27 ± 0.04	NS
Afferent arteriolar resistance (10^{10} dyn.sec.cm^{-5})	1.91 ± 0.17	3.95 ± 0.38	< 0.01
Efferent arteriolar resistance (10^{10} dyn.sec.cm^{-5})	1.30 ± 0.10	2.08 ± 0.12	< 0.01
Glomerular capillary ultrafiltration coefficient [n/(sec.mmHg)]	0.043 ± 0.008	0.032 ± 0.009	< 0.005

ceramide has been postulated to play a role in inducing apoptosis in several cell types [67]. Although the role of these changes in nephrotoxicity is still uncertain, these findings suggest that the interaction of AmB with cell membranes is not limited to a physicochemical interaction with sterols leading to pore formation and changes in permeability, but may also involve other complex effects that lead to alteration in production or function of membrane associated signaling molecules.

An alternative postulated mechanism of AmB induced cell damage involves oxidative stress with the formation of free radical intermediates [68, 69, 70]. Evidence against this hypothesis has been provided by recent studies that evaluated the anti- or pro-oxidant effects of AmB by examining its effects on phospholipid pattern in aortic smooth muscle cells [71] as well as on lipid-peroxidation of cis-Parinaric acid in liposomes [72]. These studies provided evidence for an antioxidant role for AmB rather than a pro-oxidant role and suggesting that oxidative stress is not involved in AmB-induced toxicity.

In addition, alternative factors may modulate the direct cellular toxicity of AmB. For example, maintaining kidney epithelial cells in an acidic environment enhances the permeability changes induced by AmB in an irreversible fashion [73]. This suggests that the low pH characteristic of the distal tubule makes those cells more vulnerable to the toxic effects of AmB, and may explain the protective effect of alkalinizing agents [35].

Effects on physiological parameters: Whole animal studies

Acute studies (Single dose)

Infusions of AmB, intravenously or into the renal artery, induce short-term reduction in renal blood flow (RBF) and GFR, and an increase in renal vascular resistance, in both rats and dogs [74-76]. The effects of short term infusions of AmB on the renal microcirculation in rats revealed that the single nephron GFR was decreased by 2 mechanisms (Table 1): 1) a decrease in single nephron plasma flow, due to vasoconstriction of the afferent and efferent arterioles, and 2) a reduction in the glomerular capillary ultrafiltration coefficient (Kf), an effect probably mediated by mesangial cell contraction [77]. Previous micropuncture studies demonstrated a similar vasoconstriction of the afferent arteriole but also an increased permeability of the tubular epithelium to inulin [64]. Thus, the reduction in GFR after acute AmB infusions can be attributed to contraction of afferent smooth muscle cells, efferent smooth muscle cells and glomerular mesangial cells, as well as increased tubular permeability with backleak into the interstitial space.

The mechanisms responsible for the contractile responses to AmB have not been identified. Theoretically, the drug can act either directly on the vascular smooth muscle or through release of secondary mediators. A large number of studies have examined putative indirect mechanisms of action. Those studies have revealed that neither renal denervation nor angiotensin II receptor blockade prevent the renal vasoconstriction or

the reduction in GFR [78, 79]. Although Cutaia et al [80] demonstrated a toxic effect of AmB on endothelial cells, endothelin does not appear to be involved in the acute responses to AmB [79, 81] and reduced nitric oxide synthesis, consequent to endothelial injury is not involved in modulation of AmB-induced renal vasoconstriction [79].

It has also been suggested that activation of tubuloglomerular feedback (TGF) may play a role in the acute renal effects of this compound. That hypothesis suggested that the tubular toxicity of AmB resulted in impaired reabsorption of sodium and chloride ions by the proximal tubule, which increased distal tubular delivery of these ions, thus activating TGF [82]. Indirect evidence in support of a role for TGF was derived from studies which demonstrated inhibition of the acute renal effects of AmB by physiological and pharmacological interventions that also blocked TGF, namely, salt loading, and administration of furosemide, theophylline or calcium channel blockers. [75-78, 83-90]. Finally, some studies suggested a protective effect of pentoxiphylline, a vascular decongestant and antagonist of tumor necrosis factor-alpha (TNF) and interleukin-1alpha, against AmB-induced acute and chronic nephrotoxicity, suggesting a role for these factors in the renal effects of the drug [91, 92].

More recent studies provided evidence against a role for TGF in acute AmB nephrotoxicity. In contrast to its inhibition of TGF activity, theophylline prevented the acute renal responses to AmB by a mechanism unrelated to adenosine receptor antagonism [93]. Furthermore, micropuncture studies revealed that the AmB-induced reduction in single nephron GFR was the same irrespective of whether TGF was active or interrupted (by measuring GFR from distal and proximal tubular collections, respectively) [94]. The latter study also showed that distal tubular chloride ion concentrations were not increased by AmB, which indicated that the signal for TGF was unchanged.

A direct effect of AmB on cell function was suggested by *in vitro* experiments, which demonstrated a vasoconstrictor action of AmB in perfused afferent arterioles and isometrically contracting rings of rabbit aorta or renal artery, effects which were prevented in Ca^{++}-free medium and by Ca^{++} channel blockers or theophylline [94]. Thus, AmB-induced reductions in RBF or GFR are not secondary to activation of TGF, but due to its direct vasoconstrictor effect. A role for thromboxane A_2 has also been suggested based upon partial inhibition of the AmB-induced vasoconstriction and reduction in GFR by pretreatment with ibuprofen or a thromboxane receptor antagonist [95].

The results obtained in isolated vessels are consistent with findings in cultured glomerular mesangial cells where AmB caused a concentration-dependent increase in intracellular calcium levels ($[Ca^{++}]i$), an effect almost completely inhibited when either Ca^{++} or Na^{+} ions were omitted from the cell medium (Figure 5) [96]. Diltiazem (20 μM) also suppressed the AmB-

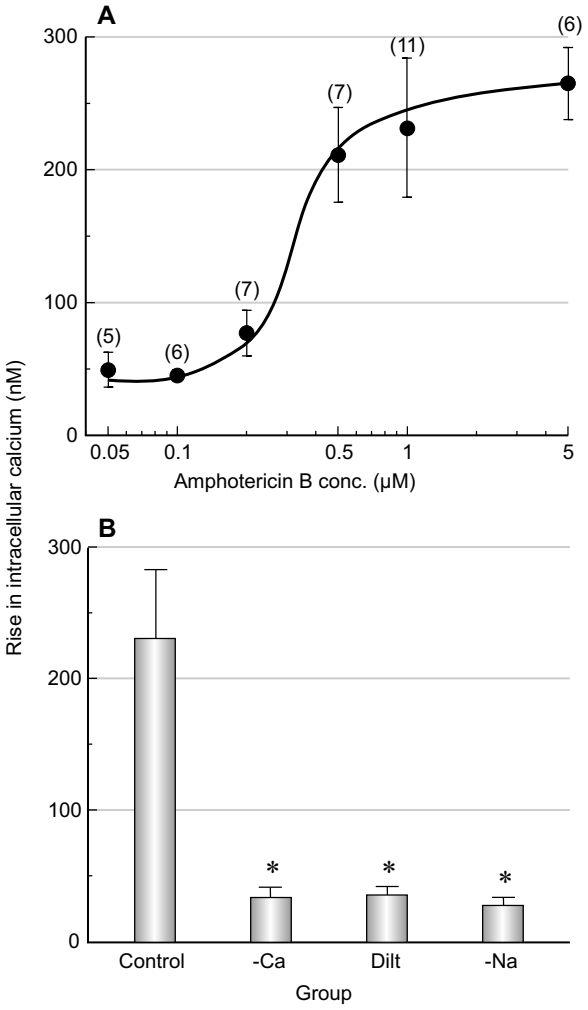

***Figure 5**. Concentration dependent increase in intracellular calcium levels in cultured glomerular mesangial cells (A) and its inhibition by removal of Ca^{++} and Na^{+} ions from the medium (-Ca and -Na, respectively), and by addition of 20 μM diltiazem (Dilt) (B). (Used with permission from [96]).*

induced rise in $[Ca^{2+}]i$. These results indicated that the reduction in K_f observed *in vivo* was most likely due to contraction of mesangial cells. Thus, the contractile effects of AmB in the nephron is probably due to a direct interaction with cell membranes, leading to formation of pores. One possibility is that these pores allow entry of Na^+ ions into the cells along the electrochemical gradient leading to depolarization-induced opening of voltage-dependent calcium channels and contraction.

Chronic studies (Multidose)

Animal models of chronic nephrotoxicity have also shown that certain interventions can modify the nephrotoxicity of AmB. Rats co-treated with sodium bicarbonate sustain smaller reductions in GFR compared with rats treated with AmB alone for 3 weeks [35]. Oral NaCl supplementation also attenuates the decrease in GFR and the elevation in renovascular resistance induced by daily administration of AmB over 3 weeks [87, 89]. In addition, renal impairment following a 7-day course of AmB in rats was less severe when theophylline was co-administered [90]. These interventions also attenuate the acute renal responses to AmB, suggesting that similar mechanisms contribute to its chronic nephrotoxicity. Similar logic would suggest that salt supplementation and theophylline are protecting the kidney by a mechanism unrelated to TGF. It is, however, possible that the latter does contribute to AmB nephrotoxicity but only at later stages of therapy, when severe damage to the tubules may have taken place. Interestingly, the protection by salt loading is associated with lower concentrations of drug in the kidney despite similar concentrations in plasma and liver tissue [87]. This raises an alternative possible mechanism of protection by salt loading involving a pharmacokinetic interaction with AmB, which limits its uptake into the kidney.

Studies on the effects of chronic administration of calcium channel blockers on chronic AmB induced nephrotoxicity have been discordant. Nifedipine does not offer a significant protective effect [97], but diltiazem blunts the increase in serum creatinine and the decreases in GFR and RPF [88]. It is possible that these differences relate to the heterogeneity of calcium channels and the differential activity of calcium channel blockers on them. However, no specific studies have addressed this question.

The co-administration of 5-flucytosine with AmB, which is commonly used clinically to obtain a synergistic antifungal effect, protects against acute and chronic nephrotoxicity [98]. The mechanisms by which flucytosine influences the renal response to AmB are not clear but may relate to (i) its administration in 0.9% NaCl, which itself is protective, (ii) a renal vasodilator effect of flucytosine that antagonizes AmB-induced vasoconstriction, and (iii) reduction in renal uptake of AmB [98].

Cell death induced by AmB in the medullary thick ascending limb is prevented by ouabain [99]. A reasonable explanation for this observation is that ouabain, by inhibiting transport, decreases the oxygen demand of an area of the nephron that already has a limited oxygen supply. This is consistent with the observation that AmB exhibits preferential damage to the medullary ray, an area that is vulnerable to hypoxic injury [38]. It is also conceivable that AmB-induced renal vasoconstriction and ischemia to this section of the nephron enhances cell death produced by a direct toxic action. Thus, any maneuver that improves renal perfusion, or decreases oxygen demand, would be expected to be protective. This may explain the salutary effect of salt loading, theophylline, calcium channel blockers, pentoxiphylline, dopamine or dopamine prodrugs such as fenoldopam on AmB nephrotoxicity [100, 101, 102). All these interventions can be expected to improve renal perfusion. Furosemide protection could be explained on a basis that it not only inhibits solute transport in the thick ascending limb to reduce oxygen demand, but also enhances renal perfusion to increase oxygen supply.

Measures to reduce nephrotoxicity

Despite being considered one of the most toxic antimicrobial drugs in use today [103], AmB remains the drug of choice for otherwise uniformly fatal systemic fungal infections [4, 5]. Consequently, it will remain in use despite the predictable occurrence of severe systemic and renal toxicity. Therefore, therapeutic interventions that decrease AmB toxicity assume critical importance. Among the early interventions that were examined was the administration of mannitol. Studies suggested a protective effect of mannitol in dogs and renal transplant recipients [104-106]. Unfortunately, these were either case-reports or poorly controlled since later reports failed to detect any protective effect of

mannitol in dogs, and ascribed the protective findings to the lower doses of AmB used [107]. In addition, a small controlled trial of mannitol co-administration in humans failed to document any beneficial effect [24].

Theoretically, any of the protective interventions mentioned in the previous section may be applicable to a clinical setting, but few have actually been studied. In some instances because there are practical limitations to their use. For example, the duration of protection conferred by furosemide is brief, being confined to the time furosemide is present in the renal tubule. Furosemide would exacerbate electrolyte imbalance by causing sodium and potassium depletion, which, if not adequately monitored and replaced, would be expected to potentiate AmB-induced nephrotoxicity. Furthermore, none of the advocated drug interventions are innocuous. Of all the alternatives, manipulation of sodium status offers a simple intervention that can be readily and usually safely be introduced into clinical practice [82]. An alternative approach is to use a liposomal formulation of amphotericin B.

Salt supplementation

The demonstration of a renal protective effect of salt loading on AmB-induced nephrotoxicity in animal models has provided a rational basis to evaluate this simple intervention in patients. Clinical evidence supporting the ability of sodium loading to attenuate AmB-induced nephrotoxicity is derived from three sources: case reports, retrospective studies and prospective studies.

One of the earliest case reports was by Butler and colleagues who reported a patient in whom a low sodium diet (9 mEq/d) exacerbated renal dysfunction, increased urinary sodium loss, and caused postural hypotension [11]. Administration of supplemental oral sodium chloride promptly reversed the defect within 12 hours. These abnormalities were confirmed on rechallenge during treatment, but were absent 13 months after completion of AmB therapy.

In a subsequent study, 5 patients were receiving AmB in clinical situations where salt-conserving states could be identified. These included dietary salt restriction, vomiting, diuretic therapy, Addison's disease and cirrhosis with ascites. In each patient sustained increases in BUN and serum creatinine levels were observed within 6 to 12 days after starting AmB [108]. Four to 12 days after liberalization of dietary sodium intake, administering intravenous saline, and/or discontinuation of diuretic therapy, renal function improved in all patients. Improvement was sustained and the full course of AmB was successfully completed after a brief interruption (range: 1 to 5 days), without permanent renal impairment.

A retrospective study revealed that only two of 17 patients (12%) receiving ticarcillin (with its obligatory sodium load of 150 mEq/day) had a nephrotoxic response to AmB, compared with 14 of 21 patients (67%) not receiving ticarcillin (Figure 2) [15]. Anecdotally, withdrawal of ticarcillin in patients continuing to receive AmB led to deterioration of renal function over a one-week period. In a companion study, the benefit of routine intravenous saline (1 L of 0.9% saline) was as-

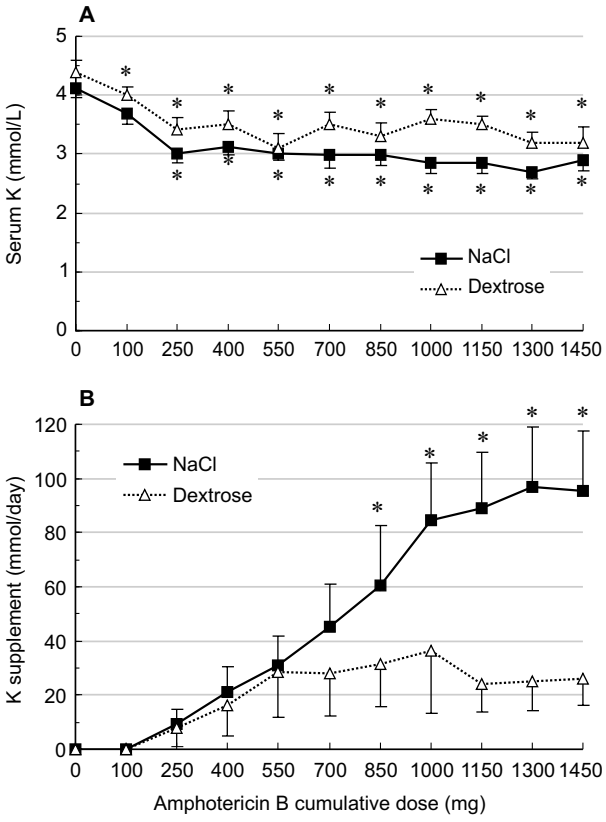

***Figure 6**. Serum K levels (A) and K supplements given to maintain serum K levels at or above 3 mmol/l (B) in patients receiving AmB with either 1 liter of 0.9% NaCl (solid line) or 1 liter of 5% dextrose in water (dotted line). Notice the difficulty in maintaining serum K levels despite significantly higher amounts of supplements in the former group. *: P<0.05 compared with baseline. (Used with permission from [28]).*

sessed prospectively in leukemic patients receiving a 28-day course of AmB for persistent fever of unknown origin. Only two of 20 patients (10%) developed mild renal dysfunction, which, however, did not necessitate interruption of therapy. The full course of high-dose AmB was successfully completed in all patients, including four with mild pretreatment renal impairment.

The question of the influence of salt supplementation was addressed using a prospective, randomized, placebo controlled trial of the influence of salt supplementation on the course of renal function during therapy with AmB [28]. AmB administration was preceded by 1 liter of either 0.9% saline or 5% dextrose in water administered i.v. over 4 hours. While the mean serum creatinine increased over time in the dextrose group, it remained unchanged in the saline group. Similarly, creatinine clearance decreased in the dextrose group, but remained unchanged in the saline group. The beneficial effect of salt loading however occurred at the expense of greater hypokalemia, since the saline group required significantly higher amounts of potassium supplementation to maintain a normal serum K level (Figure 6). Therefore, based upon these studies, it is reasonable to recommend routine salt supplementation with administration of AmB, with special attention being paid to maintaining potassium balance.

More recently, Mayer and coworkers [109] have expanded the notion that early introduction of potassium and magnesium supplements in equal amounts to that lost through the kidneys results in decreased incidence of AmB infusion-related side effects and decreased frequency of an increase in serum creatinine. This study provided further evidence to support that sufficient hydration and timely supplementation of sodium, potassium and magnesium reduces the renal toxicity of AmB.

Despite not being strictly a liposomal formulation, the preparation of Fungizone in Intralipid 20% instead of in glucose, was associated with a reduction in renal toxicity [110]. HIV positive patients with oral candidiasis were considered for treatment. The 22 subjects had comparable characteristics regarding HIV status infection and candidiasis, renal function and concomitant use of other nephrotoxic drugs. They were randomized to receive Fungizone prepared either in Intralipid 20% or in glucose. Four of 11 AmB-glucose treatments were discontinued due to renal abnormalities or intolerable acute effects, whereas all the 11 AmB-Intralipid 20% were treated without serious problems. Four of the 7 patients that completed AmB-glucose regimen had at least one serum creatinine exceeding 1.5 mg/dl versus one of 11 patients that completed AmB-Intralipid 20%. Clinical side effects were noted in 36/38 infusions with AmB-glucose but only in 10/44 with AmB-Intralipid 20%. All patients responded to therapy with AmB as assessed by an oral candidiasis score. Thus, this formulation was effective and induced less adverse reactions than the conventional formulation.

Other formulations: Liposomal formulations

The narrow therapeutic index of conventional AmB led to the development of new formulations of the drug that utilize either liposomes or complex phospholipids as drug carriers to decrease untoward effects, enhance activity and to provide site specific delivery of doses of this drug [111, 112].

Liposomes are microscopic vesicles consisting of one or more phospholipid membranes surrounding a discrete water compartment. The lipid layer is composed of amphipathic phospholipids whose hydrophobic tails associate, while the polar hydrophilic heads align toward the bulk of the water phase. A variety of liposomes with unique physical and chemical structure can be manufactured by altering non-polar and polar groups. Excellent reviews have been recently published [112-114].

Different mixtures using several lipoproteins and combined in different ratios have appeared during the last decade. At least 5 formulations have been tested in man. They include dimyristoyl phosphatidylcholine (DMPC) / phosphatidylglycerol (DMPG) liposomes, amphotericin B in lipid complex (ABLC; Abelcet, The Liposome Company, Princeton, NJ), intralipid AmB, AmB colloidal dispersion (ABCD; Amphotec, Alza Corporation, Palo Alto, CA), and amphotericin B liposome (L-Amph; AmBisome, Fujisawa Healthcare, Deerfield, IL) (Table 2). In the last decade, these preparations have been extensively examined *in vitro*, whole animal and clinical studies [111, 115-140]. When conventional AmB is used as a frame of reference, it is clear that there are substantial differences in the pharmacokinetic disposition between these formulations. However, for equally effective doses, the toxicity profile is disappointingly similar (Table 2). ABLC was the first agent

approved by the FDA. ABLC consist of two phospholipids in a 1:1 drug-to-lipid molar ratio. Electron microscopy reveals ribbon-like, macromolecular structures, ranging from 2 to 5 microns in diameter. L-Amph is a unilamellar liposomal preparation, consisting of phosphatidylcholine, cholesterol, distearoylphosphatidylglycerol and AmB in a 2:1:0.8:0.4 molar ratio; with an average diameter of 60 to 70 nm. Amphotec is created by complexing amphotericin B with cholesteryl sulfate in a 1:1 molar ratio to form a colloidal suspension in aqueous solution. The two components form a bilayer in a disk shape, with amphotericin B forming a shield at the disk edges. The disk size is uniform (about 115 nm in diameter and 4 nm thick) and very stable, with the lyophilized form retaining stability for months to years.

To evaluate whether incorporation of AmB into liposomes reduces nephrotoxicity, any comparison of conventional AmB with a new formulation of the drug should address the following questions: 1) do the different formulations have the same or different actions? 2) if they have the same action, what is the dose ratio between antifungal and toxic effects, especially nephrotoxicity? and 3) is there a selective advantage in the dose ratios indicating a wider therapeutic margin, i.e., is the dose ratio of liposomal formulation of AmB/AmB lower for the antifungal effect compared to the nephrotoxic effect?

Table 2(a). Comparative pharmacokinetics of Amphotericin B and liposomal formulations.

Agent	AmB	L-AmB	ABLC	ABCD
Distribution compared to AmB:				
in liver:		higher	higher	higher
in lungs:		similar	higher	similar
in kidney:		similar	similar	similar
Cmax	2.9 mcg/ml	higher	lower	lower
Vd	4 L/kg ml/min.kg	lower	similar	higher
Cl	0.43 mcg.h/ml	lower	higher	similar
AUC	8.6 mcg.h/ml	higher	lower	lower

2(b). Adverse events - percentage of population with response.

	AmB	L-AmB	ABLC	ABCD
mg/kg/day:	1.5-6	5	4-6	0.8-1
Chills	53	48	18	77
Hypokalemia	20	43	5	17
Nausea	6	40	9	8
Vomiting	6	32	8	9
Dyspnea	4	23	7	8
Creatinine increase	28	22	11	20
Hyperbilirubinemia	17	18	4	16
Hypotension	6	14	8	12
Hypertension	6	8	5	6

Collective evidence in the literature suggests that L-AmB and conventional AmB have a similar action on fungal and mammalian cells. Very few studies, however, have established a dose ratio for antifungal and nephrotoxic effects. In most, only one aspect of the activity or only one formulation was studied. Thus, comparisons between studies are difficult, and inferences should be made with caution. There is evidence that the fungicidal activity of liposomal formulations of AmB is influenced by several properties of the liposomes including lipid composition, physical size, the molar ratio of lipids, and the presence or absence of sterols [117-119]. Furthermore, the tests used to assess *in vivo* toxicity have rarely examined renal function adequately. The testing of new formulations by obtaining the acute LD_{50} of the drug, does not necessarily relate to the nephrotoxic potential of chronic therapy [118, 120-122]. Finally, Phase II-III studies in man are complicated by difficulties in the diagnosis of fungal infection, the underlying clinical condition of the patients, and frequent concomitant use of other nephrotoxic agents.

Cell studies

The binding of AmB to various compounds or formulations may result in reduced bioavailability of free AmB with a consequent reduction in toxicity to mammalian and /or fungal cells. Thus, the different formulations may act as a reservoir for free AmB. Since it is recognized that AmB has a higher affinity for ergosterol (the main sterol in fungal cell membranes) than for cholesterol (that is found in human cells) it is possible that the reduced amounts of free AmB is sufficient to be toxic to fungal cells and not to mammalian cells. A selective rate of transfer may be related not only to changes in the sterol components of cell membranes but also to different level of expression of lipoprotein receptors in the target cells.

Much has been learned from *in vitro* studies focused

to address the relationship between either free AmB or liposomal formulations of AmB with both high- and low-density lipoproteins (HDLs, LDLs). In a series of elegant studies by Lopez-Berenstein and coworkers, it was shown that AmB predominantly associates with HDL and that this effect is enhanced when it is incorporated into positively and negatively charged liposomes [141, 142]. These investigators have evaluated the influence of HDLs and LDLs on the toxicity of AmB to fungal and renal cells and observed a selective protective effect of HDL associated AmB for mammalian cells. The minimum inhibitory concentration of AmB and DMPC:DMPG liposome on *Candida albicans* fungal cells was not modified whether or not HDLs or LDLs were added to the incubation plates. However, HDL-associated AmB was less toxic than free AmB to LLC-PK1 cells, while LDL-associated AmB was as toxic as free AmB. In addition, DMPC:DMPG liposomes and both HDL- and LDL-associated DMPC:DMPG liposomes were less toxic to LLC-PK1 cells than was AmB [142]. Examination of HDL and LDL receptors in the LLC-PK1 cells revealed a high-affinity and low-affinity LDL receptors but only a low-affinity HDL receptor. After trypsinization of the LLC-PK1 renal cells to reduce the LDL receptor, LDL associated AmB was also less toxic than free AmB. Thus, the reduced level of toxicity of HDL-associated AmB and of DMPC:DMPG may be explained by the low level of expression of HDL receptors in LLC-PK1 [142]. Taking this information into account, it appears that AmB in liposomal formulations may exist in a complex system that includes free drug, lipoprotein-bound drug and liposomal bound drug (Figure 7). Since the dynamic of the equilibrium is not known, it is difficult to conclude whether doses of AmB in liposomal formulations result in comparable free drug concentration as the conventional

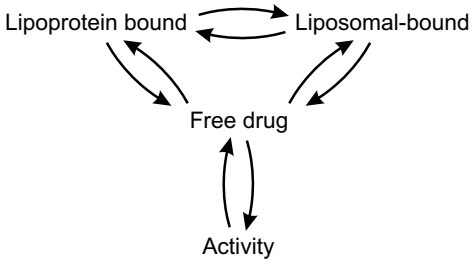

***Figure 7**. Relationships between free and bound forms of amphotericin B to activity.*

preparation. These results also support the notion that the relative distribution of AmB among the serum lipoproteins is a major factor influencing the therapeutic index of AmB incorporated into liposomes.

Ralph et al. demonstrated that DMPC:DMPG is generally less active than AmB on yeast cells, and has a slower onset of action [123]. The authors suggest that this liposomal formulation of AmB acts as a reservoir for free AmB, which is the active moiety. Others found either an equivalent or 3-4 fold less efficacy for a lipid-complexed form of AmB [118, 121, 124]. These differences may be attributed to the different preparations used and/or to the different strains of fungi examined.

An *in vitro* evaluation of a therapeutic ratio between mammalian and fungal cells serves as a basis of comparison to a ratio of dose required to induce *in vivo* renal toxicity to determine if nephrotoxicity is selectively diminished by the incorporation of AmB into liposomes. One study has calculated a concentration ratio for the actions of liposomal formulation of AmB and conventional AmB on mammalian and fungal cells. Juliano et al. compared the *in-vitro* toxicity of AmB and L-AmB to *Candida albicans* and mammalian erythrocytes [119]. While the two formulations were equipotent in their effects on ion fluxes in yeast cells (indicating formation of membrane pores), only AmB induced such an effect in erythrocytes, despite achieving concentrations of L-AmB that were 10 to 20-fold higher than those of AmB. The time required to achieve this effect in fungi was the same for the two formulations, which suggested that L-AmB did not constitute a slow-release form of the drug. The reason for the reduced toxicity of L-AmB in mammalian cells was proposed to be preferential transfer of AmB from liposomes to fungal cells compared with its transfer to mammalian cells.

Other studies have also directly compared the antifungal and toxic effects of the two formulations to confirm a wider therapeutic index. A greater toxicity to kidney epithelial cell structures of AmB was apparent when compared with AmB in DMPC:DMPG liposomes. LLC-PK1 renal cells, exposed to short exposure times (2 hours), with different formulations of DMPC:DMPG liposomes, exhibited different EC_{50}'s, the most potent having an EC_{50} 13 times that of AmB [125]. Taken in concert with previous studies [133], this indicates that the renal epithelial cell toxic concentration ratio of DMPC:DMPG liposomes/AmB (13-20:1)

is higher than its antifungal concentration ratio (1:1). Further confirmation of differential toxicity was provided by the acute administration of liposomal AmB to primary cultures of rabbit proximal tubule cells and to LLC-PK1 cells (a kidney epithelial cell line) [125]. Joly et al. suggested that higher concentrations of AmB combined to liposomes did not induce the changes elicited by smaller concentrations of conventional AmB. Evidence used to support this was increased K^+ efflux and LDH release with AmB but not L-AmB despite achieving 4 to 8 fold higher concentrations of the latter [115]. Unfortunately, antifungal activity was not assessed in this study and no dose ratio can be calculated.

Acute studies require cautious interpretation as chronic exposure of LLC-PK1 cells to L-AmB (1-2 days), which is more representative of events that occur clinically, resulted in profound toxic effects at concentrations similar to those of AmB (L-AmB/AmB =1:1), manifested by changes in cellular transport processes and in morphology [125]. This finding raises questions as to the applicability and relevance of results derived from short-term *in-vitro* experiments to whole animal or clinical situations.

Whole animal studies

Animal studies suggest that liposomal formulations of AmB are effective in the treatment of fungal infections, but usually require higher doses than AmB [117, 120, 121, 128, 129, 137, 138]. The lack of concomitant assessment of renal function in many of these studies makes it impossible to determine a dose ratio, although most reports confirm that the drug was well-tolerated. As mentioned previously, AmB can be distributed in the tissue and serum as free drug, protein-bound drug and liposome-bound drug (Figure 7). However, most studies have not discriminated between these components, so interpretation of serum and tissue concentrations of the drug remains uncertain. This is further complicated by the fact that in *ex vivo* blood samples, a significant proportion of the total AmB concentration in blood settles as a sediment during the centrifugation process [113]. Thus, it is difficult to interpret the measured free AmB fraction in serum, which may differ between formulations due to physicochemical characteristics of the mixture or to artifactual processes involved after blood sampling.

Detailed pharmacokinetic studies confirm the tissue distribution and pharmacokinetics of L-AmB differ from the conventional drug formulation [113, 122, 143] (Table 2a). In general, a greater volume of distribution and greater systemic clearance is apparent when AmB is incorporated into liposomes, suggesting substantial penetration into many organs. After parenteral administration, the total concentration in liver and spleen were higher for the liposomal formulations than with conventional AmB due to accumulation in the tissues of the reticuloendothelial system [144]. In contrast, concentrations of AmB in the kidney are lower at equivalent doses. For both AmBisome and ABLC, the results of the biodistribution multidose studies are consistent with reduced kidney toxicity of these formulations [122, 143]. Higher doses of AmBisome (5 mg/kg) and ABLC (10 mg/kg), are required to result in comparable kidney concentrations of AmB to that obtained after 1 mg/kg of conventional AmB.

The acute nephrotoxicity of the two formulations has been examined in rabbits, where AmB induced a fall in GFR and a rise in urinary sodium and potassium excretion rates, while L-AmB, at 2.5 times the dose, did not affect these parameters [126]. Here again, antifungal activity was not assessed. Unexpectedly, however, and in contrast to AmB, L-AmB increased the excretion of N-acetylglucosaminidase in these animals indicating renal tubular toxicity. The authors suggested that increased delivery of AmB to renal tubular cells by the liposomes led to an interaction with lysosomes and provided an additional mechanism of injury. In support of this conclusion is the finding that the tubular toxicity induced by repeated administration of AmB and L-AmB over 5 days was similar when a high enough dose of L-AmB (2.4 times that of AmB) was used [127].

A clear reduction in the single dose toxicity for AmBisome and ABLC has been described. The LD_{50} of AmBisome in mice was found to be greater than 175 mg/kg compared to 2.3 mg/kg for the conventional preparation. In rats the LD_{50} was more than 30 fold greater than the value of 1.6 mg/kg for Fungizone [122]. Similarly ABLC could be successfully administered up to 10 mg/kg whereas the maximum non-lethal dose that could be administered to normal mice was 1 mg/kg of Fungizone.

Human studies

Similar findings have been reported in patients,

with several studies showing that in patients who failed to respond to conventional AmB, or develop nephrotoxicity, had either a complete or partial responders to liposomal formulation of AmB, without associated deterioration in renal function [111, 130-136, 138]. However, subsequent studies have questioned the renoprotective effect for therapeutic equivalent doses.

All of the three lipid formulations of amphotericin B available in North America and Western Europe, in controlled trials, have demonstrated significantly lower nephrotoxicity than amphotericin B [145]. Differences in biochemical, pharmacokinetic and pharmacodynamic properties among the lipid products have been documented (Table 2). The clinical significance, however, of pharmacokinetic differences between the liposomal preparations is unknown because of the lack of comparative trials.

The clinical experience with these products has been mainly in patients with intolerance to conventional AmB. To date, there is no convincing evidence that any of the lipid-based formulations confers superior efficacy when prospectively compared with AmB in the treatment of documented fungal infections. Administration of liposomal formulations of AmB are associated with fewer adverse effects, and are generally well-tolerated. Comparative rates of toxicity are lacking because of different patient populations, different methods of measured infusion related toxic effects, and different dose scheduling. When used prophylactically for the empiric treatment of febrile neutropenia, Ambisome did significantly reduce the incidence of proven emergent fungal infections but did not improve short-term survival rates, as compared to conventional AmB. Carriagan [145] in a recent commentary, suggested that if ABLC therapy is administered at higher doses than recommended by the Food and Drug Administration (5 mg/kg/day) then a similar degree of nephrotoxicity to conventional AmB can be anticipated.

Despite early enthusiasm, more recent studies have yielded more equivocal information. In a randomized, double blind study, Wingard et al [146] have compared the safety of two lipid formulations of amphotericin B in febrile neutropenic patients. Subjects were randomized to receive amphotericin B lipid complex (ABLC) at a dose of 5 mg/kg/d (n=78), liposomal amphotericin B (L-Amph) at a dose of 3 mg/kg/d (n=85), or L-Amph at a dose of 5 mg/kg/d (n=81). They found that the incidence of nephrotoxicity (doubling of the baseline serum creatinine concentration) was 42% in the group of patients that received ABLC, 15% in the group that received L-Amph at a dosage of 3 mg/kg/d, and 14% in the group that received L-Amph at a dose of 5 mg/kg/d. Thus, even though L-Amph presented a superior safety profile in comparison with ABLC, along with better tolerance (fewer infusion-related reactions and lower nephrotoxicity), each L-AmB did have a clinically relevant adverse profile.

A further multicenter, randomized study comparing amphotericin B lipid complex with conventional amphotericin B (AmB) as empiric therapy for febrile neutropenic patients, found a similar incidence of nephrotoxicity (defined as a doubling of the baseline serum creatinine concentration) and infusion-related reactions (fever and chills) in both treatment arms [147].

More recently, the National Institute of Allergy and Infectious Diseases Mycoses Study Group reported their head to head comparison of L-Amph against conventional AmB in a randomized, double-blind multicenter study in 344 patients with persistent fever and neutropenia as empiric therapy for occult invasive fungal infections [148]. As in previous trials, efficacy was similar with respect to survival (93% vs. 90%, respectively) and resolution of fever during neutropenia (58% vs. 58%, respectively). Discontinuation of therapy due to drug related side effects was also similar (14% vs 19%, respectively). However, the liposomal preparation had a lower rate of increase serum creatinine by two times upper limit of normal (19% vs. 34%, respectively and infusion related fever (17% vs 44%, respectively). In this study, the authors noted a significantly lower frequency of breakthrough fungal infections (3% vs. 8%, respectively $p<0.009$). This same multicenter research team also undertook a subsequent comparison of L-Amph in comparison to voriconazole in 837 patients with the same entry criteria [149]. In that study, voriconazole proved equally effective to L-Amph, but less toxic with respect to nephrotoxicity and fever. However, it had a higher CNS toxicity causing visual disturbances and hallucinations. The ability to administer voriconazole both parenterally and orally did confer an advantage in allowing somewhat earlier patient discharge. Interestingly, this study suggested a lower documented breakthrough with voriconazole having a breakthrough rate of 2% while L-Amph had a breakthrough rate of 5% ($p=0.02$). This latter rate is higher than in the first study and not very different from con-

ventional AmB, thus the relatively small numbers in each study with breakthrough are too small to provide confidence in our ability to discriminate differences.

Experience of using L-Amph in pyrexia of unknown origin has been extended to children and to considering the dose of L-Amph required in a further randomized study of 134 adults and 204 children from the Royal Free Hospital in London [150]. In this study, a low dose L-Amph arm (1 mg/kg/day) was compared to the same dose used in prior studies (3 mg/kg/day). In this study, efficacy was observed in 49% with conventional AmB, 58% with low-dose L-Amph and 64% with high dose L-Amph. A difference was also observed for adverse events, for example doubling of serum creatinine occurred in 24% on conventional AmB versus a 10% and 12% in the two L-Amph arms. A high frequency of fever and rigors with conventional AmB was not observed with L-Amph, but rare cases of CNS toxicity (encephalopathy and seizures) only occurred in L-Amph group. These authors concluded the L-Amph does offer a therapeutic advantage over conventional amphotericin, and that the reduced toxicity profile is particularly valuable in children.

Given the relative rather than absolute differences in nephrotoxic potential between formulations that have equal efficacy, it becomes relevant to weigh the benefits of decreased nephrotoxicity against the cost of therapy. At our institution, using 2001 drug acquisition costs, a full course of 29 days of therapy in a 70 kg patient with AmB currently costs approximately $360 to reach a total dose of 2 grams [151]. In contrast, a full course of ABLC for 14 days for a total of 5 grams costs approximately $6,500. This differential raises the question of the relative value between the alternative therapies. This issue has been explored by Cagnoni et al [152] in a randomized, double-blind, comparative, multicenter trial in persistently febrile neutropenic patients treated as first-line empirical therapy with either liposomal versus conventional AmB. By using itemized hospital billing data on 414 patients, hospital costs from the time of first dose to discharge were significantly higher for all patients who received liposomal AmB ($48,962 vs. $43,183; p=0.022) without any difference in clinical outcome assessed by major clinical events [152a].

In the opinion of these authors, until superior efficacy is clearly shown (for proved infections) or pharmacoeconomic analyses document the value of these drugs, current use of such expensive agents should be restricted to patients with preexisting renal dysfunction, patients who do not respond clinically to AmB, or in patients who have a significant decrease in their renal function while receiving conventional formulations AmB.

Clinical use

General underlying condition of the patient

The indication for AmB is the presence of proved or suspected systemic fungal disease. As previously mentioned, patients who receive AmB are usually immunocompromised and severely ill, with some degree of malnutrition, multiple organ failure and life threatening infections. The clinical condition of these patients may, therefore, confer an increased risk of nephrotoxicity. Once the decision to implement AmB has been made, an algorithm can be used to optimize therapy (Figure 8) [82]. The first step is to assess renal function, the sodium status of the patient, and to correct overt sodium depletion. It is important to realize that milder sodium depleted states which are not clinically apparent can substantially enhance the nephrotoxic potential of AmB. It is, therefore, important to assess whether the patient can tolerate sodium supplementation in addition to a normal salt intake. Otherwise healthy subjects usually tolerate a supplement of 150 mEq/d over and above the normal sodium intake of 150 to 200 mEq/d without difficulty. Increased sodium intake, however, may exacerbate cardiac failure, cirrhosis with ascites, or renal failure.

Another factor to consider is whether the patient will receive sodium supplementation as a consequence of concomitant antibiotic therapy (e.g., ticarcillin). When the opportunity to choose among several antibiotics arises, the alternative with the highest obligatory sodium load should be selected whenever possible.

Finally, it is prudent to check for the presence of potassium and magnesium deficits prior to therapy since AmB will invariably cause loss of these electrolytes during therapy. Correction of these abnormalities, before or concomitantly with the start of therapy, should delay or avoid the early development of electrolyte disturbances and possible additional toxicity (e.g., arrhythmia, rhabdomyolysis) that sometimes

necessitate the early discontinuation of therapy.

In our opinion, the elective use of L-AmB at the outset should be restricted to only those patients who have impaired renal function, who have clinical contraindications to salt supplementation or who are children. Otherwise, the use of AmB is generally advocated.

Amphotericin B administration

AmB therapy should be started with a low dose, gradually escalating to a full therapeutic dose according to patient tolerance. Traditionally AmB has been administered as an IV infusion over 4-6 hours as recommended in the insert package. However, continuous infusion over 24 hours provides a reasonable alternative as it induces fewer side effects and significantly decreases nephrotoxicity [17].

If a 4-hour infusion is to be used in conjunction with ticarcillin, we advocate administration between doses of ticarcillin. If ticarcillin is not indicated, we advocate that AmB should be given between two 30-min infusions of 0.5 L normal saline, intravenously. This amount of supplementation is based on empiric observation, and further studies are needed to ascertain whether lower amounts confer an equivalent degree of protec-

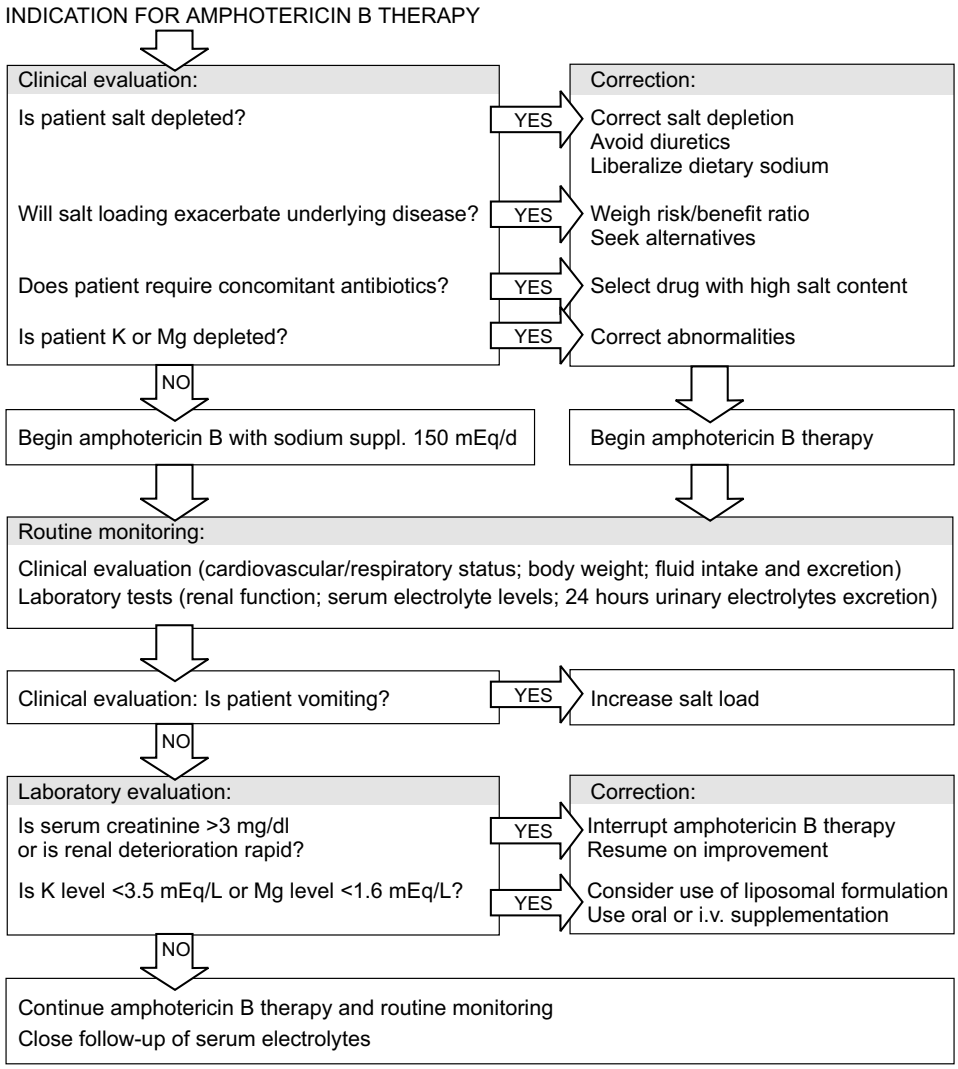

***Figure 8**. Proposed approach for management of amphotericin B therapy*

tion. If vomiting occurs, sodium supplementation should be increased.

Concomitant use of nephrotoxic drugs

If AmB is to be used in conjunction with another nephrotoxic agent, several measures can be taken in order to minimize the potential synergistic toxicity of amphotericin B. For example, if an aminoglycoside or cyclosporine is to be used, monitoring of their serum concentrations will help avoid toxic levels. It is also imperative to evaluate electrolyte losses closely and be aggressive in their replacement, since cyclosporine A nephrotoxicity may be exacerbated by dehydration [153], and to follow magnesium levels closely since both drugs cause hypomagnesemia.

Potassium and magnesium supplementation

Urinary potassium and magnesium losses are anticipated consequences of AmB therapy. Some of the losses can be compensated for with increased dietary intake, while others will require oral or intravenous replacement. It should be recognized that the serum levels of these ions do not necessarily correlate with the total deficit, as the plasma levels tend to be conserved while cellular stores are becoming depleted. In general, potassium and magnesium supplements should be given to all patients and the amounts increased if the potassium level falls below 3.5 mEq/l or the magnesium level falls below 1.6 mEq/l, with either dietary or pharmacological supplementation. Amiloride, in low doses, is an alternative therapy in patients who need high dose intravenous potassium replacement [154, 155]. Frequent monitoring of serum electrolytes (potassium and magnesium) with adequate hydration and ion supplementation corresponding to amounts lost by kidneys concomitant to AmB therapy provides an effective intervention for prophylaxis of AmB-induced renal toxicity [109].

Follow-up

In patients with mild renal dysfunction prior to AmB therapy, sodium supplementation has proved to be safe and effective. In patients not receiving sodium supplementation who develop renal impairment during AmB therapy, initiation of sodium supplementation may permit continued therapy with AmB. However if renal function continues to deteriorate, or if the rate of deterioration is rapid, temporary discontinuation of AmB therapy may be required. Therapy can be resumed in rehydrated patients when serum creatinine concentrations begin to return toward baseline values or L-AmB can be used in place of AmB.

Conclusion

AmB remains the most effective antifungal agent. Nephrotoxicity is a well-recognized dose-limiting complication, leading to interruption or discontinuation of the therapy. It is commonly expressed as azotemia and decreased GFR, however tubular abnormalities are also important. The underlying mechanisms include direct vasoconstrictor effects and direct cytotoxicity, as a reflection of its action on cell membranes leading to alteration of cell permeability. These effects are amenable to modulation. In the clinical setting, the use of salt supplementation lowers incidence and severity of nephrotoxicity; however, requires careful attention to potassium and magnesium replacement. The information accumulated to this date support the notion that liposomal formulations of AmB are at least as effective as AmB. Early reports suggested that liposomal formulations are less nephrotoxic. However, recent reports suggest that they could have similar rates of nephrotoxicity for therapeutic equivalent doses.

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References

1. Denning DW. Epidemiology and pathogenesis of systemic fungal infections in the inmunocompromised host. J Antimicrob Chemother 1991; 28(Suppl B): 1-16.
2. Horan TC, White JW, Jarvis WR, Emori TG, Culver DH, Munn VP, Thornsberry C, Olson DR, Hughes JM. Nosocomial infection surveillance, 1984. MMWR Surveill Summ 1986; 35: 17SS-29SS.
3. Bodey GP. Fungal infections complicating acute leukemia. J Chronic Dis 1966; 19: 667-687.
4. Hay RJ. Overview of the treatment of disseminated fungal infections. J Antimicrob Chemother 1991; 28(Suppl B): 17-25.
5. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. Rev Infect Dis 1990; 12(2): 308-329.
6. Warnock DW. Amphotericin B: an introduction. J Antimicrob Chemother 1991; 28(Suppl B): 27-38.
7. Brajtburg J, Powderly WG, Kobayashi GS, Medoff G. Amphotericin B: current understanding of mechanisms of action. Antimicrob Agents Chemother 1990; 34(2): 183-188.
8. Brajtburg J, Elberg S, Schwartz DR, Vertut-Croquin A, Schlessinger D, Kobayashi GS, Medoff G. Involvement of oxidative damage in erythrocyte lysis induced by amphotericin B. Antimicrob Agents Chemother 1985; 27: 172-176.
9. Sokol-Anderson M, Brajtburg J, Medoff G. Amphotericin B-induced oxidative damage and killing of *Candida albicans*. J Infect Dis 1986; 154: 76-83.
10. Sabra R, Branch RA. Amphotericin B nephrotoxicity. Drug Saf 1990; 5(2): 94-108.
11. Butler WT, Bennett JE, Alling DW, Wertlake PT, Utz JP, Hill GJ. Nephrotoxicity of amphotericin B, early and late events in 81 patients. Ann Intern Med 1964; 61: 175-187.
12. Medoff G, Koyabashi GS. Strategies in the treatment of systemic fungal infections. N Engl J Med 1980; 302: 145-155.
13. Clements JS, Peacock JE. Amphotericin B revisited: reassessment of toxicity. Am J Med 1990; 88: 22N-27N.
14. Miller RP, Bates JH. Amphotericin B toxicity: a follow-up report of 53 patients. Ann Intern Med 1969; 71: 1089-1095.
15. Branch RA, Jackson EK, Jacqz E, Stein R, Ray WA, Ohnhaus EE, Meusers P, Heidemann H. Amphotericin-B nephrotoxicity in humans decreased by sodium supplements with coadministration of ticarcillin or intravenous saline. Klin Wochenschr 1987; 65: 500-506.
16. Winn WA. Coccidiomycosis and amphotericin B. Med Clin North Am 1963; 47: 1131-1148.
17. Eriksson U, Seifert B, Schaffner A. Comparison of effects of amphotericin B deoxycholate infused over 4 or 24 hours: randomized controlled trial. Br Med J 2001; 322: 579-582.
18. Littman ML, Horowitz PL, Swadey JG. Coccidiomycosis and its treatment with amphotericin B. Am J Med 1958; 24: 568-592.
19. Rubin SI, Krawiec DR, Gilbers H, Shanks RD. Nephrotoxicity of amphotericin B in dogs: a comparison of two methods of administration. Can J Vet Res 1989; 53: 23-28.
20. Stein RS, Albridge K, Lenox RK, Ray W, Flexner JM. Nephrotoxicity in leukemic patients receiving empirical amphotericin B and aminoglycosides. South Med J 1988; 81: 1095-1099.
21. Fisher MA, Talbot GH, Maislin G, McKeon BP, Tynan KP, Strom BL. Risk factors for amphotericin B-associated nephrotoxicity. Am J Med 1989; 87: 547-552.
22. Luber AD, Maa L, Lam M, Guglielmo BJ. Risk factors for amphotericin B-induced nephrotoxicity. J Antimicrob Chemother 1999; 43: 267-271.
23. Holeman CW, Einstein H. The toxic effects of amphotericin B in man. Calif Med 1963; 99: 90-93.
24. Bullock WE, Luke RG, Nuttal CE, Bhathena D. Can mannitol reduce amphotericin B nephrotoxicity ? Double blind study and description of a new vascular lesion in kidneys. Antimicrob Agents Chemother 1976; 10: 555-563.
25. Barbour GL, Straub KD, O'Neal BL, Leatherman JW. Vasopressin-resistant diabetes insipidus, a result of amphotericin B therapy. Arch Intern Med 1979; 139: 86-88.
26. Rose BD. Hypokalemia. In: Clinical physiology of acid-base and electrolyte disorders. Rose BD (editor). McGraw Hill, Inc. 1989; p. 715-756.
27. Burgess JL, Birchall R. Nephrotoxicity of amphotericin B with emphasis on changes in tubular function. Am J Med 1972; 53: 77-84.
28. Llanos A, Cieza J, Bernardo J, Echevarría J, Biaggioni I, Sabra R, Branch RA. Effect of salt supplementation on amphotericin B nephrotoxicity. Kidney Int 1991; 40: 302-308.
29. Sellin JH, De Soigne R. Ion transport in human colon *in vitro*. Gastroenterology 1987; 93: 441-448.
30. Bernardo JF, Murakami S, Branch RA, Sabra R. Potassium depletion potentiates amphotericin-B-induced toxicity to renal tubules. Nephron 1995; 70: 235-241.
31. Barton CH, Pahl M, Vaziri ND, Cesario T. Renal magnesium wasting associated with amphotericin B therapy. Am J Med 1984; 77: 471-474.
32. Douglas JB, Healy JK. Nephrotoxic effects of amphotericin B, including renal tubular acidosis. Am J Med 1969; 46: 154-162.
33. Steinmetz PR, Lawson LR. Defect in urinary acidification induced *in vitro* by amphotericin Br J Clin Invest 1970; 49: 596-601.
34. Gil FZ, Malnic G. Effect of amphotericin B on renal tubular acidification in the rat. Pflugers Arch 1989; 413: 280-286.

35. Gouge TH, Andreoli VT. An experimental model of amphotericin B nephrotoxicity with renal tubular acidosis. J Lab Clin Med 1971; 78: 713-724.
36. McCurdy DK, Frederic M, Elkinton JR. Renal tubular acidosis due to amphotericin B. Clin Res 1964; 12: 471.
37. Takacs FJ, Tomkiewicz ZM, Merrill JP. Amphotericin B nephrotoxicity with irreversible renal failure. Ann Intern Med 1963; 59: 716-724.
38. Heyman SN, Stillman IE, Brezis M, Epstein FH, Spokes K, Rosen S. Chronic amphotericin nephropathy: morphometric, electron microscopic, and functional studies. J Am Soc Nephrol. 1993; 4: 69-80.
38a. Mayer J, Doubek M, Doubek J, Horky D, Scheer P, Stepanek M. Reduced nephrotoxicity of conventional amphotericin B therapy after minimal nephroprotective measures: animal experiments and clinical study. J Infect Dis 2002; 186: 379-388.
39. Porter GA. Extrapolation of animal data to man: the concordance between toxicity screening and clinical consequence. In: Nephrotoxicity in the experimental and clinical situation Part 2. Bach PH, Lock EA (editors). Martinus Nijhoff Publishers 1987: p. 613-641.
40. Gruda I, Nadeau P, Brajtburg, Medoff G. Application of different spectra in the UV-visible region to study the formation of amphotericin B complexes. Biochim Biophys Acta 1980; 602: 260-268.
41. Vertut-Croquin A, Bolard J, Chabert M, Gary-Bobo C. Differences in the interaction of the polyene antibiotic amphotericin B with cholesterol-or ergosterol-containing phospholipid vesicles. A circular dichroism and permeability study. Biochemistry 1983; 22: 2939-2944.
42. Caltrider PG, Gottlieb D. Capacidin: a new member of the polyene antibiotic group. Antibiot Chemother 1961; 10: 702-708.
43. Kinsky SC. The effect of polyene antibiotics on permeability in *Neurospora crassa*. Biochem Biophys Res Commun 1961; 4: 353-357.
44. Kinsky SC. Alterations in the permeability of *Neurospora crassa* due to polyene antibiotics. J Bacteriol 1961; 82: 889-897.
45. Marini FP, Arnow P, Lampen JO. The effect of monovalent cations on the inhibition of yeast metabolism by nystatin. J Gen Microbiol 1961; 24: 51-62.
46. Sutton DD, Arnow PM, Lampen JO. Effect of high concentrations of nystatin upon glycolysis and cellular permeability in yeast. Proc Soc Exp Biol Med 1961; 108: 170-175.
47. Gottlieb D, Carter HE, Sloneker JH, Amman A. Protection of fungi against polyene antibiotics by sterols. Science 1958; 128: 361.
48. Kotler-Brajtburg J, Medoff G, Schlessinger D, Kobayashi GS. Characterization of the binding of amphothericin B to Saccharomyces cerevisiae and relationship to antifungal effects. Antimicrobial Agents Chemother 1974; 6: 770-776.
49. Andreoli TE. On the anatomy of amphotericin B-cholesterol pores in lipid bilayer membranes. Kidney Int 1973; 4: 337-345.
50. DeKruijiff B, Demel RA. Polyene antibiotic-sterol interactions in membranes of *Acholeplesma laidlawii* cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic-cholesterol complexes. Biochim Biophys Acta 1974; 339: 57-70.
51. Holz RW. The effects of the polyene antibiotics nystatin and amphotericin B on thin lipid membranes. Ann N Y Acad Sci 1974; 235: 469-479.
52. Gatzy JT, Reuss L, Finn AL. Amphotericin B and K^+ transport across excised toad urinary bladder. Am J Physiol 1979; 237: F145-F156.
53. Lichtenstein NS, Leaf A. Effect of amphotericin B on the permeability of the toad bladder. J Clin Invest 1965; 44: 1328-1342.
54. Lichtenstein NS, Leaf A. Evidence for a double series permeability barrier at the mucosal surface of the toad bladder. Ann NY Acad Sci 1966; 137: 556-565.
55. Butler WT, Alling DW, Cotlove E. Potassium loss from human erythrocytes exposed to amphotericin B. Proc Soc Exp Biol Med 1965; 118: 297-300.
56. Teerlink T, DeKruijiff B, Demel RA. The action of piramicin, etruscomycin, and amphotericin B on liposomes with varying sterol content. Biochim Biophys Acta 1980; 599: 484-492.
57. Kinsky SC. Comparative responses of mammalian erythrocytes and microbial protoplast to polyene antibiotics and vitamin A. Arch Biochem Biophys 1963; 102: 180-188.
58. Kinsky SC, Avruch J, Permutt M, Rogers HB, Schonder AA. The lytic effects of polyene fungal antibiotics on mammalian erythrocytes. Biochem Biophys Res Commun 1962; 9: 503-507.
59. Finn JT, Cohen LH, Steinmetz PR. Acidifying defect induced by amphotericin B: comparison of bicarbonate and hydrogen ion permeabilities. Kidney Int 1977; 11: 261-266.
60. Capasso G, Schuetz H, Vickermann B, Kinne R. Amphotericin B and amphotericin B methylester: effect on brush border membrane permeability. Kidney Int 1986; 30: 311-317.
61. Schell RE, Tran NV, Bramhall JS. Amphotericin B-induced changes in membrane permeation: a model of nephrotoxicity. Biochem Biophys Res Commun 1989; 59: 1165-1170.
62. Hoeprich PD. Chemotherapy of systemic fungal diseases. Annu Rev Pharmacol Toxicol 1978; 18: 205-231.
63. Joly V, Saint-Pierre-Chazalet M, Saint-Julien L, Bolard J, Carbon C, Yeni P. Inhibiting cholesterol synthesis reduces the binding and toxicity of amphotericin B against rabbit renal tubular cells in primary culture. J Infect Dis 1992; 165: 337-343.

64. Cheng JT, Witty RT, Robinson RR, Yarger WE. Amphotericin B nephrotoxicity: Increased renal resistance and tubule permeability. Kidney Int 1982; 22: 626-633.
65. Varlam DE, Siddiq MM, Parton LA, Russmann H. Apoptosis contributes to amphotericin B-induced nephrotoxicity. Antimicrob Agents Chemother 2001; 45: 679-685.
66. Zager RA. Polyene antibiotics: relative degrees of *in vitro* cytotoxicity and potential effects on tubule phospholipid and ceramide content. Am J Kidney Dis 2000; 36: 238-249.
67. Hannun YA, Luberto C. Ceramide in the eukaryotic stress response. Trends Cell Biol 2000; 10: 73-80.
68. Sokol-Anderson ML, Brajtburg J, Medoff G. Amphotericin B-induced oxidative damage and killing of *Candida albicans*. J Infect Dis 1986; 154: 76-83.
69. Lamy-Freund MT, Ferreira VFN, Schreier S. Mechanism of inactivation of the polyene antibiotic amphotericin B. J Antibiot (Tokyo) 1985; 38: 753-757.
70. Brajtburg J, Elberg S, Schwartz DR, Vertut-Croquin A, Schlessinger D, Kobayashi GS, Medoff G. Involvement of oxidative damage in erythrocyte lysis induced by amphotericin B. Antimicrob Agents Chemother 1985; 27: 172-176.
71. Osaka K, Tyurina YY, Dubey RK, Tyurin VA, Ritov VB, Quinn PJ, Branch RA, Kagan VE. Amphotericin B as an intracellular antioxidant: protection against 2,2'-azobis(2,4-dimethylvaleronitrile)-induced peroxidation of membrane phospholipids in rat aortic smooth muscle cells. Biochem Pharmacol 1997; 54: 937-945.
72. Osaka K, Ritov VB, Bernardo JF, Branch RA, Kagan VE. Amphotericin B protects *cis*-parinic acid against peroxyl radical-induced oxidations: amphotericin B as an antioxidant. Antimicrob Agents Chemother 1997; 41: 743-747.
73. Walev I, Bhakdi S. Possible reason for preferential damage to renal tubular epithelial cells evoked by amphotericin B. Antimicrob Agents Chemother 1996; 40: 1116-1120.
74. Butler WT, Hill GJ, Szwed CF, Knight V. Amphotericin B renal toxicity in the dog. J Pharmacol Exp Ther 1964; 143: 47-56.
75. Gerkens JF, Heidemann HTh, Jackson EK, Branch RA. Aminophylline inhibits renal vasoconstriction induced by intrarenal hypertonic saline. J Pharmacol Exp Ther 1983; 225: 609-615.
76. Heidemann HTh, Gerkens GF, Jackson EK, Branch RA. Effect of aminophylline on renal vasoconstriction produced by amphotericin B in the rat. Naunyn Schmiedebergs Arch Pharmacol 1983; 324: 148-152.
77. Sabra R, Takahashi K, Branch RA, Badr KF. Mechanisms of amphotericin B-induced reduction of the glomerular filtration rate: a micropuncture study. J Pharmacol Exp Ther 1990; 253(1): 34-37.
78. Tolins JP, Raij L. Adverse effect of amphotericin B on renal hemodynamics in the rat: Neurohumoral mechanisms and influence of calcium channel blockade. J Pharmacol Exp Ther 1988; 245: 594-599.
79. Sabra R, Zeinoun N, Sharaf LH, Ghali R, Beshara G, Serhal H. Role of humoral mediators in, and influence of a liposomal formulation on, acute amphotericin B nephrotoxicity. Pharmacol Toxicol 2001; 88: 168-175.
80. Cutaia M, Bullard SR, Rounds S. Characteristics of amphotericin B-induced endothelial cell injury. J Lab Clin Med 1993; 121: 244-256.
81. Heyman SN, Clark BA, Kaiser N, Epstein FH, Spokes K, Rosen S., Brezis M. *In-vivo* and *in-vitro* studies on the effect of amphotericin B on endothelin release. J Antimicrob Chemother 1992; 29: 69-77.
82. Branch RA. Prevention of Amphotericin B-induced renal impairment: a review on the use of sodium supplementation. Arch Intern Med 1988; 148: 2389-2394.
83. Schnermann J. Regulation of single nephron filtration rate by feedback: facts and theories. Clin Nephrol 1975; 3: 75-81.
84. Thurau K. Modification of angiotensin-mediated tubuloglomerular feedback by extracellular volume. Kidney Int 1975; 8: S202-S207.
85. Wright FS, Schnermann J. Interference with feedback control of glomerular filtration rate by furosemide, triflocin, and cyanide. J Clin Invest 1974; 53: 1695-1708.
86. Gerkens JF, Branch RA. The influence of sodium status and furosemide on canine acute amphotericin B nephrotoxicity. J Pharmacol Exp Ther 1980; 214: 306-311.
87. Ohnishi A, Ohnishi T, Stevenhead W, Robinson RD, Glick A, O'Day DM, Sabra R, Jackson EK, Branch RA. Sodium status influences chronic amphotericin B nephrotoxicity in rats. Antimicrob Agents Chemother 1989; 33(8): 1222-1227.
88. Tolins JP, Raij L. Chronic amphotericin B nephrotoxicity in the rat: protective effect of calcium channel blockade. J Am Soc Nephrol 1991; 2(1): 98-102.
89. Tolins JP, Raij L. Chronic amphotericin B nephrotoxicity in the rat, protective effect of prophylactic salt loading. Am J Kidney Dis 1988; 11(4): 313-317.
90. Heidemann HTh, Bolten M, Inselmann G. Effect of chronic theophylline administration on amphotericin B nephrotoxicity in rats. Nephron 1991; 59: 294-298.
91. Wasan KM, Vadiel K, Lopez-Berenstein G, Verani RR, Luke DR. Pentoxifylline in amphotericin B toxicity rat model. Antimicrob Agents Chemother 1990; 34(2): 241-244.
92. Luke DR, Wasan KM, Verani RR, Brunner LJ, Vadiei K, Lopez-Berestein G. Attenuation of amphotericin B nephrotoxicity in the candidiasis rat model. Nephron 1991; 59: 139-144.

93. Kuan CJ, Branch RA, Jackson EK. Effect of an adenosine receptor antagonist on acute amphotericin B nephrotoxicity. Eur J Pharmacol 1990; 178: 285-291.
94. Sawaya BP, Weihprecht H, Campbell WR, Lorenz JN, Webb RC, Briggs JP, Schnermann J. Direct vasoconstriction as a possible cause for amphotericine B-induced nephrotoxicity in rats. J Clin Invest 1991; 87: 2097-2107.
95. Hardie W, Ebert J, Takahashi K, Badr KF. Thromboxane A_2 receptor antagonism reverses amphotericin B-induced renal vasoconstriction in the rat. Prostaglandins 1993; 45: 47-56.
96. Sabra R, Branch RA. Effect of amphotericin B on intracellular calcium levels in cultured glomerular mesangial cells. Eur J Pharmacol 1992; 226: 79-85.
97. Soupart A, Decaux G. Nifedipine and amphotericin B nephrotoxicity in the rat. Nephron 1989; 52: 278-280.
98. Heidemann HT, Brune KH, Sabra R, Branch RA. Acute and chronic effects of flucytosine on amphotericin B nephrotoxicity in rats. Antimicrob Agents Chemother 1992; 36(12): 2670-2675.
99. Brezis M, Rosen S, Silva P, Spokes K, Epstein F. Polyene toxicity in renal medulla: injury mediated by transport activity. Science 1984; 224: 66-68.
100. Nichols AJ, Koster PF, Brooks DP, Ruffolo RR Jr. Effect of fenoldopam on the acute and subacute nephrotoxicity produced by amphotericin B in the dog. J Pharmacol Exp Ther 1992; 260(1): 269-274.
101. Brooks DP, Mitchell MP, Short BG, Ruffolo RR, Nichols AJ. Attenuation of amphotericin B nephrotoxicity in the dog by the fenoldopam prodrug, SK&F R-105058. J Pharmacol Exp Ther 1991; 2577(3): 1243-1247.
102. Reiner NE, Thompson WL. Dopamine and saralasin antagonism of renal vasoconstriction and oliguria caused by amphotericin B in dogs. J Infect Dis 1979; 140: 564-575.
103. Utz JP. Chemotherapy of the systemic mycoses. Med Clin North Am 1982; 66: 221-234.
104. Hellesbusch AA, Salama F, Eadie E. The use of mannitol to reduce the toxicity of amphotericin B. Surg Gynecol Obstet 1972; 134: 241-243.
105. Olivero TJ, Lozano-Mendez L, Ghafary EM, Eknoyan G, Suki WN. Mitigation of amphotericin B nephrotoxicity with mannitol. Br Med J 1975; 1: 550-551.
106. Rosch JM, Pazin GJ, Fireman P. Reduction of amphotericin B nephrotoxicity with mannitol. JAMA 1976; 235: 1995-1996.
107. Said R, Martin P, Anicama H, Quintanilla A, Levin ML. Effect of mannitol on acute amphotericin B nephrotoxicity. Res Exp Med 1980; 177: 85-90.
108. Heidemann HTh, Gerkens GF, Spickard WA, Jackson EK, Branch RA. Amphotericin B nephrotoxicity in humans decreased by salt repletion. Am J Med 1983; 75: 476-481.
109. Mayer J, Doubek M, Vorlicek J. Must we really fear toxicity of conventional amphotericin B in oncological patients ? Support Care Cancer 1999; 7(1): 51-55.
110. Chavanet PY, Garry I, Charlier N, Caillot D, Kisterman JP, D'Athis M, Portier H. Trial of glucose versus fat emulsion in preparation of amphotericin for use in HIV infected patients with candidiasis. Br Med J 1992; 305: 921-925.
111. Lopez-Berestein G, Fainstein V, Hopfer R, Mehta K, Sullivan MP, Keating M, Rosenblum MG, Mehta R, Luna M, Hersh EM, et al. Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study. J Infect Dis 1985; 151: 704-710.
112. Gates C, Pinney RJ. Amphotericin B and its delivery by liposomal and lipid formulations. J Clin Pharmacy Ther 1993; 18: 147-153.
113. Janknegt R, de Marie S, Bakker-Wondenberg IAJM, Crommelin DJA. Liposomal and lipid formulations of amphotericin B. Clinical pharmacokinetics. Clin Pharmacokinet 1992; 23: 279-291.
114. Gregoriadis G. Overview of liposomes. J Antimicrob Chemother 1991; 28: 39-48.
115. Joly V, Saint-Julien L, Carbon C, Yeni P. Interaction of free and liposomal amphotericin B with renal proximal tubular cells in primary culture. J Pharmacol Exp Ther 1990; 255: 17-22.
116. Colette C, Van der Auwerz P, Lopez P, Heymans C, Meunier F. Tissue concentrations and bioactivity of amphotericin B in cancer patients treated with amphotericin B-deoxycholate. Antimicrob Agents Chemother 1989; 33: 362-368.
117. Payne NI, Cosgrove RF, Green AP, Liu L. *In-vivo* studies of amphotericin B liposomes derived from proliposomes: effect of formulation on toxicity and tissue disposition of the drug in mice. J Pharm Pharmacol 1987; 39: 24-28.
118. Szoka FC, Milholland D, Barza M. Effect of lipid composition and liposome size on toxicity and *in vitro* fungicidal activity of liposome-intercalated amphotericin B. Antimicrob Agents Chemother 1987; 31: 421-429.
119. Juliano RL, Grant CWM, Barber KR, Kalp MA. Mechanism of the selective toxicity of amphotericin B incorporated into liposomes. Molecular Pharmacology 1987; 31: 1-11.
120. Clemons KV, Stevens DA. Comparative efficacy of amphotericin B colloidal dispersion and amphotericin B deoxycholate suspension in treatment of murine coccidiodomycosis. Antimicrob Agents Chemother 1991; 35: 1829-1833.
121. Hostetler JS, Clemons KV, Hanson LH, Stevens DA. Efficacy and safety of amphotericin B colloidal dispersion compared with those of amphotericin B deoxycholate suspension for treatment of disseminated murine cryptococcosis. Antimicrobial Agents Chemother 1992; 36: 2656-2660.

122. Proffitt RT, Satorius A, Chiang SM, Sullivan L, Adler-Moore JP. Pharmacology and toxicology of a liposomal preparation of amphotericin B (AmBisome) in rodents. J Antimicrob Chemother 1991; 28: 49-61.
123. Ralph ED, Khazindar AM, Barber KR, Grant CW. Comparative *in vitro* effects of liposomal amphotericin B, amphotericin B-deoxycholate and free amphotericin B against fungal strains determined by using MIC and minimal lethal concentration susceptibility studies and time-kill curves. Antimicrob Agents Chemother 1991; 35: 188-191.
124. Anaissie E, Paetznick V, Proffitt R, Adler-Moore J, Bodey GP. Comparison of the *in vitro* antifungal activity of free and liposome-encapsulated amphotericin B. Eur J Clin Microbiol Infect Dis 1991; 10: 665-668.
125. Krause HJ, Juliano RL. Interaction of liposome-incorporated amphotericin B with kidney epithelial cell structures. Mol Pharmacol 1987; 34: 287-297.
126. Joly V, Dromer F, Barge J, Yeni P, Seta N, Molas G, Carbon C. Incorporation of amphotericin B (AMB) into liposomes alter AMB-induced acute nephrotoxicity in rabbits. J Pharmacol Exp Ther 1989; 251: 311-316.
127. Longuet P, Joly V, Amirault P, Seta N, Carbon C, Yeni P. Limited protection by small unilamellar liposomes against the renal tubular toxicity induced by repeated amphotericin B infusion in rats. Antimicrob Agents Chemother 1991; 35: 1303-1308.
128. Gondal JA, Swartz RP, Rahman A. Therapeutic evaluation of free and liposome-encapsulated amphotericin B in the treatment of systemic candidiasis in mice. Antimicrob Agents Chemother 1989; 33: 1544-1548.
129. Lopez-Berestein G, Mehta R, Hopfer RL, Mills K, Kasi L, Mehta K, Fainstein V, Luna M, Hersh EM, Juliano R. Treatment and prophylaxis of disseminated infection due to Candida albicans in mice with liposome-encapsulated amphotericin B. J Infect Dis 1983; 147: 939-944.
130. Lopez-Berestein G, Bodey GP, Frankel LS, Mehta K. Treatment of hepatosplenic candidiasis with liposomal-amphotericin B. J Clin Oncol 1987; 5: 310-317.
131. Lopez-Berestein G. Liposomes as carriers of antifungal drugs. Ann N Y Acad Sci 1988; 544: 590-597.
132. Lopez-Berestein G, Bodey GP, Fainstein V, Keating M, Frankel LS, Zeluff B, et al. Treatment of systemic fungal infections with liposomal amphotericin B. Arch Intern Med 1989; 149: 2533-2536.
133. Meunier F, Sculier JP, Coune A, Brassinne C, Heyman C, Laduron C, Collette N, Hollaert C, Bron D, Klastersky J. Amphotericin B encapsulated in liposomes administered to cancer patients. Ann NY Acad Sci 1988; 544: 598-610.
134. Llanos-Cuentas A, Chang J, Cieza J, Echevarria J, Garcia P, Lentnek A. Safety and tolerance of amphotericin B lipid complex vs Fungizone in patients with mucocutaneous leishmaniasis. Antimicrob Agents Chemother 1990 (Abstract).
135. Meunier F, Prentice HG, Ringden O. Liposomal amphotericin B (AmBisome): safety data from a Phase II/III clinical trial. J Antimicrob Chemother 1991; 28: 83-91.
136. Ringden O, Meunier F, Tollemar J, Ricci P, Tura S, Kuse E. Efficacy of amphotericin B encapsulated in liposomes (AmBisome) in the treatment of invasive fungal infections in immunocompromised patients. J Antimicrob Chemother 1991; 28: 73-82.
137. Taylor R, Williams DM, Craven PC, Graybill JR, Drutz DJ, Magee WE. Amphotericin B in liposomes: a novel therapy for histoplasmosis. Am Rev Respir Dis 1982; 125: 610-611.
138. Meunier F. New methods for delivery of antifungal agents. Rev Infect Dis 1989; 11: S1605-S1612.
139. Graybill JR, Craven PC, Taylor RL, Williams DM, Magee WE. Treatment of murine cryptococcosis with liposome-associated amphotericin B. J Infect Dis 1982; 145: 748-752.
140. Wasan KM, Brazeau GA, Keyhani A, Hayman AC, Lopez-Berestein G. Roles of liposome composition and temperature in distribution of amphotericin B in serum lipoproteins. Antimicrob Agents Chemother 1993; 37: 246-250.
141. Wasan KM, Rosenblum MG, Cheung L, Lopez-Berestein G. Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. Antimicrob Agents Chemother 1994; 38: 223-227.
142. Wasan KM, Lopez-Berestein G. Modification of amphotericin B's therapeutic index by increasing its association with serum high-density lipoproteins. Annals NY Acad Sci 1994; 730:93-106
143. Olsen SJ, Swerdel MR, Blue B, Clark JM, Bonner DP. Tissue distribution of amphotericin B lipid complex in laboratory animals. J Pharm Pharmacol 1991; 43: 831-835.
144. Poste G. Liposome targetting *in vivo*: problems and opportunities. Biol Cell 1983; 47: 19-37.
145. Carriagan C, Hanf-Kristufek L. Comparison of amphotericin B products. BMJ 2001;322(7286):579.
146. Wingard J, White M, Anaissie E, et al. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. L Amph/ABLC Comparative Study Group. Clin Infect Dis 2000; 31: 1155-1163.
147. Winston DJ, Schiller GJ. Controlled trials of amphotericin B lipid complex and other lipid-associated formulations (letter). Clin Infect Dis 2000; 30:2367.
148. Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, Pappas P, Seibel N, Greenberg RN, Dummer S, Schuster M, Holcenberg JS, Dismukes WE. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. N Engl J Med 1999; 340: 764-771.

149. Walsh TJ, Pappas P, Winston DJ, Lazarus HM, Petersen F, Raffalli J, Yanovich S, Stiff P, Greenberg R, Donowitz G, Lee J. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. N Engl J Med 2002; 346: 225-234.
150. Prentice HG, Hann IM, Herbrecht R, Aoun M, Kvaloy S, Catovsky D, Pinkerton CR, Schey SA, Jacobs F, Oakhill A, Stevens RF, Darbyshire PJ, Gibson BES. A randomized comparison of liposomal versus conventional amphotericin B for the treatment of pyrexia of unknown origin in neutropenic patients. Br J Haematology 1997; 98: 711-718.
151. UPMC Pharmacy and Therapeutics Committee Formulary Review. 1997.
152. Cagnoni PJ, Walsh TJ, Prendergast MM, Bodensteiner D, Hiemenz S, Greenberg RN, Arndt CA, Schuster M, Seibel N, Yeldani V, Tong KB. Pharmacoeconomic analysis of liposomal amphotericin B versus conventional amphotericin B in the empirical treatment of persistent febrile neutropenic patients. J Clin Oncol 2000;18:2476-2483.
152a. Harbarth S, Burke JP, Lloyd JF, Evans RS, Pestotnik SL, Samore MH. Clinical and economic outcomes of conventional amphotericin B associated nephrotoxicity. Clin Infect Dis 2002; 35: e120-e127.
153. Gerkens JF, Bhagwandeen SB, Dosen PJ, Smith AJ. The effect of salt intake on cyclosporine-induced impairment of renal function in rats. Transplantation 1984;38:412-417.
154. Smith SR, Galloway MJ, Reilly JT, Davies JM. Amiloride prevents amphotericin B related hypokalemia in neutropenic patients. J Clin Pathol 1988;41:494-497.
155. Ugur UA, Avcu F, Cetin T, Beyan C, Kaptan K, Nazaroglu NK, Yalcin A. Spironolactone: is it a novel drug for the prevention of amphotericin B related hypokalemia in cancer patients? Eur J Clin Pharmacol 2002; 57: 771-773.

11 Sulfonamides, sulfadiazine, trimethoprim-sulfamethoxazole, pentamidine, pyrimethamine, dapsone, quinolones

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Introduction

A number of drugs of miscellaneous class are capable of producing various degrees of renal damage and will be reviewed in this Chapter. Some have been used extensively in the past for the treatment of general infections (sulfonamides), others have had specific indications (pentamidine, dapsone), and others such as quinolones are of more recent application. Many of these, however,are of current interest because of their use in treating the complications occurring in patients with acquired immunodeficiency syndrome (AIDS).

Sulfonamides

These compounds were extensively used in the 40's through the 60's to treat pulmonary and other systemic infections. Reports of acute renal failure, most secondary to crystalluria were common [1-3]. Rarely, the sulfonamides can cause acute interstitial nephritis, necrotizing arteritis or hemoglobinuric acute renal failure due to massive acute hemolytic anemia [4, 6].

Data from the decade of 1940-1950 reviewed by Simon *et al* [7] in 1990 indicate an incidence of crystalluria of 0.4 to 49%, hematuria (with or without flank pain) of 1 to 32%, oliguria, anuria, or azotemia of 0.4 to

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29%, and renal stones of 0.4 to 20%, for an overall incidence of renal toxicity (excluding crystals) between 1 and 32%. For a number of reasons detailed elsewhere [7], these early data are difficult to assess. However, even with the use of preventive measures such as urine alkalinization, renal toxicity was 2% [7], and the incidence of gross hematuria and microscopic hematuria despite high fluid intake were 2-3% and 24%, respectively [8]. A major limiting factor in the use of the sulfonamides was their limited solubility and the need of relatively large dosage, both favoring crystallization. This complication of sulfonamide therapy was well known at the time. In the ensuing decades more soluble sulfa compounds became available and the appearance of effective antibiotics resulted, with few exceptions, (i.e., sulfadiazine), in the replacement of the older sulfas as chemotherapeutic agents. Thus, the notion of the possibility of sulfonamide-induced nephrotoxicity was somewhat lost or became a rarity. The emerging use over the last 15 years of two sulfa compounds, sulfadiazine and trimethoprim-sulfamethoxazole, in the treatment of opportunistic infections of AIDS, has again brought to the attention of nephrologists and physicians the "old and perhaps forgotten" problem of sulfa nephrotoxicity. The review of the potential nephrotoxicity of sulfasalazine (5-aminosalicylic acid and sulfapyridine), a drug extensively used in patients with inflammatory bowel disease can be found in Chapter 13. Aside from the renal failure associated with sulfasalazine attributed initially to the intratubular precipitation of sulfapyridine crystals [9], currently sulfasalazine toxicity is considered secondary to its 5-aminosalicylic moiety.

Sulfadiazine

A large bibliography exists from the 1940's related to crystalluria and acute renal failure associated with the use of sulfadiazine [1, 10, 11]. More recently, the number of reports has increased substantially because of the use of sulfadiazine and pyrimethamine, as the treatment of choice for cerebral toxoplasmosis associated with AIDS, other immunosuppressive states or specific infections [7, 12-34].

It is apparent that most recent cases of sulfadiazine-induced nephrotoxicity are not reported, thus the current incidence of sulfadiazine nephrotoxicity is unknown. In 1987, a study of 57 patients with AIDS treated for toxoplasma encephalitis indicated a renal toxicity of 6% [26]. A more recent international bibliographic search (1987 to 1995) reported 35 patients with AIDS and toxoplasma encephalitis with sulfadiazine nephrotoxicity [27]. These patients were compared to those who received sulfadiazine in the 1940's and 1950's. The prevalence of sulfadiazine nephrotoxicity was 1.9 to 7.5% in the AIDS group and 1 to 4% in the non-AIDS patients. On average, renal dysfunction was evident after three weeks of treatment in the AIDS patients who received the larger cumulative dose of sulfadiazine (84 g) compared to the control group (40 g). Renal densities or stones were found by ultrasonography in 77% of the AIDS patients. The majority of the patients recovered rapidly (median of 6 days) with appropriate treatment.

Sulfadiazine is a short-acting sulfonamide derivative that undergoes acetylation in the liver to a variable degree (10-40%). The acetylation product has no antibacterial activity but retains its toxic potential. Indeed, the acetylated forms of older sulfas are less soluble and thus, more prone to crystalluria. About 30 to 55% of the drug is protein bound, and the binding decreases in renal failure. The kidney is the major route of excretion. Both, the free (60-85%) and acetylated (15-40%) forms are rapidly excreted in the urine in high concentration. Alkalinization increases the excretion of both forms by diminishing their tubular reabsorption. Because of the rapid excretion, large doses are required for the treatment of toxoplasmosis: 1 to 1.5 g every 6 hours if renal function is normal; reduction in dosage is necessary if renal function is impaired [25].

Sulfadiazine, like other sulfas, has a low urinary solubility, particularly in acid urine. When the urine is alkalinized and pH rises above 7.15, the drug ionizes and forms a soluble salt that is excreted avoiding crystallization. It has been estimated that at a pH of 5.5 about 16 liters of urine will be needed to insure that the sulfadiazine is soluble when excreted following a dose of 4 g per day [5]. Indeed, the urinary solubility of sulfadiazine and its major metabolite, acetylsulfadiazine, are many times higher at a pH of 7.5 than at a pH of 6.5 (sulfadiazine 200 and 28 mg/dl, acetylsulfadiazine 512 and 75 mg/dl, respectively) [29]. The crystals of sulfadiazine and acetylsulfadiazine can be recognized by examining the urine sediment, where they resemble characteristic "sheaves of wheat" [3]. As the crystals transit through the tubular lumen they cause

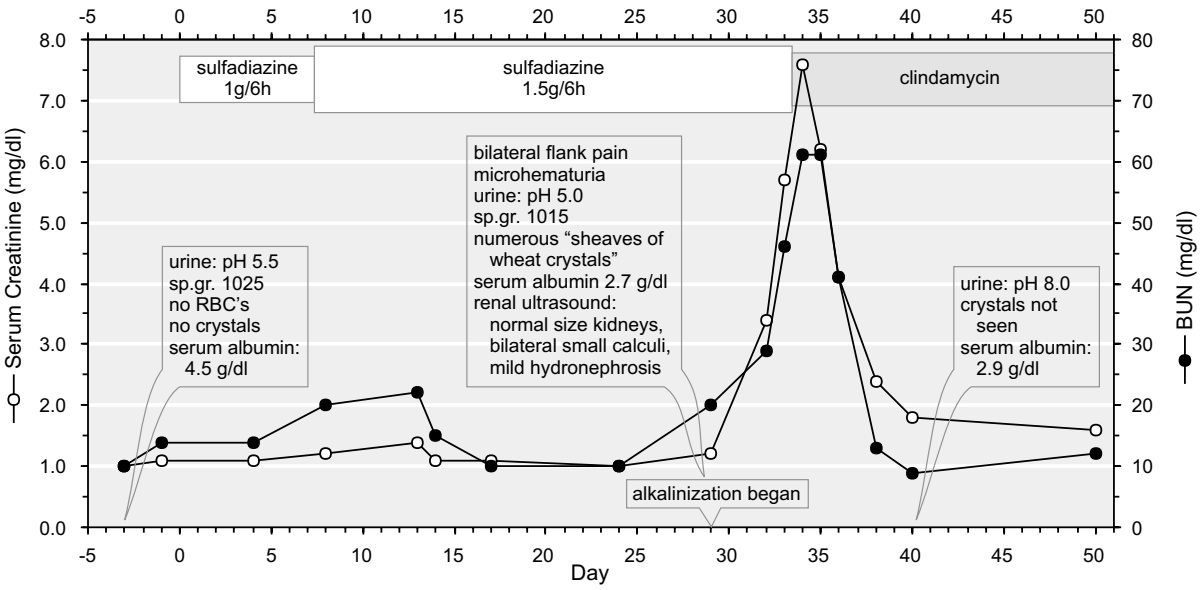

***Figure 1**. Sulfadiazine nephrotoxicity (crystalluria and acute renal failure). 35 year old man with AIDS and cerebral toxoplasmosis treated for 33 days with 4-6 g/day of sulfadiazine.The patient received oral hydration and possibly had an episode of transient renal impairment during days 8-13. By day 29 of treatment, crystalluria, hematuria, flank pain, renal calculi, and acute renal failure developed. Urine was alkalinized late in the course.*

local abrasion and chemical irritation of the collecting duct epithelium and the lining of the urinary tract. There is peritubular hemorrhage, infiltration by white cells, necrosis, and calcium deposition. The crystals can form local concretions or small calculi leading to obstruction at any level from the collecting ducts to the bladder. This explains the clinical manifestations of the crystalluria and its associated renal pathology: asymptomatic crystalluria, microhematuria, gross hematuria, renal colic, oliguria, acute urinary tract obstruction, and acute renal failure. An example of reversible sulfadiazine nephrotoxicity in a patient with AIDS and toxoplasma encephalitis is illustrated in Figure 1.

The risk factors for sulfadiazine nephrotoxicity in patients with AIDS include: (a) more prolonged courses of therapy as compared to those for community-acquired infections in normal hosts; (b) difficulty in maintaining high oral fluid intake in patients with toxoplasma encephalitis because of chronic illness, anorexia, and altered mental status; (c) concurrent fluid loses due to diarrhea; (d) levels of plasma creatinine within the range of "normal" despite impaired renal function due to AIDS-associated muscle wasting, thus masking renal insufficiency; (e) the presence of hypoalbuminemia and competition for the albumin binding sites of other antibiotics concomitantly administered, increasing the concentration of free-drug and the risk of crystalluria; and (f) the possibility of preexisting AIDS or non AIDS-related renal disease. It should be remembered that in patients with severe renal failure the serum half-life of sulfadiazine is prolonged from a normal value of 8 to 17 hours to 22 to 34 hours resulting in a marked decrease in the active form of the drug [35]. Dosing recommendations for patients with renal failure can be found in recent reviews [36, 37].

The appearance of microhematuria or gross hematuria in a patient receiving sulfadiazine should raise the suspicion of crystalluria. The recognition of classical sulfadiazine crystals in the urine sediment does not confirm renal toxicity; but this finding should increase concern regarding its impending appearance. Unfortunately urinalyses are not done regularly in these patients; therefore, the possibility of missing early microhematuria associated with sulfa crystals is real. Sulfadiazine stones are radiolucent and nicely demonstrated by ultrasonography [11, 15-17, 27, 28, 31-33]. The renal ultrasound findings include hyperechoic foci in the renal parenchyma, echogenic material in dilated and non-dilated calyces as well as dilatation of calyces and pelvis [28, 28a]. Obstruction is usually accompa-

nied by little or no hydronephrosis. Acute renal failure secondary to sulfadiazine crystalluria has been also reported in a renal transplant patient [38].

Prevention of sulfadiazine nephrotoxicity is centered in minimizing crystalluria. This can be accomplished with a high fluid intake (up to 3 liters per day). This may increase solubility of the crystals up to threefold. Nonetheless, continued alkalinization of the urine with sodium bicarbonate (6 to 12 g/day), assuring that the urine pH is 7.5 or higher, can increase solubility several fold and is very effective. As always, awareness of the possibility of this complication is the best form of prevention.

Treatment consists in stopping sulfadiazine or decreasing its dosage. The acute renal failure, however, may resolve despite continuation of the treatment [39]. Hydration and especially alkalinization are the basis for the treatment. Urinary tract obstruction may require placement of ureteral stents [13], or nephrostomy [32]. This complication is essentially reversible and dialysis is rarely needed [27].

Acute interstitial nephritis has been rarely reported in association with sulfadiazine therapy [40].

With increasing frequency, clindamycin in combination with pyrimethamine is been used as the replacement drug for sulfadiazine in the treatment of cerebral toxoplasmosis. Perhaps this new combination again may send sulfadiazine nephrotoxicity into "oblivion". Nevertheless, until this possibility occurs, primary care physicians should be aware that sulfadiazine can cause renal toxicity and that effective preventive measures are available.

Trimethoprim-sulfamethoxazole (cotrimoxazole)

The synergistic combination of trimethoprim (TMP), and sulfamethoxazole (SMZ), both folic acid antagonist antibacterial agents, was introduced over 25 years ago for its effect against a variety of infective organisms, including *Pneumocystis carinii* (PC). Prior to the AIDS era, TMP-SMZ, also referred to as cotrimoxazole, was used predominantly for the treatment of respiratory and urinary tract infections, including PC pneumonia (PCP) [40-45].

The fixed 1:5 TMP-SMZ ratio used in both the oral and intravenous preparations was chosen because it provides peak serum levels of about 1:20, which are optimally synergistic *in vitro* against susceptible pathogens [35]. TMP is 45% protein bound, whereas SMZ is 60% bound. In patients with normal renal function, the half-lifes are similar for both compounds- about 8 to 12 hours [45]. At least 50% of TMP is excreted as an unchanged form in the urine, while only 20% of SMZ is active in the urine. Both drugs undergo predominant hepatic metabolism. This compound penetrates body fluids and tissues well.

The kidney handles the two components of TMP-SMZ differently: TMP (a weak base with pKa of 7.3) is transported across the tubules by nonionic diffusion and is secreted into the urine [46], whereas SMZ undergoes glomerular filtration and tubular reabsorption [47]. Trimethoprim dissociation increases as pH falls. The unchanged base is more lipid-soluble and crosses cell membranes more readily than cations. Accordingly, TMP base diffuses passively from the peritubular fluid (pH 7.4) into the acidic urine via the tubular epithelium, and, thus, is excreted [46]. Their renal tubular accumulation is also different: In the rat [47], TMP, given alone [47, 48] or in combination with SMZ [47] achieves concentrations that are several folds greater in the renal cortex than in the serum or medium [49]. In contrast, the renal tissue concentration of SMZ is much less; SMZ concentration, when the agent is administered either alone or in combination with TMP is lower in the cortex, medulla and papilla than in the serum. Similar results were obtained in rhesus monkeys [50]. Trottier *et al* [47] have suggested that the "trapping" of TMP, in the acid tubular fluid environment, contributes to the high renal tissue accumulation of TMP. In contrast, SMZ, a weak acid with a pKa of 5.6 undergoes tubular reabsorption. Furthermore, the tissue concentrations and interactions of TMP and SMZ cannot be ascertained from their respective serum and urine levels [47]. Sulfamethoxazole is more soluble than older sulfonamides, and used at a lower dosage, resulting in a likelihood of crystalluria that is much smaller than with sulfadiazine and older sulfas.

It has been suggested by Berglund *et al* [51] that TMP interferes with the tubular secretion of creatinine resulting in mild increases in its serum concentration and decreases in the Ccr, whereas the glomerular filtration rate simultaneously measured with I^{131} iothalamate remains unchanged. This view was challenged by Shouval *et al* [52] who using the true creatinine method (Hare and Hare) for measuring Ccr found mild

decreases in Ccr in normal subjects. Nevertheless, there is convincing evidence to support the contention that the mild increase (about 15-20%) in serum creatinine (and the corresponding decrease in Ccr) reported in patients with normal renal function receiving TMP-SMZ is due to TMP interference with the tubular secretion of creatinine, as shown by simultaneous measurements of GFR by Ccr and independent methods (inulin, I^{131} iothalamate, and ^{51}Cr-EDTA) [51, 53-55]. Berglund *et al* [41] also suggested that the effects of TMP resulted from organic base inhibition of creatinine secretion. There was no evidence that SMZ affects creatinine transport [41]. Furthermore, *in vitro* studies in the rat strengthened the hypothesis that TMP inhibits creatinine secretion via the organic cation transport system [56]. The effect of TMP on serum creatinine is more pronounced in patients with decreased renal function [57, 58].

The dosage of TMP-SMZ should be reduced in patients with renal failure because the half-life of TMP becomes prolonged when GFR decreases below 30 ml/min [59], and in addition, there may be accumulation of the metabolite N-acetyl-SMZ [59, 60]. The latter may be associated with rare hypersensitivity reactions [61] or crystallization [62]. Dosing recommendations for patients with impaired renal function can be found in recent reviews [36, 37]. Because of effective removal of TMP-SMZ in patients with end-stage renal disease during hemodialysis, 50% of its maintenance dose should be supplemented after each dialysis [63]. Overall, it appears that TMP-SMZ can be given safely to patients with reduced renal function provided that the dosage is carefully reduced. Most problems arise when TMP-SMZ is given at its usual or larger dosage to patients with decreased function (serum creatinine above 2 mg/dl [57]), due to preexisting renal disease, presence of severe dehydration, or in association with other nephrotoxins [51, 64].

The clinical evaluation of the nephrotoxic effects of TMP-SMZ, notably much less frequent than other side-effects of the drug (skin, gastrointestinal) and very rarely of a serious nature (hematological, dermatological), requires keeping in mind the already described effects of TMP on serum creatinine. In practical terms, an increase in serum creatinine concentration not accompanied by increases in blood urea nitrogen concentration or other data supporting a decrease in GFR, does not indicate renal dysfunction.

In view of the extensive use of this compound around the world for the treatment of urinary tract infections, renal adverse reactions are extremely rare. Indeed, in 1982 the Boston Collaborative Drug Surveillance Program reporting on data obtained from 1966 through 1980 on 1121 hospitalized patients receiving TMP-SMZ described an overall incidence of adverse effects of 8% [65]. The most common were gastrointestinal (3.9%), and dermatological (3.3%). Four patients with elevations of serum creatinine (0.4%), and one patient with transient renal tubular acidosis were reported. Unfortunately, given the nature of this study (many hospitals, many physicians reporting) the exact causal role, rarely could be established with certainty. Nevertheless, this report emphasizes the extreme rarity of the association of renal toxicity with TMP-SMZ therapy.

The risk of developing serious renal toxicity in people receiving TMP-SMZ, TMP alone, or cephalexin was recently estimated in a large British population, and found to be extremely low [66]. Only five cases of acute parenchymal renal disease occurred in the almost 700,000 subjects evaluated, suggesting that none was likely to be caused by the study drugs. Nonetheless, since in these patients TMP-SMZ manifests considerable extrarenal toxicity, reduction of dosage according to measured blood levels should be considered in patients with impaired renal function.

In accordance with the better urinary solubility of SMZ, in comparison to older sulfas or sulfadiazine, reports of renal dysfunction secondary to crystalluria are extremely rare [67-69]. Even this theoretical risk inherited from the experience with older sulfas and sulfadiazine, can be avoided by providing adequate hydration.

When the drug is given intravenously a potential problem due to fluid volume load may arise. Because TMP-SMZ is relatively unstable in solution, it is the recommendation of the manufacturers that each ampule of TMP-SMZ (80 mg of TMP and 400 mg of SMZ) be dissolved in 75 to 125 ml of 5% dextrose in water. This relatively large water load may lead to hyponatremia, particularly in predisposed patients, such as those with impaired renal function, borderline cardiorespiratory status, or AIDS with increased AVP levels [69, 70]. The use of a smaller volume (50 ml) of isotonic sodium chloride solution as diluent for TMP-SMZ should mitigate this potential problem [71].

Non-oliguric acute tubular necrosis associated with interstitial edema and cellular infiltration was described in two patients treated with TMP-SMZ reported by Kalowski *et al* [57]. The same group [72] reported four patients with underlying renal disease and a hypersensitivity rash who developed acute renal failure when treated with TMP-SMZ. Two patients died, and in two, the renal biopsy showed acute interstitial nephritis with prominent eosinophilic infiltrates. Recurrent acute renal failure secondary to acute interstitial nephritis with mononuclear cell and eosinophilic infiltrates was described in a patient treated with TMP-SMZ but also receiving penicillin-type drugs and gentamicin [64]. Other cases of acute interstitial nephritis have been reported, including those presenting in children [73-76] and in renal transplant recipients [77]. It should be noted, that many of the patients described with renal impairment were elderly [57], had preexisting renal dysfunction, were receiving other drugs with nephrotoxic potential or received large doses, or doses inappropriate for the level of renal function. As noted, before the AIDS era, TMP-SMZ was considered a safe drug, even when administered for prolonged periods [42], or in patients with renal impairment if the daily dose is appropriately adjusted [43, 78, 79].

Rare life-threatening multisystemic reactions to TMP-SMZ with severe skin lesions and progressive renal, hepatic, and cardiac damage appearing immediately or weeks after the drug was discontinued have been described [80, 81]. In these patients the renal lesion was interstitial nephritis, and thus, the clinical picture may represent an extension of the more limited forms of hypersensitivity reactions. These severe lesions have been tentatively attributed to an inherent defect in mechanisms normally responsible for inactivating or detoxifying sulfonamide metabolites (i.e., hydroxylamine metabolite of SMZ), resulting in both direct cytotoxicity and an immune hypersensitivity reaction [82].

There are two groups of patients that currently are of particular interest to the nephrologists with respect to potential TMP-SMZ nephrotoxicity: transplant recipients and patients with AIDS. In these two groups, TMP-SMZ is usually used either for the treatment of infections or for the long-term prophylaxis against opportunistic infections.

As noted before, renal biopsy documented acute interstitial nephritis has been described in few transplant patients treated with TMP-SMZ prior to the cyclosporine era, usually for urinary tract infection or PC pneumonia [40, 57, 77]. Reports of synergistic renal toxicity between TMP-SMZ and cyclosporine also appeared [83-85, 85a]. Thompson and co-workers [83] reported that six transplant recipients receiving cyclosporine developed a marked impairment in renal function when treated with TMP or TMP-SMZ, the majority for asymptomatic bacteremia. Renal dysfunction reversed with discontinuation of the sulfa compound. A graft renal biopsy was performed in one patient, and revealed mild focal mononuclear cell infiltration in the interstitium. Five patients received TMP alone, including the one who was biopsied. In another case series, Josephson and co-workers reported five renal transplant recipients with acute renal allograft dysfunction or delayed allograft function in whom the renal biopsies showed histopathologic features of drug-induced interstitial nephritis without evidence of acute rejection, calcineurin inhibitor nephrotoxicity, or both. All the patients were receiving TMP-SMZ and other drugs associated with acute interstitial nephritis [86]. The renal biopsy findings in these patients revealed focal interstitial infiltrates of primarily mononuclear inflammatory cells with prominent involvement at the corticomedullary junction and clusters of eosinophils within the infiltrates, typical features of drug-induced acute interstitial nephritis [87].

A prospective randomized-double-blind study of prophylaxis of infections with oral TMP-SMZ in renal transplant recipients concluded that long-term prophylaxis (average 8.9 months) conferred significant protection against infection after transplantation [88]. The sulfa compound was very well tolerated by the 66 patients randomized to receive the drug, and no patient developed hypersensitivity reactions, perhaps due to the concomitant immunosuppressive therapy. Serum creatinine levels were 15% higher in the patients receiving TMP-SMZ, whereas no such increase was observed in the control group. Nevertheless, GFR, measured with ^{99m}Tc-DTPA showed no change in a crossover study performed in 17 of the patients studied with TMP-SMZ. Furthermore, the authors demonstrated that the differences in serum creatinine were not due to interference of TMP-SMZ, cyclosporine or both with the method used for the measurement of creatinine (automated Jaffe reaction). Moreover, TMP-SMZ did not influence the pharmacokinetics of cyclosporine or

result in decreased immunosuppression or increased incidence of rejection. Sulfamethoxazole may interfere with the measurement of cyclosporine by high-pressure liquid chromatography but not by radioimmunoassay [88] resulting in higher levels [89]. On the basis of their experience, the authors cautioned about the reports of putative toxicity in renal transplant patients receiving oral TMP-SMZ therapy simultaneously with cyclosporine. The prophylactic treatment was effective, safe, and cost-effective [88]. The effect of larger intravenous dosage has not been studied.

The evaluation of the possible renal toxicity of TMP-SMZ or other drugs in patients with AIDS is compounded by the many factors that can cause renal dysfunction in these patients. These include preexisting renal disease, including AIDS-related nephropathy [90-93], frequent dehydration or marked hypoalbuminemia resulting in severe volume contraction, and the concomitant or sequential use of drugs with known nephrotoxic potential. On the other hand, severe muscular wasting, by decreasing the body pool of creatinine, results in lower serum creatinine levels, thereby masking the presence of renal impairment. Of interest is the observation that the incidence of hypersensitivity reactions to drugs might be less in AIDS patients due to their immunodeficient state [88].

Prior to the recognition of AIDS as a major health problem, immunosuppressed patients with PCP (children with immune deficiency disorders or patients receiving cytotoxic or immunosuppressive drugs for lymphoreticular malignancies or organ transplantation) were treated with TMP-SMZ and/or pentamidine with varied success, depending in great measure on the underlying condition [94, 95]. Currently, in most patients with AIDS, TMP-SMZ represents the treatment of choice for PCP. Although, an increase in the incidence of side-effects attributable to TMP-SMZ —particularly dermatologic, hematologic, and hepatic toxicities —has been recognized in patients with AIDS when compared to patients without AIDS, no increase in nephrotoxicity was reported [96, 97].

When compared to pentamidine, TMP-SMZ has been associated with a lesser degree of renal impairment in the treatment of opportunistic infections in AIDS. In a prospective, randomized study of patients with AIDS, Wharton *et al* [98] reported major adverse renal reactions characterized by an increase in serum creatinine of ≥ 3.0 mg/dl in 1 of 32 patients treated with intravenous TMP-SMZ in comparison to 2 of 32 patients receiving pentamidine. Lesser increases in serum creatinine (levels between 1.5 to 3.0 mg/dl) occurred in 11 of 32 (34%) and 19 of 32 (59%), respectively. Others, however, found a greater degree of renal impairment with pentamidine than with TMP-SMZ. Sattler *et al* [99] in a prospective, randomized noncrossover study found elevations of serum creatinine in 21 of 34 (64%) patients receiving intravenous pentamidine for about 16 days. The average increase in creatinine above baseline (1.0±0.1 mg/dl) was 1.6±1.1 mg/dl; in four patients the peak serum creatinine ranged from 4.1 to 6.6 mg/dl. By contrast, in the 36 patients receiving TMP-SMZ, creatinine concentration increased only in five (14%), a value significantly different from that of the pentamidine group ($p < 0.0001$). The known effect of TMP on the tubular secretion of creatinine may have been in part responsible for the latter changes. Gordin *et al* [100] in a retrospective study reported no renal abnormalities with TMP/SMZ in 37 patients, whereas elevations in serum creatinine occurred in 6 of 30 patients treated with pentamidine. Overall, however, the incidence of non-renal side effects was higher with TMP-SMZ.

We retrospectively evaluated renal function in 38 patients (mean age 38±2 years) [101] with AIDS and PCP who were treated with intravenous TMP-SMZ for 5 to 24 days (average of 10±1 days). The dose of TMP was 19±2 (SE) and that of SMZ 95±5 mg/kg day. Risk factors for nephrotoxicity were identified: volume depletion (47%), preexisting renal dysfunction (11%), sepsis (13%), and concomitant use of known nephrotoxic agents (18%). Nephrotoxicity was defined as an increase in serum creatinine concentration of at least 0.5 mg/dl above baseline. In only three patients did serum creatinine increase above baseline. In two of these, other risk factors for nephrotoxicity were present (volume depletion, gentamicin, and amphotericin B). Thus, in only one of 38 patients (2.6%) was the renal dysfunction attributable to TMP-SMZ. Estimated creatinine clearance in 22 patients (Cockcroft and Gault formula) remained stable: at baseline, 107±10 ml/min; at peak change, 106±11 ml/min, and at end of treatment 101±9 ml/min. In four patients who have elevated serum creatinine levels at baseline, the values declined toward normal despite continuing TMP-SMZ administration (Figure 2). There was no relationship between the duration of treatment and the change in serum crea-

tinine. From these data we concluded that TMP-SMZ is a safe drug for the treatment of PCP in patients with AIDS. Even in the presence of compounding risk factors for nephrotoxicity, the risk was very low.

These findings are in accordance with our studies in the volume depleted female Sprague-Dawley rat [102]. Rats were injected intramuscularly with five times the human dose of TMP-SMZ (100/500 mg/kg/day) for nine days. Prior to treatment, the animals were placed in a low sodium diet for seven days and salt depleted by means of administration of furosemide (2 mg/kg/day) for the first three days. At baseline, experimental and control (glucose given instead of TMP-SMZ) groups have similar GFR, serum creatinine, and hematocrit and were conserving sodium maximally. Nine days of TMP-SMZ did not affect GFR, serum creatinine or electrolyte levels. Loss of body weight and anemia only developed in the rats treated with TMP-SMZ. In this study performed in female rats, known to have a lower tubular secretion of creatinine [103, 104], TMP did not appear to decrease the tubular secretion of creatinine.

In HIV-infected patients, nephrotoxicity from oral TMP-SMZ in long-term prophylaxis against opportunistic infections is not seen or is notably much less frequent than other side effects of the drug (dermatological, gastrointestinal, and hematological). Bozzette *et al* [105] in a randomized trial reported no renal abnormalities with TMP-SMZ in 276 patients with a total 690 person-years of follow-up. Similarly, in another recent randomized trial of TMP-SMZ used as primary prophylaxis for PCP, Para *et al* [106] did not report renal abnormalities, however, 33% of study subjects discontinued TMP-SMZ due to non-renal-limiting adverse effects. Most of these treatment-limiting reactions occurred within the first four weeks of beginning therapy, and the gradual initiation of TMP-SMZ was associated with significantly fewer adverse drug reactions.

The use of TMP-SMZ has been recently associated with the appearance of *hyperkalemia*. The patients either had AIDS and were receiving large doses of TMP-SMZ for the treatment of PCP [107-109] or were elderly subjects without either AIDS or PCP treated for respiratory or urinary tract infections with standard doses [110, 110a, 111]. The hyperkalemia is mild (rarely exceeding 6.5 mEq/L) and reversible on discontinuation of the drug. It appears that in patients without AIDS, hyperkalemia must be very rare since no case of

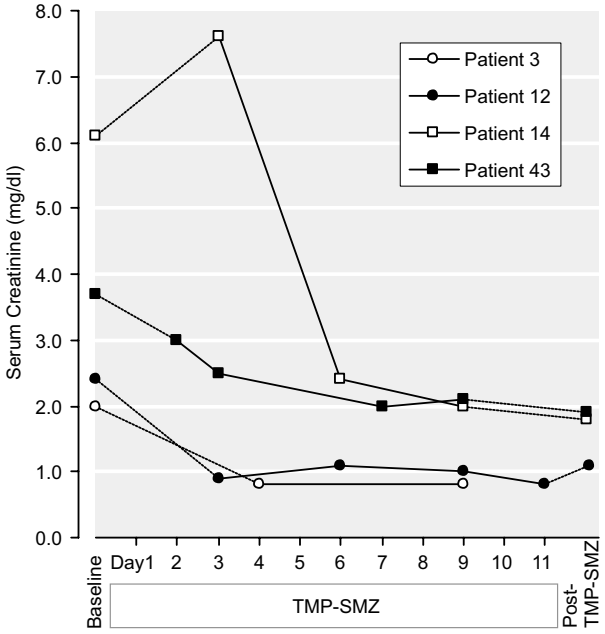

Figure 2. *Effect of intravenousTMP-SMZ in four patients with AIDS and PCP and prior impaired renal function. Patients received 20 mg of SMZ per Kg body weight per day for 9 to 11 days given intravenously. Data from Zúñiga et al [101].*

hyperkalemia was reported in 649 patients treated with standard doses of TMP-SMZ [112]. Indeed in our experimental study no changes in serum potassium occurred in rats treated with TMP-SMZ for nine days [102]: baseline, 5.24±0.06 mEq/L; day 5, 5.14±0.13 mEq/L; day 9, 5.23±0.12 mEq/L; n=7). Initially, in the patients with AIDS, the hyperkalemia was attributed to adrenal insufficiency or the syndrome of hyporeninemic hypoaldosteronism [113-115], although some patients were receiving high doses of TMP-SMZ [113, 114]. Velazquez and co-workers [116] reported 30 consecutive hospitalized patients with PCP during treatment with high dose TMP-SMZ or TMP-dapsone and noted that 27 of the 30 patients exhibited a rise in serum potassium. Fifteen of these patients developed a peak serum potassium concentration greater than 5 mEq/L, while two reached a potassium concentration more than 6 mEq/L. Seven patients were studied in detail. Among these patients, urinary potassium concentrations averaged 11.3±5.8 mEq/L, urinary sodium averaged 103±65 mEq/L, and the average transtubular potassium concentration gradient was 1.9±1.1. Three patients restudied after discontinuation of TMP-SMZ

showed normalization of their transtubular potassium concentration gradients. Their renal function was within normal limits and serum cortisol, plasma renin activity and plasma aldosterone concentrations were also normal or high during hyperkalemia. It has been suggested, however, that TMP, even at lower dosage, has an effect similar to amiloride, inhibiting apical sodium channels in the distal nephron and effectively reducing the transepithelial voltage that favors potassium secretion in a dose-dependent fashion [109, 116, 117]. A patient with renal tubular acidosis associated with TMP administration has been described [118].

In summary, taken as a whole, the clinical and experimental data indicated that TMP-SMZ when used intravenously for the treatment of infections or orally for long-term prophylaxis against opportunistic infections is safe, and it is accompanied by very low incidence of nephrotoxicity. The risk factors associated with nephrotoxicity are preexisting renal dysfunction, concomitant use of other drugs potentially nephrotoxic, advanced age, volume depletion, dose inappropriately adjusted for the level of renal function, and sepsis. TMP-SMZ pathways of nephrotoxicity are tubulo-obstructive, tubulotoxic, and immunologic.

Pentamidine

Pentamidine is a diamidine compound developed more than five decades ago [119]. Initially, it was only used for its antiprotozoal properties against African trypanosomiasis [120], and visceral leishmaniasis [121]. Subsequently, its use was extended to the treatment of PCP in immunosuppressed patients [122-124]. The use of pentamidine, which was rare in countries without tropical diseases, increased notably because of the high incidence of PCP observed in patients with AIDS [93, 97, 125-127]. Until 1984, pentamidine distribution in the USA was restricted; indeed it was only available through the Center for Disease Control. Although TMP-SMZ is regarded as the preferred treatment for PCP [98, 99], pentamidine or less frequently used combinations (trimetrexate/leucovorin, clindamycin/primaquine [128]) are reasonable alternatives when TMP-SMZ is not tolerated or is without effect [129].

Prior to the AIDS epidemic, the incidence of acute renal failure associated with intravenous pentamidine administration for the treatment of PCP was between 19 to 23% [123, 125]. During the 1980's the number of reported cases increased (Table 1). The average incidence of nephrotoxicity, the most frequent systemic adverse effect, was 41%, ranging from 20 to 94% as

Table 1. Clinical studies reporting on adverse effects of pentamidine in patients with AIDS (1984-1997).

Authors (Reference)	Nº of patients	Nº and (%) of patients with any adverse effect[a]	Nº and (%) of patients with nephrotoxicity[b]
Gordin *et al* [100]	30	13 (43%)	6 (20%)
Andersen *et al* [130]	24	20 (83%)	6 (25%)
Wharton *et al* [98]	32	32 (100%)	21 (65%)
Waskin *et al* [131]	164	94 (57%)	38 (23%)
Lachaal *et al* [132]	16	15 (94%)	5 (94%)
Sattler *et al* [99]	33	NA	21 (64%)
Chua *et al* [133]	33	NA	11 (33%)
Briceland *et al* [134]	37	NA	27 (73%)
O'Brien *et al* [128]	106	76 (72%)	48 (45%)[c]
Total	**475**	**250 (67%)**	**193 (41%)**

[a] *These include immediate reactions (hypotension, nausea/vomiting, arrythmias, etc.), local reactions (pain at injection site, phebitis, urticaria, etc.), and systemic adverse effects (hematologic, fever, liver, renal dysfunction, and electrolyte abnormalities).*

[b] *Nephrotoxicity defined as increase in serum creatinine concentration: >30% above baseline [100]; increase of 0.5 mg/dL or 50% above baseline [99, 128, 132-134], or not specified [98,130].* [c] *In 54% of these patents nephrotoxicity was associated with the concurrent use of other nephrotoxic agents.*

NA = not available

reported in 475 patients [98, 99, 128, 130-134]. If other risk factors for renal impairment are not present the majority of the cases reported are mild (see below). The acute renal failure is usually of the nonoliguric variety. There is mild proteinuria, glycosuria, pyuria, and granular casts. Gross hematuria can occur [135], as well as myoglobinuria [136]. Renal failure usually appears within the second week of treatment, and the recovery begins within few days after discontinuation of pentamidine. Nevertheless, it may take several weeks before renal function returns to baseline. Dialysis support is rarely necessary [36]. Nephrotoxicity can occur after repeated treatment courses with pentamidine [129].

From October 1988 to January 1989 we undertook a retrospective analyses of 33 consecutive patients with AIDS who received intravenous pentamidine isethionate for at least seven days for the treatment of PCP [133]. Nephrotoxicity was defined as a rise in serum creatinine greater than 0.5 mg/dl above baseline. Nephrotoxicity developed in 33%; it was mild and reversible in the majority of patients. Only one patient developed severe acute renal failure. Comparison of patients who developed nephrotoxicity (n=11) with those without renal impairment (n=22) revealed similar age, body weight, initial serum creatinine concentration, and total dose of pentamidine received. Particular attention was given to the presence of other risk factors for the development of nephrotoxicity, such as: volume depletion, sepsis, preexisting renal dysfunction, and recent or concomitant use of other nephrotoxic agents (aminoglycosides, radiocontrast, and non-steroidal anti-inflammatory agents). The risk of nephrotoxicity during pentamidine treatment was directly related to the number of risk factors present ($r=0.93$; $p=0.02$). Ten of 11 patients with pentamidine nephrotoxicity (91%) had additional risk factors. In contrast, 13 of 22 patients without nephrotoxicity (59%) had no risk factors (X^2; $p < 0.02$). The clinical data as well as our experimental results in rats [137] suggest that pentamidine has a relatively low toxicity index, and that concomitant risk factors, particularly, volume depletion are of importance in determining the appearance of nephrotoxicity in patients with AIDS. This is supported by the suggestion that patients receiving pentamidine as outpatients may have a greater risk of nephrotoxicity than those treated under inpatient conditions, perhaps because the intravenous fluid therapy that they receive is less aggressive [138]. In a recent retrospective review [128] the most significant risk factor for adverse pentamidine reactions was an increased number of concomitant medications, nonwhite ethnicity, concomitant use of nephrotoxic drugs, and daily cumulative dose of pentamidine.

Four cases of reversible acute renal failure in patient with AIDS who received both intravenous pentamidine (for PCP) and amphotericin B (for systemic mycoses) have been recently reported [139]. Of note, nephrotoxicity did not develop in three AIDS' patients treated with both TMP-SMZ and amphotericin B or in two patients who concomitantly received inhaled pentamidine and amphotericin B [139]. Reports of renal damage in patients receiving parenteral pentamidine for the treatment of non-HIV diseases continue. Reversible acute renal failure and nephrotic syndrome were documented in a young child given pentamidine mesylate and an antimonial salt for the treatment of visceral leishmaniasis [140]. In Africa (Kenya) patients with visceral leishmaniasis have developed renal toxicity during prolonged treatment (1 to 10 months) with pentamidine [141].

Currently, pentamidine in the form of aerosol is used for the prophylaxis of PCP with apparent success [142-144]. Initially no renal side effects were described with its use; two reports, however, now raise this possibility, which suggests that systemic absorption of pentamidine occurs [145, 146]. One of the patients, however, received a previous large dose of TMP-SMZ and only exhibited a mild elevation of serum creatinine, and the other appears to have had concomitant volume depletion caused by severe diarrhea. We have been unable to find additional reports of renal toxicity associated with aerosol pentamidine administration [147]. Likewise, no renal side effects were reported with the use of aerosolized pentamidine for the prophylaxis of PCP in patients who received bone marrow transplants [144].

Early experimental studies of pentamidine renal toxicity in animals were limited to few sheep [148], goats [148], and rabbits [149] given rather large doses of pentamidine (up to 40 mg/kg). The results of these studies, although indicating some degree of renal toxicity, are difficult to interpret because of the small number of animals employed, the large doses of pentamidine used, and the absence of controls or detailed renal function studies.

The exact mechanism of pentamidine renal toxicity is unknown. It has been suggested, however, that renal toxicity may result from the ability of pentamidine to react with and form insoluble precipitates with nucleotides [150].

A recent study evaluated the potential for pentamidine nephrotoxicity in the rat and explored some possible pathogenetic mechanisms [151]. The authors measured the urinary loss of tubular cells, malate dehydrogenase activity, and creatinine clearance after five daily injections of pentamidine. The tubular toxicity of pentamidine was dose-related (1, 10, or 20 mg/day/5 days) and reversible. Unfortunately, creatinine clearance was only measured once at the end of the study. As expected, tobramycin, amphotericin B, and cyclosporine increased pentamidine nephrotoxicity. On the other hand, fosfomycin and D-glucoro-1, 5-lactam (an inhibitor of lysosomal β-glucuronidase) ameliorated the renal dysfunction, and both verapamil and enalapril increased creatinine clearance reversing the effect of pentamidine. The authors suggested that pentamidine may share some of the mechanisms of tubular toxicity attributed to the aminoglycosides. Furthermore, they proposed that drugs that stabilize lysosomal membranes or inhibit lysosomal enzymes' activity or change renal hemodynamics may decrease pentamidine nephrotoxicity.

We investigated the effects of volume depletion and blockade of prostaglandin synthesis on pentamidine nephrotoxicity in the female Sprague-Dawley rat treated for a prolonged period, similar to that used in clinical protocols [137]. First, intact animals were injected for 14 days with subcutaneous pentamidine given at daily doses of 4, 10, or 20 mg/kg. With this protocol no changes in serum creatinine were observed. To mimic the state of volume depletion commonly seen in patients with AIDS, we sequentially studied groups of rats placed in a low sodium diet one week prior to injections. All groups received daily injections of indomethacin while receiving pentamidine (20 mg/kg/day) for 14 days. The creatinine clearance decreased 35% ($p<0.03$) only in the group treated with salt restriction plus indomethacin and pentamidine, and this difference appeared after the first week of pentamidine administration. The changes in the other three groups (normal salt intake + indomethacin and pentamidine; normal salt + indomethacin; and low salt + indomethacin) were minimal or not statistically significant. From this study we concluded that at least in the intact female Sprague-Dawley rat, pentamidine at a large dose appears to have minimal nephrotoxic effect. A state of sodium/volume depletion and of inhibition of prostaglandin synthesis may be necessary to reduce renal function in the rat [137].

Whereas the pharmacological properties of pentamidine have been studied for decades [152], only the recent development of high-performance liquid chromatographic and biological assays has permitted studies of the distribution and pharmacokinetics of this medication [153]. Only recently, experiments utilizing clinically relevant multiple dosing regimens of pentamidine (10 mg/kg for 14 days) in the rat have become available [154]. Following the initial injection, pentamidine appears in the urine in small amounts; however, with each subsequent injection, there is a progressive increase in the urinary excretion of pentamidine, whereas fecal excretion did not increase in a similar manner. Plasma levels of pentamidine were very low, whereas the drug accumulated in several tissues, with the kidney achieving the highest concentration, followed by the lungs, spleen, pancreas, stomach, and lesser levels in the liver, heart, and other organs [154]. Similar results have been found in the dog [155]. In the rat, fat and muscle did not contain much pentamidine raising the possibility, contrary to common belief, that metabolism of pentamidine may occur *in vivo* [156].

The pharmacokinetics of intravenous pentamidine during multiple dosing has been studied with modern technology [157] in patients with AIDS with normal renal function and in those receiving hemodialysis [158]. For a number of reasons detailed elsewhere [158], the true half-life of pentamidine is difficult to measure in humans. The true-elimination (slowest) half-life ranges from 29 hours (3 mg/kg dose) for the patients with normal renal function to 73 to 118 hours for the patients on maintenance hemodialysis (3 or 4 mg/kg dose, respectively), although these differences are not significant [157]. Little pentamidine is excreted via the kidneys [157]. Conte [158] estimated that renal clearance accounts for about two% of the plasma clearance in patients with normal renal function, and renal excretion increases only marginally with repeated dosing. Furthermore, the plasma and tissue concentrations of pentamidine associated with toxicity in man remain unknown. Tissue levels of pentamidine obtained in autopsy specimens from AIDS patients revealed that tis-

sue accumulation was usually greater in liver, kidneys, adrenal glands, and spleen than in the lung. Nevertheless, there is no correlation between tissue levels and renal dysfunction, as measured by serum creatinine levels [159]. Detectable levels of pentamidine are present in some tissues as late as one year after the last dose. Pentamidine slowly accumulates in and is slowly excreted from the major human organs [159]. As might be expected from the low renal clearance to plasma clearance ratio (2.1%), the complete absence of renal function resulted in only a marginal effect on drug disposition or ultimate total-body pentamidine burden [158].

It has been recommended that the dosing interval of pentamidine be extended to 48 hours for patients with a GFR less than 10 ml/min [160]. The recent report of Conte [158], however, suggests that dose reduction of pentamidine for renal impairment is unnecessary. The author noted, however, that his patients had mild to moderate PCP, and that it remains unknown whether the pharmacokinetics of pentamidine might be altered in more severely ill patients. It has been shown that there is minimal transfer of pentamidine to the human fetus and significant concentration of the drug in placental tissue [161]. The last mentioned finding raises an important question about placental toxicity.

Perturbations in insulin regulation both resulting in *hypoglycemia* and *diabetes mellitus* have been shown in patients treated with pentamidine [162-166]. This is not surprising considering that pentamidine accumulates in the pancreas [159], and that in 1948 the drug was considered for use as an antihypoglycemic agent [119]. The overall incidence of hypoglycemia with AIDS is several folds higher (27 to 40%) [164, 165] than previously reported for patients with other immunocompromising diseases treated with pentamidine [94]. The incidence of nephrotoxicity in patients who developed hypoglycemia was 100% [165]. The hypoglycemia, which appears early (within a week) after commencing pentamidine therapy, is associated with inappropriately high levels of insulin in the postabsorptive state [162]. The appearance of diabetes mellitus is usually delayed by several weeks. It has been suggested that pentamidine can induce hypoglycemia because of an early cytolytic release of insulin, and then diabetes mellitus because of β cell destruction and insulin deficiency [132].

Perturbations in mono- and divalent cation renal handling have been reported in association with pentamidine administration. Several reports of *hyperkalemia* in association with pentamidine therapy have been recently published [132, 134, 136, 137, 167, 168]. Lachaal and Venuto [132] in a retrospective review reported a very high incidence of hyperkalemia (5.1 to 8.7 mEq/L) in 19 of 20 patients (95%). This incidence was greater than the 5% reported earlier [123], or the 24% reported subsequently [134] in 37 patients with AIDS, and was challenged as a possible overestimation [169]. The hyperkalemia usually correlates with the presence of decreased GFR [132, 134]. In our clinical study [133] the mean serum potassium concentration tended to be higher in the AIDS patients that developed pentamidine nephrotoxicity than in those that did not (5.0±0.3 vs 4.3±0.2, respectively, $p < 0.055$). No patient, however, had a serum potassium concentration higher than 6.0 mEq/L. Hyperkalemia induced-arrhythmias occur [170], and rarely may include cardiac arrest [171]. The hyperkalemia usually reversed on discontinuation of pentamidine, and although most patients required only conservative measures, occasionally dialysis was necessary [132].

The exact mechanism of the pentamidine-induced hyperkalemia has not yet been defined. Many different mechanisms can impair the renal handling of potassium and thus favor hyperkalemia in patients with AIDS. These include: decreased renal function secondary to volume depletion, presence of underlying renal disease, including tubular dysfunction with the possibility of hyporeninemic hypoaldosteronism, hypoadrenalism, and the administration of drugs with potential for impairing renal potassium excretion (nonsteroidal anti-inflammatory agents, ACE inhibitors, potassium-sparing diuretics, β-blockers, TMP-SMZ). In our studies regarding pentamidine nephrotoxicity in the rat, however, there were no statistically significant differences observed between any of the groups [137], suggesting the possibility that extrarenal mechanisms or a more severe degree of renal dysfunction may be necessary to induce hyperkalemia. Recent *in vivo* experiments [172], however, have shown that the application of pentamidine to amphibian or mammalian distal nephron cells results in inhibition of amiloride-sensitive sodium channels and sodium reabsorption, and decrease in the electrochemical gradient that drives secretion of distal potassium into the urine. This renal

tubular effect of pentamidine may be the mechanisms for the induced hyperkalemia.

Symptomatic *hypocalcemia* and *hypomagnesemia with renal magnesium wasting* associated with pentamidine therapy was described recently in a patient with AIDS [173]. Three other cases have been reported [174-176]. Another previous report [177] described severe hypocalcemia with tetany in patients with AIDS concomitantly receiving pentamidine and foscarnet. The hypocalcemia, however, was attributed to the administration of foscarnet. Despite magnesium replacement, magnesium wasting may persist up to two months after the discontinuation of pentamidine, suggesting that anatomic renal tubular injury may be responsible [173, 175]. Both abnormalities developed within 6 to 10 days of pentamidine administration. Because life-threatening arrhythmias can develop, especially at serum magnesium levels less than 1.6 mg/dl, early replacement therapy is clinically warranted.

In summary, parenteral pentamidine administration for the treatment of PCP is associated with the development of usually mild, reversible acute renal failure in about one-third of patients with AIDS. Compounding risk factors, of which volume depletion is the most important, are found in the majority of cases of pentamidine nephrotoxicity. There is no convincing evidence that the aerosol route of pentamidine administration for PCP prophylaxis results in nephrotoxicity. Hypocalcemia and hypomagnesemia with renal magnesium wasting, and particularly, hyperkalemia are seen occasionally with pentamidine therapy. Taking into consideration other commonly associated risk factors in AIDS patients, TMP-SMZ induces less renal impairment than does pentamidine. TMP itself mildly increases the serum creatinine level by interfering with its tubular secretory mechanism. Hyperkalemia can also be induced by TMP. Considering the extensive worldwide use of TMP-SMZ, however, this combination should be considered as a safe drug.

Pyrimethamine

Pyrimethamine is a folic acid antagonist that for many years has been used as an antimalarial drug [178-180]. Due to its synergistic activity, pyrimethamine in combination with sulfadiazine or dapsone has been recently used for the treatment or prophylaxis of cerebral toxoplasmosis or PCP in patients with AIDS [181]. Pyrimethamine does not belong to the group of known nephrotoxic agents [36]. Because pyrimethamine and trimethoprim have a similar 2, 4-diaminopyrimidine molecular structure, Opravil *et al* [182] have recently evaluated its effects on renal function, with particular emphasis on the tubular secretion of creatinine. In six healthy volunteers and nine patients with AIDS, pyrimethamine caused a reversibly, small, similar, but statistically significant increase (26%) in serum creatinine concentration with a concomitant decrease in creatinine clearance. Of importance, these changes occurred without a decrease in the simultaneously measured inulin clearance. The authors concluded that pyrimethamine – like trimethoprim and other compounds (cimetidine, probenecid), reversibly and mildly (at least in patients with normal renal function) inhibits renal tubular secretion of creatinine without affecting the glomerular filtration rate. Thus, physicians using this medication should be aware of the possibility of pyrimethamine elevating serum creatinine concentration. Prospective studies on the effect of pyrimethamine on patients with impaired renal function would be welcomed.

Pyrimethamine has a long half-life (83±14 hours) [182] with only a small fraction been excreted during the first days of administration, but drug and metabolites will appear slowly in the urine for one to two months [36]. Dosage adjustment is usually not recommended for patients with renal failure. It is not known, however, if metabolite accumulation with potential hematologic toxicity may occur with the prolonged use of pyrimethamine in patients with cerebral toxoplasmosis at doses higher than dose employed for prophylaxis of malaria [36]. It appears that dialytic removal of pyrimethamine must be small because of its high protein binding (85-90%) and large volume of distribution [179, 180].

Dapsone

Dapsone, a sulfone with chemical similarities to sulfapyridine, has been used for over 50 years for the treatment of susceptible forms of leprosy, as well as for quinine-resistant *Plasmodium falciparum* malaria. Dapsone is currently used for the primary treatment of dermatitis herpetiform, and can be replaced by sulfapyridine in patients with intolerance to the sulfone [183]. Dapsone in combination with trimethoprim it is also used

for the treatment of mild to moderate first episodes of PCP, or alone for PCP prophylaxis [107, 183]. Dose-related hemolysis is the most common adverse effect in patients with or without G6PD deficiency.

Dapsone is well reabsorbed when given by the oral route, is extensively protein and tissue bound, and is metabolized by N-oxidation and acetylation. The serum half-life averages 24 to 28 hours, and about 65% of an oral dose appears in the urine after few days, mostly as metabolites. No specific guidelines for dosage modifications in patients with renal failure are available [36, 184]. When dapsone and trimethoprim (with SMZ) are used together, higher plasma levels of both drugs are achieved, than when either drug is used alone [185].

Very rarely, renal adverse effects attributable to dapsone have been reported [186-188]. A single case of nephrotic syndrome following a three-week course of treatment with dapsone at 100 mg daily for a pruritic rash was reported [186]. Although a causative effect for dapsone seemed plausible, no renal histology or long-term follow-up was available. Bilateral renal cortical necrosis developed in a patient treated for dermatitis herpetiform for several years with large doses of dapsone [187]. He had hemolytic anemia and normal G6PD levels. Two fatal cases of acute renal failure associated with intravascular hemolysis secondary to G6PD deficiency were described in Indian patients treated with dapsone [188]. Acute renal failure associated with massive dapsone overdose also was reported [189]. In a recent bibliographic review, we could not find other reports of renal dysfunction associated with dapsone. Furthermore, in the recent study of Opravil *et al* [182], administration of dapsone alone to healthy volunteers or to patients with AIDS did not result in changes in renal function. Likewise, dapsone alone did not cause hyperkalemia in patients with AIDS treated for PCP [107]. Thus, it appears that dapsone is a safe drug when used in standard dosage. Perhaps, in patients with G6PD deficiency, hemolytic complications including renal involvement should be watched for when administering dapsone [188].

Quinolones

The newer quinolones are a class of antibacterial agents with broad-spectrum activities against both gram-negative and gram-positive bacteria. They have proved to be effective against infections in the urinary tract, respiratory tree, gastrointestinal tract, as well as skin, soft tissue and bone, and for sexually transmitted bacterial diseases [190]. Nalidixic acid introduced in 1962, was the first in this series of agents [191]. Subsequently, many 4-fluoroquinolones have been introduced into clinical practice including ciprofloxacin, ofloxacin, lomefloxacin, norfloxacin, enoxacin, gatifloxacin, moxifloxacin, trovafloxacin, sparfloxacin, and grepafloxacin. However, many others are already in use or undergoing trials [190]. We will consider ciprofloxacin as the prototypical agent for the new 4-fluoroquinolones.

Nalidixic acid

Nalidixic acid is a highly protein bound oral quinolone (>90%), that undergoes major hepatic metabolism (80%) to active (hydroxynalidixic acid) and inactive metabolites [192]. The parent drug and its metabolites are rapidly excreted in the urine [193]. Most of the antibacterial effect is due to the biologically active hydroxynalidixic acid, which is 16 times more active than the parent compound [190]. Nalidixic acid has a terminal half-life of about two hours. The drug does not accumulate in tissues even after prolonged administration; the kidney is the only organ in which this may occur. Furthermore, nalidixic acid does not diffuse into prostatic fluid [190].

This drug is essentially devoid of renal toxicity. Although increased toxicity has not been reported in patients with renal failure given the usual dosage, nalidixic acid, preferably, should not be used in patients with a decreased GFR (less than 50 ml/min) or in patients with liver disease, because of the risk of enhancing gastrointestinal or dermatological adverse-effects [160]. Overdosage with nalidixic acid induces metabolic acidosis [194]. In the past the use of this drug was limited to treatment of urinary tract infections. Currently, other newer quinolones, as well as other chemotherapeutic agents, have replaced nalidixic acid.

Ciprofloxacin

Ciprofloxacin, probably the most powerful and undoubtedly the most thoroughly studied of the newer oral quinolones, is rapidly absorbed from the gastrointestinal tract. A parenteral preparation is also

available. Protein binding is low, about 35%, and the serum half-life is 3-4.5 hours. About 30-60% of the active drug and 10% of its metabolites are excreted in the urine during 24 hours; 15% appears in the feces, and less than 1% in the bile [195, 196]. Table 2 illustrates that the newer quinolones exhibit differences – sometimes important – in their pharmacokinetics, which might affect their individual behavior. The quinolones undergo hepatic metabolism and renal excretion. Hepatic metabolism includes conjugation with glucuronic acid as well as carboxylation, hydroxylation, and demethylation.

Tissue penetration, particularly in the kidneys and prostate, is excellent (Table 2). Those quinolones with longer half-life have smaller penetration ratios [196]. Available studies suggest that penetration of the newer quinolones into all extravascular sites (large-volume spaces [i.e., ascites, pleural fluid, etc.], secretory fluids [urine, prostatic secretions, sputum, etc.], barrier fluids [CSFL], and whole tissues) is high relative to the penetration reported for most other categories of antimicrobial agents, particularly, the penicillins, cephalosporins, and aminoglycosides [197]. Renal elimination is by glomerular filtration and active tubular secretion, which can be blocked by probenecid. As noted in Table 2, urinary recovery of the quinolones is variable. The antibacterial activity of these compounds is reduced at low urinary pH [190].

The bioavailability of oral or parenterally administered ciprofloxacin was not affected in patients and rats with renal insufficiency [198]. The renal clearance of the quinolone, however, was reduced resulting in a prolonged half-life [199-201]. Thus, a reduction of 50% in the dose of ciprofloxacin has been recommended when the creatinine clearance is between 10 and 30 ml/min/1.73 m^2 [200]. Of interest, it has been suggested that there may be a compensatory transintestinal elimination of ciprofloxacin in patients and rats with reduced renal function [200, 201].

A study of the pharmacokinetics of orally administered ciprofloxacin in elderly (63-76 years) and young volunteers (22-34 years) without renal impairment, revealed in the elderly group a decreased renal clearance of the quinolone with no differences detected in the terminal half-life (3.5 hours). This was accompanied, however, by a surprising increase in the absolute availability of the drug [202]. The authors cautioned about the need for a reduction of oral dosage of ciprofloxacin in the elderly population.

The newer fluoroquinolones (ciprofloxacin, norfloxacin, enoxacin, pefloxacin, gatifloxacin and moxifloxacin) have similar toxicities and incidence of adverse effects. In general, compared to other antibiotics, these are relatively safe agents [190]. Gastrointestinal side-effects are the most common (0.8 to 6.8% of patients), followed by central nervous system manifestations (0.9 to 1.8%), and skin reactions (0.6 to 2.4%). Rare cases of increased serum creatinine levels have been reported [203]. Indeed, in a study of 133 febrile episodes in neutropenic patients comparing the effectiveness and safety of high-dose oral ciprofloxacin versus azlocillin and netilmicin, there were no renal ad-

Table 2. Pharmacokinetics of selected newer quinolones after single oral dosage*.

Drug	Half-life (h)		Urinary Excretion (%)		Tissue Penetration**		Removal by Dialysis	
	NRF	ESRD	Unchanged	Metabolites	Kidney	Prostate	HD (%)	PD (%)
Ciprofloxacin	3-4.5	6-9	30-60	10	5+	3+	<10	<10
Enoxacin	4-6	NA	50-55	15	4+	2+	<5	NA
Fleroxacin	9-13	21-28	70	NA	NA	NA	NA	NA
Lomefloxacin	8	44	70	10	NA	NA	<10	NA
Norfloxacin	3-4.5	8	20-40	20	5+	2+	<10	NA
Ofloxacin	5-6	28-37	70-90	5-10	5+	4+	10-30	2-10
Pefloxacin	10-11	12-15	5-15	55	NA	NA	NA	NA
Sparfloxacin	15-20	38.5	10	NA	NA	NA	NA	NA

* *Adapted from references [184, 190 and 192].*
** *Scale of 1+ to 5+.*
NRF= normal renal function; ESRD= end-stage renal disease; NA = not available; HD = Hemodialysis; PD = Peritoneal dialysis.

verse effects reported in the quinolone group, whereas nephrotoxicity developed in 3% of the patients treated with the combination azlocillin/netilmicin [204]. Ball [205] described only one case of acute renal failure in his review of almost 6,000 patients worldwide. In another review of 2,829 patients, minor increases in serum creatinine and blood urea nitrogen were reported, but only one patient each with acute renal failure and interstitial nephritis were described [206]. Thus, initially it was thought that ciprofloxacin was almost devoid of renal toxicity. Nevertheless, since 1987 at least 49 patients with acute renal failure and a clinical presentation compatible with acute interstitial nephritis have been reported [207-235, 235a]. The diagnosis has been confirmed by renal biopsy in 15 patients [208, 214, 217, 218, 222, 224-235], and by postmortem examination in one [212]. The age of the patients ranged from 11 to 88 (data on 25 patients) with an average of 59 years. There was a predominance of elderly patients (48% were 65 or older), and, of note, 58% were women (ages 21 to 88).

The nonoliguric variety of acute renal failure was common (76%), but oliguria (23%) or anuria were also observed. The average duration of quinolone therapy prior to the recognition of nephrotoxicity was seven days. In the 20 patients reviewed by La et al [223], skin rashes were uncommon, five patients had eosinophiluria, six had eosinophilia, abnormal urinary sediment was not always present, and the duration of therapy with the quinolone prior to the diagnoses of renal failure ranged from 3 to 18 days. In only six of the 20 patients (30%) was ciprofloxacin the only drug given with the potential for causing renal dysfunction. The majority of the patients received a variety of other medications with nephrotoxic potential: aminoglycosides in six, penicillins-cephalosporins in five, amphotericin B, cisplatin and non-steroidal anti-inflammatory agents in two each, and others. Thus, although the chronological sequence of events and the observed improvement after stopping the quinolone strongly favors a causative role for ciprofloxacin, it is not possible to be absolute certain about the cause of the nephrotoxicity. Renal function improved in 14 patients after discontinuation of ciprofloxacin therapy. It is not possible to evaluate the beneficial effect of prednisone, which was given to only 4 of 21 patients. Only one patient required dialytic support [223].

Although the pathogenesis of ciprofloxacin-induced acute interstitial nephritis is not clear, it has been attributed to an inflammatory interstitial response secondary to the crystalluria associated with the quinolone (foreign body response) [208, 214, 224]. Crystalluria and the presence of crystals of ciprofloxacin in the renal tissue has been shown in animal experiments. The species studied (rats, monkeys, dogs), however, have alkaline urine, and because the quinolone solubility is poor at a neutral or alkaline pH, crystallization may occur under those circumstances, with ciprofloxacin precipitating in the tubular lumen with magnesium and protein but only in an alkaline urine. Indeed, at an acid pH crystallization does not occur [236]. It has been argued, however, that only uncommonly and intermittently, is the human urine highly alkaline [237]. By supplementing the diet of normal volunteers with sodium bicarbonate it was possible to demonstrate crystals of ciprofloxacin in the urine of individuals who received large doses of the quinolone; nevertheless, no adverse renal effects developed [238]. Because the majority of the patients with acute interstitial nephritis secondary to ciprofloxacin are assumed to or have acid urine, it has been suggested that an idiosyncratic reaction rather than intratubular crystallization, might be involved in the pathogenesis of acute interstitial nephropathy [214]. Of importance, a report on four cases of acute interstitial nephritis and two cases of hepatitis induced by quinolone [235a], revealed by immunoblotting analysis that all sera from these patients contained autoantibodies that recognize a 65-kDa protein expressed in normal human kidney and liver microsomes. Only 6% of sera from healthy individuals who did not ingest quinolone recognized the same protein. These findings sugest that a modification of microsomal proteins by quinolone itself or by a metabolite could generate an autoimmune response, and that the presence of autoantibodies could be used as a sensitive marker.

Patients who received bone marrow [239] and heart transplants [240] did not show any evi-dence of nephrotoxicity when receiving ciprofloxacin. Contrary to previous preliminary findings [241, 242], more recent data suggest lack of relevant pharmacokinetic interaction of ciproflox-acin with cyclosporine [240]. Similar preliminary claims of norfloxacin- [243], ofloxacin- [244], and pefloxacin-cyclosporine [245] interactions have been made.

Other quinolones

Early reports of acute interstitial nephritis [246] and of acute tubular necrosis [247] associated with the use of *piromidic acid* (a non-fluorinated quinolone available in Europe) have been published. At large dosage, crystals of *norfloxacin* can be occasionally seen in freshly voided urine, this, however, does not occur when low doses are used [196]. One patient treated with norfloxacin developed acute renal failure compatible with allergic interstitial nephritis [248]. No crystalluria or crystal formation was reported in acute toxicity studies with *temafloxacin* in mice, rats, or dogs. Furthermore, no nephrotoxicity was observed in rats, or dogs, when temafloxacin was administered orally for six months [249]. Finally, pre-marketing data obtained in 5,300 patients revealed no crystalluria or clinically important nephrotoxicity with the use of temafloxacin [250]. However, prior to its withdrawal from the world market, temafloxacin was associated with a syndrome of immune hemolytic anemia and renal failure based on 95 spontaneous reports of hemolysis sent to the Food and Drug Administration. New- onset renal dysfunction was noted in 54 cases (57 %), and dialysis was required in 34 cases (63%) [235]. Pre-marketing animal and clinical studies with *ofloxacin* revealed the absence of renal toxicity [251]. Likewise, no adverse renal effects were reported in a comparative study of *lomefloxacin* with TMP-SMZ [252]. The renal handling of *flexoracin*, a trifluorinated quinolone, in humans occurs by glomerular filtration, and both renal tubular secretion and reabsorption [253]. Animal studies indicated that the tubular transport processes of some of the quinolones have a considerable species dependency [253].

It is reasonable to conclude that in general, quinolones are safe drugs from the renal point of view. It is often difficult, however, to ascertain the exact causative role of these agents in the appearance of nephrotoxicity. Judging by the recent accumulated experience with ciprofloxacin, physicians using quinolones should be alert for the development of acute interstitial nephritis leading to renal failure. This concern should be extended to the other newer fluoroquinolones despite the paucity of reports dealing with nephrotoxicity.

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References

1. Dowling HF, Lepper MH. Toxic reactions following therapy with sulfapyridine, sulfa-thiazole and sulfadiazine. JAMA 1943; 121: 1190-1194.
2. Vilter CF, Blankehorn MA. Toxic reactions of the newer sulfonamides. JAMA 1944; 126: 691-695.
3. Lehr D. Clinical toxicity of sulfonamides. Ann NY Acad Sci 1957; 69: 417-447.
4. Weinstein L, Madoff MA, Samet CM. The sulfonamides. N Engl J Med 1960; 263: 793-800 and 952-957.
5. Dorfman LE, Smith JP. Sulfonamide crystalluria: A forgotten disease. J Urol 1970; 104: 482-483.
6. Appel GB, Kunis CL. Acute interstitial nephritis. In: Tubulointerstitial nephropathies. Cotran RS, Brenner BM, Stein JH (editors). Churchill, New York 1983; p. 151-185.
7. Simon DI, Brosius III FC, Rothstein DM. Sulfadiazine crystalluria revisited. The treatment of Toxoplasma encephalitis in patients with acquired immunodeficiency syndrome. Arch Intern Med 1990; 150: 2379-2384.
8. Nissen NI, Aagaard K, Flindt-Hansen E. Sulfonamide hematuria frequency of injury to the urinary tract as estimated on the basis of 6084 cases treated with different sulfonamide-preparations. Acta Med Scand 1950; 138: 301-314.
9. Dawarakanath AD, Michael J, Allan RN. Sulphasalazine induced renal failure. Gut 1992; 33: 1006-1007.
10. Finland M, Strauss E, Peterson OL. Sulfadiazine: therapeutic evaluation and toxic effects in four hundred and forty-six patients. JAMA 1941; 16: 2641-2647.
11. Fox CL Jr., Jensen OJ, Jr., Mudge GH. The prevention of renal obstruction during sulfadiazine therapy. JAMA 1943; 121: 1147-1150.
12. Yap HK, Yip WCL, Tay SH, Lee BW. Reversible ultrasound findings in the kidneys of a patient with sulfonamide induced obstructive uropathy. J Singapore Pediatr Soc 1984; 26: 105-106.
13. Goadsby PJ, Donaghy AJ, Lloyd AR, Wakefield D. Acquired immunodeficiency syndrome (AIDS) and sulfadiazine-associated acute renal failure. Ann Int Med 1987; 107: 783-784.

14. Sahai J, Heimberger T, Collins K, Kaplowitz L, Polk R. Sulfadiazine-induced crystalluria in a patient with the acquired immunodeficiency syndrome: a reminder. Am J Med 1988; 84: 791-792.
15. Ventura MG, Wybram J, Farber CM. Sulfadiazine revisited. J Inf Dis 1989; 160: 556-557.
16. Carbone LG, Bendixen B, Appel GB. Sulfadiazine-associated obstructive nephropathy occurring in a patient with the acquired immunodeficiency syndrome. Am J Kidney Dis 1988; 12: 72-75.
17. Christin S, Baumelou A, Bahri S, Ben Hmida M, Deray G, Jacobs C. Acute renal failure due to sulfadiazine in patients with AIDS. Nephron 1990; 55: 233-234.
18. Antopol W, Robinson H. Urolithiasis and renal pathology after oral administration of 2-sulfanilylamino (sulfapyridine). Proc Soc Exp Biol Med 1939; 40: 428-430.
19. Molina J-M, Belenfant X, Doco-Lecompte T, Idatte J-M, Modai J. Sulfadiazine-induced crystalluria in AIDS patients with toxoplasma encephalitis. AIDS 1991; 5: 587-589.
20. Filks IN, Follador CR, Barbas Filho JV, Kairalla RA, Oliveira-Vianna E dos S, Stavale ML, de Carvalho CR. Kidney failure as a complication of the treatment of pulmonary paracoccidioidomycosis (in Portuguese). Rev Hosp Clin Fac Med Sao Paulo 1992; 47: 103-105.
21. Marques LP, Silva MT, Madeira EP, Santos OR. Obstructive renal failure due to therapy with sulfadiazine in an AIDS patient. Nephron 1992; 62: 361 (letter).
22. De Jaureguiberry JP, Reboul P, Vey N, Jaubert D. Acute renal failure caused by sulfadiazine crystalluria in AIDS. An historical resurgent iatrogenic complication (in French). Ann Med Interne (Paris) 1992; 143: 218-219 (letter).
23. Corbella X, Mascaró J, Santín M, Carratalà J. Insuficiencia renal aguda por sulfadiazina en el tratamiento de una toxoplasmosis cerebral. Rev Clin Esp 1992; 190: 330-331.
24. Farinas MC, Echevarria S, Sampedro I, Gonzalez A, Perez del Molino A, Gonzalez-Macias J. Renal failure due to sulphadiazine in AIDS patients with cerebral toxoplasmosis. J Intern Med 1993; 233: 365-367.
25. Hein R, Brunkhorst R, Thon WF, Schedel I, Schmidt RE. Symptomatic sulfadiazine crystalluria in AIDS patients: a report of two cases. Clin Nephrol 1993; 39: 254-256.
26. Haverkos HW - TE Study Group. Assessment of therapy for Toxoplasma encephalitis. Am J Med 1987; 82: 907-914.
27. Becker K, Jablonowski H, Häusssinger D. Sulfadiazine-associated nephrotoxicity in patients with the acquired immunodeficiency syndrome. Medicine (Balt.) 1996; 75: 185-194.
28. Kane D, Murphy JM, Keating S, Wilson GF, Mulcahy FM. Renal ultrasonic findings in sulfadiazine-induced renal failure. Br J Radiol 1996; 69: 925-928.
28a. Colebunders R, Depraetere K, De Droogh E, Kamper A, Corthout B, Bottiau E. Obstructive nephropathy due to sulfa crystals in two HIV seropositive patients treated with sulfadiazine. Jbr-Btr: Organe de la Société Royale Belge de Radiologie 1999; 82: 153-154.
29. Goodman LS, Gilman A. The Pharmacological Basis of Therapeutics, 8th Ed, New York: McGraw Hill, 1991; p 1047.
30. Díaz F, Collazos J, Mayo J, Martínez E. Sulfadiazine-induced multiple urolithiasis and acute renal failure in a patient with AIDS and toxoplasma encephalitis. Ann Pharmacother 1996; 30:41-42.
31. De Sequera P, Albalate M, Hernández J, Vazquez A, Abad J, Ramiro E, Fernández Guerrero M, Caramelo C, Casado S, Ortíz A. Acute renal failure due to sulphadiazine crystalluria in AIDS patients. Postgr Med J 1996; 72: 557-558.
32. Mir N, O'Farrell N, Creagh TA, Knowles C. Obstructive renal failure requiring surgical intervention in an AIDS patient being treated with sulphadiazine. Int J STD & AIDS 1997; 8: 61-62.
33. Dong BJ, Rodríguez RA, Goldsmith RH. Sulphadiazine-induced crystalluria and renal failure in a patient with AIDS. JABFP 1999; 12: 243-248.
34. Crespo M, Quereda C, Pascual J, Rivera M, Clemente L, Cano T. Patterns of sulfadiazine acute nephrotoxicity. Clin Nephrol 2000; 54: 68-72.
35. Bergan T, Brodwall EK, Vik-Mo H, Anstad U. Pharmacokinetics of sulphadizine, sulphamethoxazole and trimethoprim in patients with varying renal function. Infection 1979; 7(Suppl 4): S382-S387.
36. Berns JS, Cohen RM, Stumackher RJ, Rudnick MR. Renal aspects of therapy of human immunodeficiency virus and associated opportunistic infections. J Am Soc Nephrol 1991; 1: 1061-1080.
37. Swan SK, Bennett WM. Use of drugs in patients with renal failure. In: Diseases of the Kidney, 6th Ed. Schrier RW, Gottschalk CW (editors). Little, Brown & Co., Boston, 1996; p. 1061-1080.
38. Portoles J, Torralbo A, Prats D, Blanco J, Barrientos A. Acute renal failure and sulfadiazine crystalluria in kidney transplant. Nephrol Dial Transplant 1994; 9: 180-181.
39. Oster S, Hutchison F, McCabe R. Resolution of acute renal failure in toxoplasmic encephalitis despite continuance of sulfadiazine. Rev Inf Dis 1990; 12:618-620.
40. Robson M, Levi J, Dolber L, Rosenfeld JB. Acute tubulo-interstitial nephritis following sulfadiazine therapy. Isr J Med Sci 1970; 4: 561-566.

41. Nanra RS, Anderton JL, Evans M, Fairley KF, Kincaid-Smith P. The use of trimethoprim and sulfamethoxazole in the management of chronic and recurrent upper and lower urinary tract infection. Med J Aust 1971; 1: 25-26.
42. Rosenfeld JB, Najenson T, Grosswater Z. Effect of long-term co-trimoxazole therapy on renal function. Med J Aust 1975; 2: 546-548.
43. Bennett WM, Craven R. Urinary tract infections in patients with severe renal disease. Treatment with ampicillin and trimethoprim-sulfamethoxazole. JAMA 1976; 236: 946-948.
44. Lawson DH, Paice BJ. Adverse reactions to trimethoprim-sulfamethoxazole. Rev Inf Dis 1982; 4: 429-433.
45. Foltzer MA, Reese RE. Trimethoprim-sulfamethoxazole and other sulfonamides. Med Clin North Amer 1987; 71: 1177-1194.
46. Bergan T, Brodwall EK. Kidney transport in man of sulfamethoxazole and trimethoprim. Chemotherapy 1972; 17: 320-333.
47. Trottier S, Bergeron MG, Lessard C. Intrarenal distribution of trimethoprim and sulfa-methoxazole. Antimicrob Agents Chemother 1980; 17: 383-388.
48. Tu Y-H, Allen LV Jr., Fiorica VM, Albers DD. Pharmacokinetics of trimethoprim in the rat. Am Pharmaceutical Sc 1989; 78: 556-560.
49. Cacini W, Myre SA. Uptake of trimethoprim by renal cortex. Biochem Pharmacol 1985; 34: 3483-3488.
50. Craig WA, Kunin CM: Distribution of trimethoprim-sulfamethoxazole in tissues of rhesus monkeys. J Infect Dis 1973; 128: S575-S579.
51. Berglund F, Killander J, Pompeius R. Effect of trimethoprim-sulfamethoxazole on the renal excretion of creatinine in man. J Urol 1975; 114: 802-808.
52. Shouval D, Ligumsky M, Ben-Ishary D. Effect of cotrimoxazole on normal creatinine clearance. Lancet 1978; 1: 244-245.
53. Brautigam M, Forese P, Baethke R, Kessel M. Zur kreatininaus scheidung beim Menschen nach Gabe von cotrimoxazole. Klin Wochenschr 1979; 57: 95-96.
54. Trollfers B, Wahl M, Alestig K. Co-trimoxazole, creatinine and renal function. J Infect 1980; 2: 221-226.
55. Kainer G, Rosenberg AR. Effect of co-trimoxazole on the glomerular filtration rate of healthy adults. Chemotherapy 1981; 27: 229-232.
56. Lee J, Hollyer R, Rodelas R, Preuss HG. The influence of trimethoprim, sulfa-methoxazole, and creatinine on renal organic anion and cation transport in rat kidney tissue. Tox Appl Pharmacol 1981; 58: 184-193.
57. Kalowski S, Nanra RS, Mathew TH, Kincaid-Smith P. Deterioration in renal function in association with co-trimoxazole therapy. Lancet 1973; 1: 394-397.
58. Myre SA, McCann J, First MR, Cluxton RJ Jr. Effect of trimethoprim on serum creatinine in healthy and chronic renal failure volunteers. Ther Drug Monit 1987; 9: 161-165.
59. Paap CM, Nahata MC. Clinical use of trimethoprim-sulfamethoxazole during renal dysfunction. Drug Intell Clin Pharm 1989; 23: 646-654.
60. Siber GR, Gorham CC, Ericson JF, Smith AL. Pharmacokinetics of intravenous trimethoprim-sulfamethoxazole in children and adults with normal and impaired renal function. Rev Inf Dis 1982; 4: 566-578.
61. Eknoyan G. Acute renal failure associated with tubulointerstitial nephropathies. In: Acute Renal Failure, 2nd Ed. Brenner BM, Lazarus JM (editors). New York: Churchill Livingstone, 1988; p. 491-534.
62. Siegel WH. Unusual complications of therapy with sulfamethoxazole-trimethoprim. J Urol 1977; 117: 397.
63. Nissenson AR, Wilson C, Holazo A. Pharmacokinetics of intravenous trimethoprim-sulfamethoxazole during hemodialysis. Am J Nephrol 1988; 7: 270-274.
64. Saltissi D, Pusey CD, Rainford DJ. Recurrent acute renal failure due to antibiotic-induced interstitial nephritis. Br Med J 1979; 1: 1182-1183.
65. Jick H. Adverse reactions to trimethoprim-sulfamethoxazole in hospitalized patients. Rev Infect Dis 1982; 4: 426-428.
66. Jick H, Derby LE. A large population-based follow-up study of trimethoprim-sulfamethoxazole, trimethoprim, and cephalexin for uncommon serious drugs toxicity. Pharmacotherapy 1995; 15: 1747-1758.
67. Hanley T. The synergy of trimethoprim and sulfonamides. Postgrad Med J 1969; 45(Suppl): 85.
68. Buchanan N. Sulfamethoxazole, hypoalbuminemia, crystalluria, and renal failure. Br Med J 1978; 2: 172.
69. Ahn YH, Goldman JM. Trimethoprim-sulfamethoxazole and hyponatremia. Ann Intern Med 1985; 103: 161-162 (letter).
70. Wofsy CB. Use of trimethoprim-sulfamethoxazole in the treatment of Pneumocystis carinii pneumonia in patients with acquired immunodeficiency syndrome. Rev Infect Dis 1987; 92: S184-S194.
71. Deans KW, Lang JR, Smith DE. Stability of trimethoprim-sulfamethoxazole injection in five infusion fluids. Am J Hosp Pharm 1982; 39: 1681-1684.
72. Richmond JM, Whitworth JA, Fairley KF, Kincaid-Smith P. Co-trimoxazole nephrotoxicity. Lancet 1979; 1: 493.
73. Kraemer MJ, Kendall R, Hickman RO, Hass JE, Bierman CW. A generalized allergic reaction with acute interstitial nephritis following trimethoprim-sulfamethoxazole use. Ann Allergy 1982; 49: 323-325.
74. Chandra M, Chandra P, McVicar M, Susin M, Teichberg S. Rapid onset of co-trimoxazole induced interstitial nephritis. Int J Pediatr Nephrol 1985; 8: 289-292.

75. Payne FE, Jr., Giesecke TF. Multiple system reaction to trimethoprim-sulfamethoxazole. South Med J 1987; 80: 275-276.
76. Dutertre JP, Despert F, Jonville AP, Autret E, Nivet H. Nécrose tubulaire aiguë au cours d'un traitement par Bactrim. Arch F Pediatr 1991; 48: 441-442.
77. Smith EJ, Light JA, Filo RS, Yum MN. Interstitial nephritis caused by trimethoprim-sulfamethoxazole in renal transplant recipients. JAMA 1980; 244: 360-361.
78. Tasker PR, MacGregor GA, de Wardener HE. Use of co-trimoxazole in chronic renal failure. Lancet 1975; 1: 1216-1218.
79. Bourgault AM, Van Scoy RE, Brewer NS, Rosenow EC. Trimethoprim with sulfamethoxazole for treatment of infection with *Pneumocystis carinii* in renal insufficiency. Chest 1978; 74: 91-92.
80. Brockner J, Boisen E. Fatal multisystem toxicity after co-trimoxazole. Lancet 1978; 1: 831.
81. Finlayson WB, Johnson G. Multisystem toxicity after co-trimoxazole. Lancet 1978; 2: 682-683.
82. Epstein M, Wright JM. Severe multisystem disease caused by trimethoprim-sulfamethoxazole: possible role of an *in vitro* lymphocyte assay. J Allergy Clin Immunol 1990; 86: 416-417.
83. Thompson JF, Chalmers DH, Hunnisett AG, Wood RF, Morris PJ. Nephrotoxicity of trimethoprim-and co-trimoxazole in renal allograft recipients treated with cyclosporine. Transplantation 1983; 36: 204-206.
84. Ringdén O, Myrenfors P, Klintmalm G, Tydén G, Ost L. Nephrotoxicity by co-trimoxazole and cyclosporin in transplant patients. Lancet 1984; 1: 1016-1017.
85. Berg KJ, Nordby G, Rootwelt K, Droseland O, Fauchald P, Mehl A, Narverud J, Talseth T. Effects on renal function of combined treatment with trimethoprim and cyclosporine A in kidney transplant patients. Transplant Proc 1988; 20: 413-415.
85a. Sen S, Bayrak R, Ok E, Bademir G. Drug-induced acute interstitial nephritis and vasculitis or vasculary rejection in renal allografts. Am J Kidney Dis 2001; 37: E4.
86. Josephson MA, Chiu MY, Woodle ES, Thistlethwaite JR, Haas M. Drug-induced acute interstitial nephritis in renal allografts: histopathologic features and clinical course in six patients. Am J Kidney Dis 1999; 34(3): 540-548.
87. Colvin RB, Fang LST. Interstitial nephritis. In: Renal pathology, 2nd Ed. Tisher CC, Brenner BM (editors). Lippincott, Philadelphia 1994; p. 723-768.
88. Maki DG, Fox BC, Kuntz J, Sollinger HW, Belzer FO. A prospective, randomized, double-blind study of trimethoprim-sulfamethoxazole for prophylaxis of infection in renal transplantation. Side-effects of trimethoprim-sulfamethoxazole, interaction with cyclosporine. J Lab Clin Med 1992; 119: 11-24.
89. Kimmelt MS, Philips TM, Kramer NC, Thompson AM. *In vitro* and *in vivo* interaction of sulfamethoxazole with cyclosporine A measurement by high-pressure liquid chromatography (HRLC). Kidney Int 1985; 27: 343A.
90. Bourgoignie JJ. Renal complications of human immunodeficiency virus type 1. Kidney Int 1990; 37: 1571-1584.
91. Rao TK, Friedman EA. Renal syndromes in the acquired immunodeficiency syndrome (AIDS): lessons learned from analysis over 5 years. Artif Organs 1988; 12: 206-209.
92. Humphreys MH, Schoenfeld PY. Renal complications in patients with the acquired immune deficiency syndrome (AIDS). Am J Nephrol 1987; 7: 1-7.
93. Bourgoignie JJ, Ortiz-Butcher C, Green DF, Pardo V. Renal disease and AIDS. In: Textbook of AIDS Disease. Broder S, Merigan TC Jr., Bolognesi D (editors). Williams and Wilkins, Baltimore, 1993; p. 597-608.
94. Walzer PD, Perl DP, Krogstad DJ, Rawson PG, Schultz MG: *Pneumocystis carinii* pneumonia in the United States. Epidemiologic, diagnostic, and clinical features. Ann Intern Med 1974; 80: 83-93.
95. Glatt AE, Chirgwin K. *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected patients. Arch Intern Med 1990; 150: 271-279.
96. Kovacs JA, Hiemenz JW, Macher AM, Stover D, Murray HW, Shelmhamer J, Lane HC, Urmacher C, Honig C, Longo DL, Parker MM, Natanson C, Parrillo JE, Fauci AS, Pizzo PA, Masur H. *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med 1984; 100: 663-671.
97. Wofsy CB. Use of trimethoprim-sulfamethoxazole in the treatment of *Pneumocystis carinii* pneumonitis in patients with acquired immunodeficiency syndrome. Rev Infect Dis 1987; 9: S184-S194.
98. Wharton JM, Coleman DL, Wofsy CB, Luce JM, Blumenfeld W, Hadley WK, Ingram-Drake L, Volberding PA, Hopewell PC. Trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. A prospective randomized trial. Ann Intern Med 1986; 105: 37-44.
99. Sattler FR, Cowan R, Nielsen DM, Ruskin J. Trimethoprim-sulfamethoxazole compared with pentamidine for treatment of *Pneumocystis carinii* pneumonia in the acquired immuno-deficiency syndrome. A prospective, noncrossover study. Ann Intern Med 1988; 109: 280-287.
100. Gordin FM, Simon GI, Wofsy CB, Mills J. Adverse reactions to trimethoprim-sulfamethoxazole in patients with the acquired immunodeficiency syndrome. Ann Intern Med 1984; 100: 495-499.
101. Zúñiga CA, Chua A, Alpert H, Vaamonde CA. Trimethoprim-sulfamethoxazole nephrotoxicity in patients with AIDS. Nat Kidney Found 1990; 20: A29 (abstract).

102. Chua A, Zúñiga CA, Alpert H, Vaamonde CA. Trimethoprim-sulfamethoxazole nephrotoxicity in the sodium-depleted rat. J Am Soc Nephrol 1990; 1: 608 (abstract).
103. Harvey AM, Malvin RL. Comparison of creatinine and inulin clearances in male and female rats. Am J Physiol 1965; 209: 849-852.
104. Gouvea W, Vaamonde CM, Owens B, Alpert H, Pardo V, Vaamonde CA. The protection against gentamicin nephrotoxicity in the streptozotocin-induced diabetic rat is not related to gender. Life Sci 1992; 51: 1747-1758.
105. Bozzette SA, Finkelstein DM, Spector SA, Frame P, Powderly WG, He W, Phillips L, Craven D, Van Der Host C, Feinberg J, for the NIAID AIDS clinical trial group. A randomized trial of three antipneumocystis agents in patients with advanced human immunodeficiency virus infection. N Engl J Med 1995; 332: 693-699.
106. Para MF, Finkelstein D, Becker S, Dohn M, Walawander A, Black JR. Reduced toxicity with gradual initiation of trimethoprim-sulfamethoxazole as primary prophylaxis for pneumocystis carinii pneumonia: AIDS clinical trials group 268. J AIDS 2000; 24: 337-343.
107. Medina I, Mills J, Leoung G, Hopewell PC, Lee B, Modin G, Benowitz N, Wofsy CB. Oral therapy for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. N Engl J Med 1990; 323: 776-782.
108. Greenberg S, Reiser IW, Chou SY, Porush JG. Trimethoprim-sulfamethoxazole induces reversible hyperkalemia. Ann Intern Med 1993; 119: 291-295.
109. Choi MJ, Fernández PC, Patnaik A, Coupage-Gerard B, D'Andrea D, Szerlip H, Kleyman TR. Brief report: Trimethoprim-induced hyperkalemia in a patients with AIDS. N Engl J Med 1993; 328: 703-706.
110. (a) Modest GA, Price B, Mascoli N: (b) Pennypacker LC, Mintzer J, Pitner J; (c) Canaday DH, Johnson JR; (d) Reiser IW, Chou S-Y; (e) Velazquez H, Ellison DH. Hyperkalemia in elderly patients receiving standard doses of trimethoprim-sulfamethoxazole. Ann Intern Med 1994; 120: 437-438 (letters).
110a. Perazella MA. Hyperkalemia and trimethoprim-sulfamethoxazole: a new problem emerges 25 years later. Review. Connecticut Medicine 1997; 61: 451-458.
111. Funai N, Shimamoto Y, Matsuzaki M, Watanabe M, Tokioka T, Sueoka E, Suga K, Ono K, Sano M, Yamaguchi M. Hyperkalemia with renal tubular dysfunction by sulfamethoxazole-trimethoprim for pneumocystis carinii pneumonia in patients with lymphoid malignancy. Haematologia 1993; 25: 137-141.
112. Lawson DH, Jick H. Adverse reactions to co-trimoxazole in hospitalized medical patients. Am J Med Sci 1978; 275: 53-57.
113. Kalin MF, Poretsky L, Seres DS, Zumoff B. Hyporeninemic hypoaldosteronism associated with acquired immune deficiency syndrome. Am J Med 1987; 82: 1035-1038.
114. Guy R, Turberg Y, Davidson RN, Finnerty G, MacGregor GA, Wise PH. Mineralocorticoid deficiency in HIV infection. Br Med J 1989; 298: 496-497.
115. Murphy CE, Ratnavel RG, Davidson AC. Mineralocorticoid deficiency in HIV infection. Br Med J 1989; 298: 1181.
116. Velázquez H, Perazella MA, Wright FS, Ellison DH. Renal mechanism of trimethoprim-induced hyperkalemia. Ann Intern Med 1993; 119: 296-301.
117. Schlanger LE, Kleyman TR, Ling BN. K+-sparing diuretic actions of trimethoprim: inhibition of Na+ channels in A6 distal nephron cells. Kidney Int 1994; 45: 1070-1076.
118. Murphy JL. Renal tubular acidosis in children treated with trimethoprim-sulfamethoxazole during therapy for acute lymphoid leukemia. Pediatrics 1992; 89: 1072-1074.
119. Schoenbach EB, Greenspan EM. The pharmacology, mode of action, and therapeutic potentialities of stilbamidine, pentamidine, propamidine, and other aromatic diamidines. A review. Medicine 1948; 27: 327-377.
120. Van Hoof L, Henrard C, Peel E. Pentamidine in the prevention and treatment of trypanosomiasis. Trans Roy Soc Trop Med Hyg 1944; 37: 271-280.
121. Napier LE, Sen Gupta PC. The treatment of kala-azar with diaminodiphenoxypentane. Preliminary observations on the treatment of 32 cases. Indian Med Gaz 1943; 78: 177-183.
122. Ivady G, Paldy L, Koltay M, Toth G, Kovacs Z. *Pneumocystis carinii* pneumonia. Lancet 1967; 1: 616-617.
123. Western KA, Perera DR, Schultz MG. Pentamidine isethionate in the treatment of *Pneumocystis carinii* pneumonia. Ann Intern Med 1970; 73: 695-702.
124. Hughes WT, Feldman S, Chandhary SC, Ossi MJ, Cox F, Canyal SK. Comparison of pentamidine isethionate and trimethoprim-sulfamethoxazole in the treatment of *Pneumocystis carinii* pneumonia. J Pediatr 1978; 92: 285-291.
125. Sands M, Kron MA, Brown RB. Pentamidine: a review. Rev Infec Dis 1985; 7: 625-634.
126. Pearson RD, Hewlett EL. Pentamidine for the treatment of *Pneumocystis carinii* pneumonia and other protozoal diseases. Ann Intern Med 1985; 103: 782-786.
127. Haley CJ. Pentamidine in *Pneumocystis carinii* pneumonia. Microlink Update 1987; 3: 1-4.
128. O'Brien G, Dong BJ, Coleman RL, Gee L, Balano KB. A 5-year retrospective review of adverse drug reactions and their risk factors in human immunodeficiency virus-infected patients who were receiving intravenous pentamidine therapy for *Pneumocystis carinii* pneumonia. Clin Inf Dis 1997; 24: 854-859.

129. Sattler FR, Frame P, Davis R, Nichols L, Shelton B, Akil B, Baughman R, Hughlett C, Weiss W, Boylen CT, et al. Trimetrexate with leucovorin versus trimethoprim-sulfamethoxazole for moderate to severe episodes of *Pneumocystis carinii* pneumonia in patients with AIDS: a prospective controlled multicenter investigation of the AIDS clinical trials group protocol 029/031. J Infect Dis 1994; 170: 165-172.
130. Andersen R, Boedicker M, Ma M, Goldstein EJC. Adverse reactions associated with pentamidine isethionate in AIDS patients: recommendations for monitoring therapy. Drug Intell Clin Pharm 1986; 20: 862-868.
131. Waskin H, Stehr-Green JK, Helmick CG, Sattler F. Risk factors for hypoglycemia associated with pentamidine therapy for *Pneumocystis carinii* pneumonia. JAMA 1988; 260: 345-347.
132. Lachaal M, Venuto RC. Nephrotoxicity and hyperkalemia in patients with AIDS treated with pentamidine. Am J Med 1989; 87: 260-263.
133. Chua A, Busse J, Alpert H, Vaamonde CA. Risk factors for pentamidine nephrotoxicity in AIDS patients. Nat Kidney Found 1989; 19: A5 (abstract).
134. Briceland LL, Bailie GR. Pentamidine-associated nephrotoxicity and hyperkalemia in patients with AIDS. DICP, Ann Pharmacother 1991; 25: 1171-1174.
135. Shuster M, Dunn M. Pentamidine and hematuria. Ann Intern Med 1986; 105: 106 (letter).
136. Sensakovic JW, Suarez M, Perez G, Johnson ES, Smith LG. Pentamidine treatment of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. Association with acute renal failure and myoglobinuria. Arch Intern Med 1985; 145: 2247.
137. Chua A, Busse J, Papendick R, Alpert H, Vaamonde CA. Pentamidine nephrotoxicity in the rat. Kidney Int 1990; 37: 478A.
138. Stehr-Green J, Helmick CG. Pentamidine and renal toxicity. N Engl J Med 1985; 313: 6994-6995.
139. Antoniskis D, Larsen RA. Acute, rapidly progressive renal failure with simultaneous use of amphotericin B and pentamidine. Antimicrob Agents Chemother 1990; 34: 470-472.
140. Morin D, Dumas ML, Valette H, Dumas R. Insuffisance rénale aiguë et diabète-insulino-dépéndant transitoires au décours du traitement d'un Kala-azar par pentamidine et antimoniate de N-méthylglucamine. Arch F Pediatr 1991; 48: 349-351.
141. Chunge CN, Gachihi G, Mulgai R, Wasunna K, Rashid JR, Chulay JD, Anabwani G, Oster CN, Bryceson AD. Visceral leishmaniasis unresponsive to antimonial drugs. II. Response to high dosage sodium stibogluconate or prolonged treatment with pentamidine. Trans R Soc Trop Med Hyg 1985; 79: 705-714.
142. Golden JA, Chernoff D, Hollander H, Feigal D, Conte JE. Prevention of *Pneumocystis carinii* pneumonia by inhaled pentamidine. Lancet 1989; 1: 654-657.
143. Nybo Jensen B, Nielsen TG, Lerche B, Jensen TH, Backer V, Mathiesen L, Nielsen JO, Skinhøj P. Prevention of *Pneumocystis carinii* pneumonia relapse by aerosolised pentamidine, 60 mg biweekly, using an Acorn System 22 nebuliser. Scand J Infect Dis 1990; 22: 533-536.
144. Link H, Vöhringer H-F, Wingen F, Brägas B, Schwardt A, Ehninger G: Pentamidine aerosol for prophylaxis of *Pneumocystis carinii* pneumonia after BMT. Bone Marrow Transplant 1993; 11: 403-406.
145. Miller RF, Delany S, Semple SJG. Acute renal failure after nebulised pentamidine. Lancet 1989; 1: 1271-1272.
146. Chapelon C, Raguin G, De Gennes C. Renal insufficiency with nebulised pentamidine. Lancet 1989; 2: 1045-1046.
147. Soo Hoo GW, Mohsenifar Z, Meyer RD. Inhaled or intravenous pentamidine therapy for *Pneumocystis carinii* pneumonia in AIDS. A randomized trial. Ann Intern Med 1990; 113: 195-202.
148. French MH, Milne AH. Some metabolic disturbances in domestic stock following injection of 4:4'-diamidine 1:5 diphenoxypentane. Vet Rec 1941; 53: 413-416.
149. Wien R, Freeman W, Scotcher NM. The metabolic effects produced by certain aromatic diamidines. Ann Trop Med Parasitol 1943; 37: 19-33.
150. Makula DR, Waalkes TP. Interaction between aromatic diamidines and nucleic acids: possible implications for chemotherapy. J Natl Cancer Inst 1975; 54: 305-309.
151. Feddersen A, Sack K. Experimental studies on the nephrotoxicity of pentamidine in rats. J Antimicrob Chemother 1991; 28: 437-446.
152. Waalkes TP, Denham C, DeVita VT. Pentamidine: clinical pharmacologic correlations in man and mice. Clin Pharmacol Ther 1970; 11: 505-512.
153. Bernard EM, Donnelly HJ, Maker MP, Armstrong D. Use of a new bioassay to study pentamidine pharmacokinetics. J Infect Dis 1985; 152: 750-754.
154. Berger BJ, Hall JE, Tidwell RR. The distribution of multiple doses of pentamidine in rats. Pharmacol Toxicol 1990; 66: 234-236.
155. Navim TR, Dickinson CM, Adams SR, Mayersohn M, Juranek DD. Effect of azotemia in dogs on the pharmacokinetics of pentamidine. J Infect Dis 1987; 155: 1020-1026.
156. Berger BJ, Lombardy RJ, Marbury GD, Bell CA, Dykstra CC, Hall JE, Tidwell RR. Metabolic N-hydroxilation of pentamidine *in vitro*. Antimicrob Agents Chemother 1990; 34: 167-1684.

157. Conte JE Jr, Upton RA, Phelps RT, Wofsy CB,Zurlinder E, Lin ET. Use of a specific and sensitive assay to determine pentamidine pharmacokinetics in patients with AIDS. J Infect Dis 1986; 154: 923-929.
158. Conte JE Jr. Pharmacokinetics of intravenous pentamidine in patients with normal renal function or receiving hemodialysis. J Infect Dis 1991; 163: 169-175.
159. Donnelly H, Bernard EM, Rothkotter H, Gold JWM, Armstrong D. Distribution of pentamidine in patients with AIDS. J Infect Dis 1988; 157: 985-989.
160. Gambertoglio JG, Aweeka FT, Blythe WB. Use of drugs in patients with renal failure. In: Diseases of the Kidney, 5th Ed. Schrier RW, Gottschalk CW (editors). Little, Brown and Co, Boston 1993; p. 3211-3268.
161. Fortunato SJ, Bawdon RE: Determination of pentamidine transfer in the *in vitro* perfused human cotyledon with high-performance liquid chromatography. Am J Obstet Gynecol 1989; 160: 759-761.
162. Bouchard PH, Sai P, Reach G, Caubarrere I, Ganeval D, Assan R. Diabetes mellitus following pentamidine-induced hypoglycemia in humans. Diabetes 1982; 31: 40-45.
163. Sharpe SM. Pentamidine and hypoglycemia. Ann Intern Med 1983; 99: 128 (letter).
164. Murdoch JK, Keystone JS. Pentamidine and hypoglycemia (Letter). Ann Intern Med 1983; 99: 879.
165. Stahl Bayliss CM, Kalman CM, Laskin OL. Pentamidine induced hypoglycemia in patients with the acquired immune deficiency syndrome. Clin Pharmacol Ther 1986; 39: 271-275.
166. Collins RJ, Pien FD, Houk JH. Case report: insulin-dependent diabetes mellitus associated with pentamidine. Am J Med Sci 1989; 296: 174-175.
167. DeVita VT, Emmer M, Levine A, Jacobs B, Berard C. *Pneumocystis carinii* pneumonia: successful diagnosis and treatment of two patients with associated malignant processes. N Engl J Med 1969; 280: 287-291.
168. Petz S, Hashmi S. Pentamidine-induced severe hyperkalemia. Am J Med 1989; 87: 698-699.
169. Buff DD, Aboal AA. Pentamidine-associated renal dysfunction and hyperkalemia. Am J Med 1990; 88: 552.
170. Bibler MR, Chou T, Toltzis RJH, Wade PA. Recurrent ventricular tachycardia due to pentamidine-induced cardio-toxicity. Chest 1988; 94: 1303-1306.
171. Balshev U, Berild D, Nielsen TL. Cardiac arrest during treatment of *Pneumocystis carinii* pneumonia with intravenous pentamidine isethionate. Scand J Infect Dis 1992; 24: 111-112.
172. Kleyman TR, Roberts C, Ling BN. A mechanism for pentamidine-induced hyperkalemia: Inhibition of distal nephron sodium transport. Ann Intern Med 1995; 122: 103-106.
173. Shah GM, Alvarado P, Kirschenbaum MA. Symptomatic hypocalcemia and hypomagnesemia with renal magnesium wasting associated with pentamidine therapy in a patient with AIDS. Am J Med 1990; 89: 380-382.
174. Burnett RJ, Reents SB. Severe hypomagnesemia induced by pentamidine. Drug Intell Clin Pharm 1990; 24: 239-240.
175. Gradon JD, Fricchione L, Sepkowitz D. Severe hypomagnesemia associated with pentamidine therapy. Rev Infect Dis 1991; 13: 511-521.
176. Mani S. Pentamidine-induced renal magnesium wasting. AIDS 1992; 6: 594-595.
177. Youle MS, Clarbour J, Gazzard B, Chanas A. Severe hypocalcemia in AIDS patients treated with foscarnet and pentamidine. Lancet 1988; 1: 1455-1456.
178. Hitchings GH: The use of an antimetabolite in chemotherapy of malaria and other infections. Clin Pharmacol Ther 1960; 1: 570-589.
179. Ritschel WA, Hammer GV, Thompson GA: Pharmacokinetics of antimalarials and proposals for dosage regimens. Int J Clin Pharmacol 1978; 16: 395-401.
180. White NJ. Clinical pharmacokinetics of antimalarial drugs. Clin Pharmacol 1985; 10: 187-215.
181. Opravil M, Heald A, Lazzarin A, Hirschel B, Lüthy R, and the Swiss Group for Clinical Studies in AIDS. Combined prophylaxis of pneumocystis carinii pneumonia and toxoplasmosis: dapsone + pyrimethamine vs aerolized pentamidine. AIDS 1992; 6: S8.
182. Opravil M, Keusch G, Lüthy R. Pyrimethamine inhibits renal secretion of creatinine. Antimicrob Agents Chemother 1993; 37: 1056-1060.
183. Paniker U, Levine N. Dapsone and sulfapyridine. Dermatol Clin 2001; 19: 79-86.
184. Aronoff GR, Berus JS, Brier ME, Golper TA, Morrison G, Singer I, Swan SK, Bennett WM (editors). Drug Prescribing in Renal Failure. Dosing Guidelines for Adults, 4th Ed., American College of Physicians, Philadelphia, 1999; p 48.
185. Lee BL, Medina I, Benowitz NL, Jacob P III, Wofsy CB, Mills JV. Dapsone, trimethoprim, and sulfamethoxazole plasma levels during treatment of Pneumocystis carinii pneumonia in patients with the acquired immunodeficiency syndrome (AIDS). Ann Intern Med 1989; 110: 606-611.
186. Belmont A. Dapsone-induced nephrotic syndrome. JAMA 1967; 200: 262-263.
187. Hoffbrand BI. Dapsone and renal papillary necrosis. Br Med J 1978; 1: 78.
188. Chugh KS, Singhal PC, Sharma BK, Mahakur AC, Pal Y, Datta BN, Das KC. Acute renal failure due to intravascular hemolysis in the North Indian patients. Am J Med Sc 1977; 274: 139-146.

189. Kenner DJ, Holt K, Agnello R, Chester GH. Permanent retinal damage following massive dapsone overdose. Br J Ophthalmol 1980; 64: 741-744.
190. Hooper DC. Quinolones. In: Principles and practice of infectious diseases, 4th Ed. Mandel GL, Bennett JE, Dolin R (editors). Churchill Livingstone, New York 1995; p. 364-376.
191. Lesher GY, Froelich EJ, Gruett MD, et al. 1,8-naphthyridine derivatives. A new class of chemotherapeutic agents. J Med Pharmacol Chem 1962; 5: 1063.
192. USPDI: Fluoroquinolones (systemic). In: The United States Pharmacopeial Convention, Inc. Drug Information for the Health Care Professional. 14th Ed, Rockville, 1994; p. 1392-1401.
193. Ferry N, Cuisinaud G, Pozet NV, Zech PY, Sassard J. Nalidixic acid kinetics after single and repeated oral doses. Clin Pharmacol Ther 1981; 29: 695-698.
194. Portmann GA, McChesney EW, Stander H, Moore WE. Pharmacokinetic model for nalidixic acid in man. II. Parameters for abortion, metabolism, and elimination. J Pharm Sci 1966; 55: 72-78.
195. Porter GA. Antimicrobial nephrotoxicity. In: Textbook of Nephrology, 2nd Ed. Massry SG, Glassock RJ (editors). Williams & Wilkins, Baltimore 1989; p. 812-818.
196. Bergan T: Pharmacokinetics of fluorinated quinolones. In: The Quinolones. Andreoli VT (editor). Academic Press, London 1988; p. 119-154.
197. Gerding DN, Hitt JA. Tissue penetration of the new quinolones in humans. Rev Inf Dis 1989; 11: S1046-S1057.
198. Plaisance KI, Drusano GL, Forrest A, Weir MR, Standiford HC. Effect of renal function on bioavailability of ciprofloxacin. Antimicrob Agents Chemother 1990; 34: 1031-1034.
199. Drusano GL. An overview of the pharmacology of intravenously administered ciprofloxacin. Am J Med 1987; 82: S339-S345.
200. Rohwedder R, Bergan T, Thorsteinsson SB, Scholl H. Transintestinal elimination of ciprofloxacin. Chemotherapy 1990; 36: 76-84.
201. Nouaille-Degorge B, Veau C, Dautrey S, Tod M, Laouari D, Carbon C, Farinotti R. Influence of renal failure on ciprofloxacin pharmacokinetics in rats. Antimicrob Agents Chemother 1998; 42: 289-292.
202. Ljungberg B, Nilsson-Ehle I. Pharmacokinetics of ciprofloxacin in the elderly: Increased oral bioavailability and reduced renal clearance. Eur J Clin Microbiol Infect Dis 1989; 8: 515-520.
203. Stahlmann R, Lode H. Safety overview: Toxicity, adverse effects, and drug interactions. In: The Quinolones. Andreoli VT (editor). Academic Press, London 1988; p. 201-233.
204. Johnson PRE, Liu Yin JA, Tooth JA. A randomized trial of high-dose ciprofloxacin versus azlocillin and netilmicin in the empirical therapy of febrile neutropenic patients. J Antimicrob Chemother 1992; 30: 203-214.
205. Ball P. Ciprofloxacin: An overview of adverse experiences. Antimicrob Chemother 1986; 18: S187-S193.
206. Arcieri G, Griffith E, Gruenwaldt G, Heyd A, O'Brien B, Becker N, August R: Ciprofloxacin: An update on clinical experience. Am J Med 1987; 82: S381-S386.
207. Gerritsen WR, Peters A, Henry FC, Brouwes JRB Jr. Ciprofloxacin-induced nephrotoxicity. Nephrol Dial Transplant 1987; 2: 382-383.
208. Rippelmeyer DJ, Synhavsky A. Ciprofloxacin and allergic interstitial nephritis. Ann Intern Med 1988; 109: 170.
209. Lee CW, Fialkow RZ, VanWyck D. Adverse effects associated with the newer fluoroquinolones. Am J Kidney Dis 1989; 13: 510-511 (letter).
210. Hootkins R, Fenves AZ, Stephens MK. Acute renal failure secondary to oral ciprofloxacin therapy: a presentation of three cases and a review of the literature. Clin Nephrol 1989; 32: 75-78.
211. Ying L, Johnson C. Ciprofloxacin-induced interstitial nephritis. Clin Pharm 1989; 8: 518-521.
212. Helmink R, Bunediktsson H. Ciprofloxacin-induced allergic interstitial nephritis. Nephron 1990; 55: 432-433.
213. Rastogi S, Atkinson J, McCarthy J. Allergic nephropathy associated with ciprofloxacin. Mayo Clin Proc 1990; 65: 987-989.
214. Allon M, Lopez E, Min K-W. Acute renal failure due to ciprofloxacin. Arch Intern Med 1990; 150: 2187-2189.
215. Murray K, Wilson M. Suspected ciprofloxacin-induced interstitial nephritis. DICP. Ann Pharmacother 1990; 24: 379-380.
216. Hatton J, Haagensen D. Renal dysfunction associated with ciprofloxacin. Pharmacotherapy 1990; 10: 337-340.
217. Simpson J, Watson AR, Mellersh A, Nelson CS, Dodd K. Typhoid fever, ciprofloxacin, and renal failure. Arch Dis Child 1991; 66: 1083-1084.
218. Arviewux C, Charasse C, Guilhem I, Rivalan J, Le Pogamp P, Raniee MP. Insuffisance rénale aigüé par néphropathie tubulo-interstitielle aprés traitement par ciprofloxacine. Presse Med 1991; 20: 1686.
219. Gonski PN. Ciprofloxacin-induced renal failure in an elderly patient. Med J Austral 1991; 154: 638-639.
220. George MJ, Dew RB III, Daly JS. Acute renal failure after an overdose of ciprofloxacin. Arch Intern Med 1991; 151: 620.
221. Andersen I, Sjostrom P. Ciprofloxacin can cause allergic interstitial nephritis with acute renal failure (Swedish). Lakartidningen 1991; 88: 1298-1299.
222. Bailey JR, Trott SA, Phibrick JT. Ciprofloxacin-induced acute interstitial nephritis. Am J Nephrol 1992; 12: 271-273.

223. La WK, Rolston KVI, Rubenstein EB, Bodey GP. Ciprofloxacin-induced nephrotoxicity in patients with cancer. Arch Intern Med 1993; 153: 1258-1262.
224. Lien Y-HH, Hansen R, Kern WF, Bangert J, Nagle RB, Ko M, Siskind MS. Ciprofloxacin-induced granulomatous interstitial nephritis and localized elastolysis. Am J Kidney Dis 1993; 22: 598-602.
225. Reece RJ, Nicholls A. Ciprofloxacin-induced acute interstitial nephritis. Nephrol Dial Transplant 1996; 24: 379-380.
226. Rosado LJ, Sisking MS, Copeland JG. Acute insterstitial nephritis in a cardiac transplant recipient receiving ciprofloxacin. J Thorac Cardiovasc Surg 1994; 107: 1364-1379.
227. Shih DJ, Korbet SM, Rydel JJ, Schwartz MM: Renal vasculitis associated with ciprofloxacin. Am J Kidney Dis 1995; 26: 516-519.
228. Dharnidharka VR, Nadeau K, Cannon CL, Harris HW, Rosen S: Ciprofloxacin overdose: acute renal failure with prominent apoptotic changes. Am J Kidney Dis 1998; 31: 710-712.
229. Avent CK, Krinsky D, Kirklin JK, Bourge RC, Figg WD. Synergistic nephrotoxicity due to ciprofloxacin and cyclosporine. Am J Med 1988; 85: 452-453.
230. Anand A. Ciprofloxacin nephrotoxicity. Arch Intern Med 1993; 153: 2705-2706.
231. Raja N, Miller WE, McMillan R, Mason JR. Ciprofloxacin-associated acute renal failure in patients undergoing high-dose chemotherapy and autologous stem cell rescue. Bone Marrow Transplant 1998; 21: 1283-1284.
232. Yew WW, Chau CH, Wong PC, Choi HY. Ciprofloxacin-induced renal dysfunction in patients with mycobacterial lung infections. Tuber Lung Dis 1995; 76: 173-175.
233. Hadimeri H, Almroth G, Cederbrant K, Enestrom S, Hultman P, Lindell A. Allergic nephropathy associated with norfloxacin and ciprofloxacin therapy. Report of two cases and review of the literature. Scand J Urol Nephrol 1997; 31: 481-485.
234. Ortiz A, Plaza JJ, Egido J. Ciprofloxacin-associated tubulointerstitial nephritis with linear tubular basement membrane deposits. Nephron 1992; 60: 248.
235. Blum MD, Graham DJ, McCloskey CA. Temafloxacin syndrome: review of 95 cases. Clin Infect Dis 1994; 18: 946-950.
235a. Gauffre A, Mircheva J, Glotz D, Fillastre JP, Simon P, Beaune PH, Druet P. Autoantibodies against a kidney-liver protein associated with quinolone-induced acute interstitial nephritis of hepatitis. Nephrol Dial Transplant 1997; 12: 1961-1962.
236. Schluter G. Ciprofloxain: Review of potential toxicologic effects. Am J Med 1987; 82:S91-S93.
237. Christ W, Lehnert T, Ulbrich B. Specific toxicologic aspects of the quinolones. Rev Infect Dis 1988; 10: S141-S146.
238. Thorsteinsson SB, Bergan T, Oddsdottir S, Rowwedder R, Holin R. Crystalluria and ciprofloxacin: influence of urinary pH and hydration. Chemotherapy 1986; 32: 408-417.
239. Kruger HV, Schuler U, Prokasch B, Gobel M, Ehninger G. Investigation of potential interaction of ciprofloxacin with cyclosporine in bone marrow transplant recipients. Antimicrob Agents Chemother 1990; 34: 1048-1052.
240. Robinson JA, Venezio FR, Costanzo-Nordin MR, Pifarre R, O'Keefe PJ. Patients receiving quinolones and cyclosporine after heart transplantation. J Heart Transplant 1990; 9: 30-31.
241. Avent CK, Krimsky D, Kirklin JK, Bourge RC, Fige WD. Synergistic nephrotoxicity due to cyprofloxacin and cyclosporine. Am J Med 1988; 83: 452-453 (letter).
242. Elston RA, Taylor J. Possible interaction of ciprofloxacin with cyclosporin A. J Antimicrob Chemother 1988; 21: 452-453 (letter).
243. Thompson DJ, Menkis AH, McKenzie FN. Norfloxacin-cyclosporine interaction. Transplantation 1988; 46: 312-313.
244. Vogt P, Schorn T Frei U. Ofloxacin in the treatment of urinary tract infection in renal transplant recipient. Infection 1988; 16: 175-178.
245. Gluckman E, Devergie A, Lokiec F, de Castro H, Meletis J, Traineau R, Cosset JM, Le Mercier N, Varrin F, Cavazzano M. Role of immunosuppresive drugs for prevention of graft-host disease after human HLA matched bone marrow transplantation. Transplant Proc 1987; 19: S61-S65.
246. Rossi E, Silverstri MG, Manari A, Savazzi G, Migone L. Acute renal failure and piromidic acid. Nephron 1982; 32: 80-82.
247. Godin M, Ducastelle T, Bercoff E, Dubois D, Fillastre JP, Boureille J. Renal failure and quinolone. Nephron 1984; 37: 70-72.
248. Boelaert J, de Jaegere PP, Daneels R, Schurgers M, Gordts B, van Landuyt HW. Case report of renal failure during norfloxacin therapy. Clin Nephrol 1986; 25: 272.
249. Krasula RW, Pernet AG. Comparison of organ-specific toxicity of temafloxacin in animals and humans. Am J Med 1991; 91: 38S-41S.
250. Norbby SR. Assessment of adverse events during drug development: experience with temafloxacin. J Antimicrob Chemother 1991; 28: S111-S120.
251. Davis GJ, McKenzie BE. Toxicologic evaluation of ofloxacin. Am J Med 1989; 87: 43S-46S.
252. Mouton Y, Ajana F, Chidiac C, Capron MH, Home P, Masquelier A-M. A multicenter study of lomefloxacin and trimethoprim-sulfamethoxazole in the treatment of uncomplicated acute pyelonephritis. Am J Med 1992; 92: 87S-90S.
253. Shiba K, Saito A, Shimada J, Hori S, Kaji M, Miyahara T, Kusajima H, Kaneko S, Saito S, Ooie T, Uchida H. Renal handling of fleroxacin in rabbits, dogs, and humans. Antimicrob Agents Chemother 1990; 34: 58-64.

12

Antiviral agents

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Introduction

The number of available antiviral agents has increased dramatically over the last five years, especially with the introduction of many new agents used primarily to treat human immunodeficiency virus (HIV) infection. Fortunately, most antiviral agents do not cause acute or chronic renal failure, glomerular disease, or other renal toxicity. Acute renal failure is, however, an important and potentially therapy-limiting toxicity of some antiviral agents, which are the main focus of this chapter. In this chapter, we have adopted a rather broad definition of "nephrotoxicity", and review fluid and electrolyte complications seen with these drugs as well as direct toxic effects on the kidneys. For information regarding clinical pharmacokinetics of antiviral agents and dosing guidelines for their use in patients with renal failure, which are not discussed here, the reader is referred to several recent reviews [1-3, 3a]. This discussion is limited to antiviral agents that are administered orally or parentally only; topical and intraocular applications are not addressed. The nephrotoxicity of interferon alpha is discussed in Chapter 22.

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Acyclovir

Acyclovir is a cyclic analogue of deoxyguanosine, with activity against herpes viruses. Acyclovir diffuses freely into cells, where its subsequent activation and accumulation is dependent upon a herpes virus-specific thymidine kinase. *In vitro*, acyclovir has its greatest action against herpes simplex viruses 1 and 2, compared to varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus, which are less sensitive. Intravenous acyclovir is the drug of choice for treating serious infection caused by herpes simplex virus or varicella-zoster virus, especially in immunocompromised hosts. Oral acyclovir is used to treat less serious herpes simplex infections and for suppression of herpes simplex virus recurrences.

Evidence for significant nephrotoxicity of acyclovir was apparent from preclinical toxicological studies, in which large parenteral doses resulted in the deposition of acyclovir crystals in distal renal tubules and collecting ducts of animals, causing acute renal dysfunction due to tubular obstruction [4]. Numerous reports have documented acyclovir nephrotoxicity in humans [5-15]. In a review by Brigden et al., of 354 immunocompromised patients with life-threatening herpes infections treated with intravenous acyclovir, 58 developed acute renal insufficiency [5]. Keeney and colleagues, in reviewing the early British experience with intravenous bolus acyclovir, reported that 10.3% of adults and 11.5% of children developed azotemia [7]. In another study, up to 48% of outpatients receiving high-dose intravenous therapy developed elevations in the serum creatinine concentration [8].

Renal functional deterioration usually occurs within the first few days of therapy, and may be detected after only a few doses or, more rarely, later in the course of treatment [5-15]. Patients may be asymptomatic, but nausea, vomiting, and abdominal, back, or flank pain are common. Oliguria is uncommon. The rise in the serum creatinine concentration is usually modest, and dialysis has only rarely been necessary. Most patients recover renal function within 3 to 14 days of stopping acyclovir therapy, reducing the dose, or increasing hydration [5, 6, 8-15]. Urinalysis usually shows mild proteinuria, microscopic hematuria, and variable degrees of pyuria. Birefringent needle-shaped crystals may be seen either free or within white blood cells in the urine sediment [10]. It should be noted, however, that acyclovir crystalluria has also been found in patients without acute renal failure [16]. Renal tissue from one autopsy specimen demonstrated crystal deposits in distal tubules [5]. In other cases, however, kidney biopsies from patients with acute renal failure attributed to acyclovir failed to detect such crystals [10, 12]. Instead, tubulointerstitial injury with necrosis and mitotic figures, proteinaceous tubular casts, interstitial infiltration by lymphocytes, plasma cells, and eosinophils, and occasional granulomata were seen.

In an *in vivo* animal study, at doses not causing crystalluria or tissue crystal deposition, short term exposure to acyclovir caused increased renal vasoconstriction and an associated fall in renal blood flow and single nephron plasma flow [17]. Longer-term treatment resulted in a fall in glomerular ultrafiltration coefficient. Thus, it is not clear whether the pathogenesis of acyclovir-induced acute renal failure in humans reflects an obstructive nephropathy from intratubular precipitation of acyclovir, a hemodynamic response, or a toxic, immunologic, or hypersensitivity reaction. It is possible that more than one process may be involved.

The most important risk factors for acyclovir nephrotoxicity are intravascular volume contraction, preexisting renal insufficiency, and high-dose, rapid bolus intravenous infusion [5-15]. Nephrotoxicity with oral acyclovir has been reported rarely [13].

The main non-renal toxicities of acyclovir are gastrointestinal and neurologic side effects. These toxicities tend to develop in patients on high-dose intravenous acyclovir, but since acyclovir is primarily cleared by the kidneys, lower intravenous doses and even oral administration can lead to neurotoxicity in patients with underlying chronic renal insufficiency or acute renal failure [13, 18, 19].

Valacyclovir

Valacyclovir is the L-valyl ester of acyclovir, with oral bioavailability three to five times that of oral acyclovir. Following ingestion, it is rapidly converted by intestinal and hepatic hydrolases to acyclovir. Valacyclovir has gastrointestinal and neurological side effects as seen with acyclovir. To date, significant nephrotoxicity and crystalluria as seen with acyclovir have not been reported with valacyclovir, perhaps because of more conscientious dose adjustments in high-risk patients and avoidance of the very high peak blood lev-

els seen with intravenous acyclovir.

A thrombotic microangiopathic anemia (TMA) syndrome with renal involvement and other clinical features similar to thrombotic thrombocytopenic purpura (TTP) has been reported among patients with HIV infection enrolled in a clinical trial of CMV prophylaxis and in one additional case report [20, 21]. A similar syndrome, with a microangiopathic hemolytic anemia and features of TTP or hemolytic uremic syndrome (HUS) has also been well described in HIV-infected patients not receiving valacyclovir [22, 23]. In the randomized trial, which compared high-dose valacyclovir and two doses of acyclovir, the risk of developing TTP was much greater among valacyclovir-treated patients (14 of 523) than acyclovir-treated patients (4 of 704) [20]. Many of the patients had anemia and thrombocytopenia for several weeks to months prior to the onset of renal disease. Renal failure, which varied from mild to severe, was present at the time of diagnosis or developed shortly thereafter in all but one of the patients. Renal biopsies showed evidence of a TMA in four of five patients. A more gradual onset than is seen with idiopathic or HIV-related TTP, a generally poor response to therapy, including plasmapheresis, and a poor prognosis was described. Death was attributed to HUS, TTP, or renal failure in half the patients. In more than half of the cases, the diagnosis of TTP was not made until valacyclovir had already been discontinued, and on some of the patients, it was felt that other infectious processes could have accounted for the hematologic and renal manifestations [20]. The precise role of valacyclovir and the relative risk of developing TTP with lower doses of valacyclovir remain to be more clearly defined.

Ganciclovir

Ganciclovir is an acyclic nucleoside analogue of guanine that is structurally similar to acyclovir, but is more effective in the treatment and prophylaxis of severe cytomegalovirus infection in immunocompromised hosts. Ganciclovir is myelotoxic, but has no significant nephrotoxicity.

Famciclovir & Penciclovir

Penciclovir is an acyclic guanine analogue similar to ganciclovir, with *in vitro* activity against the herpes viruses and hepatitis B virus. Poor oral availability limits it use to topical applications. Famciclovir is an analogue of penciclovir with a similar spectrum of antiviral activity that is well absorbed following oral administration. As a pro-drug, famciclovir is rapidly metabolized to penciclovir. There have not been reported cases of significant renal toxicity with famciclovir.

Cidofovir

Cidofovir is an acyclic nucleotide analogue of the monophosphate of cytosine. When phosphorylated by host cellular enzymes, the active compound cidofovir diphosphate has broad activity against the herpes viruses, including CMV, herpes simplex viruses 1 and 2, varicella zoster virus and Epstein-Barr virus. Cidofovir is used primarily in treatment of CMV retinitis in patients who have failed treatment with ganciclovir or foscarnet and in acyclovir-resistant herpes simplex infections.

Nephrotoxicity was found in preclinical studies to be the major toxicity of cidofovir, associated with histologic evidence of damage to proximal tubule epithelial cells [24]. Dose- and schedule-dependent nephrotoxicity is also the treatment limiting toxicity of cidofovir in humans [25-28]. Cidofovir is thought to be concentrated by a basolateral membrane organic anion transporter in proximal tubule epithelial cells [29]. Probenecid, an inhibitor of organic anion transport, ameliorates renal toxicity of cidofovir by reducing cellular uptake [24, 30, 31]. *In vitro*, toxicity of cidofovir has been conveyed into mammalian cells by transfection with a gene for a human renal organic anion transporter, and correlates with concomitant intracellular accumulation of the drugs [32]. Renal clearance of cidofovir exceeds creatinine clearance, suggesting that active tubular secretion contributes to renal clearance [25, 30]. At cidofovir doses of 3 mg/kg, probenecid does not appear to affect cidofovir pharmacokinetics, while at higher doses, tubular secretion and renal clearance of cidofovir are reduced [30].

Experience from clinical trials suggest that twenty five percent or more of patients receiving intravenous doses of cidofovir of 3 mg/kg or more develop ARF, often associated with a Fanconi syndrome with tubular proteinuria and evidence of proximal tubular dysfunction with glucosuria, hypophosphatemia, and urinary bicarbonate wasting, with evidence of proximal

tubular injury on renal biopsy [25-28, 33]. Volume expansion with isotonic saline and administration of probenecid substantially reduces this risk. Probenecid is routinely given along with each administration of cidofovir. Preexisting renal insufficiency, recent use of other nephrotoxic agents, and the development while on therapy of proteinuria or other tubular abnormalities predispose patients to risk of severe ARF with cidofovir, which should be avoided or discontinued in these settings. Renal failure due to cidofovir nephrotoxicity may result in the need for dialysis. Both the renal failure and proximal tubule dysfunction associated with cidofovir may be only partially reversible or irreversible [34, 35, 35a], despite discontinuation of therapy and pretreatment with intravenous saline and probenecid. Nephrogenic diabetes insipidus has also been described during therapy with cidofovir [36]. A case of ARF attributed to topical cidofovir has also been recently reported [36a].

Foscarnet

Foscarnet (trisodium phosphonoformate) is an inorganic pyrophosphate analog, which inhibits many DNA polymerases, retroviral reverse transcriptase, and some RNA polymerases, and has antiviral activity against all of the herpes viruses and HIV. Foscarnet has been used primarily for the treatment of serious cytomegalovirus infection.

Foscarnet competitively inhibits Na^+-P_i cotransport in rat, mouse, dog, rabbit, and human renal proximal tubule brush border membrane vesicles, reversibly inhibiting sodium-dependent phosphate transport [37, 38]. Renal cortical Na-K-ATPase and alkaline phosphatase activity are not inhibited by foscarnet, nor is proline, glucose, succinate, or Na^+ transport [37, 38]. Foscarnet induces isolated phosphaturia without hypophosphatemia in thyroparathyroidectomized rats maintained on a low phosphorus diet, without affecting glomerular filtration rate, urinary adenosine 3'5'-cyclic monophosphate (cAMP) activity, or urinary calcium, sodium or potassium excretion [37, 39]. Sodium-P_i cotransport in brush border membrane vesicles from human renal cortex was reported to be even more sensitive to inhibition by foscarnet than in rat renal brush border membrane vesicles [38].

Acute renal failure has been reported to occur in as many as two-thirds of patients treated with foscarnet and has been the major dose-limiting toxicity in 10-20% of cases [40-45]. Despite dose reduction or discontinuation of foscarnet, azotemia typically progresses for at least a few days before resolving. It may be possible to continue foscarnet at reduced doses in some patients with mild azotemia. Foscarnet-induced ARF is usually reversible, although temporary dialysis may be required [46]. Recovery may be slow, particularly in patients with preexisting renal insufficiency. Elevated serum creatinine concentrations may persist for several months after discontinuation of foscarnet. Foscarnet nephrotoxicity may be associated with mild proteinuria. Volume expansion with 1.5 to 2.5 liters of isotonic saline was effective in reducing the incidence of foscarnet nephrotoxicity to 13%, compared to 66% in non-hydrated historical controls, and allowed patients with prior renal insufficiency to receive foscarnet without further reduction of renal function [43, 47]. Intermittent, rather than continuous, infusion of foscarnet may also reduce the incidence of nephrotoxicity [41].

Acute tubular necrosis, tubulointerstitial nephritis, and glomerulonephritis have been described in patients with foscarnet-induced ARF [43, 48-51]. Kidney biopsy specimens from patients who had received foscarnet have, in several reports, shown the presence of crystals within glomerular capillaries and tubules [48, 50-54]. These crystals have had an appearance similar to that of crystalline foscarnet. In addition to trisodium foscarnet, crystals have also been identified as being mixed sodium-calcium and rarely pure calcium salts of foscarnet [52, 53]. The pathophysiologic role of these crystals is uncertain, as they are not seen in all patients with foscarnet-induced ARF. Disruption of glomerular basement membrane by these crystals has been suggested as a cause of non-immune glomerulonephritis seen in some foscarnet-treated patients [50, 51, 53]. Interestingly, crystalluria has not been seen in patients receiving foscarnet [54].

Nephrogenic diabetes insipidus has been described in patients receiving foscarnet [55, 56]. In fact, a recent review cited foscarnet as the second most common reported cause of drug-induced diabetes insipidus, second only to lithium [57]. In experiments using toad urinary bladders [58], serosal application of foscarnet enhanced water flow in the presence of submaximal ADH concentrations, but did not affect water transport in the absence of ADH or when maximal concentrations of ADH were used. Mucosal foscarnet did not affect

water transport. Further studies are needed to clarify the mechanisms for altered water handling by the kidneys with foscarnet.

Hypo- and hypercalcemia, hypo- and hyperphosphatemia, and hypomagnesemia have all been described in patients receiving foscarnet [44, 59-61]. Hypocalcemia is the most common and serious of these electrolyte disturbances. Severe symptomatic hypocalcemia with paresthesias, accompanied by Chvostek's and Trousseau's signs and fatal hypocalcemia have occurred with foscarnet [59]. Jacobson et al. systematically evaluated changes in the serum calcium and phosphate concentrations occurring during single and repeated doses of foscarnet [60]. Ionized calcium levels fell below the lower limit of normal in all patients receiving an infusion of 120 mg/kg and 66% of those who received 90 mg/kg. No changes in total calcium or phosphate concentrations were found. Despite normal total serum calcium concentrations, symptoms compatible with hypocalcemia occurred in two patients. No significant changes in serum phosphate, magnesium, ionized or total calcium, parathyroid hormone, or 1, 25-$(OH)_2$ vitamin D levels were found after a 14 day course of therapy, although increases in parathyroid hormone and vitamin D levels have been reported [41]. Likewise, urinary calcium, phosphorus, magnesium, and potassium excretion were unchanged during 14 days of foscarnet. *In vitro* studies showed an inverse relationship between serum or plasma foscarnet concentrations and ionized calcium concentration, but not with total calcium or phosphate concentrations [60].

Foscarnet is a phosphate analog, and can chelate calcium, as well as other metal ions [62] Since the studies of Jacobson et al. [60] cited above suggested that foscarnet did not increase calcium binding to plasma proteins, the authors concluded that ionized hypocalcemia was primarily a result of foscarnet complexing with ionized calcium. In another recent study, total calcium concentrations declined during and after foscarnet infusion, with ionized calcium concentrations falling to an even greater extent than total calcium [61]. Total magnesium levels also declined, with ionized magnesium concentrations falling to a greater extent. These data also suggest that foscarnet lowers calcium and magnesium levels primarily by binding to calcium and magnesium ions, respectively. Intravenous magnesium ameliorates the fall in ionized magnesium levels with foscarnet, but not the fall in calcium levels [63]. An experimental liposome-encapsulated foscarnet preparation did not reduce plasma calcium levels in animals [64].

Antiretroviral agents

Nucleoside reverse transcriptase inhibitors

Nucleoside analogue reverse transcriptase inhibitors (NRTIs) were the first drugs developed for treatment of HIV infection. They are structural analogues of nucleic acids. When phosphorylated intracellularly to their triphosphate forms, they are competitive inhibitors of viral reverse transcriptase. Drugs in this class include abacavir, adefovir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, and zidovudine.

Despite their widespread clinical use, no direct nephrotoxicity has been reported with zidovudine, didanosine or zalcitabine. Hypokalemia was reported in patients receiving didanosine, which may have been caused by HIV- or didanosine-related diarrhea in some patients, but was apparently not associated with diarrhea in others [65]. Symptomatic hypocalcemia, without changes in serum magnesium, phosphorus, parathyroid hormone, and vitamin D levels (or findings of pancreatitis, a known adverse effect of didanosine) has also been seen with didanosine [66]. Asymptomatic hyperuricemia is common in patients receiving didanosine, particularly at higher doses, because of metabolism of this purine analogue to hypoxanthine and then uric acid [67-69]. Reduction of the dose of didanosine and increased hydration usually correct the hyperuricemia, which has not been associated with the development of gout. Hyperuricemia is not a complication of treatment with zidovudine or zalcitabine, which are pyrimidine analogues.

Lamivudine, a weak inhibitor of organic cation transport by renal tubule epithelial cells [70], has also not been associated with significant nephrotoxicity. Acute renal failure with eosinophilic interstitial nephritis was attributed to abacavir in one patient with HIV infection who also had what appeared to be "classic" FSGS on renal biopsy [71]. The serum creatinine returned to baseline levels after treatment with prednisone and discontinuation of abacavir.

Adefovir is an acyclic analogue of the monophosphate of deoxyadenine. When phosphorylated by host

enzymes, it is a potent inhibitor of DNA polymerases, including reverse transcriptase. Adefovir is active against HIV-1 and hepatitis B virus. Poor oral absorption necessitates intravenous administration. Intravenous adefovir is not currently marketed or under active development; an oral prodrug adefovir dipivoxil is under development for treatment of hepatitis B infection. As seen also with cidofovir, *in vitro* toxicity of adefovir is conveyed into mammalian cells by transfection with a gene for a human renal organic anion transporter, and correlates with concomitant intracellular accumulation of the drugs [32]. Probenecid as well as nonsteroidal anti-inflammatory drugs reduce the cellular uptake and *in vitro* cytotoxicity of adefovir [72]. Clinical experience with adefovir is more limited than with cidofovir, so that its true nephrotoxic potential may not yet be clear. Proximal tubule dysfunction and renal failure, which may take many weeks to resolve after drug discontinuation, are common, however, in patients treated with adefovir [73, 74]. A role for depletion of proximal tubule cell mitochondrial DNA has been suggested as a possible cause of adefovir-related ARF [74a]. Adefovir has similar pharmacokinetic properties as cidofovir, suggesting that the renal toxicity of these drugs may be similar.

Reports of a myopathy developing in patients with AIDS being treated with zidovudine led to the observation that this drug could cause mitochondrial toxicity related to effects on mitochondrial DNA [75-78]. Myopathies and other neuromuscular and systemic manifestations occur in a variety of circumstances as a consequence of mutations in mitochondrial nuclear DNA. Mitochondria have their own extrachromosomal DNA that is distinct from nuclear DNA. Human mitochondrial DNA is a double-stranded, circular molecule with genes that encode proteins for four of the five complexes involved in oxidative phosphorylation and for structural and transfer RNA's required for mitochondrial translation of the protein-encoding genes. Mutations occur more frequently in mitochondrial DNA than nuclear DNA, and have been found in each of the mitochondrial DNA genes. Phenotypically, these mutations are associated most commonly with neuromuscular syndromes, but virtually any organ system can be affected [79-81]. Renal manifestations include Fanconi syndrome most commonly, but nephrotic syndrome (usually with focal and segmental glomerular sclerosis), chronic renal insufficiency with interstitial fibrosis and tubular atrophy and lactic acidosis have also been described [91]. Other significant toxicities include peripheral neuropathy, pancreatitis and hepatic steatosis with liver failure.

Subsequent reports described a syndrome of type B lactic acidosis in patients treated with zidovudine and other nucleoside reverse transcriptase inhibitors, including stavudine, lamivudine, zalcitabine, and didanosine which has also been attribute to mitochondrial DNA toxicity [82-93]. There are five types of DNA polymerase in human cells that catalyze the synthesis of new complementary DNA from the original DNA template (HIV encodes a reverse transcriptase DNA polymerase which uses RNA as the template). The active triphosphate metabolites of zidovudine, didanosine, zalcitabine, and stavudine inhibit DNA polymerase gamma in mitochondria, block the elongation of mitochondrial DNA, and deplete mitochondrial DNA [78-80, 87, 92-94, 94a]. The link between NRTI effects on mitochondrial DNA and lactic acidosis is not entirely clear but is most likely related to disturbances of oxidative phosphorylation and impaired pyruvate metabolism leading to lactate accumulation.

Perhaps one of the most dramatic examples of hepatic failure and lactic acidosis associated with nucleoside analogues was that which occurred during the course of early clinical trials with an investigational nucleoside analogue fialuridine for treatment of chronic hepatitis B [95]. Seven of fifteen study patients developed progressive liver failure and lactic acidosis. Five of the patients died with severe lactic acidosis; two patients underwent emergency liver transplantation and survived. Severe mitochondrial toxicity was proposed as the mechanism for this injury, based in part on the similarity of this presentation to that seen in individuals with inherited disorders of mitochondrial DNA and the presence of histopathological evidence of mitochondrial injury [94, 95].

The lactic acidosis seen with these drugs has ranged from mild and chronic to acute, severe, and fatal [82-93]. The acidosis generally develops after several months of therapy. Patients with NRTI-associated lactic acidosis present with symptoms of nausea, vomiting and abdominal pain. Other features often include elevated liver enzymes, hepatic steatosis, pancreatitis and elevated creatinine kinase with evidence of a myopathy, and liver failure. The lactic acidosis may persist for many weeks despite discontinuation of the

NRTI [82-93]. NRTI-related mitochondrial toxicity may also present with rhabdomyolysis and acute renal failure [96]. Mortality related to NRTI-induced acute lactic acidosis is high, in the range of 50% to 100%, despite drug discontinuation.

In addition to discontinuation of the NRTI, L-carnitine, riboflavin, and thiamine have been used in isolated reports but with unclear therapeutic role [93, 97-99]. In addition to often high-dose intravenous sodium bicarbonate, hemodialysis [100] and continuous venovenous hemodiafiltration [85] have been used to reduce the lactic acidosis, even in the absence of significant renal failure. Lactic acidosis transiently and modestly improved after administration of dichloroacetate in one report [85]. The benefit of any of these therapies is not clear.

Crowther et al. reported a patient with AIDS who was found to have proteinuria, hypokalemia, hyperchloremic metabolic acidosis, hypophosphatemia, hypouricemia, and antidiuretic hormone-resistant polyuria while receiving didanosine (and other medications) [101]. The authors suggested that these disturbances were due to renal tubular toxicities of didanosine, resulting in a Fanconi syndrome with nephrogenic diabetes insipidus. A Fanconi-like syndrome with hypokalemia, metabolic acidosis, hypophosphatemia and aminoaciduria but without glucosuria has also been reported in a patient treated with stavudine and lamivudine [102]. The metabolic acidosis in this case was partly due to lactic acidosis, perhaps related to mitochondrial dysfunction. Abacavir was recently implicated as a cause of biopsy-proven interstitial nephritis in a patient with ARF [102a]. Tenofovir has also been associated with the development of ARF, Fanconi syndrome, and nephrogenic diabetes insipidus [102b-c].

Non-nucleoside reverse transcriptase inhibitors

Drugs in this class include delavirdine, efavirenz, and nevirapine. These drugs bind to viral reverse transcriptase and block DNA polymerase activity. Unlike the NRTIs, these drugs do not require intracellular phosphorylation and are not incorporated into viral DNA. None of these drugs has been associated with any clinically significant renal toxicities or specific fluid-electrolyte complications, and they do not appear to affect mitochondrial DNA polymerases. There is a species dependent renal toxicity of efavirenz [103, 104]. In rats, this drug produces renal tubular epithelial cell necrosis, an effect not observed in humans. A unique glutathione adduct produced as a metabolite of efavirenz in rats but not humans appears to account for this toxicity [103, 104].

Protease inhibitors

Drugs in this class, which inhibit HIV protease leading to impaired maturation of noninfectious HIV virions, include amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir. There are no published reports of ARF or other direct renal toxicity due to amprenavir, nelfinavir, and lopinavir.

Indinavir has been associated with development of crystalluria and nephrolithiasis, which was recognized during initial clinical trials with this drug [105]. Daudon and colleagues first identified and characterized indinavir urinary stones [106]. Up to 20% of a dose of indinavir is excreted into the urine; the solubility of indinavir in urine is pH dependent, with greater solubility at lower pH [107]. Ten to twenty percent of individuals receiving indinavir may develop indinavir crystalluria, even in the absence of any symptoms [108-114]. In one large series, about 8% of indinavir-treated patients had symptoms related to crystal or stone formation; about two-thirds had crystalluria associated with dysuria, urinary urgency, and/or back or flank pain and about one-third had nephrolithiasis [108]. In another retrospective series, 12.4% of patients receiving indinavir developed nephrolithiasis [109]. A prospective study recently evaluated the incidence of urinary abnormalities during the first year of indinavir treatment in a cohort of 54 HIV infected individuals, all of whom were specifically directed to maintain a high fluid intake, evaluated with monthly urinalyses [110]. Crystals first began to appear in urine samples after 1 to 2 weeks of indinavir, beyond which time about 25% of urine specimens contained crystals. Crystals were seen in at least one urine sample from two-thirds of patients. Hypovolemia, a concentrated urine and perhaps a high urine pH appear to be risk factors for indinavir crystalluria and nephrolithiasis [110, 111].

Patients with indinavir crystalluria may be asymptomatic or develop clinical features of renal colic, flank or back pain, dysuria, urinary urgency, fever, nausea and vomiting. Pyuria and microscopic or, less com-

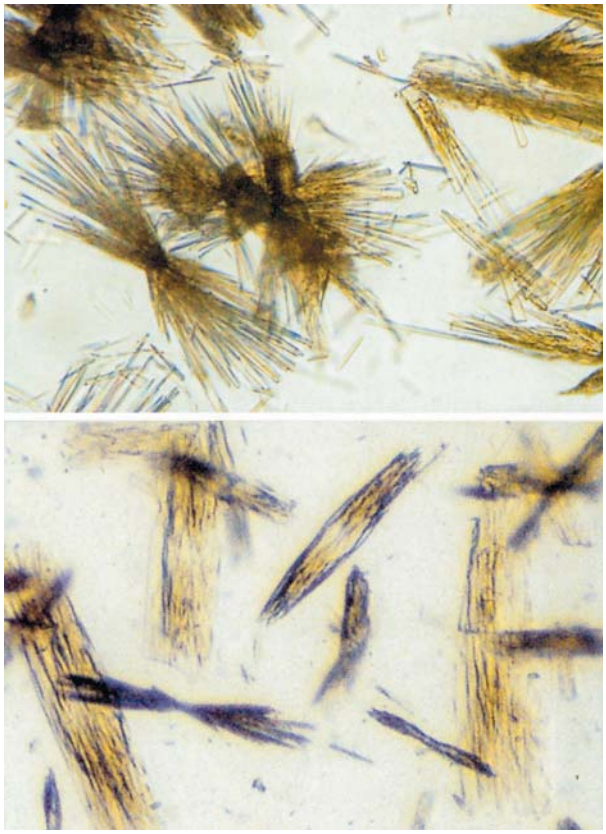

Figure 1. *Photomicrograph of unstained urine sediment showing indinavir crystals (orig. magn. x50). Reproduced with permission from [110].*

monly, gross hematuria may be seen [106, 108-110, 112-114, 114a]. Indinavir crystals (Figure 1) are variable in their appearance by microscopy, usually with needle-shaped, plate-like, fan-shaped, or starburst-like appearances [108, 110]. Renal ultrasonography may be more diagnostically helpful than abdominal radiographs, intravenous urography or CT [115]. Hydronephrosis and sludge in the renal collecting system may be seen. Fewer than 30% of the stones are radiopaque [115]. Renal parenchymal defects may be seen on contrast enhanced CT scans of the kidneys [108]. The crystals are composed of indinavir [106, 108], although calcium oxalate and calcium phosphate have also been identified, which may coexist with or serve as a nidus for indinavir crystals [106, 116]. Urologic intervention may be required for removal of stone or relief of urinary tract obstruction and associated acute renal failure [108, 109, 115-117]. After temporary discontinuation of indinavir and volume repletion, many patients are able to resume treatment with indinavir, although nephrolithiasis and crystalluria may recur. Patients receiving indinavir should be instructed to maintain a high fluid intake. Renal calculi have been reported in one patient treated with saquinavir [118] and in one treated with nelfinavir, in whom the stone was shown to be composed of 99% nelfinavir [118a].

Interstitial nephritis has been described from renal biopsies in patients treated with indinavir [119-124], in some cases with eosinophiluria and crystals (assumed to be indinavir) associated with histiocytes and giant cells in the renal tubules. Some of these patients were asymptomatic, while other had urinary symptoms as described above and crystalluria. Renal atrophy associated with long-term use of indinavir was described in two patients with hematuria, pyuria, and reversible renal insufficiency [125].

Several patients have also been reported with acute renal failure attributed to ritonavir [126-129]. Most of these patients were on other potentially nephrotoxic medications, and some had volume depletion or pre-existing kidney disease. In several patients, ARF recurred upon rechallenge with ritonavir. None of the patients had renal biopsies performed, so there is no information on the histopathologic correlates and etiology of the renal failure.

Amantadine hydrochloride and Rimatadine

Amantadine and rimatadine are tricyclic aliphatic primary amines, active only against influenza A virus, and recommended for prophylaxis in high-risk patients during community or institutional outbreaks of influenza A. In the late 1960's, amantadine was also accidentally observed to ameliorate the symptoms of Parkinson's disease. No nephrotoxicity has been reported with amantadine, although prolonged use of the drug occasionally has been associated with orthostatic hypotension. Acute overdose with amantadine has been associated with an anti-cholinergic syndrome, which may include urinary retention. Severe neurologic reactions to amantadine have been reported in patients with renal failure, including elderly patients with mild renal insufficiency [130, 131].

Rimatadine is structurally similar to amantadine, and has a similar spectrum of antiviral activity. No nephrotoxicity has been described with rimatadine.

Ribavirin

Ribavirin is a synthetic guanosine analogue, with *in vitro* activity against a broad spectrum of DNA and RNA viruses, and retroviruses, including HIV. Ribavirin has been used for treatment of a variety of viral infections, including respiratory syncytial virus bronchiolitis and pneumonia, measles, influenza types A and B, Lassa fever, hemorrhagic fever with renal syndrome (Hantaviruses), hepatitis C, and HIV infection. It is used commonly now along with interferon alpha for treatment of hepatitis C infection. There is no known direct nephrotoxicity of ribavirin.

Vidarabine

Vidarabine is a purine nucleoside analogue active against herpes viruses, influenza viruses, and some RNA viruses. Use of vidarabine for treatment of herpes simplex and varicella-zoster infections has largely been supplanted by acyclovir because of the superior efficacy, fewer adverse effects, and easier administration of the latter agent. Vidarabine has been associated with significant gastrointestinal, neurologic, and hematopoietic toxicities. Patients with renal insufficiency may be particularly susceptible to severe neurologic side effects [132]. No nephrotoxicity with vidarabine has been noted. Because of its poor solubility, vidarabine must be administered in large volumes of fluid. A syndrome of inappropriate antidiuretic hormone has been reported in a patient with disseminated herpes zoster who was being treated with intravenous vidarabine [133].

Conclusion

Fortunately, many antiviral agents do not pose a significant risk of serious nephrotoxicity. However, some of these agents have been associated with the development of acute renal failure, other renal disorders, or fluid and electrolyte disturbances. In some instances, sporadic case reports, a paucity of histological information, and undefined roles of other underlying diseases and concomitant medications make it difficult to establish a definite cause-and-effect relationship with a particular antiviral agent. Careful attention to the potential for toxic renal reactions to antiviral drugs, and in the case of patients being treated for HIV infection, combinations of drugs, is essential to more clearly define the prevalence, pathophysiology, and pathologic correlates of these toxicities.

References

1. Jayasekara D, Aweeka FT, Rodriguez R, Kalayjian RC, Humphreys MH, Gambertoglio JG: Antiviral therapy for HIV patients with renal insufficiency. JAIDS 1999; 21: 384-395.
2. Aronoff GR, Berns JS, Brier ME, Golper TA, Morrison G, Singer I, Swan SK, Bennett WM. Drug prescribing in renal failure. In: Dosing guidelines for adults, 4th edition. Philadelphia: American College of Physicians, 1999.
3. Berns JS, Cohen RM, Stumacher RJ, Rudnick MR. Renal aspects of therapy for human immunodeficiency virus and associated opportunistic infections. J Am Soc Nephrol 1991; 1: 1061-1080.

3a. Izzedine H, Launay-Vacher V, Baumelou A, Deray G. An appraisal of antiretroviral drugs in hemodialysis. Kidney Int 2001; 60: 821-830.

4. Tucker WE. Preclinical toxicology profile of acyclovir: an overview. Am J Med 1982; 73: 27-30.
5. Brigden D, Rosling AE, Woods NC. Renal function after acyclovir intravenous injection. Am J Med 1982; 73: 182-185.
6. Selby PJ, Powles RL, Jameson B, Kay HEM, Watson JG, Thornton R, Morgenstern G, Clink HM. Parenteral acyclovir therapy for herpes virus infections in man. Lancet 1979; 2: 1267-1270.
7. Keeney RE, Kirk LE, Brigden D. Acyclovir tolerance in humans. Am J Med 1982; 73: 171-181.
8. Bean B, Aeppli D. Adverse effects of high dose intravenous acyclovir in ambulatory patients with acute herpes zoster. J Infect Dis 1985; 151: 362-365.
9. Spiegal D, Lau K. Acute renal failure and coma secondary to acyclovir therapy. JAMA 1986; 255: 1882-1883.
10. Sawyer MH, Webb DF, Balow JE, Straus SE. Acyclovir induced renal failure: clinical course and histology. Am J Med 1988; 84: 1067-1071.
11. Giustina A, Romanelli G, Cimino A, Brunori G. Low dose acyclovir and acute renal failure. Ann Intern Med 1988; 108: 312.
12. Rashed A, Azadeh B, Abu-Romeh SH. Acyclovir-induced acute tubulo-interstitial nephritis. Nephron 1990; 56: 436-438.
13. Eck P, Silver SM, Clark EC. Acute renal failure and coma after a high dose of oral acyclovir. N Engl J Med 1991; 325: 1178-1179.
14. Bianchetti M, Roduit C, Oetliker OH. Acyclovir-induced renal failure: course and risk factors. Pediatr Nephrol 1991; 5: 238-239.

15. Becker BN, Fall P, Hall C, Milam D, Leonard J, Glick A, Schulman A. Rapidly progressive acute renal failure due to acyclovir: Case report and review of the literature. Am J Kid Dis 1993; 22(4): 611-615.
16. Potter JL, Krill CE. Acyclovir crystalluria. Pediatr Infect Dis 1986; 5: 710-712.
17. DosSantos MDeFF, DosSantos OFP, Boim MA, Razvickas CV, DeMoura LAR, Ajzen H, Schor N. Nephrotoxicity of acyclovir and ganciclovir in rats: evaluation of glomerular hemodynamics. J Am Soc Nephrol 1997; 8: 361-367.
18. MacDiarmaid-Gordon AR, O'Connor M, Beaman M, Ackrill P. Neurotoxicity associated with oral acyclovir in patients undergoing dialysis. Nephron 1992; 62: 280-283.
19. Almond MK, Fan S, Dhillon S, Pollock AM, Raftery MJ. Avoiding acyclovir neurotoxicity in patients with chronic renal failure undergoing hemodialysis. Nephron 1995; 69: 428-432.
20. Bell, WR, Chulay JD, Feinberg JE. Manifestations resembling thrombotic microangiopathy in patients with advanced human immunodeficiency virus (HIV) disease in a cytomegalovirus prophylaxis trial (ACTG 204). Medicine 1997; 76: 369-380.
21. Rivaud E, Massiani M-A, Vincent F, Azoulay E, Couderc L-J. Valacyclovir hydrochloride therapy and thrombotic thrombocytopenic purpura in an HIV-infected patient. Arch Int Med 2000; 160: 1705-1706.
22. Leaf AN, Laubenstein LJ, Raphael B, Hochster H, Baez L, Karpatkin S. Thrombotic thrombocytopenic purpura associated with human immunodeficiency virus type 1 (HIV-1) infection. Ann Intern Med 1988; 109: 194-197.
23. Berns J. Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura associated with HIV infection. In: Renal and Urologic Aspects of HIV Infection. Kimmel PL, Berns JS (editors). Churchill Livingstone, Inc., Philadelphia, PA 1995; p. 111-133.
24. Lacy SA, Hitchcock MJ, Lee WA, Tellier P, Cundy KC. Effect of oral probenecid coadministration on the chronic toxicity and pharmacokinetics of intravenous cidofovir in cynomolgus monkeys. Toxicol Sci 1998; 44: 97-106.
25. Lalezari JF, Drew WL, Glutzer E, James C, Miner D, Flaherty J, Fisher PE, Cundy K, Hannigan J, Martin JC, Jaffe HS. (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl] cytosine (Cidofovir): results of a phase I/II study of a novel antiviral nucleotide analogue. J Infect Dis 1995; 171: 788-796.
26. Lalezari JP, Stagg RJ, Kuppermann BD, Holland GN, Kramer F, Ives DV, Youle M, Robinson MR, Drew WL, Jaffe HS. Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS. A randomized, controlled trial. Ann Intern Med 1997: 126: 257-263.
27. Polis MA, Spooner KM, Baird BF, Manischewitz JF, Jaffe HS, Fisher PF, Falloon J, Davey RT Jr, Kovacs JA, Walker RE, Whitcup SM, Nussenblatt RB, Lane HC, Masur H. Anticytomegaloviral activity and safety of cidofovir in patients with human immunodeficiency virus infection and cytomegalovirus viremia. Antimicrob Agents Chemother 1996; 39: 882-886.
28. Lalezari JP, Holland GN, Kramer F, McKinley GF, Kemper CA, Ives DV, Nelson R, Hardy WD, Kupperman BD, Northfelt DW, Youle M, Johnson M, Lewis RA, Weinberg DV, Simon GL, Wolitz RA, Ruby AE, Stagg RJ, Jaffe HS. Randomized, controlled trial of the safety and efficacy of intravenous cidofovir for the treatment of relapsing cytomegalovirus retinitis in patients with AIDS. J AIDS Hum Retrovirol 1998;17: 339-344.
29. Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, Sweet DH. The antiviral nucleotide analogs cidofovir and adefovir novel substrates for human and rat renal organic anion transporter 1. Mol Pharmcol 1999; 56: 570-580.
30. Cundy KC, Petty BG, Flaherty J, Fisher PE, Polis MA, Wachsman M, Lietman PS, Lalezari JP, Hitchcock MJM, Jaffe HS. Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 1995; 39: 1247-1252.
31. Hitchcock MJM, Jaffe HS, Martin JC, Stagg RJ. Cidofovir, a new agent with potent anti-herpes virus activity. Antiviral Chem Chemother 1996; 7: 115-127.
32. Ho ES, Lin DC, Mendel DB, Cihlar T. Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. J Am Soc Nephrol 2000; 11: 383-393.
33. Ljungman P, Deliliers GL, Platzbecker U, Matthes-Martin S, Bacigalupo A, Einsele H, Ullmann J, Musso M, Trenschel R, Ribaud P, Bornhauser M, Cesaro S, Crooks B, Dekker A, Gratecos N, Klingebiel T, Tabliaferri E, Ullmann AJ, Wacker P, Cordonnier C. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. Blood 2001; 15: 388-392.
34. Kay TD, Hogan PG, McLeod SE, Johnson DW. Severe irreversible proximal renal tubular acidosis and azotaemia secondary to cidofovir. Nephron 2000; 86: 348-349.
35. Vandercam B, Moreau M, Goffin E, Marot JC, Cosyns JP, Jadoul M. Cidofovir-induced end-stage renal failure. Clin Infect Dis 1999; 29: 948-949.
35a. Meier P, Dautheville-Guibal S, Ronco PM, Rossert J. Cidofovir-induced end-stage renal failure. Nephrol Dial Transplant 2002; 17: 148-149.
36. Schliefer K, Rockstroh JK, Spengler U, Sauerbruch T. Nephrogenic diabetes insipidus in a patient taking cidofovir. Lancet 1997; 350: 413-414.
36a. Bienvenu B, Martinez F, Devergie A, Rybojad M, Rivet J, Ballenger P, Morel P, Gluckman E, Lebbe C. Topical use of cidofovir induced acute renal failure. Transplantation 2002; 73: 661-662.
37. Szczepanska-Konkel M, Yusufi ANK, VanScoy M, Webster SK, Dousa TP. Phosphonocarboxylic acids as specific inhibitors of Na^+-dependent transport of phosphate across renal brush border membrane. J Biol Chem 1986; 261: 6375-6383.

38. Yusufi ANK, Szczepanska-Konkel M, Kempson SA, McAteer JA, Dousa TP. Inhibition of human renal epithelial Na^+/P_i cotransport by phosphonoformic acid. Biochem Biophys Res Comm 1986; 139: 679-686.
39. VanScoy M, Loghman-Adham M, Onsgard M, Szcepanska-Konkel M, Homma S, Knox FG, Dousa TP. Mechanism of phosphaturia elicited by administration of phosphonoformate in vivo. Am J Physiol 1988; 255: F984-F994.
40. Ringden O, Lonnqvist B, Paulin T, Ahlmen J, Klintmalm G, Wahren B, Lernestedt J-O. Pharmacokinetics, safety and preliminary clinical experiences using foscarnet in the treatment of cytomegalovirus infections in bone marrow and renal transplant recipients. J Antimicrob Chemother 1986; 17: 373-387.
41. Jacobson MA, O'Donnell JJ, Mills J. Foscarnet treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. Antimicrob Agents Chemother 1989; 33: 736-741.
42. Cacoub P, Deray G, Baumelou A, Le Hoang P, Rozenbaum W, Gentilini M, Soubrie C, Rousselie E, Jacobs C. Acute renal failure induced by foscarnet: 4 cases. Clin Nephrol 1988; 29: 315-318.
43. Deray G, Martinez F, Katlama C, Levaltier B, Beaufils H, Danis M, Rozenheim M, Baumelou A, Dohin E, Gentilini M, Jacobs C. Foscarnet nephrotoxicity: mechanism, incidence, and prevention. Am J Nephrol 1989; 9: 316-321.
44. Jacobson MA. Review of the toxicities of foscarnet. J AIDS 1992; 5(Suppl 1): S11-S17.
45. Chatelain E, Deminiere C, Lacut JY, Potaux L. Severe renal failure and polyneuritis induced by foscarnet. Nephrol Dial Transplant 1998; 13: 2368-2369.
46. Deray G, Cacoub P, Le Hoang P, Baumelou A, Soubrie C, Rousselie F, Morer I, Jacobs C. Foscarnet-induced acute renal failure and effectiveness of haemodialysis. Lancet 1987; 2: 216.
47. Deray G, Katlama C, Dohin E. Prevention of foscarnet nephrotoxocity. Ann Intern Med 1990; 113: 332.
48. Beaufils H, Deray G, Katlama C, Dohin E, Henin D, Sazdovitch V, Jouanneau C. Foscarnet and crystals in glomerular capillary lumens. Lancet 1990; 336: 755.
49. Nyberg G, Svalander C, Blohme I, Persson H. Tubulointerstitial nephritis caused by the antiviral agent foscarnet. Transplant Int 1989; 2: 223-227.
50. Zanetta G, Maurice-Estepa L, Mousson C, Justrabo E, Daudon M, Rifle G, Tanter Y. Foscarnet-induced crystalline glomerulonephritis with nephrotic syndrome and acute renal failure after kidney transplantation. Transplantation 1999; 67: 1376-1378.
51. Trolliet P, Dijoud F, Cotte L, Cahen R, Francois B, Trepo C, Patricot LM. Crescentic glomerulonephritis and crystals within glomerular capillaries in an AIDS patient treated with foscarnet. Am J Nephrol 1995; 15: 256-259.
52. Estepa-Maurice L, Daudon M, Katlama C, Jouanneau C, Sazdovitch V, Lacour B, Beaufils H. Identification of crystals in kidneys of AIDS patients treated with foscarnet. Am J Kid Dis 1998; 32: 392-400.
53. Justrabo E, Zanetta G, Martin L, Assem M, Mousson C, Maurice-Estepa L, Rifle G, Tanter Y. Irreversible glomerular lesions induced by crystal precipitation in a renal transplant after foscarnet therapy for cytomegalovirus infection. Histopathology 1999; 34: 365-369.
54. Goldfarb DS, Coe FL. Foscarnet crystal deposition and renal failure. Am J Kid Dis 1998; 32: 519-520.
55. Farese RV Jr, Schambelan M, Hollander H, Stringari S, Jacobson MA. Nephrogenic diabetes insipidus associated with foscarnet treatment of cytomegalovirus retinitis. Ann Intern Med 1990; 112: 955-956.
56. Conn J, Colman P, Brown G, Street A, Bate K. Nephrogenic diabetes insipidus associated with foscarnet – a case report. J Antimicrob Chemother 1996; 37: 1180-1181.
57. Bendz H, Aurell M. Drug-induced diabetes insipidus: incidence, prevention and management. Drug Saf 1999; 2: 449-456.
58. Hoch, BS, Shahmehdi SJ, Louis BM, Lipner HI. Foscarnet alters antidiuretic hormone-mediated transport. Antimicrob Agents Chemother 1995; 39: 2008-2012.
59. Youle MS, Clarbour J, Gazzard B, Chanas A. Severe hypocalcemia in AIDS patients treated with foscarnet and pentamadine. Lancet 1988; 1: 1455-1456.
60. Jacobson MA, Gambertoglio JG, Aweeka FT, Causey DM, Portale A. Foscarnet-induced hypocalcemia and effects of foscarnet on calcium metabolism. J Clin Endocrinol Metab 1991; 72: 1130-1135.
61. Noormohamed FH, Youle MS, Tang B, Martin-Munley S, Gazzard BG, Lant AF. Foscarnet-induced changes in plasma concentrations of total and ionized calcium and magnesium in HIV-positive patients. Antivir Ther 1996; 1: 172-179.
62. Perrin DD, Stunzi H. Viral chemotherapy: antiviral actions of metal ions and metal-chelating agents. Pharmac Ther 1981; 12: 255-297.
63. Huycke MM, Naguib MT, Stroemmel MM, Blick K, Monti K, Martin-Munley S, Kaufman C. A double-blind placebo-controlled crossover trial of intravenous magnesium sulfate for foscarnet-induced ionized hypocalcemia and hypomagnesemia in patients with AIDS and cytomegaloviral infection. Antimicrob Agents Chemother 2000; 44: 2143-2148.
64. Omar RF, Dusserre N, Desormeaux A, Poulin L, Tremblay M, Beauchamp D, Bergeron M. Liposomal encapsulation of foscarnet protects against hypocalcemia induced by free foscarnet. Antimicrob Agents Chemother 1995; 39: 1973-1978.
65. Katlama C, Tubiana R, Rosenheim M, Valantin MA, Caumes E, Bricaire F, Gentilini M. Dideoxyinosine-associated hypokalaemia. Lancet 1991; 337: 183.

66. Connolly KJ, Allan JD, Fitch H, Jackson-Pope L, McLaren C, Canetta R, Groopman JE. Phase I study of 2'-3'-dideoxyinosine administered orally twice daily to patients with AIDS and AIDS-related complex and hematologic intolerance to zidovudine. Am J Med 1991; 91: 471-478.
67. Yarchoan R, Pluda JM, Thomas RV, Mitsuya H, Brouwers P, Wyvill KM, Hartman N, Johns DG, Broder S. Long-term toxicity/activity profile of 2', 3'-dideoxyinosine in AIDS or AIDS-related complex. Lancet 1990; 336: 526-529.
68. Lambert JS, Seidlin M, Reichman RC, Plank CS, Laverty M, Morse GD, Knupp C, LcLaren C, Pettinelli C, Valentine FT, Dolin R. 2', 3'-dideoxyinosine (ddI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex. N Engl J Med 1990; 322: 1333-1340.
69. Richardson D, Liou SH, Kahn JO. Uric acid and didanosine compliance in AIDS clinical trials: an analysis of AIDS clinical trials group protocols 116A and 116B/117. J AIDS 1993; 6: 1212-1223.
70. Takubo T, Kato T, Kinami J, Hanada K, Ogata H. Effect of lamivudine on uptake of organic cations by rat renal brush-border and basolateral membrane vesicles. J Pharm Pharmacol 2000; 52: 569-575.
71. Krishnan M, Nair R, Haas M, Atta MG. Acute renal failure in an HIV-positive 50- year old man. Am J Kidney Dis 2000; 36: 1075-1078.
72. Mulato AS, Ho ES, Cihlar T. Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. J Pharmacol Exp Ther 2000; 295: 10-15.
73. Fisher E, Brosgart C, Cohn D, Chaloner K, Pulling C, Semetter B, Alston B, El-Sadr W, CPCRA 039 Team. Safety of adefovir dipivoxil (ADV) and incidence of proximal tubular disorders (PRTD) in a placebo-controlled trial in patients with advanced HIV disease. 6th Conference on Retroviruses and Opportunistic Infections, Chicago 1999 (abstract 678).
74. Kahn J, Lagakos S, Wulfsohn M, Cherng D, Miller M, Cherrington J, Hardy D, Beall G, Cooper R, Murphy R, Basgoz N, Ng E, Deeks S, Winlsow D, Toole JJ, Coakley D. Efficacy and safety of adefovir dipivoxil with antiretroviral therapy. JAMA 1999; 282: 2305-2312.
74a. Tanji N, Tanji K, Kambham N, Markowitz GS, Bell A, D'agati VD. Adefovir nephrotoxicity: possible role of mitochondrial DNA depletion. Hum Pathol 2001; 32: 734-740.
75. Arnaudo E, Dalakas M, Shanske S, Morales CT, DiMauro S, Schon EA. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. Lancet 1991; 337: 508-510.
76. Chen SC, Barker SM, Mitchell DH, Stevens SM, O'Neill P, Cunningham AL. Concurrent zidovudine-induced myopathy and hepatotoxicity in patients treated for human immunodeficiency virus (HIV) infection. Pathology 1992; 24: 109-111.
77. Dalakas MC, Illa I, Pezeshkpour GH, Laukaitis JP, Cohen B, Griffin JL. Mitochondrial myopathy caused by long-term zidovudine therapy. N Engl J Med 1990; 322: 1098-1105.
78. Chen CH, Vazquez-Padua M, Cheng YC. Effect of anti-human immunodeficiency virus nucleoside analogs on mitochondrial DNA and its implication for delayed toxicity. Mol Pharmacol 1991; 39: 625-628.
79. Zeviani M, Tiranti V, Plantadosi C. Reviews in molecular medicine. Medicine 1999; 77: 59-72.
80. Johns DR. Mitochondrial DNA and disease. N Engl J Med 1995; 33(10):638-644.
81. Niaudet P, Rotig A. The kidney in mitochondrial cytopathies. Kidney Int 1997; 51: 1000-1007.
82. Gopinath R, Hutcheon M, Cheema-Dhadli S, Halperin M. Chronic lactic acidosis in a patient with acquired immunodeficiency syndrome and mitochondrial myopathy: biochemical studies. J Am Soc Nephrol 1992; 3: 1212-1219.
83. Chattha G, Arieff AI, Cummings C, Tierney LM Jr. Lactic acidosis complicating the acquired immunodeficiency syndrome. Ann Intern Med 1993; 118: 37-39.
84. Haupt MT, Carlson RW, Fulop M, Coyle T, Chariot P, Baram D, Chattha G. Lactic acidosis and AIDS. Ann Intern Med 1993;119: 343-345.
85. Shaer A, Rastegar A. Lactic acidosis in the setting of antiretroviral therapy for the acquired immunodeficiency syndrome. Am J Nephrol 2000; 20: 332-338.
86. Bleeker-Rovers CP, Kadir SW, vanLeusen R, Richter C. Hepatic steatosis and lactic acidosis caused by stavudine in an HIV-infected patient. Neth J Med 2000; 57: 190-193.
87. Bartley PB, Westacott L, Boots RJ, Lawson M, Potter JM, Hyland VJ, Woods ML. Large hepatic mitochondrial DNA deletions associated with L-lactic acidosis and highly active antiretroviral therapy. AIDS 2001; 15: 419-420.
88. Mokrzycki MH, Harris C, May H, Laut J, Palmisano J. Lactic acidosis associated with stavudine administration: a report of five cases. Clin Infect Dis 2000; 30: 198-200.
89. John M, Moore CB, James IR, Nolan D, Upton RP, McKinnon EJ Mal SA. Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy. AIDS 2001; 15: 717-723.
90. Miller KD, Cameron M, Wood LV, Dalakas MC, Kovacs JA. Lactic acidosis and hepatic steatosis associated with use of stavudine: report of four cases. Ann Intern Med 2000; 133: 192-196.
91. Sundar K, Suarez M, Banogon PE, Shapiro JM. Zidovudine-induced fatal lactic acidosis and hepatic failure in patients with acquired immunodeficiency syndrome: report of two patients and review of the literature. Crit Care Med 1997;25: 1425-1430.

92. Brinkman K, terHofstede HJ, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS 1998; 12: 1735-1744.
93. Moyle G. Clinical manifestations and management of antiretroviral nucleoside analog-related mitochondrial toxicity. Clin Ther 2000; 22: 911-936.
94. Swartz M. Mitochondrial toxicity – new adverse drug effects. N Engl J Med 1995; 333: 1146-1148.
94a. Côté HCF, Brumme ZL, Craib KJP, Alexander CS, Wynhoven B, Ting L, Wong H, Harris M, Harrigan PR, O'Shaughnessy MV, Montaner JSG. Changes in mitochondrial DNA as a marker of nuceloside toxicity in HIV-infected patients. New Engl J Med 2002; 346: 811-820.
95. McKenzie R, Fried M, Sallie R, Conjeevaram H, DiBisceglie AM, Park Y, Savarese B, Kleiner D, Tsokos M, Luciano C, Pruett T, Stotka JL, Straus SE, Hoofnagle JH. Hepatic failure and lactic acidosis due to fialuridine (flau), an investigational nucleoside analogue for chronic hepatitis B. N Engl J Med 1995; 333: 1099-1105.
96. Chariot P, Ruet E, Authier FJ, Levy Y, Gherardi R. Acute rhabdomyolysis in patients infected by human immunodeficiency virus. Neurology 1994; 44: 1692-1696.
97. Foulty B, Frerman F, Reves R. Riboflavin to treat NRTI-induced lactic acidosis. Lancet 1999; 352: 291-292.
98. Luzzati R, DelBravo P, DiPerri G, Luzzani A, Concia E. Riboflavine and severe lactic acidosis. Lancet 1999; 353: 901-902.
99. Claessens YE, Cariou A, Chiche JD, Dauriat G, Dhainaut JF. L-carnitine as a treatment of life-threatening lactic acidosis induced by nucleoside analogues. AIDS 2000; 14: 472-473.
100. Chodock R, Eleftherios M, Shemin D, Valgerdur R, Yodice P, Renzi R, Tashima K, Towe C, Rich J. Survival of a human immunodeficiency patient with nucleoside-induced lactic acidosis – role of haemodialysis treatment. Nephrol Dial Transplant 1999; 14: 2484-2486.
101. Crowther MA, Callaghan W, Hodsman AB, Mackie ID. Dideoxyinosine-associated nephrotoxicity. AIDS 1993; 7: 131-132.
102. Morris AAM, Baudouin SV, Snow MH. Renal tubular acidosis and hypophosphataemia after treatment with nucleoside reverse transcriptase inhibitors. AIDS 2001; 15: 140-141.
102a. Krishnan M, Nair R, Haas M, Atta MG. Acute renal failure in an HIV-positive 50-year-old man. Am J Kidney Dis 2000; 36: 1075-1078.
102b. Coca S, Perazella MA. Acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. Am J Med Sci 2002; 324: 342-344.
102c. Verhelst D, Monge M, Meynard J-L, Fouqueray B, Mougenot B, Girard P-M, Ronco P, Rossert J. Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 2002; 40: 1331-1333.
103. Mutlib AE, Chen H, Nemeth GA, Markwalder JA, Seitz SP, Gan LS, Christ DD. Identification and characterization of efavirenz metabolites by liquid chromatography/mass spectrometry and high field NMR: species differences in the metabolism of efavirenz. Drug Metab Dispos 1999; 27: 1319-1333.
104. Mutlib AE, Gerson RJ, Meunier PC, Haley PJ, Chen H, Gan LS, Davies MH, Gemzik B, Christ DD, Krahn DF, Markwalder JA, Seitz SP, Robertson RT, Miwa GT. The species dependent metabolism of efavirenz produces a nephrotoxic glutathione conjugate in rats. Toxicol Appl Pharmacol 2000; 169: 102-113.
105. Olyaei AJ, deMattos AM, Bennett WM. Renal toxicity of protease inhibitors. Curr Opin Nephrol Hypertens 2000; 9: 473-476.
106. Daudon M, Estepa L, Viard JP, Joly D, Jungers P. Urinary stones in HIV-1-positive patients treated with indinavir. Lancet 1997; 349: 1294-1295.
107. Balani SK, Woolf EJ, Hoagland VL, Sturgill MG. Disposition of indinavir, a potent HIV-1 protease inhibitor, after an oral dose in humans. Drug Metab Dispos 1995; 24: 1389-1394.
108. Kopp JB, Miller KD, Mican JM, Feuerstein IM, Vaughan E, Baker C, Pannell LK, Falloon J. Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997; 127: 119-125.
109. Reiter WJ, Pernerstorfer-Schon H, Dorfinger K, Hofbauer J, Marberger M. Frequency of urolithiasis in individuals seropositive for human immunodeficiency virus treated with indinavir is higher than previously assumed. J Urol 1999; 161: 1082-1084.
110. Gagnon, RF, Tecimer SN, Watters, AK, Tsoukas CM. Prospective study of urinalysis abnormalities in HIV-positive individuals treated with indinavir. Am J Kid Dis 2000; 36: 507-515.
111. Brodie SB, Keller MJ, Ewenstein BM, Sax PE. Variation in incidence of indinavir-associated nephrolithiasis amount HIV-positive patients. AIDS 1998; 12: 2433-2437.
112. Trainor LD, Steinberg JP, Austin GW, Solomon HM. Indinavir crystalluria: identification of patients at increased risk of developing nephrotoxicity. Arch Pathol Lab Med 1998; 122: 256-259.
113. Padberg J, Fritsche L, Bergmann F, Schurmann D, Suttorp N. Nephropathy and renal colic in patients treated with indinavir, ritonavir plus indinavir or ritonavir plus saquinavir. AIDS 1999; 13: 2173-2174.
114. Bonfanti P, Valsecchi L, Parazzini F, Carradori S, Pusterla L, Furtuna P, Timillero L, Alessi F, Ghiselli G, Gabbuti A, DiCintio E, Martinelli C, Faggion I, Landonio S, Quirino T. Incidence of adverse reactions in HIV patients treated with protease inhibitors: a cohort study. Coordinamento Italiano Studio Allergia e Infezione da HIV (CISAI) group. J AIDS 2000; 23: 236-245.

114a. Herman JS, Ives NJ, Nelson M, Gazzard BG, Easterbrook PJ. Incidence and risk factors for the development of indinavir-associated renal complications. J Antimicrob Chemother 2001; 48: 355-360.
115. Schwartz, BF, Schenkman N, Armenakas NA, Stoller ML. Imaging characteristics of indinavir calculi. Am J Urol 1999; 161: 1085-1087.
116. Kohan AD, Armenakas NA, Fracchia JA. Indinavir urolithiasis: an emerging cause of renal colic inpatients with human immunodeficiency virus. J Urol 1999; 161: 1765-1768.
117. Berns JS, Cohen RM, Silverman M, Turner J. Acute renal failure due to indinavir crystalluria and nephrolithiasis: report of two cases. Am J Kid Dis 1997; 30: 558-560.
118. Green ST, McKendrick MW, Schmid ML, Mohsen AH, Prakasam SF. Renal calculi developing de novo in a patient taking saquinavir. Int J STD AIDS 1998; 9: 555.
118a. Engeler DS, Hubert J, Rentsch KM, Ruef C, Oertle D, Suter S. Nelfinavir urinary stones. J Urol 2002; 167: 1384-1385.
119. Tashima KT, Horowitz JD, Rosen S. Indinavir nephropathy. N Engl J Med 1997; 336: 138-139.
120. Sarcletti M, Petter A, Zangerle R. Indinavir and interstitial nephritis. Ann Intern Med 1998; 128: 320-322.
121. Grabe DW, Eisele G, Miller C, Singh J, Stein D. Indinavir-induced nephropathy. Clin Nephrol 1999; 51: 181-183.
122. Perazella MA, Kashgarian M, Cooney E. Indinavir nephropathy in an AIDS patient with renal insufficiency and pyuria. Clin Nephrol 1998; 50: 194-196.
123. Jaradat M, Phillips C, Yum MN, Cushing H, Moe S. Acute tubulointerstitial nephritis attributable to indinavir therapy. Am J Kid Dis 2000; 35: 1-5.
124. Marroni M, Gaburri M, Mecozzi F, Baldellli F. Acute interstitial nephritis secondary to the administration of indivavir. Ann Pharmocother 1998; 32: 843-844.
125. Hanabusa H, Tagami H, Hataya H. Renal atrophy associated with long term treatement with indinavir. New Engl J Med 1999; 340: 392-393.
126. Duong M, Sgro C, Grappin M, Biron F, Boibieux A. Renal failure after treatment with ritonavir. Lancet 1996; 348: 693-694.
127. Witzke C, Plentz A, Schafers RF, Reinhardt W, Heemann U, Philipp T. Side effects of ritonavir and its combination with saquinavir with special regard to renal function. AIDS 1997; 11: 836-838.
128. Bochet MV, Jacquiaud C, Valantin MA, Katlama C, Deray G. Renal insufficiency induced by ritonavir in HIV-infected patients. Am J Med 1998; 105: 457.
129. Chugh S, Bird R, Alexander EA. Ritonavir and renal failure. N Engl J Med 1997; 336: 138.
130. Ing TS, Daugirdas JT, Soung LS, Klawans HL, Maharker SD, Hayashi JA, Geis WP, Hong JE. Toxic effects of amantadine in patients with renal failure. Can Med Assoc J 1979; 120: 695-698.
131. Ing TS, Rahn AC, Armbruster KFW, Oyama JH, Klawans HL. Amantadine accumulation in renal failure. N Engl J Med 1974; 291: 1257.
132. Marker SC, Howard RJ, Groth KE, Mastri AR, Simmons RL, Balfour HH Jr. A trial of vidarabine for cytomegalovirus infection in renal transplant patients. Arch Intern Med 1980; 140: 1441-1444.
133. Ramos E, Timmons RF, Schimpff SC. Inappropriate antidiuretic hormone following adenine arabinoside administration. Antimicrob Agents Chemother 1979; 15: 142-144.

13

Analgesics and 5-aminosalicylic acid

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Introduction

Classic analgesic nephropathy is a slowly progressive disease resulting from the daily use for many years of analgesic mixtures containing at least two antipyretics, anilides and salicylates, and usually caffeine or codeine (or both). The nephropathy is characterized by renal papillary necrosis/calcifications and chronic interstitial nephritis, with an insidious progression to renal failure, sometimes in association with transitional-cell carcinoma of the uroepithelium [1-10]. This type of nephropathy has never been described after the intake of single analgesic substances. Analgesic nephropathy is a facultative part of a broad spectrum of clinical findings that is summarized as 'analgesic syndrome' (see below). Historically, these analgesic mixtures most frequently contained phenacetin in combination with a further analgesic and a centrally acting agent such as caffeine or codeine, which may lead to psychological dependence [11-15].

In addition to the classical picture of analgesic nephropathy, excessive exposure to analgesics and NSAIDs may contribute to the progression of a chronic renal disease of whatever etiology towards end-stage renal failure [16-21]. In all those epidemiological studies, however, it is impossible to rule out bias caused by the consumption of these analgesics for symptoms of the conditions that predisposed patients to renal failure.

Renal failure induced by abuse of analgesic mixtures, was identified as a serious problem in several countries in the second part of the 20th century. The initial reports [1, 22-24], observing that phenacetin was present in most abused analgesics, held this substance solely responsible for the development of what was

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 263-278

called "phenacetin nephritis". In the late 1970's, it became apparent that the abuse of different kinds of analgesic mixtures may induce renal damage, whether they contained phenacetin or not. Consequently, the disease was more appropriately named 'analgesic nephropathy'.

Apart from classic analgesic nephropathy, this chapter will also handle the possible nephrotoxic role of 5-aminosalicylic acid (5-ASA) used in patients with chronic inflammatory bowel disease (IBD). During the last decade, 5-ASA replaced sulfasalazine as first-line therapy for mildly to moderately active IBD. For decades, sulphasalazine, an azo-compound derived from sulphapyridine and 5-aminosalicylic acid (5-ASA), has been the only valuable non-corticosteroid drug in the treatment of inflammatory bowel disease. Azad Kahn et al. [25] showed that the pharmacologically active moiety in sulphasalazine for the treatment of these diseases was 5-ASA. Consequently, this resulted in a number of new 5-ASA formulations (mesalazine, olsalazine, balsalazine) for topical and oral use. Since the metabolite sulphapyridine was largely responsible for the side effects of sulfasalazine, the primary advantage of the newer 5-ASA agents is their improved adverse effect profile.

In recent years, however, several case reports have been published, suggesting an association between the use of 5-ASA and the development of a particular type of chronic tubulo-interstitial nephritis, characterized by an important cellular infiltration of the interstitium [26, 27]. In some cases, it was shown that this cellular infiltration was not disappearing upon arrest of the drug, even after a period of more than one year [28]. Although acute renal failure under non-steroidal anti-inflammatory drugs (NSAID) is well documented, the risk for developing chronic lesions remains controversial.

Chronic renal effects associated to the use of non-steroidal anti-inflammatory drugs (NSAID) is discussed in chapter 14.

Analgesics

Epidemiological observations

Association between analgesic abuse and analgesic nephropathy

Clinical evidence linking the abuse of analgesic mixtures with the development of renal failure was well documented from the 1960's on. There are numerous reports demonstrating a high incidence of heavy analgesic consumption in patients with renal failure or papillary necrosis [14]. Also the observed deterioration of renal function in patients with analgesic nephropathy who continued their abuse, and in contrast the stabilization of renal function after discontinuation of the abuse, is in favor of this association [29-32].

Epidemiological evidence of the association between analgesic abuse and the development of renal impairment is documented in several case-control studies published in the 1980's. The studies were conducted in Australia [33], the United States [16, 20, 34], Germany [35, 36] and Spain [18]. Methodological details are summarized in Table 1. In all studies, the matched controls were randomly selected except in McCredie's study. A history of analgesic consumption was obtained by direct interview of the subjects, except in the studies of Sandler and Perneger where telephone interviews were used. Moreover, in the study of Sandler the interviews were conducted using proxies for 55% of the cases and 10% of the controls.

It is clear that these case control studies are not able to establish cause and effect. Moreover, one needs to take into account that flaws in the study design, inaccurate renal diagnosis and confounding by indication have to be considered when interpreting the results [39, 40]. In table 1, results shown are odds ratios estimating the relative risk for developing the disease under study when particular types of analgesics were taken in the minimum dose defined. The studies by Sandler [16], Pommer [36] and Morlans [18] resulted in comparable odds ratios showing a two-threefold increased risk for developing chronic renal failure after analgesic abuse. McCredie's study [33] showed a considerable higher ratio by defining the more specific lesion of renal papillary necrosis as disease. The negative results obtained in Murray's study [34] can possibly be attributed to the low prevalence of analgesic nephropathy in the area investigated and the low amount of analgesic use in the definition of analgesic abuse employed.

The most firmly based evidence of association is provided by the prospective studies reported. Dubach [37] followed a cohort of 623 working women aged 30-49 years with objective evidence of the intake of phenacetin-containing analgesics and 621 age-matched controls during a 10 years follow-up period. Urine speci-

Table 2.1. Epidemiological studies demonstrating the nephrotoxicity of different kind of analgesics.

STUDY DESIGN			
Case-control studies	**Cases**	**Controls**	**Definition of minimal abuse**
McCredie et al, Australia, 1982	80 women with RPN	80 healthy women	3 units/week for one year
Murray et al, USA, 1983	527 p. with ESRD	1047 hospitalized p.	almost daily for 30 days
Sandler et al, USA, 1989	554 p. with newly diagnosed CRF	516 population based	daily for one year
Pommer et al, West Berlin, 1989	517 p. with ESRD	517 outpatient clinic p.	15 units/month for one year
Morlans et al, Barcelona,1990	340 p. with ESRD	673 hospitalized p.	15 units/month for 30 days
Perneger et al, USA,1994	716 p. with ESRD	361 population based	daily for one year
Prospective studies	**Cases**	**Controls**	**Definition of minimal abuse**
Dubach et al, Switzerland, 1983	623 healthy women followed during 10 years	621 healthy women	NAPAP in urine positive
Elseviers & De Broe, Belgium, 1995	200 healthy subjects followed during 7 years	200 population based	daily for one year overall at least 1000 units

RESULTS							
	Any analgesic	**Single analgesics *(3)***			**Analgesic mixtures**		
		Any	Aspirin	Acetaminophen	Any	Phenacetin	Acetaminophen
Case-control studies (1)							
McCredie et al	17.2	-	-	-	-	18.1	ns
Murray et al	ns	-	-	-	-	-	-
Sandler et al	2.79	-	ns	3.21	-	7.59	6.9
Pommer et al	2.44	ns	-	-	2.69	4.76	4.06
Morlans et al	2.89	-	2.54	-	-	19.05	-
Perneger et al	-	-	ns *(4)*	-	-	-	2.1 *(4)*
Prospective studies **(2)**							
Dubach et al	-	-	-	-	-	8.10	-
Elseviers & De Broe		-	-	-	6.10	-	-

(1) Results are odds' ratios showing significant differences between cases and controls.
(2) Results are relative risks for the development of renal failure during the study period.
(3) Results are obtained after adjustment for the use of other analgesics.
(4) Analgesic component either or not taken in mixture.
Abbreviations: NAPAP= N-acetyl-p-aminophenol (the main metabolite of phenacetin); ns= not significant.

mens of the women under study were analyzed for the presence of N-acetyl-p-aminophenol (NAPAP), the main metabolite of phenacetin. Subjects with a positive result for NAPAP in their urine (2 x 3 samples) at least twice were considered as analgesic abusers. Compared to the controls, abusers showed a more frequent raised serum creatinine (6.7% vs. 0.9%) and a low specific gravity of urine (23 vs. 7%). The two groups did not differ in development of bacteriuria, hematuria or proteinuria [37]. Based on Dubach's data, the relative risk for developing renal failure after regular analgesic consumption of phenacetin-containing products in young women could be estimated to be 8.1 (95% CI: 2.8-23.2) [41].

A second prospective study, investigating a cohort of 200 active analgesic abusers and 200 matched controls during seven years, extended the results of Dubach, including abusers of different sex and age categories, abusing different kinds of analgesic mixtures all or not containing phenacetin. A relative risk of 6.1 (95% CI: 1.4-25.9) was observed for the development of a decreased renal function. Moreover, the re-

nal impairment was found to be compatible with the diagnosis of analgesic nephropathy in most of the cases, in the absence of other forms of renal disease [38].

Nephrotoxicity of different kinds of analgesics

In the majority of the early analgesic nephropathy reports, phenacetin was singled out as the nephrotoxic culprit on the basis of association and circumstantial evidence. Nearly all patients initially reported had taken large amounts of analgesic mixtures containing phenacetin. Prescott [14], was the first to evaluate the nephrotoxic role of phenacetin and other analgesics. He stated that in the past insufficient attention had been given to the possible nephrotoxicity of the other analgesics invariably taken with phenacetin, and that the common belief that phenacetin is the primary cause of analgesic nephropathy can be challenged on many counts. He argued that numerous chronic toxicity studies in animals with phenacetin have failed to produce renal papillary necrosis, that the removal of phenacetin in some countries has not been followed by the expected fall in mortality from analgesic nephropathy and that analgesic nephropathy has a poor prognosis if phenacetin is discontinued but other analgesics are abused further.

The withdrawal of phenacetin from analgesic mixtures in Western Europe and the United States, however, gives rise to question the nephrotoxic potency of the different kinds of products without phenacetin, currently on the market [10]. Although in the 1980's new case series of analgesic nephropathy were reported from all over the world, evidence of the nephrotoxic effect of these newer drugs remained mainly limited to local observations, with or without controlling for the previous intake of phenacetin containing products. However, the nephrotoxic potency of this type of analgesic mixtures could be demonstrated using different kinds of epidemiological observations [42].

First, the case-control studies of the 1980's could confirm the nephrotoxic potency of analgesic mixtures and the different substances worked-up in these mixtures (table 1). Interpretation of the presented odds ratios per substance however, remains difficult since they were seriously influenced by the additional effect of other substances invariably taken with it. The nephrotoxic effect of single analgesics could only be documented in the studies of Sandler et al. [16] and Morlans et al. [18]. Their observations remain however debatable since a rigorous control for additional consumption of other analgesics was lacking in these studies [16, 18, 43, 44].

Moreover, a cohort of 226 patients with a clear diagnosis of analgesic nephropathy was investigated regarding their analgesic consumption. Patients were recruited within the framework of diagnostic criteria studies in Belgium (n=130) and eleven other European countries (n=96) [45, 46]. In all patients, analgesic nephropathy was diagnosed using the same validated renal imaging criteria with high diagnostic performance [47, 48]. In all included patients, the history of abuse was documented by the same methodology using the same structured questionnaire accompanied by a color picture book showing the analgesics with a high sales volume in each particular country. Results clearly showed that analgesic nephropathy was associated with the abuse of different kind of analgesic mixtures mostly containing phenacetin. However, 46 out of the 226 patients never consumed phenacetin-containing analgesics. Their documented analgesic nephropathy was associated with the abuse of the following combinations: aspirin and acetaminophen, aspirin and a pyrazolone, acetaminophen and a pyrazolone, and two pyrazolones all of which were combined with caffeine, codeine or both. Additionally, the minimal analgesic consumption for developing analgesic nephropathy could be defined as a daily consumption for at least five years. None of the subjects with a daily use of analgesic mixtures for less than 5 years (n=16) or those with a weekly but not a daily consumption for more than 5 years (n=19) met the renal imaging criteria of analgesic nephropathy [49].

Furtheron, a broad range of other clinical and epidemiological observations is in support with the previous results. For single analgesics, abuse is only poorly documented and the nephrotoxic potency of single analgesics can be considered as minimal. Even in patients with rheumatoid arthritis in which high dose salicylate therapy was the mainstay of treatment, analgesic nephropathy seldom developed [14]. For single analgesics combined with caffeine/codeine, the example of Sweden is of particular interest. Although, Sweden has a high sales volume of this type of analgesics (40% of the total volume), prevalence of analgesic nephropathy remained at the low level of 1-2% during the last decade [50]. Moreover, it is of interest to note that in countries with a low prevalence of analgesic nephr-

opathy such as Sweden and France, analgesic mixtures containing two analgesic substances combined with caffeine/codeine are not available (Sweden) or not sold (France), despite the fact that in both countries the total volume of analgesics sold is higher than in Belgium (Figure 1).

Quantification of the problem

Detailed information concerning the extent of the problem in analgesic nephropathy is limited, particularly for recent years. National annual data are collected in Australia/New Zealand by the Australian and New Zealand Dialysis and Transplant Registry (ANZDATA) [51] and in the United States by the United States Renal Data System (USRDS) [52]. In Europe, the registration system of the European Dialysis and Transplant Association (EDTA) [53] published regularly incidence and prevalence data of analgesic nephropathy for all European countries in the past.

Australian incidence rates showed a significant decline after the restriction of over-the-counter analgesic sales in 1979. During the 1970's, Australia had the highest incidence rate in the world (up to 22%). The incidence declined to 15% in 1985 and to 11% in 1990 [54, 55]. In recent years the incidence remained at a level of 5-6% with a peak incidence of 10% for Queensland [51]. In Flanders, a region with well-documented high incidence of analgesic nephropathy in end stage renal failure patients, the incidence fell from 17% in the mid eighties to 5.4% in 2000 and 2001.

In the United States the national prevalence of analgesic nephropathy is not well documented. In the 1980's, local studies showed incidences, ranging from 1.7 in Philadelphia and 2.8% in Washington D.C. to 10% in Northwest North Carolina [34, 56, 57]. According the USRDS annual data report, current incidence of analgesic nephropathy is 0.2% for all patients starting renal replacement therapy in the period 1994-1998 (USRDS 2000) [52]. In Canada 2.5% of dialysis patients had analgesic nephropathy in 1976. The recent prevalence can be expected to be lower [58]. In South Africa in the early eighties, 33% of the white patients starting chronic renal replacement therapy in Durban were diagnosed with analgesic nephropathy [59]. In Kuala Lumpur, Malaysia, 8% of the 180 dialysis patients had consumed excessive quantities of analgesics and in 4% signs of renal papillary necrosis were observed [60]. More recently high analgesic abuse of 7-10% in rural areas was reported [61]. Incidence of analgesic nephropathy in ESRD population of Thailand is however unknown.

On the other hand, Central and Eastern European countries are confronted nowadays with an increasing incidence of the disease partly due to the increasing number of older patients accepted for renal replacement therapy in recent years. Moreover, in these countries analgesic mixtures containing two analgesic substances combined with caffeine/codeine are still available and have an important market share. In 1992, Matousovic et al. [62] measured an incidence rate of 9.1% of analgesic nephropathy in the Czech and Slovak Republics using renal imaging criteria [63]. The same methodology was used in Hungary where an incidence up to 13% was noted in 1996 [64]. In contrast, in the southwest region of Poland not any case of analgesic nephropathy could be identified in the period 1991-1992. The investigators concluded, however, that a reassessment of the incidence after 5-10 years should be mandatory because in the early 1990's only 40% of (younger) ESRD patients received dialysis treatment [65].

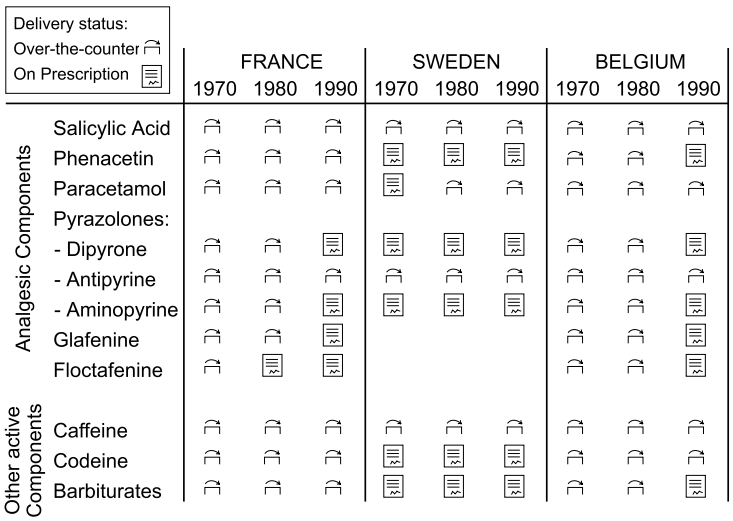


Figure 1. *For each country, the delivery status in 1970, 1980 and 1990 is presented for all single ingredients. To know the status of analgesic mixtures, it is the status of the component whoese availability is the most stringent that applies. Exceptions are indicated [50].*

Pathophysiology

The exact pathophysiological mechanism(s) of analgesic nephropathy is unknown. The disease is characterized by capillary sclerosis of the vessels of the renal pelvis and ureteral mucosa, renal papillary necrosis and calcification, interstitial infiltration fibrosis, progressive cortical atrophy next to zones with hypertrophy of the remaining nephrons, aspecific glomerular changes. The main pathological lesion strongly indicates the more distal parts of the nephron as the predilected target for analgesic toxicity (Figure 2).

The potentiating effect of aspirin with both phenacetin and acetaminophen may be related to two factors:

- Acetaminophen undergoes oxidative metabolism by prostaglandin H synthase to reactive quinoneimine that is conjugated to glutathione. If acetaminophen is present alone, there is sufficient glutathione generated in the papillae to detoxify the reactive intermediate. However, if acetaminophen is ingested with aspirin, the aspirin is converted to salicylate, which becomes highly concentrated and depletes glutathione in both the cortex and papillae of the kidney. With the cellular glutathione depleted, the reactive metabolite of acetaminophen then produces lipid peroxides and arylation of tissue proteins, ultimately resulting in necrosis of the papillae [9, 66].
- Aspirin and NSAID suppress prostaglandin production by inhibiting cyclooxygenase enzymes. Renal blood flow, particularly within the renal medulla, is highly dependent upon systemic and local production of vasodilatory prostaglandins. Thus, this region, in the setting of combined aspirin and NSAID use, is more prone to ischemic damage. Loss of proteoglycans and glycosaminoglycans, essential constituents of medullary matrix may occur.

Clinical aspects

Clinically, analgesic nephropathy is characterized by its slow and stealthy progression. Most analgesic nephropathy patients only attended the outpatient nephrology clinic when renal failure reached a chronic and advanced stage. End-stage renal failure due to analgesic abuse was observed after consumption for approximately 20 years and most patients with analgesic nephropathy entered renal replacement therapy in their fifth-sixth decade.

An increased occurrence of anemia and an increased risk of developing vascular diseases and ischemic heart disease are mentioned in patients with analgesic nephropathy [67]. Gastrointestinal manifestations occur in more than half of analgesic abusers and particularly gastric ulcerations are frequently reported [68, 69]. Psychological and psychiatric manifestations are common in analgesic abusers and this is reflected in the frequency of associated addictive habits such as smoking, alcoholism and the excessive use of psychotropic drugs. Also the prematurely aged appearance of these patients has been emphasized. These observations pointed to the fact that analgesic nephropathy is part of a much wider syndrome called "the analgesic syndrome" [30, 70, 71].

Moreover, in 1965 a first publication from Sweden drew attention to the increasing incidence of tumoral degeneration of the kidney and the urinary tract ob-

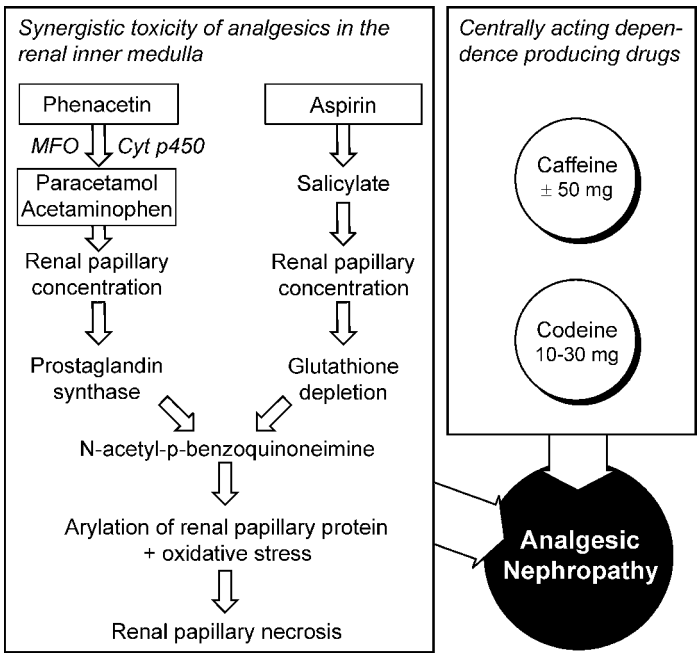


Figure 2. *Analgesic nephropathy is caused by abuse of analgesic mixtures plus caffeine/codeine. Left: the potentiating effect of aspirin with both phenacetin and acetaminophen (see text). Adapted from [5, 9], with permission.*

served in analgesic abusers [72-74]. Additional case reports were published in Switzerland, Australia, and Belgium [75-77]. Although, the risk for developing tumors of the urinary tract after the abuse of different kinds of analgesics is not clearly established, the abuse of phenacetin containing products showed a four-to tenfold increased risk [76]. The tumors generally become apparent after 15 to 25 years of analgesic abuse [78], usually but not always in patients with clinically evident analgesic nephropathy [79]. Most patients are still taking the drug at the time of diagnosis, but clinically evident disease can first become apparent several years after cessation of analgesic intake and even after renal transplantation has been performed [78]. It is presumed that the induction of malignancy results from the intrarenal accumulation of N-hydroxylated phenacetin metabolites that have potent alkylating action [79]. Because of urinary concentration, the highest concentration of these metabolites will be in the renal medulla, ureters, and bladder, possibly explaining the predisposition to carcinogenesis at these sites. The major presenting symptom of urinary tract malignancy in analgesic nephropathy is microscopic or gross hematuria. Thus, continued monitoring is essential, and new hematuria should be evaluated with urinary cytology, and, if indicated, cystoscopy with retrograde pyelography [78]. It may also be prudent to obtain yearly urine cytology for the first several years if analgesics are discontinued or indefinitely if drug intake persists. The incidence of urothelial carcinoma after renal transplantation in patients with analgesic nephropathy is comparable to the general incidence of up to 10% of urothelial carcinomas in end-stage renal failure patients with analgesic nephropathy. Removal of the native kidneys prior to renal transplantation has also been suggested, but the efficacy of this regimen has not been proven [78].

Diagnosis

Until recently, the diagnosis of analgesic nephropathy was difficult to obtain. The disease is associated with a large number of mainly aspecific clinical symptoms [80]. Renal papillary necrosis, considered as the hallmark of analgesic nephropathy, can only be directly demonstrated by autopsy, after nephrectomy or in the exceptional case of a patient eliminating a papilla [81]. In a large part of cases, the diagnosis was mainly based on a documented history of abuse after a process of exclusion of other causes of renal failure. Since, furthermore, several authors [82, 83] have noted that a clear history of abuse is difficult to obtain, the need for diagnostic criteria with a well-defined performance became mandatory.

In Belgium, a prospective controlled multicentre study started in 1988, aiming to select diagnostic criteria for analgesic nephropathy with well-defined performance in patients with end-stage renal failure. In a cohort of 60 analgesic abusers and 188 controls, all starting renal replacement therapy, a large number of clinical, laboratory and radiological signs reported to be associated with analgesic nephropathy were tested. It was found that renal imaging investigations (sonography and tomography) demonstrating a decrease in length of both kidneys combined with either bumpy contours or signs of renal papillary necrosis were the only ones which showed a high sensitivity and specificity for diagnosing the disease. Other signs frequently mentioned such as hypertension, anemia, sterile pyuria and bacteriuria showed low sensitivity and/or specificity [47].

In a separate study, the diagnostic value of CT scan without contrast media was compared to the previously used renal imaging techniques (sonography and tomography) (Figure 3). A cohort of 40 analgesic abusers (= daily use of mixtures during at least 5 years) and 40 controls, all end-stage renal failure patients without a clear renal diagnosis were investigated with sonography, tomography and CT scan without contrast, searching for the renal imaging signs of analgesic nephropathy. Using CT scan the renal size and contour could be evaluated with comparable results while this technique scored better for the detection of papillary calcifications (Figure 3) [46, 63].

In an additional controlled study, the diagnostic performance of CT scan in patients with incipient/moderate renal failure was studied. In a cohort of 53 analgesic abusers with a serum creatinine between 1.5 and 4 mg/dl and in the absence of a clear renal diagnosis, a CT scan was performed. It was found that the renal image of analgesic nephropathy on CT scan in an early stage of renal failure is comparable with the observations made in end-stage renal failure patients (Figure 3). Especially the demonstration of bilateral papillary calcifications showed a high sensitivity of 92% with a specificity of 100% for the early diagnosis of

analgesic nephropathy (Figure 3) [63].

Prevention

Analgesic nephropathy is one of the few renal diseases currently suitable for primary prevention.

Informative campaigns focused on the population at risk did not solve the problem of analgesic nephropathy. In Belgium, it was clearly demonstrated that in most abusers, sustained analgesic consumption was no longer related to a physical complaint but analgesics were mainly taken for their mood-altering capacities. Most analgesic abusers admitted to having been informed of the health risks related to long-standing analgesic abuse and even if renal impairment occurred, only a part of the cases stopped their analgesic abuse [84].

Also the withdrawal of phenacetin from analgesic mixtures did not solve the problem. When phenacetin was withdrawn from most analgesic mixtures in Australia (1970's), no decline in the occurrence of analgesic nephropathy could be observed [80, 85]. A declining incidence rate was only observed after restriction of the over-the-counter sales of all analgesic mixtures in 1979-1980 [55, 51, 86]. Some countries in Europe, particularly Sweden, have succeeded in controlling the disease after legislative measures were taken. As early as the sixties, Sweden elaborated legislation that only a few years later became very effective.

The legislation was simple and clear: all analgesic containing, even the slightest dose of phenacetin became prescription limited. This resulted in a prescription status of almost all combined analgesics, hence a dramatic drop in their sale. In spite of the substantial total increase of consumption of single analgesics between 1980-1990, analgesic nephropathy belongs nowadays to the history of medicine-nephrology (< 1% of Swedish dialysis population) [50].

In contrast, in many other European countries, no effective legislative measurements were taken. In Belgium, Germany and Switzerland, the pharmaceutical industry spontaneously removed phenacetin from their products. Phenacetin was replaced by another analgesic substance such as pyrazolone maintaining a high volume of analgesic mixtures still containing two or more analgesic substances. In Belgium, the Ministry of Health decided in 1988 that when obtaining analgesic mixtures in the pharmacy users had to sign a request and received an information sheet warning for possible renal consequences of extensive analgesic consumption. This resulted obviously in a fall of the sale/consumption of analgesic mixtures. Although these measures were only effective during one year, their indirect effect was more important. After 1988, several pharmaceutical companies modified their analgesic products, resulting in a reduction of the mixtures from two analgesic components to one analgesic plus caffeine and/or codeine.

Analgesic nephropathy gained recognition in recent years in several Central and Eastern European countries. Abuse of analgesic mixtures is also reported in several third world countries without any knowledge about the extent of the problem of analgesic nephropathy. Moreover, in many countries there are no legislative limitations to introduce analgesic mixtures containing two analgesic substances combined with caffeine/codeine onto the market.

In view of prevention, it would be advisable to obtain legislative measures worldwide in order to limit the over-the-counter availability of all analgesics containing two analgesic components plus caffeine/codeine. This is formally asked in Europe [49] as well as in the United States [7] by a large group of investigators active in the field.

5-Aminosalicylic acid (5-ASA)

Epidemiological observations

Case reports

The association between the use of 5-ASA and the development of chronic tubulointerstitial nephritis in patients with inflammatory bowel disease (IBD) gained recognition in recent years by the publication of several case reports [26, 28, 87-94] (Figure 4). Reported cases are summarized in Table 2. The disease was more prevalent in males with a male/female ratio of 16:3. The age of reported cases ranged from 14 to 45 years. In contrast with analgesic nephropathy where renal lesions were only observed after several years of analgesic abuse, interstitial nephritis associated to 5-ASA was already observed during the first year of treatment in 8 out of 19 reported cases. Most cases started 5-ASA therapy with a documented normal renal function. Complete recovery upon arrest of the drug however, was only observed in 6 out of 19 published cases and

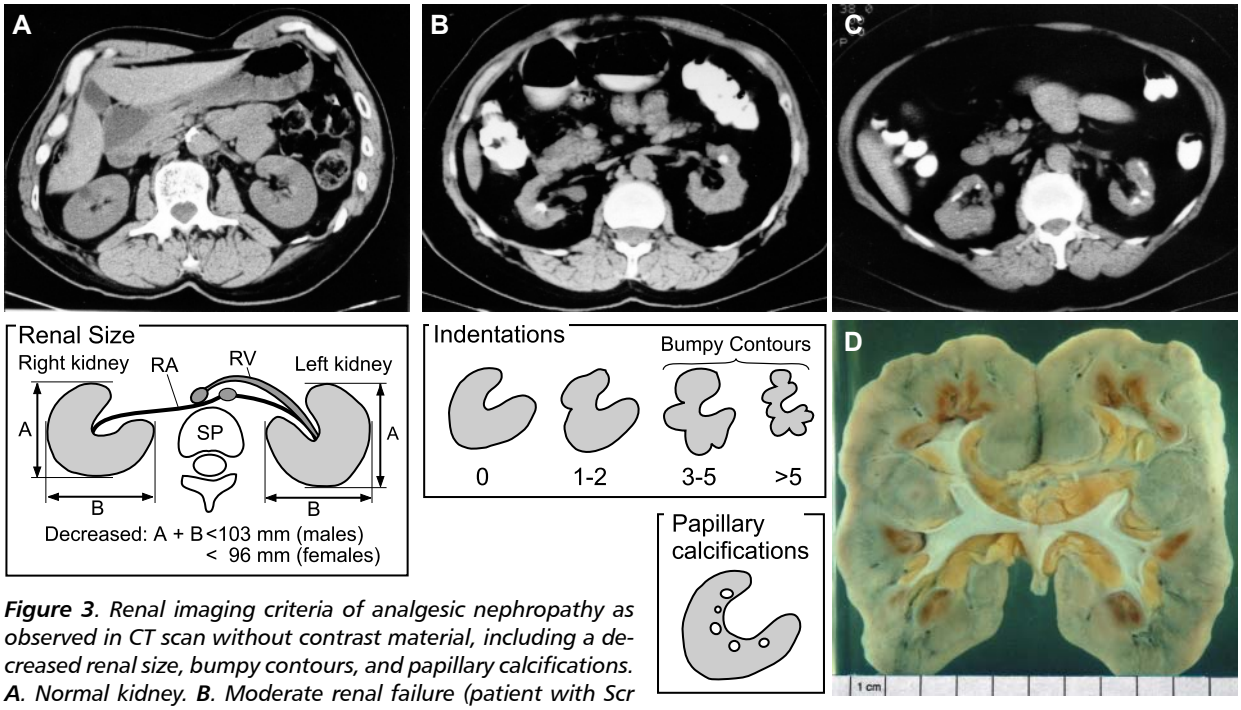
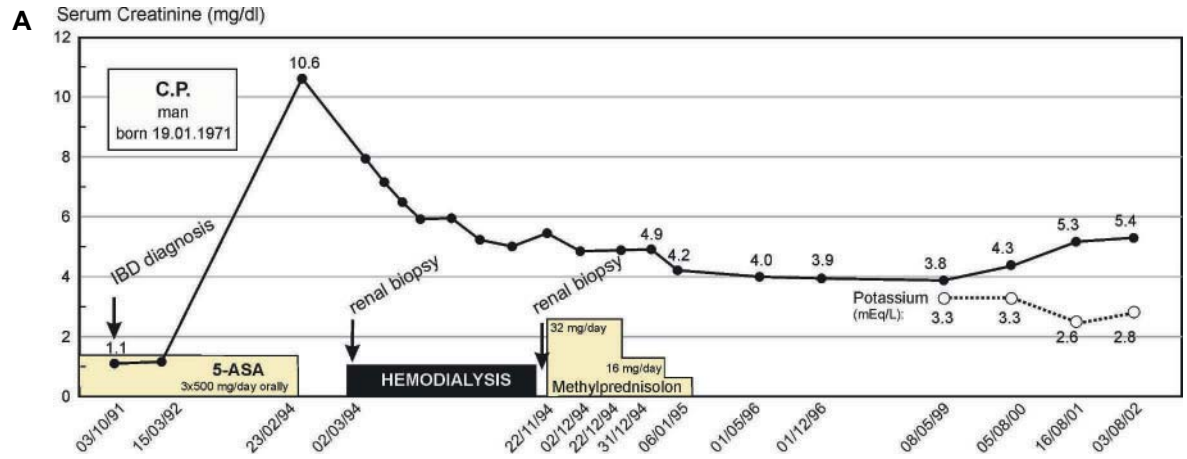


***Figure 3**. Renal imaging criteria of analgesic nephropathy as observed in CT scan without contrast material, including a decreased renal size, bumpy contours, and papillary calcifications. **A**. Normal kidney. **B**. Moderate renal failure (patient with Scr 2.3 mg/dl). **C**. End-stage renal failure. **D**. Post-mortem kidney of an analgesic nephropathy patient with moderate renal failure. Irregular contours of the kidney, fibrotic-necrotic foci of papillae. RA= renal artery; RV= renal vein; SP= spine.*



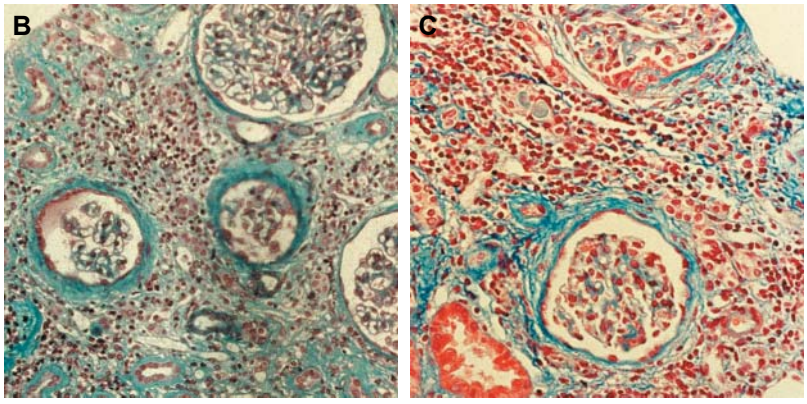


***Figure 4.** Case report of nephrotoxicity of 5-aminosalicylic acid (5-ASA) in inflammatory bowel disease.*
***A**. Evolution of renal failure.*
***B**. First renal biopsy.*
***C**. Second renal biopsy.*
Note the important cellular infiltration in both biopsies. Normal aspect of glomeruli.
B-C: H&E staining, orig. magn. x350.

dependent on the degree of renal damage at diagnosis.

Retrospective study

A retrospective study was performed aiming to obtain more insight in the frequency of this disease [27]. Nephrologists of Belgium, France and the Netherlands were asked to report all cases of inflammatory bowel disease (IBD) showing signs of renal impairment associated or not with 5-ASA therapy. Questionnaires were completed and returned by 71 nephrologists. Among them, 44 reported that they had no such cases. The remaining 27 nephrologists sent detailed information on 40 cases of IBD with renal failure. Among 40 reported cases 26 used 5-ASA including 15 with biopsy proven interstitial nephritis (Figure 4). It is worthwhile to notice that on the one hand a few cases with chronic tubulointerstitial nephritis never used 5-ASA and on the other hand some cases with 5-ASA therapy showed renal failure diagnosed as glomerulonephritis or amyloidosis.

Prospective studies

Until now, the risk ratio of 5-ASA associated renal failure in patients with inflammatory bowel disease is not known. It will be rather difficult to obtain more insight in this risk. Drug usage in these patients is irregular and acute episodes of inflammation result in either increasing drug regimen and/or increasing number of drugs prescribed, including some with known nephrotoxic potential. Furthermore, the kidney is an extra-intestinal target of the disease as shown in Table 2.

In recent years several attempts were made to measure early signs of renal impairment in patients with IBD treated with 5-ASA. Schreiber et al. [95] investigated 223 IBD patients using sensitive markers of glomerular and tubular dysfunction. Patients receiving high amounts of 5-ASA showed an increased prevalence of tubular proteinuria. He concluded that the possibility exists that high doses of 5-ASA may be associated with proximal tubular proteinuria but that his study design was not able to dissect the possible impact of chronic inflammation on the development of renal impairment. In contrast, K.R. Herlinger et al. [96] performed an investigation on 95 IBD patients carefully assessing disease activity. He concluded that tubular proteinuria occurred in the majority of IBD patients and was re-

Table 2. Published case reports of interstitial nephritis in patients with inflammatory bowel disease using 5-ASA.

Reference	Sex	Age	5-ASA use duration (months)	Creatinine onset therapy	Clearance lowest level	Follow-up upon arrest 5-ASA (months)	Recovery?
von Mühlendal [87]	m	14	5	?	31*	1	complete
Henning [88]	m	31	42	normal	17	4	partial
Ruf-Ballauf [89]	m	45	7	80*	33	12	complete
Mehta [90]	m	29	5	normal	38	2	complete
Masson [91]	m	26	18	normal	55*	3	complete
Thulavath [92]	m	28	26	normal	45	3	no
	m	24	36	121*	47	>12	no
Smilde [93]	m	42	20	90	18	54	partial
	f	37	5	51	25	?	complete
	m	25	26	78	14	12	partial
	m	30	5	116	54	36	no
	m	34	8	73	13	5	no
World [94]	m	31	42	?	33	2	no
	m	43	28	?	25	12	no
	m	24	22	?	17	27	no
	f	30	3	?	<10*	44	no
Stolear [28]	m	24	23	104*	11*	27	partial

:calculated creatinine clearance

lated to disease activity rather than to 5-ASA treatment.

Recently, a European prospective study aiming to register all IBD patients with renal impairment and to control for a possible association with 5-ASA therapy was performed [97]. During a one-year observation period, gastroenterologists of Belgium, France, Italy, Republic of Macedonia and Yugoslavia registered 1529 patients with IBD seen at their outpatient clinic. At the start of the study a questionnaire was filled in focused on medical and drug history. Additional data were collected at baseline, after 6 months and after 12 months, including activity of IBD, actual medication and results of the serum creatinine determination. Only 34 patients (2.2%) showed at least once a decreased creatinine clearance. Consecutive decreased creatinine clearances were observed in 13 patients (0.9%). Dehydration due to low body mass combined with active IBD was the main reason for an intermittent decrease in renal function in most of these patients. Particularly, the observation of 5-ASA therapy in 5 patients with renal impairment of unknown origin is suggestive for a possible etiological role of 5-ASA. Comparing patients with and without renal impairment, the presence of a stoma revealed the highest increased risk.

The number of renal impairment cases observed in this prospective study is highly comparable with the estimations made by World et al. 5 years ago. They stated that the available evidence suggested that renal impairment of any severity may occur in up to one in 100 patients, but clinically significant interstitial nephritis occurs in less than one in 500 patients [26].

Pathophysiology

This particular form of chronic tubulo-interstitial nephritis is characterized by an important cellular infiltration of the interstitium with macrophages, T-cells but also B-cells. Furthermore, after arrest of the drug, there is improvement of the renal function in some cases [26, 93]. In those in which there is a delayed diagnosis of renal damage, recovery of renal function does not occur. Instead, several of those patients needed one or another form of renal replacement therapy. An important aspect of this type of toxic nephropathy is the documented persistence of the inflammation of the renal interstitium even several months after arrest of drug intake [28].

The molecular structure of 5-ASA is very close of

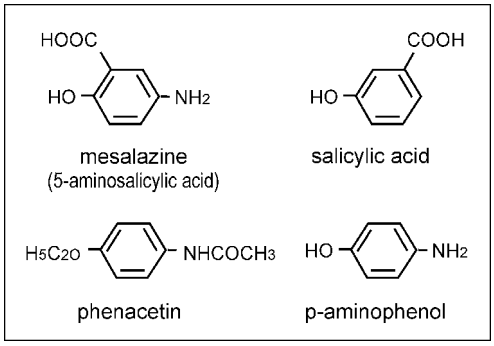


Figure 5. *Molecular structure of 5-aminosalicylic acid, salicylic acid, phenacetin and p-aminophenol.*

that of salicylic acid, phenacetin and aminophenol, drugs with a well-documented nephrotoxic potential (Figure 5). In rats, it is demonstrated that after a single intravenous injection of 5-ASA, at doses of 1.4, 2.8, 5.7 mM per kg body weight (high pharmacological doses), necrosis of the proximal convoluted tubules and papillary necrosis developed [98].

The mechanism of renal damage caused by 5-ASA may be analogue to that of salicylates by inducing hypoxia of renal tissues either by uncoupling oxidative phosphorylation in renal mitochondria, by inhibiting the synthesis of renal prostaglandins, or by rendering the kidney susceptible to oxidative damage by a reducing renal glutathione concentration after inhibition of the pentose phosphate shunt.

5-ASA is taken up by the gastrointestinal tract, particularly in the acetylated form and eliminated as such in the urine. The colon is the predilected place for this acetylation since in the small bowel there is a lack of the responsible bacterial flora. Hence, there is a limited readily absorption of 5-ASA as such in the small bowel. How far this may form a rationale for a possible difference in nephrotoxicity for the different preparations remains to be determined. Indeed, experimental evidence has shown that free 5-ASA is more nephrotoxic than the acetylated form [99, 100].

Clinical aspects

A typical case report is shown in Figure 4 (p. 271).

An association between the use of 5-ASA in patients with chronic inflammatory bowel disease and the development of a particular type of chronic tubulo-interstitial nephritis is difficult to interpret since renal involvement in chronic inflammatory bowel disease

may be an extra-intestinal manifestation of the underlying disease [101]. Extra-intestinal manifestations of chronic inflammatory bowel disease are well recognized. The most frequent renal complications are oxalate stones and their consequences such as pyelonephritis, hydronephrosis and on the long-term amyloidosis [102, 103]. As for many drugs, reversible acute interstitial nephritis has been described [90].

Glomerulonephritis may be associated with chronic inflammatory bowel disease and has a heterogeneous expression [104]. Minimal change glomerulonephritis, membranous, membranoproliferative, focal glomerulosclerosis, and proliferative crescentic glomerulonephritis have been described and a summary of these case reports is available in the paper of Wilcox et al. [105]. In almost half of these cases, there was no relationship with drug intake such as sulphasalazine or 5-ASA.

That 5-ASA seems to be implicated in the generation/development/maintenance of this particular reaction at the level of the kidney however, is supported by a large number of case reports appearing in recent literature of patients with IBD using 5-ASA as the only medication, the improvement at least partial of the impaired renal function arrest of the drug and a worsening after resuming 5-ASA use [87].

Prevention

The efficacy of 5-ASA as first-line treatment for IBD is clearly documented and generally accepted [106, 107]. Preventive measures need to be taken into consideration however, in order to avoid nephrotoxic adverse effects. Although the incidence and risk ratio's of 5-ASA associated chronic tubulo-interstitial nephritis are not well known, the link established by case reports and the demonstration that recovery of renal function was observed only in patients with limited renal damage necessitates preventive measures [26].

Patients receiving 5-ASA should be screened regularly in order to detect signs of renal impairment. It is suggested that serum creatinine concentration should be measured each month for the first 3 months of treatment, three monthly for the remainder of the first year and annually thereafter [26]. The use of concurrent immunosuppressive therapy may necessitate extension to the period of intensive monitoring. Moreover, it is shown that IBD patients with a stoma and patients with extreme dehydration are more susceptible to develop renal impairment (Table 3).

Table 3. Risk factors for renal impairment in inflammatory bowel disease patients.

	Risk ratio (95% CI)
Stoma	6.2 (1.8-20.9)
Male sex	3.1 (1.1-8.6)
Duration of IBD symptoms (weeks)	1.06 (1.01-1.12)

References

1. Spuehler O, Zollinger HU. Die chronisch-interstitielle Nephritis. Zeitschr Klin Medizin 1953; 151: 1–50.
2. Gloor F. Die doppelseitige chronische nichtobstruktive interstitielle Nephritis. Ergebnisse der allgemeinen Pathologie und Pathologischen Anatomie 1961; 41: 63–207.
3. Burry AF, de Jersey P, Weedon D. Phenacetin and renal papillary necrosis: results of a prospective autopsy investigation. Med J Australia 1966; 53: 873–879.
4. Kincaid-Smith P. Pathogenesis of the renal lesion associated with the abuse of analgesics. Lancet 1967; i: 859–862.
5. Kincaid-Smith P, Nanra RS. Lithium-induced and analgesic-induced renal diseases. In: Diseases of the kidney, 5th edition. Schrier RW, Gottschalk CW (editors). Little Brown, Boston 1993; p. 1099-1129.
6. Mihatsch MJ, Torhorst J, Amsler B, Zollinger HU. Capillarosclerosis of the lower urinary tract in analgesic (phenacetin) abuse. Virchows Archiv A. Pathol Anat Histol 1978; 381: 41–47.
7. Henrich WL, Agodoa LE, Barrett B, Bennett WM, Blantz RC, Buckalew VM Jr, D'Agati VD, De Broe ME, Duggin GG, Eknoyan G. Analgesics and the kidney: summary and recommendations to the Scientific Advisory Board of the National Kidney Foundation from an Ad Hoc Committee of the National Kidney Foundation. Am J Kidney Dis 1996; 27(1): 162-165.

8. De Broe ME, Elseviers MM, Bengtsson U, Mihatsch MJ, Molzahn M, Pommer W, Ritz E, Schwarz A. Analgesic nephropathy. Nephrol Dial Transplant 1996; 11: 2407-2408.
9. Duggin GG. Combination analgesic-induced kidney disease: the Australian experience. Am J Kidney Dis 1996; 28 (suppl 1): S39-S47.
10. De Broe ME, Elseviers MM. Analgesic nephropathy. N Engl J Med 1998; 338: 446-452.
11. Haschek H (editor). International symposium on problems of phenacetin abuse. Egermann, Vienna 1973.
12. Gsell O. Nephropathie durch Analgetika. Ergebnisse der inneren Medizin und Kinderheilkunde 1974; 35: 68–175.
13. Carro-Ciampi G. Phenacetin abuse: a review. Toxicology 1978; 10: 311–339.
14. Prescott LF. Analgesic nephropathy: a reassessment of the role of phenacetin and other analgesics. Drugs 1982; 23: 75-149.
15. Kincaid-Smith P. Effects of non-narcotic analgesics on the kidney. Drugs 1986; 42(4): 109-128.
16. Sandler DP, Smith JC, Weinberg CR, Buckalew VM Jr, Dennis VW, Blythe WB, Burgess WP. Analgesic use and chronic renal disease. N Engl J Med 1989; 320(19): 1238-1243.
17. Sandler DP, Burr FR, Weinberg CS. Nonsteroidal anti-inflammatory drugs and the risk for chronic renal disease. Ann Int Med 1991; 115(3): 165-172.
18. Morlans M, Laporte JR, Vidal X, Cabeza D, Stolley PD. End-stage renal disease and non-narcotic analgesics: a case-control study. Br J Clin Pharmacol 1990; 30(5): 717-723.
19. Pommer W. Clinical presentation of analgesic-induced nephropathy. In: Analgesic and NSAID-induced kidney disease. Stewart JH (editor). Oxford University Press, Oxford 1993; p. 108-118.
20. Perneger TV, Whelton PK, Klag MJ. Risk of kidney failure associated with the use of acetaminophen, aspirin, and nonsteroidal antiinflammatory drugs. N Engl J Med 1994; 331: 1675–1679.
21. Fored CM, Ejerblad E, Lindblad P, Fryzek JP, Dickman PW, Signobello LB, Lipworth L, Elinder CG, Blot WJ, McLaughlin JK, Zack MM, Nyrén O. Acetaminophen, aspirin, and chronic renal failure. N Engl J Med 2001; 345: 1801-1808.
22. Larsen K, Moller CE. A renal lesion caused by abuse of phenacetin. Acta Med Scand 1959; 164: 53-71.
23. Jacobs LA, Morris JG. Renal papillary necrosis and the abuse of phenacetin. Med J Australia 1962; 2(14): 531-538.
24. Grimlund K. Phenacetin and renal damage at a Swedish factory. Acta Med Scan 1963; 174 (suppl 405): 1-26.
25. Azad Khan AK, iris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulphasalazine. Lancet 1977; 1: 892-895.
26. World MJ, Stevens PE, Ashton MA, Rainford DJ. Mesalazine-associated interstitial nephritis. Nephrol Dial Transplant 1996; 11: 614-621.
27. De Broe ME, Stolear JC, Nouwen EJ, Elseviers MM. 5-Aminosalicylic acid (5-ASA) and chronic tubulo-interstitial nephritis in patients with chronic inflammatory bowel disease: is there a link ? Nephrol Dial Transplant 1997; 12: 1839-1841.
28. Stolear JC, Georges B, Gossun A, Ros Ph, Nouwen EJ, De Broe ME. Néphrite interstitiëlle associée à la Mésalazine. Confrontations anatomo-cliniques en pathologie renale et vasculaire; Hôpital Tenon, Paris, 10 Janvier 1996.
29. Murray RM. Analgesic nephropathy: removal of phenacetin from proprietary analgesics. Brit Med J 1972; 4: 131-132.
30. Nanra RS, Stuart-Taylor J, De Leon AH, White KH. Analgesic nephropathy: etiology, clinical syndrome, and clinicopathologic correlation in Australia. Kidney Int 1978; 13: 79-92.
31. Schwarz A, Kunzendorf U, Keller F, Offerman G. Progression of renal failure in analgesic-associated nephropathy. Nephron 1989; 53: 244-249.
32. Hauser AC, Derfler K, Balcke P. Progression of renal insufficiency in analgesic nephropathy: Impact of continuous drug abuse. J Clin Epidemiol 1991; 44: 53-56.
33. McCredie M, Stewart JH, Mahony JF. Is phenacetin responsible for analgesic nephropathy in New South Wales ? Clin Nephrol 1982; 17(3): 134-140.
34. Murray TG, Stolley PD, Anthony JC, Schinnar R, Hepler-Smith E, Jeffreys JL. Epidemiologic study of regular analgesic use and end-stage renal disease. Arch Intern Med 1983; 143: 1687-1693.
35. Pommer W, Glaeske G, Molzahn M. The analgesic problem in the Federal Republic of Germany: analgesic consumption, frequency of analgesic nephropathy and regional differences. Clin Nephrol 1986; 26: 273-278.
36. Pommer W, Bronder E, Greiser E, Helmert U, Jesdinsky HJ, Klimpel A, Borner K, Mohlzahn M. Regular analgesic intake and the risk of end-stage renal failure. Am J Nephrol 1989; 9: 403-412.
37. Dubach UC, Rosner B, Pfister E. Epidemiologic study of abuse of analgesics containing phenacetin. Renal morbidity and mortality (1968-1979). N Engl J Med 1983; 308: 357-362.
38. Elseviers MM, De Broe ME. A long-term prospective controlled study of analgesic abuse in Belgium. Kidney Int 1995; 48: 1912-1919.
39. McLaughlin JK, Lipworth L, Chow W-H, Blot WJ. Analgesic use and chronic renal failure: A critical review of the epidemiologic literature. Kidney Int 1998; 54: 679-686.
40. Delzell E, Shapiro S. A review of epidemiologic studies of nonnarcotic analgesics and chronic renal disease. Medicine 1998; 77(2): 102-121.

41. Buckalew VM, Schey HM. Renal disease from habitual antipyretic analgesic consumption: an assessment of the epidemiologic evidence. Medicine 1986, 11: 291-303.
42. Elseviers M.M., De Broe M.E. Analgesic nephropathy: Is it caused by multi-analgesic abuse or single substance use ? Drug Safety 1999; 20(1): 15-24.
43. Gates TN, Temple AR. Analgesic use and chronic renal disease. N Engl J Med 1989; 321: 1125.
44. McCredie M. Analgesic use and chronic renal disease. N Engl J Med 1989; 321: 1125.
45. Elseviers MM, De Broe ME. Epidemiology of analgesic nephropathy. J Nephrol 1992; 5: 94-98.
46. Elseviers MM, De Schepper A, Corthouts R, Bosmans JL, Cosyn L, Lins RL, Lornoy W, Matthys E, Roose R, Van Caesbroeck D, Waller I, Horackova M, Schwarz A, Svrcek P, Bonucchi D, Franek E, Morlans M, De Broe ME. High diagnostic performance of CT scan for analgesic nephropathy in patients with incipient to severe renal failure. Kidney Int 1995; 48: 1316-1323.
47. Elseviers MM, Bosteels V, Cambier P, De Paepe M, Godon JP, Lins R, Lornoy W, Matthys E, Moeremans C, Roose R, Theelen B, Van Caesbroeck D, Verbanck J, De Broe ME. Diagnostic criteria of analgesic nephropathy in patients with end-stage renal failure: results of the Belgian study. Nephrol Dial Transplant 1992; 7: 479-486.
48. Elseviers MM, Waller I, Nenov D, Levora J, Matousovic K, Tanquerel T, Pommer W, Schwarz A, Keller E, Thieler H, Köhler H, Lemoniatou H, Cresseri D, Bonucchi D, Fiocchi O, Jordans J, Franek E, Silva FJ, Fernandez Ruiz EJ, Morlans M, Hellström M, Wauters JP, Felle D, Cassi HV, De Broe ME. Evaluation of diagnostic criteria for analgesic nephropathy in patients with end-stage renal failure: results of the ANNE study. Nephrol Dial Transplant 1995; 10: 808-814.
49. Elseviers MM, De Broe ME. Combination analgesic involvement in the pathogenesis of analgesic nephropathy. Am J Kidney Dis 1996; 28(6): 958-962 (letter to the editor).
50. Noels LM, Elseviers MM, De Broe ME. Impact of legislative measures on the sales of analgesics and the subsequent prevalenceof analgesic nephropathy: a comparative study in France, Sweden and Belgium. Nephrol Dial Transplant 1995; 10: 167-174.
51. Primary renal disease. ANZDATA Registry 2000 Report.
52. United States Renal Data System. 1993 Annual Data Report. The National Institute of Health, The National Institute of Diabetes and Digestive and Kidney Diseases, Division of Kidney, Urologic and Hematologic Diseases, Bethesda, M.D.
53. Wing AJ, Brunner FP, Geerlings W, Broyer M, Brynger H, Fassbinder W, Rissoni G, Selwood NH, Tufveson G. Contribution of toxic nephropathies to end-stage renal failure in Europe: a report from the EDTA-ERA Registry. Toxicol Lett 1989; 46: 281-292.
54. Nanra RS. Analgesic nephropathy in the 1990's: an Australian perspective. Kidney Int 1993; 42 (suppl 44): 86-92.
55. Stewart JH, McCredie M, Disney APS, Mathew TH. Trends in incidence of end-stage of end-stage renal failure in Australia, 1972-1991. Nephrol Dial Transpl 1994; 9: 1377-1382.
56. McAnally JF, Winchester JF, Schreiner GE. Analgesic nephropathy. An uncommon cause of end-stage renal disease. Arch Intern Med 1983; 143: 1897-1899.
57. Gonwas TA, Hamilton RW, Buckalew VM. Chronic renal failure and end-stage renal disease in Northwest North Carolina. Importance of analgesic-associated nephropathy. Arch Intern Med 1981; 141: 462-465.
58. Wilson DR, Gault MH. Declining incidence of analgesic nephropathy in Canada. Can Med Assoc J 1982; 127: 500-503.
59. Seedat YK, Naicker S, Rawat R, Parsoo I. Racial differences in the causes of end-stage renal failure in Natal. S Afr Med J 1984; 65: 956-958.
60. Segasothy M, Suleimam AB, Puvaneswary M, Rohana A. Paracetamol: a cause for analgesic nephropathy and end-stage renal disease. Nephron 1988; 50: 50-54.
61. Kantachuvesiri S, Kaojarern S, Kitayaporn D, Phanichphant S, Sumethkul V, Wananukul W, Pongskul C, Arkaravichien W, Jankriengkri S, Chanchairujira T. Risk factors between analgesic use and chronic nephropathy in Thailand. Southeast Asian J Trop Med Public Health 1996; 27(2): 350-355.
62. Matousovic K, Elseviers MM, Devecka D, Horackova M, Turek T, De Broe ME, and the Group of Czech and Slovak Nephrologists. Incidence of analgesic nephropathy among patients undergoing renal replacement therapy in Czech republic and Slovak republic. Nephrol Dial Transplant 1996; 11: 1048-1051.
63. Elseviers MM, De Broe ME. Is analgesic nephropathy still a problem in Belgium ? Nephrol Dial Transplant 1988; 2: 143-149.
64. Matyus J, Ujhelyi L, Karpati I, Ben T, Kakuk G. Increase in the incidence of analgesic nephropathy in Hungary. Nephrol Dial Transplant 1997; 12(8): 1774-1775.
65. Franek E, Kokot F, Grzeszczak W, Gajos L. Is analgesic nephropathy a problem in the South-West Region of Poland ? Nephron 1996; 72: 353 (letter to the editor).
66. Bennett WM, De Broe ME. Analgesic nephropathy – a preventable renal disease. N Engl J Med 1989; 320: 1269-1271.
67. Mihatsch MJ, Staehelin HB, Musfeld D, Perret E, Oberholzer M. Phenacetin-Abusus: Kardiovaskuläre Risikofactoren. Nieren Hochdruck 1983; 3: 83-92.
68. Ivey KJ. Gastrointestinal intolerance and bleeding with non-narcotic analgesics. Drugs 1986; 32(suppl 4): 71-89.
69. Laporte J-R, Carne X, Vidal X, Moreno V, Juan J. Upper gastrointestinal bleeding in relation to previous use of analgesics and non-steroidal anti-inflammatory drugs. Lancet 1991; 337: 85-89.

70. Mihatsch MJ. Empfohlene Massnahmen zur Bekampfung des Analgetikamisbrauchs. In: Das Analgetikasyndrom. Mihatsch MJ (editor). George Theme Verlag, Stuttgart 1986; p. 134-135.
71. Stewart JH. The analgesic syndrome. In: Analgesic and NSAID-induced kidney disease. Stewart JH (editor). Oxford University Press, Oxford 1993; p. 58-66.
72. Hultengren N, Lagergren C, Ljungqvist A. Carcinoma of the renal pelvis in renal papillary necrosis. Acta Chir Scand 1965; 130: 314-320.
73. Bengtsson U, Johansen S, Angervall L. Malignancies of the urinary tract and their relation to analgesic abuse. Kidney Int 1978; 13: 107-113.
74. Kliem V, Thon W, Krautzig S, Kolditz M, Behrend M, Pichlmayr R, Koch KM, Frei U, Brunkhorst R. High mortality from urothelial carcinoma despite regular tumor screening in patients with analgesic nephropathy after renal transplantation. Transpl Int 1996; 9: 231-235.
75. Mihatsch MJ, Manz T, Knüsli C, Hofer HO, Rist M, Guetg R, Rutishauser G, Zollinger HU. Phenacetinabusus III. Maligne Harnwegtumoren bei Phenacetinabusus in Basel 1963-1977. Schweiz Med Wschr 1980; 110: 255-264.
76. McCredie M, Ford JM, Stuart Taylor J, Stewart JH. Analgesics and cancer of the pelvis in New South Wales. Cancer 1982; 49: 2617-2625.
77. Lornoy W, Becaus I, Morelle V, Fonteyne E. Néphropathies chroniques par abus d'analgésiques: fréquence, pathogenèse, aspects cliniques, développement de tumeurs malignes. In: Séminaires d'uro-néphrologie. Chatelain C (editor). Masson, Paris 1988; p. 99-105.
78. Blohme I, Johansson S. Renal pelvic neoplasms and atypical urothelium in patients with end-stage analgesic nephropathy. Kidney Int 1981; 20(5): 671-675.
79. McCredie M, Stewart JH, Carter JJ, Turner J, Mahony JF. Phenacetin and papillary necrosis: independent risk factors for renal pelvic cancer. Kidney Int 1986; 30(1): 81-84.
80. Kincaid-Smith P. Renal toxicity of non-narcotic analgesics. At-risk patients and prescribing applications. Med Toxicol 1986; 1 (suppl 1): 14–22.
81. Gloor FJ. Changing concepts in pathogenesis and morphology of analgesic nephropathy as seen in Europe. Kidney Int 1978; 13: 27-33.
82. Murray RM. Genesis of analgesic nephropathy in the United Kingdom. Kidney Int 1978; 13: 50-57.
83. Schwarz A, Faber U, Borner K, Keller F, Offerman G, Molzahn M. Reliability of drug history in analgesic users. Lancet 1984; 2:1163-1164.
84. Elseviers MM, De Broe ME. Analgesic nephropathy in Belgium is related to the sales of particular analgesic mixtures. Nephrol Dial Transplant 1994; 9: 41-46.
85. Kincaid-Smith P. Analgesic nephropathy in Australia. Contrib Nephrol 1979; 16: 57-64.
86. Nanra RS, Kincaid-Smith P. Experimental evidence for nephrotoxicity of analgesics. In: Analgesic and NSAID-induced kidney disease. Stewart JH (editor). Oxford University Press, Oxford 1993, p. 17-31
87. von Mühlendahl KE. Nephritis durch 5-aminosolicylsäure. Deutsch Med Wschr 1989; 114: 236.
88. Henning HV, Meinhold J. Chronische interstitielle nephritis durch 5-aminosalicylsäure ? Deutsch Med Wsch 1989; 114: 1091.
89. Ruf-Ballauf W, Hofstädter F, Krentz K. Acute interstitielle nephritis durch 5-aminosalicylsäure ? Internist 1989; 30: 262-264.
90. Mehta RP. Acute interstitial nephritis due to 5-aminosalicylic acid. Can Med Assoc J 1990; 143(10): 1031-1032.
90. Masson EA, Rodhes JM. Mesalazine associated nephrogenic diabetes insipidus presenting as weight loss. Gut 1992; 33: 563-4.
92. Thuluvath PJ, Ninkovic M, Calam J, Anderson M. Mesalazine induced interstitial nephritis. Gut 1994; 35: 1493-1496.
93. Smilde TJ, van Liebergen FJHM, Koolen MI, Gerlag PGG, Assmann KJM, Berden JHM. Tubulo-interstitiële nefritis door mesalazine (5-ASA)-preparaten. Ned Tijdschr Geneesk 1994; 138: 2557-2561.
94. Calvino J, Romero R, Pintos E, Losada E, Novoa D, Güimil D, Mardaras J, Sanchez-Guisande D. Mesalazine-associated tubulo-interstitial nephritis in inflammatory bowel disease. Clin Nephrol 1998; 49(4): 265-257.
95. Schreiber S, Hämling J, Zehnter E, Howaldt S, Daerr W, Raedler A, Kruis W. Renal tubular dysfunction in patients with inflammatory bowel disease treated with aminosalicylate. Gut 1997; 40: 761-766.
96. Herrlinger KR, Noftz MK, Fellermann K, Schmidt K, Steinhoff J, Stange EF. Minimal renal dysfunction in inflammatory bowel disease is related to disease activity but not to 5-ASA use. Aliment Pharmacol Ther 2001; 15: 363-369.
97. Elseviers M.M., De Broe M.E., the 5-ASA-Study Group. Risk of chronic interstitial nephritis in patients with inflammatory bowel disease treated with 5-ASA therapy. J Am Soc Nephrol 11: 128A, 2000.
98. Calder IC, Funder CC, Green CR, Ham KN, Tange JD. Nephrotoxic lesions from 5-aminosalicylic acid. Brit Med J 1972; 1: 152-154.
99. Calder IC, Funder CC, Green CR, Ham KN, Tange JD. Comparative toxicity of aspirin and phenacetin derivatives. Brit Med J 1971; 4: 518-521.
100. Dwarakanath AD, Michael J, Allan RN. Sulphasalazine induced renal failure. Gut 1992; 33: 1006-1007.

101. Izzedine H, Simon J, Piette AM, Lucsko M, Baumelou A, Charitanski D, Kernaonet E, Baglin AC, Deray G, Beaufils H. Primary chronic interstitial nephritis in Crohn's disease. Gastroenterology 2002; 123(5): 1436-1440.
102. Glickman RM. Inflammatory bowel disease (ulcerative colitis and Crohn's disease). In: Harrison's Principles of Internal Medicine (13th edition, volume 2). Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL (editors). McGraw-Hill, In, New York, p. 1403-1417.
103. Greenstein AJ, Janowitz HD, Sachar DB. The extra-intestinal complications of Crohn's disease and ulcerative colitis: a study of 700 patients. Medicine 1976; 55(4): 401-412.
104. Moayyedi P, Fletcher S, Harnder P, Axon ATR, Brownjohn A. Mesangiocapillary glomerulonephritis associated with ulcerative colitis: case report of two patients. Nephrol Dial Transplant 1995; 10: 1923-1924.
105. Wilcox GM, Aretz HT, Roy MA, Roche JK. Glomerulonephritis associated with inflammatory bowel disease. Gastroenterology 1990; 98: 786-791.
106. Järnerot G. New salicylates as maintenance treatment in ulcerative colitis. Gut 1994; 35: 1155-1158.
107. Clemett D, Markham A. Prolonged-release mesalazine: a review of its therapeutic potential in ulcerative colitis and Crohn's disease. Drugs 2000; 59(4): 929-956.

14

Non-steroidal anti-inflammatory drugs

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Introduction

To assign a precise, reliable numerical frequency to the renal functional disorders induced by non-steroidal anti-inflammatory drugs (NSAID) is next to impossible. This is due, in part, to the heterogeneity of the individuals who consume these agents and the variability in social customs that strongly influence the per capita ingestion of analgesic-anti-inflammatory drugs. Nonetheless, in most unselected populations in developed countries who seek care from their family physicians, approximately 1-3% of persons ingesting a NSAID will manifest one of a variety of renal functional abnormalities typically requiring physician intervention [1-5]. Although this percentage is relatively low, the number of "at risk" individuals are very high because of the current use profile of NSAIDs and their availability either by prescription or as over-the-counter medications. In view of the enormous number of patients consuming these compounds, the frequency with which patients expected to develop some variety of renal functional abnormality is substantial

Over 30 billion tablets of non-steroidal anti-inflammatory drugs (NSAID) were dispensed in the United States in 2000; approximately 16% represent prescriptions for NSAIDs [1]. One in seven inhabitants of the North American (~ 50 million) is likely to be treated with an NSAID for a rheumatologic disorder in any given year [3]. These compounds enjoy a remarkable benefit/risk ratio when used in the treatment of acute self-limited pain syndromes. Unfortunately, when taken for prolonged periods of time, either by the elderly or individuals with certain co-morbid conditions, the frequency of adverse reactions rises dramatically.

The NSAID-induced abnormalities of renal function, in descending order of clinical frequency, are (i) fluid and electrolyte disturbances; (ii) destabilization of controlled hypertension (iii) decompensated congestive heart failure; (iv) acute deterioration of renal function; (v) nephrotic syndrome with interstitial nephritis; and (vi) chronic renal failure/papillary necrosis [1, 3-5, 7].

Most of the renal abnormalities that are clinically encountered as a result of NSAIDs can be attributed to the inhibitory action of these compounds upon prostaglandin production within the kidney. Hence, a brief overview of the influence of prostaglandins on renal function will be presented, followed by an analysis of the pathophysiologic mechanisms in the production of renal disturbances, the clinical manifestations of these abnormalities, the patient risk factors involved and the preventive approaches to NSAID related renal syndromes.

Prostaglandins and renal function

The prostaglandin pathway

Renal prostaglandins serve a critical role in regulating both glomerular hemodynamics and tubular function [8,9]. For this process to occur, an intact arachidonic acid cascade is crucial. Prostaglandins are derived from deacylated arachidonic acid derived from cell membranes (Figure 1). The cellular release of arachidonic acid is controlled by a variety of vasoactive hormones including: norepinephrine, angiotensin, bradykinin and vasopressin [10, 11]. Once released, cyclooxygenase [COX-1 and -2] facilitates the addition of molecular oxygen to arachidonic acid creating endoperoxide PGG_2. The key role that COX's occupies in the cascade revolves around the regulation of the rate and amount of prostaglandin precursors that is converted to prostacyclin, prostaglandin and thromboxane (Figure 1).

Prostaglandins are ubiquitous substances that influence renal function along with the function of other body systems [8, 9, 11]. Prostaglandins are local hormones or 'autocoids' because they act in a paracrine or autocrine fashion. Biologic activity is characteristically limited to their site of production and interaction with the associated prostanoid receptors (Figure 1), the latter being responsible for activating the cellular response mechanisms. Because of the short circulatory half-life of prostaglandins, they are without significant systemic effect. In addition, prostaglandins are not stored in tissue but, rather, are synthesized on demand.

Arachidonic acid can also be metabolized to a variety of mediators, depending on the cell type. For example, lipoxygenase catalyzes the production of leukotrienes, and mixed-function oxygenases catalyze the production of epoxyeicosatrienoic acids. Collectively, these oxygenated metabolites may play a critical role in NSAID-induced nephrotic syndrome by shunting arachidonic acid metabolism from prostaglandins to lipoxygenase products, a shift that favors production of eicosanoid, an endogenous product that

increase capillary permeability [12]. Prostaglandins act as autocoids at either cortical and medullary sites of renal production [13]. Prostaglandins produced in the renal cortex modulate vascular resistance [RVR] and renin secretion, while those produced in the medulla have a major influence on salt and water balance. The major prostaglandins with renal action include: PGE_2, PGI_2 and TxA_2. PGE_2, produced in the greatest amounts, is found in both tubular and interstitial cells. Prostaglandins undergo rapid local metabolized to inactive products by a 15-prostaglandin dehydrogenase [10].

Renal prostanoid receptors

Four EP receptor sub-types have been identified (table 1) [14]. Since prostaglandins are autocoids with a short half-life, interaction with specific EP receptors within the nephron activates the biologic effect of PGE_2. Three of the four E-prostanoid receptors, EP2, EP3, and EP4, exert their biologic effect by the coupling of G proteins to cAMP, whereas, EP1 receptor action is coupled by increasing intracellular calcium. The existence of EP2 receptor in the kidney remains to be confirmed. Breyer et al. [14] has recently reviewed the distribution of the EP receptors known to exist in the kidney. EP4, IP, and possible EP2 are located in the glom-

Table 1. E-prostanoid (EP) receptor characteristics.

E-receptor	Function	Signal	mRNA
EP1	Contracts	IP3/DAG/PKC	CD/musc. Mucosa
EP2	Relaxes	↑ cAMP	Utreus Arteries
EP3	Contracts	↓ cAMP	CD/cTal Stomach
EP4	Dilates	↑ cAMP	Kidney Bladder

Adapted from Breyer et al. [14]

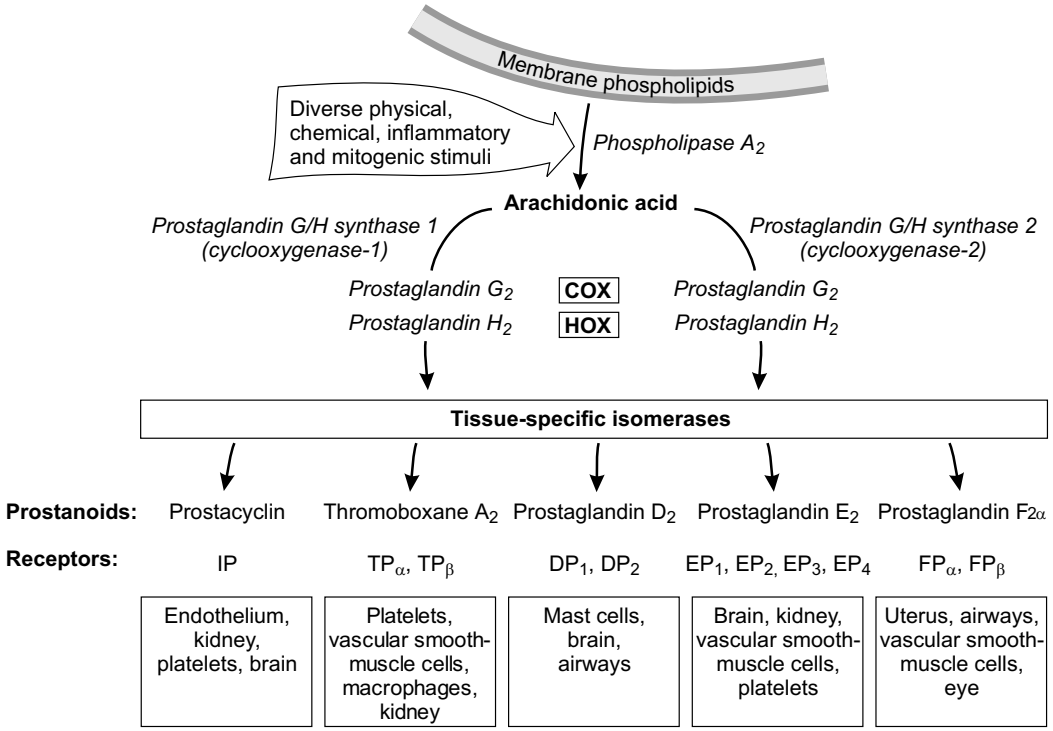

Figure 1. *Arachidonic acid is cleaved from membrane phospholipids by the action of phospholipase A_2. The liberated arachidonic acid is then acted upon by prostaglandin G/H synthase to produce the unstable intermediate PGH2. PGH2 is converted to the multiple prostanoids shown by tissue specific isomerases. The resulting prostanoids then activate cell-membrane receptor which couple G proteins leading to the terminal effect designated in each of the boxes (with permission from [11]).*

erular area. IP and EP4 probably mediate afferent arteriolar dilatation, while EP4 is involved with renin release. EP3 in the mTAL is thought to modify intense active Cl transport and reduce NaCl reabsorption. EP1, EP3, EP4 coexist in the both the cortical collecting duct (CCD) and medullary collecting duct (MCD). EP3 inhibits basolateral water reabsorption, while EP1 inhibits basolateral Na reabsorption. EP4, which is located at both the luminal and basolateral cell surfaces, stimulates water reabsorption. The relative expression of each of the receptors at the various intrarenal sites will determine the extent of the biologic modulation induced by local prostaglandin production.

Cyclooxygenase isoforms

Factors regulating isoform expression

Two isoforms of human cyclooxygenase (COX-1 and -2), possessing similar molecular weights (70-kDa) have been cloned, sequenced, and identified as being expressed in various human cells and tissues but possessing different mechanisms of regulation [9, 15]. COX-1 has been referred to as a constitutive enzyme, responsible for maintenance of normal cellular processes such as platelet function, protection of the gastrointestinal mucosa, and renal function under conditions of hemodynamic stress or decreased renal perfusion [16]. COX-2 was initially thought to be solely an inducible enzyme, activated to mediate the inflammatory response and pain perception [17]. It is now recognized that COX-2 also plays a constitutive role within the kidney [18, 19] although its specific functions have yet to be fully characterized. Nonetheless, COX-2 appears to play an important role in regulating renal salt and water homeostasis and renal hemodynamics and is induced during the inflammatory response thus contributing to the development of interstitial fibrosis [20-22]. In general, inhibition of COX-2 probably accounts for many of the desired therapeutic abilities of a NSAID while inhibition of COX-1 explains many of the undesirable gastrointestinal side effects of a NSAID.

The relative proportion of COX-1/COX-2 inhibition exhibited by both non-selective and selective NSAID has become an important clinical issue since the selective inhibition of the COX-2 isoforms offers the opportunity for a drug that possesses exclusive anti-arthritic therapeutic benefit without the drawback of gastric and renal side effects. Recently, FitzGerald and Patrono [11] have summarized the *in vitro* COX-1/COX-2 IC-50 inhibitory actions of a variety of NSAIDs. While the whole-blood assay for COX inhibitory action has improved the predictability of a similar result in human applications, the ultimate test remains the clinical trial. In this regard several large efficacy and safety trials have been conducted using the COX-2 inhibitors, celecoxib, rofecoxib and valdecoxib, and these are reviewed in detail later in this chapter.

The results of whole-blood assays identify indomethacin as the most potent inhibitor of cyclooxygenase-1, being 60 times more potent against this isoform than against cyclooxygenase-2 [23]. Aspirin was 166 times more active against cyclooxygenase-1 than cyclooxygenase-2, but was less potent than indomethacin on each of the isoforms. Acetaminophen was only a weak inhibitor of both isoforms. Some of the NSAIDs were virtually equally potent in their effects upon cyclooxygenase-1 and cyclooxygenase-2 (ibuprofen and naproxen). Of the currently available COX-2 inhibitors, rofecoxib and valdecoxib are the most potent inhibitor of cyclooxygenase-2 and also demonstrated the greatest selectivity for cyclooxygenase-2 inhibition [23a].

Distribution within the kidney

Distribution of the COX-2 isoform in the adult human kidney is based upon *in-situ* hybridization and immunolocation studies [14]. COX-2 has been detected in both the macula densa and medullary interstitial cells in patients with Bartter's syndrome and Congestive Heart Failure [24] as well as in elderly patients. COX-1, in addition to being expressed in the glomerulus, is constitutively expressed in both the cortical and medullary collecting ducts [18, 19] (Figure 2). The exact role of the duel expression of both COX isoforms in the medullary collecting duct remains to be elucidated.

Mechanism of action of NSAIDs

All NSAIDs act by inhibiting COX and thereby preventing prostaglandin synthesis [25]. The interaction between aspirin and cyclooxygenase (acetylation) is irreversible, whereas with other NSAIDs this binding is reversible. Traditional NSAIDs are non-selective blockers of both the COX-1 and COX-2 isoforms,

whereas celecoxib, rofecoxib and valdecoxib are specific inhibitors of COX-2 [23a].

The kidney is a frequent target of adverse effects from NSAIDs use [1-5, 12]. Much of this relates to the pharmacological action of NSAIDs in the presence of a stimulated endogenous prostaglandin system. NSAIDs therapeutic action derives from the 70-95% inhibition of the key regulatory enzyme COX. This inhibition has a profound effect on renal function since it eliminates the possible production of compensatory prostaglandins. This is especially true for the hemodynamically stressed individual where compensatory prostaglandin production acts to preserve renal function in the face of a systemic reduction in blood flow. Renal blood flow [RBF] is regulated by changes in RVR, which ultimately is determined by the balance between the amount of vasodilatory PGE_2 and PGI_2 and vasoconstrictive vasopeptides, e.g. TxA_2, angiotensin II, endothelin [4, 5]. Glomerular filtration rate (GFR) also responds to these prostaglandins, increasing with PGE_2 and PGI_2 and declining with TxA_2. Because of the reduction in RVR, which follows vasodilatation, prostaglandins can directly influence renin secretion, with PGI_2, PGE_2 increasing it, and TxA_2 either without effect or decreasing it. Medullary salt and water regulation are strongly influenced by PGE_2, which has both a natriuretic and diuretic action, while PGI_2 action is limited to natriuresis [26].

Renal prostaglandin production is minimal during non-stress conditions, and thus do not play a significant role in the maintenance of renal function under normal conditions. However, their production and release are substantially increased during hemodynamic instability being called forth to preserve both glomerular perfusion and tubular function [1]. A reduction in effective blood volume initiates secretion of the various vasoconstrictive peptides, which can initiate arachidonic acid release from the membrane (Figure 1). If, during such a stimulated state, NSAIDs are administered a marked reduction in production of vasodilatory prostaglandins PGE_2 and PGI_2 will result in a predictable imbalance causing a decreased renal perfusion and an increased tubular sodium reabsorption. Interruption of PG's production by NSAIDs is manifested by a variety of renal syndromes [1-5, 27, 28].

Renal syndromes associated with NSAIDs

Several renal syndromes can complicate NSAID use [1-5, 27, 28]. Generally, individuals who have normal renal function and are properly hydrated, are not at risk for developing adverse renal effects [1]. NSAID-induced deterioration in renal function depends on the specific drug, the dose and duration of pharmacologic effect and the state of health of the recipient [29]. Patients who have prostaglandin-dependent states associated with co-morbid diseases, such as high renin states or chronic renal insufficiency, are especially susceptible to NSAID-induced renal toxicities. Renal prostaglandins, by initiating counterregulatory vasodila-

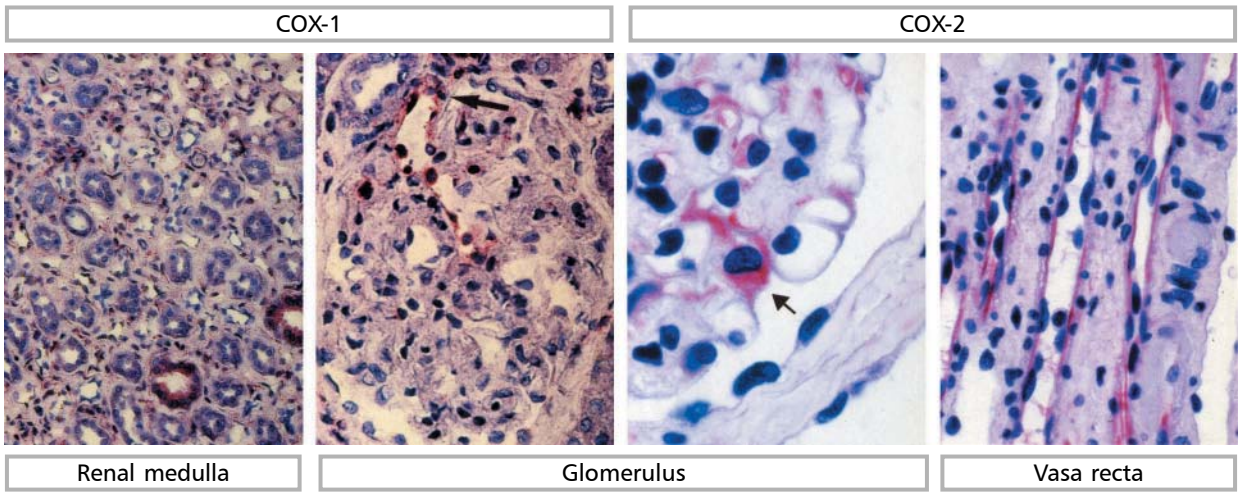

Figure 2. *Localization of COX-1 and COX-2 immunoreactive protein in adult and fetal human kidney (reproduced with permission from [18].*

Table 2. Effects of NSAIDs on the kidney (adapted from Whelton [30]).

Syndrome	Mechanism	Risk factors	Treatment
Sodium retention and edema	↓ PG, ↓ RBF, ↓ GFR, ↑ chloride absorption	NSAID use, hepatic disease, renal disease, HTN, DM, diuretic use, circulatory compromise, dehydration, advanced age	Discontinue NSAID
Hyperkalemia	↓ PG, ↓ RAA axis activity, ↓ K+ delivery to renal tubule	Renal disease, CHF, type 2 DM, multiple myeloma, use of K+ supplements, K+ sparing diuretics, ACE inhibitors	Discontinue NSAID, avoid indomethacin in patients at risk
Acute renal failure	↓ PG, ↓ RBF, ↓ GFR Hemodynamic disruption	CHF, renal disease, hepatic disease, diuretic use, advanced age, dehydration, SLE, shock, sepsis, hyperreninemia, hyperaldosteronemia	Discontinue NSAID, support with dialysis and steroids, if needed
Proteinuria/ Interstitial nephritis*	↑ recruitment and activation of lymphocytes, likely through leukotriene formation, affecting glomerular and peritubular permeability	Fenoprofen use, possibly female gender, advanced age	Discontinue NSAID, support with dialysis and steroids, if needed
Renal papillary necrosis*	Direct toxicity ↓ PG	Massive NSAID ingestion Dehydration	Discontinue NSAID Rehydrate

Abbreviations: NSAID = nonsteroidal anti-inflammatory drugs, PG = prostaglandin, RBF = renal blood flow, GFR = glomerular filtration rate, HTN = hypertension, DM = diabetes mellitus, K^+ = potassium, RAA = renin-angiotensin- aldosterone, CHF = congestive heart failure, ACE = angiotensin-converting enzyme, SLE = systemic lupus erythematosis.

**: distinctly unusual*

tion, are crucial in maintaining perfusion in 1) individuals with parenchymal renal disease and renal impairment, and 2) when circulating volume is decreased, such as in dehydrated patients or in individuals with a decrease in their "effective" circulating volume such as CHF or significant liver disease associated with ascites [1, 27]. The renal syndromes associated with NSAIDs can be predicted based upon inhibition of COX, which modifies the compensatory actions of prostaglandins. These modifications lead to a fall in both RBF and GFR with concomitant abnormal water and electrolyte excretion [28]. In addition, nephrotic syndrome, papillary necrosis and chronic tubulo-interstitial disease can complicate NSAIDs use [2]. These syndromes are summarized on Table 2.

Acute deterioration of renal function

NSAID-induced acute renal deterioration occurs in the setting of severe vasoconstrictive renal ischemia and can be attributed to interruption of the delicate balance between hormonally mediated pressor mechanisms and prostaglandin-associated vasodilatory effects (Figure 3). During NSAID inhibition of renal prostaglandin synthesis, unopposed vasoconstriction occurs by eliminating crucial counter-regulatory vasodilation [4, 5]. Similar to traditional NSAIDs, both celecoxib, rofecoxib and valdecoxib have been shown to reduce renal prostaglandin synthesis [22, 31, 32, 32a]. High-risk individuals (Table 3) can develop ARF within days of starting traditional NSAID therapy. Fortunately, the incidence of such an event is low, ranging from 0.5% to 1.0% of patients [27].

There is an apparent association between the relatively rapid onset of ARF and ingestion of NSAIDs with short half-lives (e.g. ibuprofen) [33]. In a crossover study, involving 11 days of active treatment, renal decompensation appeared within a few days of initiation of ibuprofen therapy, whereas no evidence of ARF was reported from NSAIDs with prolonged half-lives (e.g. sulindac and piroxicam) [33]. Although NSAIDs do not reduce glomerular filtration in normal individuals [34, 35], they are capable of induce acute renal decompensation in "at risk" patients with various renal and extrarenal clinical conditions that cause a decrease in blood perfusion to the kidney (Table 3). Renal prostaglandins play an important role in the maintenance of homeostasis in these patients, so disruption of

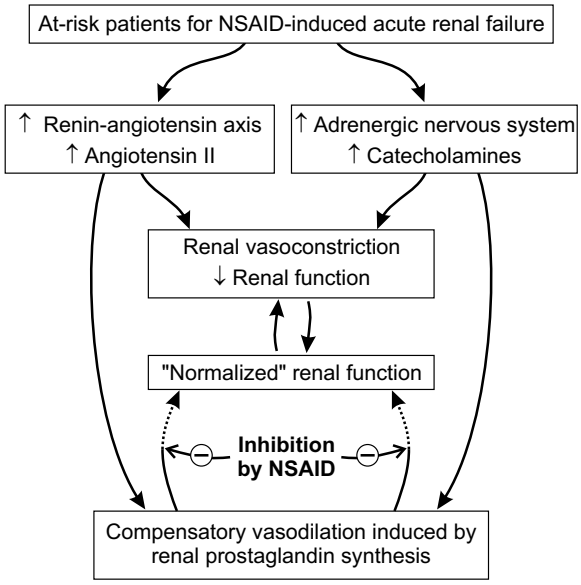

Figure 3. *Mechanism by which NSAIDs disrupt the compensatory vasodilation response of renal prostaglandins to vasoconstrictor hormones in patients with prerenal conditions.*

counter-regulatory mechanisms can produce clinically important and even severe deterioration in renal function [4, 5]. Typically, the addition of a NSAID increases the risk of hemodynamically mediated ischemic damage to the kidney by removing the protective effects of vasodilatory prostaglandins and allowing unopposed vasoconstriction. In the hemodynamically stressed patient a reduction in effective arterial blood volume initiates a neuroendocrine cascade, which has both renal and extrarenal consequences that require intact prostaglandin production [7].

Fortunately, the ARF usually reverses once the NSAID is stopped but in the high risk patient, can occur with any COX inhibitor.

Epidemiology and Incidence

Recently, Perez Gutthann et al. [36] evaluated the incidence of NSAID-induced ARF from a population-based, case control study using data from Canada. Over 200,000 health plan members were included because they had filled at least 1 prescription for NSAIDs during the 5-year interval. The crude incidence for ARF requiring hospitalization was 1.7/100,000 persons. Current NSAID use increased risk of ARF 4 fold, a risk that equaled the risk associated with other known nephrotoxins, e.g. aminoglycosides, contrast media. The risk of ARF was especially high during the first month of NSAID therapy and a direct dose relationship was observed. While the incidence of ARF is low as compared to other clinical settings, the outcome is serious since nearly half the patients died.

Table 3. "At risk" patients for NSAID-induced acute renal failure.

Severe heart disease (congestive heart failure)
Severe liver disease (cirrhosis)
Nephrotic syndrome (low oncotic pressure)
Chronic renal disease
Elderly population (age 80 or >)
Dehydration (protracted - several days)

Evans et al. [37] conducted a case-control study involving a population base of 420,600 individuals searching for any relationship between ARF and NSAID ingestions. ARF was confirmed by analysis of individual hospital charts. These authors found that the risk of ARF was doubled for patients who ingested NSAIDs within 90 days of hospitalization for ARF and also for patients taking therapeutic doses of Aspirin. Interestingly, they could not identify any interaction between NSAID use in patients with chronic renal failure and subsequent hospitalization for ARF.

Griffin et al. [38] reported a nested, case-control study involving 1799 ARF cases compared to 9899 controls. Chart survey was used to confirm both the diagnosis of ARF and the use of NSAIDs. 18.1% of the ARF patients were NSAID-users compared to only 11.3% of the controls (OR 1.58; 1.34-1.86). They estimated that NSAID-use was associated with 25 excess hospitalizations/10,000 years of use. The NSAIDs with odds ratio that were significantly correlated with ARF included ibuprofen, piroxicam, fenoprofen, and indomethacin. Based on these finding and the results of three other case-control studies, they concluded that the odds ratio significantly favored a direct relationship between NSAID ingestion and ARF, especially in the elderly.

To summarize, the risk characteristics, based upon these epidemiological studies include: 1) Patients taking NSAIDs develop ARF 2 to 4 times more frequently than non-users; 2) ARF is more common within the

first month of starting NSAIDs; 3) Elderly patients and patients taking ibuprofen, piroxicam, fenoprofen, and indomethacin are at greater risk of developing ARF. However, these case control studies have methodological limitations. Limitations include: confounding by indication for the drug use, the bias introduced by difficulty in establishing the time-order of exposure, and, in some cases, the bias introduced by recall. The failure of Evans and co-workers [37] to demonstrate an increased risk of NSAID-induced ARF in patients with pre-existing renal impairment should prompt a more focused study of this selected risk category.

Clinical features of NSAID-induced acute renal failure

At onset, NSAID-induced renal impairment is of moderate severity and is characterized by increasing blood urea nitrogen, serum creatinine, serum potassium, and weight with variable decrease in urinary output. With early detection and drug discontinuation, this form of NSAID-induced acute renal failure is usually reversible over 2-7 days. Indomethacin-induced acute renal failure may take longer to reverse following drug discontinuation, but reversal is the rule [33]. If NSAID-induced renal failure is not recognized early, severe morbid consequences occur. Continued NSAID therapy in the setting of deteriorating renal function may advance rapidly to the point wherein dialysis support is needed [39]. While this profound level of renal failure is often designated as "acute tubular necrosis", it often is only the extreme end of the spectrum of a hemodynamic insult and probably does not deserve identification as a separate clinical entity. Fortunately, even this profound level of renal functional impairment will nonetheless recover several days to weeks after discontinuation of the NSAID.

A possible relationship between the parenteral administration of the NSAID, ketorolac, and ARF has been evaluated in a multi-center study by Feldman et al. [40]. These authors found no difference in the frequency of ARF for patients receiving either ketorolac or morphine sulfate during the first 5 post-operative days; however, a significant, preferential increase in ARF frequency occurred when ketorolac treatment was extended beyond 5 days.

Clinical risk factors for acute renal failure

A tabulation of patients at risk for NSAID induced ARF is presented on Table 3. Thus, conditions associated with reduced RBF, e.g. CHF, cirrhosis, shock, and volume contraction, triggers pressor responses via adrenergic and renin-angiotensin pathways that is referred to as the neuro-endocrine cascade. The risk of NSAID-induced acute renal deterioration is greatest in patients with liver disease, pre-existing renal impairment including the nephrotic syndrome, cardiac failure, volume contraction due to protracted intercurrent dehydration or diuretic therapy, and old age. For example, NSAID-induced renal decompensation has been well documented in patients with cirrhosis, particularly when ascites is present [10]. This sensitivity can be traced to increased urinary excretion of prostaglandin E_2, prostacyclin metabolites, and thromboxane A_2 in these patients [41, 42]. An analogous prostaglandin dependent renal function exists in patients with underlying congestive heart failure [43], nephrotic syndrome [44, 45], or lupus nephritis [46, 47]. A drug-drug interaction to be aware of is that combining NSAIDs with triameterene which significantly increases the risk of ARF [48].

Patients with chronic renal impairment because of diminished renal prostaglandin production may also be at increased risk of NSAID-induced renal failure. NSAID-induced acute renal failure has been documented in patients with asymptomatic, but mild chronic renal failure, defined as a recruitment serum creatinine between 133 μmol/L and 265 μmol/L (1.5 and 3.0 mg/dl) [49]. Baseline excretion of urinary prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ was quantitatively lower in the individuals who developed NSAID-induced renal decompensation than in those who did not. Upon initiation of ibuprofen, urinary prostaglandin excretion fell in all patients, but trough concentrations were quantitatively lower in the subset of patients who experienced acute renal failure.

Volume contraction due to diuretic therapy or an intercurrent disease represents another important risk factor for the development of NSAID-induced acute deterioration of renal function [1, 3, 37]. Elderly patients are also at increased risk. It is estimated that, in the absence of other disease entities, the age of 80 years or greater is an independent risk factor since 50% of the population at age 80 have a 50% loss of glomerular function primarily as a result of the progression of arteriolonephrosclerosis [50].

To summarize patient a risk of NSAID-induced ARF. Frequency will be greater in patient populations

with restricted renal blood flow, e.g. CHF, cirrhosis, nephrotic syndrome, shock. However, for absolute numbers, the elderly are probably most at risk since they are the primary group who take NSAIDs for relieve rheumatic complaints [3].

Hyperkalemia/Hyporenin-Hypoaldosteronism sydrome

Hyperkalemia is an unusual complication of NSAID ingestion, presumably because of the multiplicity of factors that are capable of maintaining potassium homeostasis, even in the absence of prostaglandins. However, NSAID-induced hyperkalemia can occurs in up to 46% of high-risk individuals, but is reversible upon cessation of therapy [51] Patients at risk to develop hyperkalemia include those with: pre-existing renal impairment [52, 53], cardiac failure [54], diabetes [54], multiple myeloma [55], concomitant potassium supplementation [56], potassium-sparing diuretic therapy [57] or taking an angiotensin converting enzyme inhibitor [27]. The interaction of NSAIDs with ACE inhibitors is an important and common form of drug-drug interaction. In particular, this interaction must be recognized when an arthritic patient, who is receiving long term NSAID treatment, develops hypertension that requiring drug therapy. If, in addition, the patient has mild renal impairment, our experience suggests that a baseline serum creatinine value of 180 μmol/L or greater (2.0 mg/dl or >) at least doubles their risk for NSAID related acute deterioration of renal function. In this clinical situation the angiotensin converting enzyme (ACE) inhibitor-NSAID drug combination should be avoided because of the potential for the development of both hyperkalemia and acute renal failure [27, 49]. The general interaction of NSAIDs with antihypertensive drugs will be addressed later in this chapter.

Indomethacin appears to be the NSAID most frequently associated with the development of hyperkalemia, including patients without apparent risk factors [58]. In addition to the known effects of NSAIDs on potassium delivery to the distal tubule and their inhibition of the renin-angiotensin and aldosterone pathways, indomethacin may have a direct effect to limit cellular uptake of potassium [59].

As noted above, hyperkalemia often complicates the NSAID-induced acute renal deterioration. However, the severity of hyperkalemia can be disproportionate to the degree of renal impairment. Tan et al. [60] have reported a patient who had a serum potassium of 6.2 mEq/L in spite of only mildly abnormal renal function. In this patient, plasma renin and aldosterone levels were suppressed and failed to respond to furosemide or postural changes. Urinary prostaglandin E_2 was also suppressed. Discontinuation of indomethacin resulted in normalization of potassium, prostaglandin E_2, and a rebound of renin and aldosterone.

The COX-2 inhibitor, Celecoxib, appears to have little effect on serum potassium, even in patients receiving diuretic therapy [61-63] and similarly, there does not appear to be any significant effect of rofecoxib on serum potassium [64].

In conclusion, hyperkalemia associated with the use of traditional NSAIDs or the coxibs becomes a clinical risk in individuals with significantly decreased renal function and/or in those with the combination of decreased renal function and use of an ACE inhibitor.

Pharmacodynamic and pharmacokinetic relationships in NSAID-induced acute renal failure

NSAID-induced acute renal decompensation is a pharmacologically predictable phenomenon that possesses a dose-dependent component. In a triple-crossover study of 12 females with mild renal failure, ibuprofen (800 mg three times daily) was discontinued in 3 patients after 8 days because of worsening renal function (≥ 133 μmol/L - ≥ 1.5 mg/dl increase in serum creatinine) or hyperkalemia (potassium ≥ 6 mmol/ml). When these 3 patients were rechallenged at a 50% lower dose of ibuprofen, two developed evidence of acute renal deterioration [49].

An additional important finding from this study was the time of onset of acute renal decompensation. Ibuprofen-induced renal failure occurred rapidly (within 8 days), but piroxicam and sulindac were not associated with any deterioration of renal function during the 11-day treatment period [43]. A pharmacokinetic analysis of the drugs used in these patients suggested the following: Ibuprofen, which has a short elimination half-life, reached maximum serum concentrations quickly; in contrast, piroxicam and sulindac have longer half-lives and continued to accumulate throughout the treatment period. These findings are consistent with basic pharmacologic principles and suggest that NSAIDs having short elimination half-

lives will reach steady-state and exert maximum pharmacologic effects before this occurs with NSAIDs having longer half-lives.

Salt and water retention

Electrolyte abnormalities

NSAID, by inhibiting both cortical and medullary prostaglandin production, cause a variety of electrolyte abnormalities include sodium, potassium and water retention [28, 65, 66]. While sodium retention is usually transient with escape after several days, occasionally a patient will develop significant edema [67]. Water retention secondary to NSAIDs is manifest as hyponatremia [68] and occurs when the basal prostaglandin antagonism of antidiuretic hormone is removed, allowing unmodified water reabsorption in the collecting duct of the kidney. When this action is coupled with the NSAID-induced enhanced sodium chloride reabsorption in the thick ascending limb of Henle, free water clearance is virtually eliminated causing even more profound hyponatremia.

Edema Formation

Edema due to NSAIDs induced sodium and fluid retention usually occurs in susceptible individuals within the first week of therapy. Furthermore, these effects are reversible when the drug is discontinued. Clinically evident peripheral edema occurs in up to 5% of patients [27], likely as a result of decreased renal blood flow, possible redistribution of intrarenal blood flow, and increased reabsorption of sodium chloride in the thick ascending loop of Henle. In elderly patients this increased sodium chloride reabsorption coupled with increased water reabsorption is more likely to result in the edema.

Diuretics and NSAIDs

The renal saluretic response to loop diuretics is partially dependent on intact intrarenal prostaglandin production in the thick ascending loop of Henle. The decrease in the response to loop diuretics is mediated both by removing the inhibition of sodium chloride reabsorption and an increase in renal medullary blood flow causing a reduction in renal concentrating capacity. The net result is that the concurrent use of a NSAID may blunt the diuresis induced by loop diuretics.

For the practicing physician, this interaction is not of major clinical importance since either increasing the diuretic dose or, if possible, discontinuation of the NSAID will permit reinstitution of the desired diuretic response. In patients who are well controlled on a stable regimen of chronic loop diuretics use, the intercurrent need for long term use of an NSAID will typically lead to increasing the dosage of the loop agent, or the addition of a diuretic that acts in the distal nephron.

Thiazide diuretics do not stimulate or require prostaglandins to produce their desired effect and they do not directly interact with NSAIDs. The magnitude of increased risk of NSAID-induced ARF with concomitant triamterene can not be estimated based on sporadic case reports [48].

Antihypertensive drugs and NSAIDs

Three recent reports have provided insight regarding the interaction between antihypertensive therapy and NSAIDs. The first is a case control study by Gurwitz et al. [69] involving 9411 medicare patients and examined the frequency with which antihypertensive therapy was required following initiation of NSAID therapy. Based on odds ratio, NSAID users were nearly 70% more likely to require antihypertensive drugs and this requirement correlated with the NSAID dose. The need for antihypertensive treatment was evident within the first 3 months of NSAID administration. The other two study of interest are both meta-analysis of NSAIDs effect on blood pressure. Pope et al. [70] included 54 short-term studies encompassing 1324 patients, 92% being hypertensive. The adverse influence of NSAIDs on blood pressure (3.5 mm Hg – 6.2 mm Hg increase) was limited to hypertensive patient taking indomethacin, naproxen or piroxicam. These authors could not eliminate the confounding effect of dietary sodium. The meta-analysis reported by Johnson et al. [71] included 50 clinical trials encompassing 771 patients only 80% of who were hypertensive. NSAID administration resulted in a mean increase in blood pressure of 5 mmHg in the hypertensive patients, but no significant increase in the normotensive patients. While NSAIDs antagonized the antihypertensive action of β-blockers =ace-inhibitors > vasodilators > diuretics = calcium channel blockers, significant alteration of body weight, daily urinary sodium excretion, creatinine clearance, plasma renin activity, or the urinary excretion of either PGE_2 or 6-keto-$PGF_{1\alpha}$ were absent. Thus, in hypertensive patients, especially the

elderly, NSAIDs will interfere with antihypertensive treatment especially if β-blockers, ACE inhibitors or angiotensin receptor blockers [71a] are the principle drugs used. The interaction between NSAIDs and antihypertensive medications is likely due to the fact that certain antihypertensive medications exert a substantial part of their therapeutic effect via prostaglandin-mediated mechanisms [72]. Calcium channel blockers are not dependent on the prostaglandin pathway [73], however, β-blockers, vasodilators, and ACE inhibitors seem to be particularly affected by NSAID therapy [4]. The lack of an interaction between chronic NSAID-treatment and the anti-hypertensive action of CCB's has been confirmed by a large prospective study [73a].

Proposed mechanism of blood pressure destabilization

Prostaglandins, in concert with nitric oxide, act as a renal vasodilator-natriuretic system [74] whose action is to offset the vasoconstrictive-sodium retaining effects of the renin-angiotensin system. Because of these interactions, significant destabilization of blood pressure control can occur during systemic administration of NSAIDs. PGE_2 and PGI_2 possess both prohypertensive and antihypertensive actions on blood pressure [72]. The prohypertensive actions involve increasing renin release and raising cardiac output. The antihypertensive action includes vasodilatation, reversing vasopeptide-induced vasoconstriction and inducing a negative sodium balance. Recent evidence has identified a decline in nitric oxide availability in both elderly [75] and hypertensive patients [76]. In addition, the plasma nitric oxide response to alterations in dietary sodium intake is distinctly abnormal in elderly salt-sensitive hypertensive patients [77]. When these observations are combined with the recent studies of Perinotto et al. [78] which confirmed that endogenous prostaglandin will counteract the renal actions of endogenous angiotensin II in the face of NO inhibition, a mechanistic explanation for NSAIDs-induced hypertension can be formulated. The speculation involves the following: The decline in NO production in elderly, hypertensive patients puts additional requirement on the endogenous renal PG to counteract the intrinsic action of the RAS. When NSAIDs are given, the vasodilator-natriuretic action of PG is removed and thus the RAS is unopposed leading to destabilization of BP control.

The interaction between sodium intake, blood pressure and NSAIDs has been studied by Mulkerrin et al. [79]. These authors measured the change in blood pressure and sodium excretion in five young normotensive females and five elderly females to an intravenous saline load before and after 1800 mg of ibuprofen was given for 3 days. Saline loading induced a consistent 25mmHg rise in systolic pressure with or without ibuprofen in the elderly patients, while ibuprofen alone caused a 14mmHg rise from baseline in the elderly patients before saline. The naturiesis associated with saline loading in both groups was significantly blunted in the elderly after treatment with ibuprofen. They concluded that aging increases the susceptibility to salt retention and hypertension from NSAIDs. This may well be due to unmasking the diminished activity of nitrous oxide synthetase, which characterizes elderly patients who are salt sensitive.

Alam et al. [80] used chronic salt loading to examine the interaction between blood pressure, salt and NSAIDs. Thirty-one healthy individuals, age 60 or more, were enrolled in a randomized, placebo-controlled, crossover study. Patients were stratified as to normotensive or isolated systolic hypertension based on their blood pressure response after 6 weeks of a controlled 150 mEq/d sodium diet. Crossover involved a two-week interval receiving either low sodium (90 mEq/d) diet or high sodium diet (240 mEq/d) diet and placebo or indomethacin 75 mg/d. For all patients, high salt diet was associated with a 6mmHg rise in systolic pressure and 3 mmHg in diastolic pressure. Indomethacin administration increased systolic but not diastolic pressure. High salt diet and indomethacin had an additive effect on blood pressure, but failed to demonstrate any interaction. Indomethacin significantly elevated the blood pressure in normotensive individuals but did not in patients classified as salt-sensitive. They concluded that salt-sensitive patients with isolated systolic hypertension were resistant to the pressor effect of indomethacin but normotensive elderly patients were not.

The duration and magnitude of salt loading between these two studies may account for the different conclusion reached by each set of authors.

The concept of "renal sparing" NSAIDs

While all NSAIDs have the potential for inducing

renal failure, there has been speculation of quantitative differences among the individual NSAIDs. Sulindac was thought to be renal sparing, possibly because of its unusual metabolic pathway [33, 81-83]. The parent compound, sulindac sulphoxide, is an inactive prodrug, which undergoes hepatic metabolism to sulindac sulphide, the metabolite responsible for its anti-inflammatory activity. Sulindac sulphoxide is also metabolized to a much lesser extent to an inactive metabolite, sulindac sulphone. It was hypothesized that, within the human kidney, sulindac sulphide was reversibly oxidized to the inactive parent compound, sulindac sulphoxide, with the result that renal prostaglandin production was not perturbed [33, 82].

In clinical studies, urinary prostaglandin levels and renal effects were unchanged in patients with normal renal function [33, 34] and patients with proteinuria [83]. However, the duration of sulindac exposure in these studies may not have been sufficient to allow the full pharmacologic effect of sulindac. Also, NSAID-induced changes may not have been detectable because of the presence of only very mild renal impairment or the absence of co-existing renal failure in this study [84]. Longer courses of sulindac in patients with slightly more severe renal impairment have been associated with statistically significant reductions in urinary prostaglandins [49, 84] and GFR [85].

The ability of sulindac to inhibit prostaglandin synthesis and impair renal function has been confirmed in a different high-risk group, namely patients with hepatic cirrhosis and ascites [86]. We have also identified the development of profound acute renal failure in risk prone patients who received sulindac for several days to weeks. Collectively, these studies suggest caution in accepting any NSAID as being "renal sparing".

Nephrotic syndrome with interstitial nephritis

This rare complication of NSAID use may develop at any time during treatment, but typically occurs months to years after therapy has been initiated, and generally resolves upon discontinuation of therapy [1, 87].

Fenoprofen, on a per capita use basis, has been associated with interstitial nephritis more frequently than other traditional NSAIDs [87, 88]. To date, there have been three reports of coxib-induced acute interstitial nephritis [88a-c]. All were biopsy proven and cleared after stopping the coxib.

Clinical presentation

The features of this NSAID-induced renal syndrome are somewhat variable. The patient may experience edema, oliguria, and clinical signs indicative of significant proteinuria [89, 90]. Systemic signs of allergic interstitial nephritis such as fever, drug rash, peripheral eosinophilia, and eosinophiluria are typically absent. The urine sediment contains microscopic hematuria and cellular elements reported as pyuria [12, 90]. In a recent discussion of NSAID-induced acute interstitial nephritis, Rossert [87] confirmed that proteinuria, usually in the nephrotic range, occurs in 70% of cases [88]. The occurrence of acute renal failure parallels the nephrotic syndrome. For patients without the nephritic syndrome the functional extent of renal deterioration can range from minimal to requiring hemodialysis. The onset of NSAID-induced nephrotic syndrome is usually delayed, having a mean time of onset of 5.4 months after initiation of NSAID therapy and ranging from 2 weeks to 18 months [12, 89]. NSAID-induced nephrotic syndrome is usually reversible between 1 month and 1 year after discontinuation of NSAID therapy. During the recovery period, some 20% of patients require dialysis. Steroids have been used empirically, but it is not certain that they hasten recovery. If proteinuria is not significantly reduced within two weeks of discontinuation of the putative NSAID, we recommend a standard 2-month trial of corticosteroids as would be employed in an adult patient with idiopathic minimal change glomerulonephritis. While pyuria and eosinophiluria develop in ≈40% of patients who present with nephrotic syndrome, gross hematuria occurs in less than 10% of patients [87].

Histologic features of NSAID-induced nephrotic syndrome

NSAID-induced acute interstitial nephritis is a recognized cause of ARF [20], the frequency of which appears to be increasing [91]. In a recent series reported by Schwarz [91] of 64 biopsy-proven cases of acute interstitial nephritis, 85% were drug induced. The responsible drugs included: antibiotics, analgesics, NSAIDs and diuretics. Characteristically, the histology of this form of NSAID-induced nephrotic syndrome consists of minimal change glomerulonephritis with tubulo-

interstitial nephritis. This is an unusual combination of findings and, when noted in the clinical setting of protracted NSAID use, is virtually pathognomic of NSAID-related nephrotic syndrome. Nephrotic syndrome without apparent interstitial disease has been reported in a handful of patients taking fenoprofen, sulindac, or diclofenac. Conversely, interstitial disease without nephrosis has been reported in a few patients, but this may, possibly, represent allergic interstitial nephritis [90].

In spite of the nephrotic range proteinuria, the most impressive histopathologic findings in NSAID-induced nephrotic syndrome involve the interstitium and tubules [92]. A focally, diffuse inflammatory infiltrate can be found around the proximal and distal tubules. While this infiltrate consists primarily of cytotoxic T lymphocytes, it also contains other T cells, some B cells, and plasma cells [93]. Changes in the glomeruli in these patients were minimal and resembled those of classic minimal change glomerulonephritis with marked epithelial-foot process fusion. These findings are consistent with reports by other investigators [11, 39, 40, 94].

Of the 14 cases of biopsy proven drug-induced allergic nephritis reported by Shibasaki et al. [95], 4 were ascribed to NSAIDs; while in the series reported by Schwarz et al. [91], 16 of 68 biopsies were ascribed to NSAIDs. While 3 of the patients had taken the offending agent for less than 1 week, the forth had received aspirin for 3 months. All presented with oliguric renal failure without systemic signs of rash or fever. Positive 67Ga scintigrams were obtained in both patients in whom it was performed. In 3 of the 4 patients serum creatinine returned to normal range at follow-up. The authors conclude that 67Ga scintigram combined with a lymphocyte stimulation test can confirm a diagnosis of suspect drug-induced allergic nephritis without resorting to a renal biopsy.

NSAID induced nephrotic syndrome is suspected of being immunologically mediated and idiosyncratic. It has a distinct presentation when compared to that ascribed to acute interstitial nephritis. The nephrotic syndrome is not associated with hemodynamically stressed patients. Recently Radford et al. [96] published a retrospective study of NSAIDs induced membranous nephropathy using the Mayo Clinic biopsy registry. They reported that >10% of biopsy proven membranous glomerulonephritis [stage I/II] was attributable to NSAIDs. They summarized the clinical features of NSAID-induced nephrotic syndrome as having no consistent clinical predisposition, with a median duration of 43 weeks of drug ingestion, and nephrotic range proteinuria that was present for <8 weeks but reversed with discontinuation of the drug. The clinical features, absence of risk factors, and pathophysiology distinguish this from other NSAID-induced renal syndromes and from classic drug-induced allergic interstitial nephritis.

Risk factors for NSAID-induced nephrotic syndrome

The risk factors associated with NSAID-related nephrotic syndrome are not well identified. Underlying renal impairment does not appear to be a risk factor. Old age has been identified as a risk factor [37,89], but this may also be a reflection of the usual candidate for chronic NSAID therapy. The syndrome has been more frequently reported with fenoprofen, so the actual NSAID itself may be critical. However, the syndrome has been attributed to virtually all NSAIDs, including those from structurally distinct classes [11, 39, 89, 90, 93].

Mechanism of NSAID-induced nephrotic syndrome

The mechanism of NSAID-induced nephrotic syndrome has not been fully characterized, but some likely contributing mechanisms are under evaluation. While the mechanism of toxicity is unknown, it is theorized to be the result of leukotrienes, which are formed from arachidonic acid via the lipooxygenase pathway when the cyclooxygenase pathway is blocked [93]. Leukotrienes increase glomerular and peritubular permeability, which may lead to the induction of interstitial nephritis and proteinuria. The association of this syndrome with structurally distinct NSAIDs suggests a common pathophysiologic denominator [92]. It is conceivable that T lymphocytes function as immune mediators instead of the humoral factors that are responsible for classic drug-induced allergic interstitial nephritis. In keeping with this hypothesis, NSAID-induced prostaglandin inhibition may play an indirect role. By inhibiting cyclooxygenase, NSAIDs may promote metabolism of arachidonic acid to non-prostaglandin eicosanoids. Indeed, leukotrienes, the products of the interaction between lipoxygenase and arachidonic acid, are known to recruit T lymphocytes and promote the inflammatory process. As noted above, leukotrienes may contribute to proteinuria by increas-

ing vascular permeability [11, 89, 90].

Chronic renal failure/papillary necrosis

Epidemiology

There is limited information regarding any link between long-term NSAIDs ingestion and development of chronic renal failure. In the case control study reported by Perneger et al. [97], individuals judged to be taking an average annual dose of NSAID were not at additional risk of developing ESRD (adjusted RR 1.0, 95% CI 0.5 to 2.0). However, the relative risk of ESRD was concluded to be 8.8 for individuals who took in excess of 5000 doses of NSAID. On the other hand, in a multicenter case control study conducted by Sandler et al. [98], the relative risk of chronic renal failure was found to be 2.1 (95% CI 1.1 to 4.1) with one year's use of daily NSAIDs. Fields et al. [99] enrolled 4099 patients 70 years or older in a cross-sectional analysis. The serum creatinines were separated into quartiles and chronic NSAIDs users were significantly more prevalent in the highest quartile (Scr ≥ 1.4mg/dl or 124 mmol/L), OR 1.7 (95% CI 1.3 to 2.3). More recently, Sturmer et al. [100] conducted a cross-sectional study of 802 patients regarding the association between NSAIDs and impaired renal function. Detailed questionnaires were used to define NSAID use and renal function measurements included serum creatinine and calculated creatinine clearance. Overall, while impaired renal function was slightly more common in NSAID users than non-users (16% vs. 14%), the difference was not significant. However, individuals who used longer half-life NSAIDs (> 4 hours) had a significantly greater prevalence of impaired renal function. Interestingly, diuretics were associated with a significant incidence of renal impairment, OR 3.5 (95% CI 1.6 to 7.6), but no additional interaction with NSAIDs could be identified [101]. No significant interaction between ACE inhibitors and NSAIDs was evident from their data. The authors concluded that elderly patients taking long half-life NSAIDs are at increased risk for impaired renal function. While the information to date is suggestive of an association between high dose and/or long duration NSAID use and ESRD, additional epidemiological studies are needed [100].

Calvo-Alen et al. [102] evaluated creatinine clearance, osmolar clearance, free water clearance, sodium excretion and urinalysis in 104 arthritic patients whose treatment with NSAIDs exceeded 2 years compared to 123 health controls. The major abnormal finding was restrict to impaired renal concentrating capacity in the arthritic patients as manifested by a decreased osmolar clearance, increased free water clearance and a decreased urinary density. Compared to controls, no significant differences in either sodium excretion or creatinine clearance were recorded. However, Murray et al. [29] determined the incidence and risk factors for ibuprofen-associated renal impairment by analyzing 1908 computerized patient records. Multivariable analysis of the 343 patient records with renal impairment identified: age, prior renal insufficiency, coronary artery disease, male gender, elevated systolic blood pressure and diuretic use as risk factors. Only two subsets of at risk patients, age > 65 and coronary artery disease, were at greater risk to develop renal insufficiency when compared to acetaminophen.

The observation by Schwarz et al. [91] are germane to the influence of NSAID-induced ARF on the development of chronic renal failure. While NSAIDs accounted for only 20% of the cases of acute interstitial nephritis [91], nearly 2 out of every 3 patients from the NSAID subgroup was found to have permanent renal impairment at follow-up which represented the greatest frequency of any of the drug-induced acute interstitial nephritis.

Papillary necrosis

In a prospective study by Segasothy et al. [103] conducted over 11 years, IVP confirmed NSAID-induced papillary necrosis was reported to occur in 27% of heavy analgesic users. In over half of the cases (55%), the offending analgesic was excess NSAIDs consumption more often a single type rather than multiple agents. In over 80% of the cases the NSAID was prescribed for an arthritic condition, with males:female ratio of 1.9:1. Coexisting additive behavior was rare in the patients include in this study. Because of the wide differences in relative risk noted in these limited studies, plus questions that have been raised as to their validity [12], a precise risk can not be stated. Papillary necrosis is the least common type of NSAID-induced renal toxicity, but unlike the other types, it is irreversible. Volume depleted patients who ingest large quantities of NSAIDs may be at higher risk for developing papillary necrosis and parenchymal damage is permanent [104-107]. Its cause is likely a combination of de-

creased renal papillary perfusion and excessive papillary parenchymal NSAID and NSAID-metabolite concentrations [107].

Definition and differentiation of acute versus chronic papillary necrosis lesion

By definition, papillary necrosis represents the development of irreversible damage within the parenchyma of the renal papillae. The papillae of the kidney contain the tip portions of the long loops of Henle, together with the terminal portions of the collecting duct complexes, which open in to the minor calyces. The minor calyces of the kidneys representing the first location in the upper renal outflow tract into which urine is collected before it travels into the renal pelvis and into the urinary bladder via the ureters.

The mechanism of NSAID-induced acute papillary necrosis is often not clear and the causative role of the NSAID in question may be difficult to delineate because of the presence of confounding factors such as underlying disease, urinary tract infection, and/or concomitant medications. Selected NSAIDs may exert a direct toxic effect on renal papillae and may become highly concentrated in the medullary-papillary region of the kidney. Aspirin depletes cellular glutathione, which would otherwise neutralize the acetaminophen metabolite, N-acetyl-benzo-quinoneimine. Without glutathione, this highly reactive metabolite could lead to cell death [108]. Prostaglandin inhibition may also play a role [12]. Medullary ischemia, a possible precipitating factor in development of papillary necrosis, results from NSAID-induced reduction of blood into the renal medulla in experimental models [109, 110].

The development of acute papillary necrosis, as a consequence of the use of a single NSAID, at recommended dosing levels, is an extremely rare event. In preclinical studies, nearly all of the NSAIDs produced papillary necrosis in experimental animal models. Although, as already identified, clinical toxicity is exceedingly rare it has been reported for ibuprofen [111], phenylbutazone [112, 113], fenoprofen [114], and mefenamic acid [115] and, according to prescribing information, several other NSAIDs.

The chronic progression of events that lead to NSAID/analgesic related papillary necrosis are well known since the days of the first descriptions of chronic combined analgesics abuse nephropathy and the subsequent extensive investigations which defined the consequences of chronic (5-20 years) exposure of the kidney to high doses of analgesic combinations such as salicylate and acetaminophen (the metabolite of phenacetin) often with the addition of caffeine [108]. Fortunately, the incidence of this form of chronic analgesic abuse nephropathy has diminished because of a better understanding of the drugs involved, patient education, and in some countries thanks to efficient regulatory measures. The topic of chronic papillary necrosis related to analgesic-NSAID mixtures is reviewed in detail elsewhere in the text and will not be further discussed here.

The clinical circumstances that lead to chronic "analgesic abuse" nephropathy [116] are quite distinct to the rare occurrence of acute papillary necrosis associated with exposure of the patient to a single NSAID and often with only a short period of drug exposure. In these acute circumstances, the patient will typically present clinically with gross hematuria and may have flank pain suggestive of ureteric obstruction consequent to the passage of a sloughed papilla.

Other NSAID-induced renal syndromes

Phenylbutazone, suprofen, and benoxaprofen produce unique renal syndromes that are of historic interest. Fortunately, the use of phenylbutazone use has diminished because of the availability of safer drugs, and suprofen and benoxaprofen have been voluntarily removed from the market.

Two mechanisms responsible for phenylbutazone-induced acute oligo-anuric renal failure include: 1) inhibition of uric acid reabsorption, leading to hyperuricosuria and, ultimately, bilateral ureteral obstruction due to uric acid stones [117]; 2) an idiosyncratic reaction has been reported that results in acute tubular injury without uric acid precipitation [118].

Suprofen-induced ARF is characterized by acute flank and/or abdominal pain. In series of 16 patients, Hart et al. [119] described that the mean peak serum creatinine was 3.6 mg/dl (range: 2 to 8 mg/dl), which returned to normal limits at follow-up. Suprofen is know to have uricosuric activity leading Hart and colleagues [119] to suggest that this renal syndrome may have resulted from ureteral or tubular precipitation of uric acid.

Benoxaprofen, an NSAID with a very long half-life, was removed from the market in the early 1980s be-

cause of severe hepatic toxicity that occasionally resulted in death; however, renal failure was a contributing factor. Risk factors for benoxaprofen-induced toxicity were old age, concomitant diuretic therapy, and likely excessive drug administration.

Renal Effects of COX-2 inhibitors

Vane published the seminal work on the mechanism of action of aspirin-like drugs in the early 1970's [120]. Since that time, the goal in NSAID research has been to formulate agents with increased potency and limited toxicity. The elderly comprise the majority of patients who use high doses of NSAIDs for their analgesic and anti-inflammatory effects. However, the gastrointestinal toxic effects of the traditional NSAIDs and underlying disease states, such as hypertension and congestive heart failure (CHF), may preclude their use. Hence, these agents must be used cautiously in this population.

The most recent advance in NSAID pharmacology are agents that specifically block the cyclooxygenase-2 (COX-2) isoform while sparing the effect of COX-1 related activities [121] (Figure 4). These drugs have been designated by the WHO as a new pharmacologic category of NSAIDs, namely the 'coxibs' [122]. By blocking COX-2, the intent is to spare toxicity in organs such as the gut and kidney, thereby increase their utility, especially in elderly patients. All of the currently available COX-2 specific inhibitors, i.e. celecoxib, rofecoxib and valdecoxib, have established their safety advantage with respect to clinically important reductions in gastrointestinal toxicity and platelet-sparing characteristics [32a, 121-128]. Bleeding complications as seen with aspirin and traditional NSAIDs have essentially been eliminated. However, the clinical impact of the coxibs upon renal and cardiovascular function is an area of evolving information, especially now that it is known that the COX-2 isoenzyme is expressed within the human kidney [18, 20, 129]. The nephrotoxic effects of traditional NSAIDs are well recognized and have been the subject of extensive reviews [4, 30].

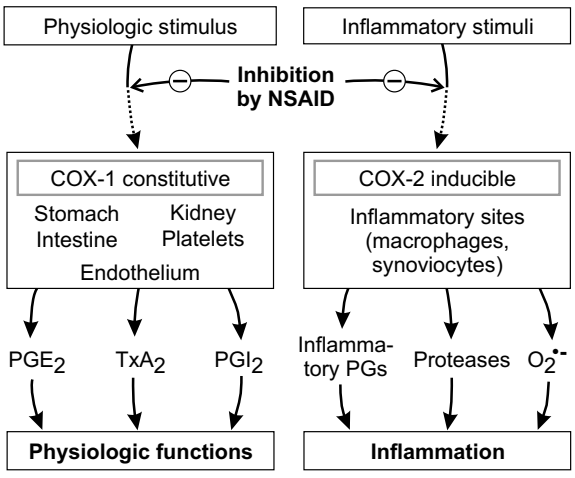

Figure 4. *Different functions of COX-1 and COX-2 in prostaglandin synthesis.*

Effects on renal function: GFR/urinary sodium excretion

The effect of COX-2 specific inhibitors on renal function, including sodium excretion, has been assessed in prostaglandin dependent patients. Catella-Lawson et al. [31] enrolled 36 healthy elderly patients for her study, which evaluated not only sodium excretion and glomerular filtration rates, but also changes in body weight, blood pressure and the urinary metabolites of thromboxane. Patients were randomized to either rofecoxib, indomethacin or placebo while receiving a isocaloric diet containing 200 mEq of sodium. Both NSAIDs induced a significant, but transient, decrease in sodium excretion during the first 72 hours of ingestion. Following this sodium excretion returned to pretreatment levels despite continued administration of the NSAIDs. Only indomethacin caused a significant reduction in GFR after 14 days of treatment. Body weight and blood pressure did not change significantly for any of the treatment groups. Inhibition of platelet thromboxane synthesis was limited to indomethacin treated patients, while both rofecoxib and indomethacin were associated with a significant reduction in urinary excretion of the prostacyclin metabolite, 2,3-dinor-6-keto prostaglandin $F_{1\alpha}$. Because of the later finding, the possibility of a prothrombotic state resulting from the administration of coxibs was suggested by these authors. The basis for the speculation is as follows: Activation of platelet aggregation is thromboxane dependent and under the control of the COX-1 isoform. Production of prostacyclin by vascular endothelium is a COX-2 dependent step. By inhibiting the production of prostacyclin, a anti-platelet aggregation factor, this would leave thromboxane mediated platelet aggrega-

tion unopposed and could result in a prothrombotic state. However, the anti-platelet aggregation action of endothelin would be unaffected during COX-2 inhibition [11].

Rossat et al. [130] conducted their renal assessment of COX-2 inhibition using health male volunteers rendered prostaglandin-dependent by a combination of low salt diet and administration of a loop diuretic. Their trial consisted of a parallel, randomized study involving giving either celecoxib, 200 mg bid or 400 mg bid, naproxen 500 mg bid or placebo for seven days. Blood pressure, renal hemodynamics, urinary salt and water excretion were measured before and 3 hours after ingestion of the test drug. The urinary excretion of sodium, potassium, lithium, and water were significantly decreased on both day one and day seven at the second and third hour after administration of either celecoxib or naproxen. Accumulative sodium excretion was significantly reduced during the first 3 days of NSAID dosing, but then subjects escaped from the effect. Glomerular filtration rates were transiently decreased following the 800 mg dose of celecoxib on day one, and the naproxen dose on day seven. These same author confirmed that lack of an effect of celecoxib on platelet thromboxine synthesis. They concluded that COX-2 inhibition in salt-depleted subjected induced retention of sodium and potassium.

Whelton et al. [22] enrolled 29 healthy elderly individuals in a single blind, randomized, cross-over study to determine the effects of celecoxib on prostaglandin dependent renal function. Either celecoxib or naproxen was given for 10 days followed by a 7 day washout period and then 10 days of the alternate drug. During the first 5 days, celecoxib 200 mg bid was given, then the dose was increased to 400 mg bid for the final 5 days of the trial. Naproxen dose as 500 mg bid throughout the 10 days. Only the –7.5 ml/min/1.73 m^2 decrease in GFR on day 6 of naproxen proved to be significant. Transient sodium retention was noted with both celecoxib and naproxen treatments, returning to baseline within the first 3 days. Both NSAIDs caused a significant reduction in urinary PGE_2 and 6-keto-$PGF_{-1\alpha}$ throughout the 10 days of administration. The authors concluded that, like conventional NSAIDs, celecoxib effects the urinary excretion of both sodium and prostaglandin E2. However, in elderly patients, unlike conventional NSAIDs, celecoxib spares renal hemodynamics.

Swan et al. [131] conducted a multi-center that involved both a randomized, single-dose crossover study and a randomized, parallel group, multidose study involving elderly, salt-depleted subjects. The single dose study involved 15 subjects who where crossed over between rofecoxib 250 mg or indomethicin 75 mg. For the multidose trial 60 subjects received either rofecoxib 12.5 or 25 mg/d, indomethacin 150 mg/d, or placebo for 6 days, with measurement performed during the last 6 hours of study day 6. Peak GFR, measured by either inulin or iothalamate clearance, fell by nearly 40% following acute administration of either rofecoxib or indomethacin. For the multidose trial, the reduction in GFR, while still significant, was less than 10%. While sodium excretion was reduced by both drug following acute administration, only 12.5 mg rofecoxib was associated with significant sodium retention after 6 days of drug. These author concluded that the effects of rofecoxib on renal function resembled nonselective NSAIDs and that COX-2 plays an important role in human renal function.

Collectively, these studies suggest that COX-2 plays a dominate role in the regulation of salt and water excretion in prostaglandin dependent patient, while the role of COX-1 seems to involve the regulation of renal hemodynamics, including GFR. The Swan et al. [131] study suggests that COX-2 may also play a role in regulating GFR; however, the combination of elderly patients who are salt depleted may have provided a more severe hemodynamic stress than was present in the other three studies.

Incidence of adverse cardio-renal events

Serum Electrolytes and Creatinine

In the recent 8000-patient celecoxib long-term arthritis safety study [123], significantly more patients receiving traditional NSAIDs (ibuprofen or diclofenac) experienced clinically significant elevations in serum creatinine and/or serum urea nitrogen levels when compared to celecoxib. In an equally large gastrointestinal safety trial with rofecoxib, the incidence of adverse effects related to renal function for rofecoxib was similar to naproxen (1.2% versus 0.9%, respectively) [124]. When rofecoxib and celecoxib were directly evaluated in elderly hypertensive OA patients who manifested "normal" serum creatinine at the time of study recruitment, the overall incidence of clinically

significant increases in serum creatinine, blood urea nitrogen, and serum potassium was 1.5% for both agents [132]. In post-marketing surveillance, ARF has been reported for both coxib compounds. Uniformly, this complication has been reported in patients with significant pre-existing renal impairment (serum creatinine ≥ 3.0 mg/dl [250 mmol/L] prior to coxib treatment). Details of 4 cases of ARF associated with COX-2 specific inhibitor use have been reported in the literature [141, 142]. In each of these cases, creatinine clearances returned to baseline after cessation of COX-2 specific inhibitor therapy. Ahmad et al [142a], reported 264 cases of renal failure due to either celecoxib or rofecoxib based on voluntary reported submitted to the FDA AER system. 122 cases occurred with celecoxib and 142 with rofecoxib. Hypertension, diabetes, congestive heart failure and renal insufficiency were shared risk factors for both drugs. However, concomitant use of diuretics, ACE inhibitors and other NSAID's occurred more frequently in patients with renal failure attributed to rofecoxib. No correlation with dose was evident. In of the 122 cases of celecoxib associated renal failure initial renal function was normal, while in 12 of 142 cases of rofecoxib initial renal function was reported to be normal. Zhao et al [142b] compared the renal-related adverse drug reactions between rofecoxib and celecoxib as reported to WHO Safety Monitoring Center. The center uses a statistical parameter, e.g. information component (IC), from a Bayesian confidence propagation neural network method to calculate each drug-ADR combination. When the IC values for rofecoxib were compared to celecoxib, an statistically significant adverse renal impact of rofecoxib was present for: water retention (R 1.97 vs. C 1.18, $p<0.01$), abnormal renal function (R 2.38 vs. C 0.70, $p<0.01$), renal failure (R 2.22 vs. C 1.09, $p<0.01$), cardiac failure (R 2.39 vs. 0.48, $p<0.01$), hypertension (R 2.15 vs. C 1.33, $p<0.01$). As with the report of Ahmad et al [142a], information as to dosage is missing.

Peripheral edema

In a post-hoc analysis of over 9500 patients with osteoarthritis (OA) or rheumatoid arthritis (RA) enrolled in 12 well-controlled trials, the incidence of celecoxib-induced edema was similar to that observed with the traditional NSAIDs (2.1%) and significantly different from placebo (1.1%) [61]. No correlation was evident between weight gain or blood pressure increase and peripheral edema. There was also no evidence of a dose-related increase in the frequency of edema with celecoxib. No clinically important differences in peripheral edema incidence were found between OA and RA patients who received celecoxib 100-400 mg twice daily (BID) [61]. Unlike celecoxib, the incidence of lower extremity edema with rofecoxib appears to be dose-related. In OA clinical trials to determine the general safety of rofecoxib 50 mg once daily (QD), the incidence of lower extremity edema was 6.3% compared to 3.7% at the recommended OA doses of 12.5 mg and 25 mg QD [64]. In a six-weeks study of 810 older, stable hypertensive patients with OA, those treated with rofecoxib 25 mg QD had significantly more edema than patients treated with celecoxib 200 mg QD (9.5% versus 4.9%; $p = 0.014$) [132]. Similar results were observed in a recent study of the same design in over 1100 patients [133].

Hypertension

Traditional NSAIDs are well known to cause peripheral edema and increases in blood pressure [70, 71, 80]. Two large metaanalyses found increases in mean arterial pressure of 3.5 mm Hg to 6.2 mm Hg [70, 71]. However, the same metaanalyses have concluded that NSAID-induced changes in blood pressure are almost exclusively limited to patients with pre-existing hypertension. This concept may have to change following the report of Dedier et al. [133a]. These authors accumulated 381,078 patient years over an 8 year follow-up to determine if non-narcotic analgesic use was associated with the development of hypertension. Over 10,000 incident cases of hypertension were identified. Analgesic use was determined by questionnaire. After adjusting for potential confounders, significantly high frequency of hypertension occurred in women taking aspirin 1.21 (95% CI, 1.13 to 1.30); acetaminophen 1.20 (95% CI 1.08 to 1.33); and NSAID's 1.35 (95% CI, 1.25 to 1.46). In addition they identified an increased risk of hypertension with increasing frequency of analgesic use ($p<0.001$). A prolonged increase in diastolic blood pressure of 5-6 mm Hg has been associated with a 67% increased risk of stroke and a 15% increased risk of coronary heart disease [134]. Less well established are the cardiovascular effects on the COX-2 specific inhibitors (celecoxib and rofecoxib), and how they may differ from each other and from traditional NSAIDs. Recent data suggest, however, that

there may be differences.

In two clinical studies directly compared celecoxib and rofecoxib in large (> 800 patients), well-controlled trials in older hypertensive subjects with OA increases in blood pressure were observed in 17% of rofecoxib and 11% of celecoxib treated individuals [132]. Similar increases in blood pressure, i.e. Rofecoxib > celecoxib were observed in a recent study of the same design including over 1100 patients [133].

No significant change in blood pressure was noted in a study of 36 healthy normotensive older adults on a fixed-sodium diet when rofecoxib was compared with indomethacin and placebo [72]. Similarly, no effect on systolic or diastolic blood pressure was observed in another study (n=67) of healthy normotensive elderly volunteers that compared in-house administration of celecoxib 400 mg QD, rofecoxib 25 mg QD, and naproxen 500 mg BID for 14 days under a strict weight-maintaining isocaloric diet [73]. Finally, two studies compared the COX-2 specific inhibitors with traditional NSAIDs, one in hypertensive subjects [62] and one in normal subjects [73]. In summary, rofecoxib, unlike celecoxib, is associated with dose-related increases in blood pressure (12.5-25 mg incidence rate = 3.5 %; 50 mg incidence rate = 8.2%) [61, 64]. This differential effect of rofecoxib on blood pressure may be traced to elimination of the diurnal dip. Reitblat et al. [134b] compared the effect of rofecoxib and nabumetone on diurnal blood pressure patterns in OA patients with stable hypertension. Nabumetone induced a moderate increase in both day and night blood pressure without changing the biological diurnal variation. Rofecoxib, on the other hand, had no effect on daytime blood pressure but raised nighttime systolic BP +15.7 and diastolic BP +8.5, thus eliminating the biologic diurnal variation.

As noted above, it is suggested from the results of the two large comparator studies in higher risk individuals, that patients who receive celecoxib 200 mg QD will experience significantly less edema and less destabilization of SBP than patients receiving rofecoxib 25 mg QD [132, 133]. The design of these studies mimicked real life conditions (i.e. involving doses commonly prescribed for the management of OA, blood pressure was measured by standard cuff methodology, and there was no control of diet or sodium intake other than that recommended by the treating physician). In contrast, a placebo-controlled study in 67 healthy elderly, normotensive volunteers that compared in-house administration of celecoxib 400 mg QD, rofecoxib 25 mg QD, and naproxen 500 mg BID for 14 days under a strict weight-maintaining isocaloric diet, found no difference among groups in systolic or diastolic blood pressure changes, and reported no incidences of edema [73]. Recently, Dilger et al [134a] compared the effects of celecoxib vs. diclofenac on blood pressure and renal function in young and elderly normotensive patients. Using standard arthritic doses of each, they were unable to demonstrate any adverse effect of either drug on blood pressure or renal function on either age group during the 15 days of treatment.

Two studies that compared the interaction of celecoxib with ACE inhibitors found no difference in blood pressure effects compared to placebo [62, 63]. In one study (n=359), the blood pressure (systolic and diastolic) effects of celecoxib 200 mg BID and nabumetone 1 g BID were found to be similar to placebo, but significantly different from ibuprofen 800 mg TID [62]. In the second study (n=178), the effects of celecoxib 400 mg daily and placebo on 24-hour blood pressure in hypertensive patients controlled on lisinopril 10-40 mg daily was evaluated [63]. No difference between groups was observed in 24-hour ambulatory SBP. The difference between groups in 24-hour diastolic BP was only 1.4 mm Hg. The change from baseline in 24-hour blood pressure (1.8 mm Hg/ 1.4mm Hg) is less than what has been the effect of NSAIDs on the SBP (defined as an increase >20 mm Hg with an absolute value of >140 mm Hg) reported for traditional NSAIDs in ACE inhibitor-treated patients. On the other hand, co-administration of rofecoxib 25 mg daily with benazepril 10-40 mg for 4 weeks in patients with mild to moderate hypertension was associated with a 3 mm Hg increase in mean arterial pressure [64].

These changes take on significance since one out of every 4 adults has hypertension, and only 27% of this group is on anti-hypertensive medication and have well-controlled blood pressure [135]. The remaining 73% of patients are either unaware of their hypertension, are not taking medication, or are uncontrolled on their current medication [135]. Nearly 50% of people who have a first heart attack and 66% of those who have a first stroke have blood pressures > 160/95 mm Hg [135].

Elevated SBP has been shown to be associated with

an increased risk of stroke, CHF, myocardial infarction and death [136, 137]. The authors of the ALLHAT study suggested that a 3 mm Hg increase in SBP could explain a 10% to 20% increase in the incidence of CHF [138]. In a meta-analysis of 15,693 older patients with isolated systolic hypertension from 8 trials, a 10 mmHg higher initial SBP was associated with relative hazard rates of 1.26 (p=0.001) for total mortality, 1.22 (p=0.007) for cardiovascular mortality, and 1.22 (p=0.02) for stroke [139].

It has been estimated that approximately 20 million Americans are currently taking concomitant NSAIDs and anti-hypertensive medications [140]. Thus, the potential for destabilization of controlled hypertension or worsening hypertension in those already uncontrolled is of great public health concern.

Congestive Heart Failure

When the NSAID induced decrease of therapeutic efficacy of diuretics is combined with NSAID-induced retention of salt and water, the development of CHF is promoted. Patients with a history of CHF are particularly prone to worsening heart failure when taking traditional NSAIDs. Hospitalizations due to CHF were increased 2-fold in elderly patients who reported concomitant use of diuretics and NSAIDs when compared to those taking diuretics alone [143]. A second study also found a 2-fold increased risk of hospitalization for CHF among elderly patients reporting use of traditional NSAIDs within the week prior to admission [144]. Thus, in susceptible patients, high doses of traditional NSAIDs with prolonged half-lives were associated with increased risk of developing CHF. However, both reports [143,144] have been criticized for not excluding pre-existing ventricular dysfunction as a risk factor. Feenstra et al. [144a], using the Rotterdam population based cohort, conducted a prospective 6½ year study of both 1) the association of NSAID treatment and initial hospitalization for heart failure and 2) the risk of subsequent cardiac decompensation and hospitalization when NSAID are used. These authors could not confirm NSAID-induced heart failure in patients without co-existing ventricular dysfunction. However, in patients with prevalent heart failure, the use of NSAIDs was associated with a significant risk of relapse, adjusted relative risk 9.9 (95% CI, 1.7-57.0). While NSAIDs were not associated with increased heart failure incidence, in heart failure patients, NSAIDs substantially increased the risk of relapse.

In the 6-week comparative study of rofecoxib and celecoxib in elderly hypertensive patients with OA, 4 patients (1.0%) in the rofecoxib group and none in the celecoxib group developed CHF during the study [132].

Concurrent use of an oral synthetic prostaglandin analog with a NSAID

The synthetic prostaglandin E1 analogue, misoprostal, has been used in combination with NSAIDs to prevent the NSAID-induced complication of gastric ulcers. It is well tolerated in patients with rheumatologic conditions and does not interfere with NSAID anti-inflammatory activity [145].

Misoprostol appears to exert renal vasodilatory effects in experiment models and in humans. In experimental models, exogenously administered prostaglandin E_1 has renal effects comparable to those of prostaglandin E_2, a potent vasodilatory prostaglandin [146-149]. In rats, misoprostol has mitigated cyclosporine-induced acute nephrotoxicity [150], which is thought to be mediated partly by prostaglandins. Misoprostol minimized NSAID-induced reductions in GFR in a double-blind, crossover study [151]. Six of 12 females with normal renal function experienced at least a 10% decrease in GFR following a 3-day course of indomethacin (25 mg four times daily) ($p<0.05$). When misoprostol was added, four of these six NSAID-sensitive patients experienced no change in GFR. Misoprostol also blunted indomethacin-induced decreases in creatinine clearance and natriuresis in another at-risk group, patients with alcoholic cirrhosis and ascites [152].

In contrast to the above results, Boers and colleagues [153] failed to detect any beneficial effects of misoprostol in a double-blind, crossover study of diclofenac-treated patients with renal insufficiency (creatinine clearance < 80 ml/min/1.73 m^2). Renal prostaglandin production was not measured in this study, which precludes any conclusions regarding the interactions between misoprostol and NSAID on prostaglandins. It is conceivable that the dose of misoprostol (200 μg three times daily) used was inadequate to prevent NSAIDs from suppressing renal prostaglandin production. Alternatively, the dose (50 mg three times daily) and duration (14 to 21 days) of diclofenac may not have been sufficient to suppress urinary prostag-

landins or renal function. Furthermore, as noted by the authors of this study, NSAID therapy was not withdrawn, so the effect of diclofenac on renal function is unclear.

Two prospective, crossover, placebo controlled, double-blind evaluations of the nephroprotective role of misoprostol in patients with mild stable chronic renal failure, taking either ibuprofen or indomethacin have been reported [154]. The mean baseline GFR of the patients at the time of entry into the study was 53 ml/min (misoprostol)/55 ml/min (placebo), and 57 ml/min (misoprostol)/57 ml/min (placebo) in Study I (ibuprofen) and Study II (indomethacin) respectively. At this level of renal functional impairment, the use of the non-selective NSAIDs did not produce additional significant impairment of renal function, hence a renal protective role for misoprostol could not be demonstrated. The findings from study I indicate that a numerically small, but significant, improvement in serum creatinine took place during the first week of the study when misoprostol treatment was compared with placebo. A similar trend was noted for the GFR and effective RPF results, but it was not significant.

Wiegmann and colleagues have reported that misoprostol does have a potential nephroprotective effect in patients undergoing radiocontrast procedures [155].

In the setting of chronic renal failure in which NSAIDs are being intercurrently used, we conclude that the nephroprotective role of misoprostol has not yet been satisfactorily resolved and additional controlled trial of misoprostol-NSAID effect in patients with more pronounced chronic renal failure could resolve this quandary.

Conclusions and future challenges

The NSAIDs are correctly considered as safe and effective therapeutic agents for the management of a variety of acute and chronic conditions. The risk of inducing acute deterioration renal function after the initiation of any given NSAID is low, nonetheless, the number of at-risk patients is high because of the widespread use of these drugs. Similarly, the risk of inducing other renal syndromes, such as the nephrotic syndrome, is rare, but in view of the massive number of individuals who consume NSAIDs the associated must always be considered in the evaluation of new onset nephrotic range proteinuria.

When selecting a NSAID, it is prudent to consider the potential effect of seemingly minor elevations in SBP. In the one study of elderly treated hypertensive patients with OA, a 3.1 mm Hg increase in mean SBP was measured after 6 weeks of therapy in a rofecoxib-treated group compared to a celecoxib-treated group [132]. Russell et al. estimated the impact of this increase in mean SBP on the occurrence and associated costs of coronary heart disease (CHD) and stroke over a 4-year period [156]. They estimated that a 3.07 mm Hg increase in mean SBP might be associated with 21,800 additional CHD events and 22,100 additional stroke events. Treating patients with these events was estimated to cost US$650 million.

By understanding the pathophysiology involved in NSAID-related renal disorders, preventive clinical measures can be put into operation. Risk factors have been identified for most NSAID-induced renal syndromes (Table 3). It is prudent to avoid high-dose, chronic NSAID therapy in patients with underlying renal impairment (Scr > 1.5 mg/dl), congestive heart failure, cirrhosis, volume contraction due to aggressive diuretic therapy or prolonged dehydration associated with intercurrent illnesses. Unfortunately, this is not always possible. If NSAIDs are necessary in these high-risk groups or in elderly patients, the patient serum creatinine and potassium should be monitored closely and receive appropriate counseling. Monitoring should begin within a week after initiation of a short-acting NSAID such as ibuprofen and continue indefinitely for signs of syndromes having a more delayed onset, such as the nephrotic syndrome with interstitial nephritis.

In the event of NSAID-induced renal failure, the NSAID should be discontinued promptly. The patient should receive supportive care, including temporary dialysis if needed. Beware that after stabilization of renal function, rechallenge with the same or even a structurally different NSAID is likely to reproduce the undesirable side effect. Hence, if anti-inflammatory therapy is essential, underlying risk factors should be identified and eliminated. If this is not possible, as in the case of old age or chronic kidney or liver failure, the patient may require alternative therapy using corticosteroids or other supportive drugs, such as acetaminophen and/or opioids.

The future development and clinical testing of selective COX-2 inhibitors will undoubtedly have far

reaching therapeutic consequences. It appears likely that other isoforms of cyclooxygenase may be identified in the future and could possibly explain differential effects of NSAIDs upon various organ systems, such as intracranial/hypothalamic effects that modulate temperature regulation.

In summary, it is clear that massive amounts of traditional NSAIDs and COX-2 specific inhibitors will continue to be consumed worldwide. Because these agents inhibit renal prostaglandin synthesis, they affect salt and water homeostasis and renal hemodynamics. This inhibition will have little clinical effect in the majority of patients who are well-hydrated, have good renal function, and no concomitant disease states. However, both traditional NSAIDs and COX-2 specific inhibitors must be used judiciously in patients with compromised renal blood flow. In general, the COX-2 specific inhibitors are well tolerated by the kidney and it is only in the clinical setting of significant pre-existing renal impairment that these agents should be avoided or at least used with very careful monitoring of renal function. With respect to destabilization of blood pressure in treated hypertensive patients or the development of edema in susceptible older individuals, it appears that there is a safety gradient in the progression of these adverse effects - the least being seen with celecoxib and the most with high-dose rofecoxib while the traditional NSAIDs are bracketed by the two coxibs. Seemingly minor elevations in SBP caused by these agents can potentially have catastrophic cardiovascular complications. Prior to initiation of therapy, each patient should be carefully assessed, weighing the benefit of using these agents against their risks. Thereafter, patients should be closely followed so that appropriate preventive clinical therapeutic strategies can be instituted. Future studies will need to clarify the inherent mechanistic differences that seem to account for the differentiation of cardiorenal safety profiles of the currently available COX-2 specific inhibitors.

References

1. Bennet WM, Henrich WL, Stoff JS. The renal effects of nonsteroidal anti-inflammatory drugs: summary and recommendations. Am J Kidney Dis 1996; 28(Suppl 1): 56-62.
2. Bennett WM, Porter GA. Analgesic nephropathy and the use of nonsteroidal anti-inflammatory drugs in renal patients: new insights. J Nephrol 1998; 11: 70-75.
3. Whelton A. Nonsteroidal anti-inflammatory drugs: effects on kidney function. In: Primer On Kidney Diseases. Greenberg A (editor). Academic Press, San Diego 1994; p 163-167.
4. Whelton A. Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiological foundations and clinical implications. Am J Med 1999; 106: 13S-24S.
5. Brater DC: Effects of nonsteroidal anti-inflammatory drugs on renal function: focus on cyclooxygenase-2–selective inhibition. Am J Med 1999; 107: 65S-71S.
6. Personal communication, Evans T, McNeil Consumer Health Care, 2000.
7. Whelton A, Hamilton CW. Nonsteroidal anti-inflammatory drugs: effects on kidney function. J Clin Pharmacol 1991; 31: 588-598.
8. Patrono C, Dunn MJ. The clinical significance of inhibition of renal prostaglandin synthesis. Kidney Int 1987; 32: 1-12.
9. Breyer MD, Harris RC. Cyclooxgenase-2 and the kidney. Curr Opin Nephrol Hypertens 2001; 10: 89-98.
10. Oates JA, FitzGerald GA, Branch RA, Jackson EK, Knapp HR, Roberts LJ II. Clinical implications of prostaglandin and thromboxane A_2 formation (2 parts). N Engl J Med 1988; 319: 689-698 and 761-767.
11. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001; 6: 433-442.
12. Clive DM, Stoff JS. Renal syndromes associated with nonsteroidal anti-inflammatory drugs. N Engl J Med 1984; 310: 563-572.
13. Stokes JB. Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle: selective inhibition of the medullary portion. J Clin Invest 1979; 64: 495-502.
14. Breyer MD, Zhang YH, Guan YF, Hao CM, Herbert RL, Breyer RM. Regulation of renal function by prostaglandin E receptors. Kidney Int 1998; 54: S88-S94.
15. O'Banion MK, Winn VD, Young DA. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc Natl Acad Sci 1992; 89: 4888-4892.
16. Crofford LJ. COX-1 and COX-2 tissue expression: implications and predictions. J Rheumatol 1997; 24(Suppl 49): 15-19.
17. Hla T, Nielson K. Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci 1992; 89: 7384-7386.

18. Kömhoff M, Gröne HJ, Klein T, Seyberth HW, Nusing R. Localization of cyclooxygenase-1 and –2 in adult and fetal human kidney: implication for renal function. Am J Physiol 1997; 272(4 Pt 2): F460-468.
19. Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, Breyer MD. Cyclooxygenase 2 is associated with the macula densa of rat kidney and increases with salt restriction. J Clin Invest 1994; 94: 2504-2510.
20. Whelton A. Renal aspects of treatment with conventional nonsteroidal anti-inflammatory drugs versus cyclooxygenase-2-specific inhibitors. Am J Med 2001;110 (3 Suppl 1): 33-42.
21. Blume C, Heise G, Muhlfeld A, Bach D, Schrork K, Gerhardz CD, Grabensee B, Heering P. Effect of flosulide, a selective cyclooxygenase inhibitor, on passive Heymann nephritis in the rat. Kidney Int 1999; 56: 1770-1778.
22. Whelton A, Schulman G, Wallemark C, Drower EJ, Isakson PC, Verburg KM, Geis S. Effects of celecoxib and naproxen on renal function in the elderly. Arch Intern Med 2000; 160: 1465-1470.
23. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci 1994; 90: 11693-11697.
23a. McMurray RW, Hardy KJ: COX-2 inhibitors: Today and tomorrow. Am J Med Sci. 2002;323:181-189.
24. Harris RC. Cyclooxygenase – 2 in the kidney. J Am Soc Nephrol 2000; 11: 2387-2394.
25. Patrono C, Dunn MJ. The clinical significance of inhibition of renal prostaglandin synthesis. Kidney Int 1987; 32: 1-12.
26. Stokes JB, Kokko JP. Inhibition of sodium transport by prostaglandin E_2 across the isolated, perfused rabbit collecting tubule. J Clin Invest 1977; 59: 1099-1104.
27. Whelton A, Hamilton CW: Nonsteroidal anti-inflammatory drugs: effects on kidney function. J Clin Pharmacol 1991; 31: 588-598.
28. Schlondorf D. Renal complications of nonsteroidal anti-inflammatory drugs. Kidney Int 1993; 44: 643-653.
29. Murray MD, Brater DC, Tierney WM, Hui SL, McDonald CJ. Ibuprofen-associated renal impairment in a large general internal medicine practice. Am J Med Sci 1990; 229: 222-229.
30. Whelton A. Renal and related cardiovascular effects of conventional and COX-2 specific NSAIDs and non-NSAID analgesics. Am J Ther 2000; 7:63-74.
31. Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, Fitzgerald GA. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. J Pharmacol Exp Ther 1999; 289: 735-741.
32. Van Hecken A, Schwartz JI, Depre M, De Lepeleire I, Dallob A, Tanaka W, Wynants K, Buntinx A, Arnout J, Wong PH, Ebel DL, Gertz BJ, De Schepper PJ. Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. J Clin Pharmacol 2000; 40: 1109-1120.
32a. Ormrod D, Wellington K, Wagstaff AJ: Valdecoxib . Drugs 2002;62:2059-2071.
33. Sedor JR, Williams SL, Chremos AN, Johnson CL, Dunn MJ. Effects of sulindac and indomethacin on renal prostaglandin synthesis. Clin Pharmacol Ther 1984; 36: 85-91.
34. Berg KJ. Acute effects of acetylsalicylic acid on renal function in normal man. Eur J Clin Pharmacol 1977; 11: 117-123.
35. Donker AJ, Arisz L, Brentjens JR, van der Hem GK, Hollemans HJ. The effect of indomethacin on kidney function and plasma renin activity in man. Nephron 1976; 17: 288-296.
36. Perez Gutthann S, Garcia Rodriguez LA, Raiford DS, Duque Oliart A, Ris Romeu J. Nonsteroidal anti-inflammatory drugs and the risk of hospitalization for acute renal failure. Arch Intern Med 1996; 156: 2433-2439.
37. Evans JMM, McGregor E, McMahon AD, McGilchrist MM, Jones MC, White G, McDevitt DG, MacDonald TM. Non-steroidal drugs anti-inflammatory drugs and hospitalization for acute renal failure. QJMed 1995;88: 551-557.
38. Griffin MR, Yared A, Ray WA. Nonsteroidal anti-inflammatory drugs and acute renal failure in elderly persons. Am J Epidemiol 2000; 151: 488-496.
39. Blackshear JL, Napier JS, Davidman M, Stillman MT. Renal complications of nonsteroidal anti-inflammatory drugs: identification and monitoring of those at risk. Semin Arthritis Rheum 1985; 14: 163-175.
40. Feldman HI, Kinman JL, Berlin JA, Hennessy S, Kimmel SE, Farrar J, Carson JL, Strom BL. Parenteral ketorolac: the risk of acute renal failure. Ann Intern Med 1997; 126: 193-199.
41. Zipser RD, Hoefs JC, Speckart PF, Zia PK, Horton R. Prostaglandins: modulators of renal function and pressor resistance in chronic liver disease. J Clin Endocrinol Metab 1979; 48: 895-900.
42. Zipser RD, Radvan GH, Kronborg IJ, Duke R, Little TE. Urinary thromboxane B_2 and prostaglandin E_2 in the hepatorenal syndrome: evidence for increased vasoconstrictor and decreased vasodilator factors. Gastroenterology 1983; 84: 697-703.
43. Walshe JJ, Venuto RC. Acute oliguric renal failure induced by indomethacin: possible mechanisms. Ann Intern Med 1979; 91: 47-49.
44. Arisz L, Donker AJM, Brentjens JRH, van der Hem GK. The effect of indomethacin on proteinuria and kidney function in the nephrotic syndrome. Acta Med Scand 1976; 199: 121-125.
45. Kleinknecht C, Broyer M, Gubler MC, Palcoux JB. Irreversible renal failure after indomethacin in steroid-resistant nephrosis. N Engl J Med 1980; 302: 691.

46. Kimberley RP, Gill JR Jr, Bowden RE, Keiser HR, Plotz PH. Elevated urinary prostaglandins and the effects of aspirin on renal function in lupus erythematosus. Ann Intern Med 1978; 89: 336-341.
47. Fong HJ, Cohen AH. Ibuprofen-induced acute renal failure with acute tubular necrosis. Am J Nephrol 1982; 2: 28-31.
48. Favre L, Glasson P, Vallotton MB. Reversible acute renal failure from combined triamterene and indomethacin: a study in healthy subjects. Ann Intern Med 1982; 96: 317-20.
49. Whelton A, Stout RL, Spilman PS, Klassen DL. Renal effects of ibuprofen, piroxicam, and sulindac in patients with asymptomatic renal failure: a prospective, randomized, crossover comparison. Ann Intern Med 1990; 112: 568-576.
50. Whelton A. Renal effects of over-the-counter analgesics. J Clin Pharmacol 1995; 35: 454-463.
51. Perazella MA. Drug-induced hyperkalemia: old culprits and new offenders. Am J Med 2000; 109: 307-314.
52. Galler M, Folkert VW, Schlondorff D. Reversible acute renal insufficiency and hyperkalemia following indomethacin therapy. JAMA 1981; 246: 154-155.
53. Findling JW, Beckstrom D, Rawsthorne L, Kozin F, Itskovitz H. Indomethacin-induced hyperkalemia in three patients with gouty arthritis. JAMA 1980; 244: 1127-1128.
54. Nicholls MG, Espiner EA. Indomethacin-induced azotaemia and hyperkalemia: a case study. N Z Med J 1981; 94: 377-379.
55. Paladini G, Tonazzi C. Indomethacin-induced hyperkalemia and renal failure in multiple myeloma. Acta Haematol 1982; 68: 256-260.
56. Akbarpour F, Afrasiabi A, Vaziri ND. Severe hyperkalemia caused by indomethacin and potassium supplementation. South Med J 1985; 78: 756-757.
57. Mor R, Pitlik S, Rosenfeld JB. Indomethacin- and moduretic-induced hyperkalemia. Isr J Med Sci 1983; 19: 535-537.
58. Goldszer RC, Coodley EL, Rosner MJ, Simons WM, Schwartz AB. Hyperkalemia associated with indomethacin. Arch Intern Med 1981; 141: 802-804.
59. MacCarthy EP, Frost GW, Strokes GS. Indomethacin-induced hyperkalemia. Med J Aust 1979; 1: 550.
60. Tan SY, Shapiro R, Franco R, Stockard H, Mulrow PJ. Indomethacin-induced prostaglandin inhibition with hyperkalemia. Ann Intern Med 1979; 90: 783-785.
61. Whelton A, Maurath CJ, Verburg KM, Geis GS: Renal safety and tolerability of celecoxib, a novel cyclooxygenase-2 inhibitor. Am J Ther 2000; 7: 159-175.
62. Palmer RH, Haig AE, Flavin SK, Iyengar NK. Effects of ibuprofen, nabumetone and celecoxib on blood pressure control in hypertensive patients on ACE inhibitors. Am J Hypertens 2001; 14: 85A.
63. White WB, Kent J, Taylor A, Verburg KM, Lefkowith JB, Whelton A: Effects of celecoxib on ambulatory blood pressure in hypertensive patients on ACE inhibitors. Hyperten 2002;39:929-934.
64. Vioxx[â] [package insert]. Whitehouse Station, NJ: Merck & Co, March 2000.
65. Orloff J, Handler JS, Bergstrom S. Effect of prostaglandin (PGE_1) on the permeability response of the toad bladder to vasopressin, theophylline and adenosine 3', 5'-monophosphate. Nature 1965; 205: 397-398.
66. Anderson RJ, Berl T, McDonald KM, Schrier RW. Evidence for an *in vivo* antagonism between vasopressin and prostaglandin in the mammalian kidney. J Clin Invest 1975; 56: 420-426.
67. Schooley RT, Wagley PF, Lietman PS. Edema associated with ibuprofen therapy. JAMA 1977; 237: 1716-1717.
68. Blum M, Aviram A. Ibuprofen induced hyponatremia. Rheumatol Rehabil 1980; 19: 258-259.
69. Gurwitz JH, Avorn J, Bohn RL, Glynn RJ, Monane M, Mogun H. Initiation of anti-hypertensive treatment during nonsteroidal anti-inflammatory drug therapy. JAMA 1994; 272: 781-786.
70. Pope JE, Anderson JJ, Felson, DT: A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. Arch Intern Med 1993;153:477-484.
71. Johnson AG, Nguyen TV, Day RO. Do nonsteroidal anti-inflammatory drugs affect blood pressure? A meta-analysis [see comments]. Ann Intern Med 1994; 121: 289-300.
71a. Fogari R, Zoppi A, Carretta R Veglio F, Salvetti A: Effect of indomethacin on the antihypertensive efficacy of valsartan and lisinopril: a multicenter study. J Hyperten 2002;20:1007-1014.
72. Ruoff GE. The impact of nonsteroidal anti-inflammatory drugs on hypertension: alternative analgesics for patients at risk. Clin Ther 1998; 20: 376-387.
73. Schwartz JI, Malice MP, Kalyani R, Lasseter K, Holmes GB, Gertz BJ, Gottesdiener K, Laurenzi M, Brune K. Effect of rofecoxib, celecoxib, and naproxen on blood pressure in elderly volunteers. Am J Hypert 2001; 14: 177A.
73a. Celis H, Thijs L, Staessen JA, Birkenhager WH, Bulpitt CJ, deLeeuw PW, Leonetti G, Nachev C, Tuomilehto J, Fagard RH: Interaction between nonsteroidal anti-inflammatory drug intake and calcium channel blocker-based antihypertensive treatment in Syst-Eur trial. J Human Hyperten 2001;15:613-8.
74. Raij L, Baylis C. Nitric oxide and the glomerulus. Kidney Int 1995; 48: 20-32.
75. Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Basile Fasolo C, Sudano I, Salvetti A. Aging and endothelial function in the normotensive subjects and patients with essential hypertension. Circulation 1995; 91: 1981-1987.

76. Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N. Basal nitric oxide synthesis in essential hypertension. Lancet 1997; 349: 837-842.
77. Campese VM, Amar M, Anjali C, Medhat T, Wurgaft A: Effect of L-arginine on systemic and renal haemondynamics in salt-sensitive patients with essential hypertension. J Hum Hyperten. 1997; 11: 527-532.
78. Perinotto P, Biggi A, Carra N, Orrico A, Valmadre G, Dall'aglio P, Novarinin A, Montanari A. Angiotensin II and prostaglandin interactions on systemic and renal effects of L-NAME in humans. J Am Soc Nephrol 2001; 12: 1706-1712.
79. Mulkerrin EC, Clark BA, Epstein FH: Increased salt retention and hypertension from non-steroidal agents in the elderly. Q J Med 1997; 90: 411-415.
80. Alam S, Purdie DM, Johnson AG. Evaluation of the potential interaction between NaCl and prostaglandin inhibition in elderly individuals with isolated systolic hypertension J Hyperten 1999; 17: 1195-1202.
81. Brater DC, Anderson S, Baird B, Campbell WB. Effects of ibuprofen, naproxen, and sulindac on prostaglandings in men. Kidney Int 1985; 27: 66-73.
82. Bunning RD, Barth WF. Sulindac: a potentially renal-sparing nonsteroidal anti-inflammatory drug. JAMA 1982; 248: 2864-2867.
83. Ciabattoni G, Cinotti GA, Pierucci A, Simonetti BM, Manzi M, Pugliese F, Barsotti P, Pecci G, Taggi F, Patrono C. Effects of sulindac and ibuprofen in patients with chronic glomerular disease: evidence for the dependence of renal function on prostacyclin. N Engl J Med 1984; 310: 279-283.
84. Klassen DK, Stout RL, Spilman PS, Whelton AW. Sulindac kinetics and effects on renal function and prostaglandin excretion in renal insufficiency. J Clin Pharmacol 1989; 29: 1037-1042.
85. Mistry CD, Lote CJ, Gokal R, Currie WJ, Vandenberg M, Mallick NP. Effects of sulindac on renal function and prostaglandin synthesis in patients with moderate chronic renal insufficiency. Clin Sci 1986; 70: 501-505.
86. Quintero E, Gines P, Arroya V, Rimola A, Camps J, Gaya J, Guevara A, Rodamilans M, Rodes J. Sulindac reduces the urinary excretion of prostaglandins and impairs renal function in patients with cirrhosis and ascites. Nephron 1986; 42: 298-303.
87. Rossert J. Drug-induced acute interstitial nephritis. Kidney Int 2001; 60: 804-817.
88. Porile JL, Bakris GL, Garella S. Acute interstitial nephritis with glomerulonephropathy due to nonsteroidal anti-inflammatory agents: a review of its clinical spectrum and effects of steroid therapy. J Clin Pharmacol 1990; 30: 468-475.
88a. Rocha JL, Fernandez-Alonso J: Acute interstitial nephritis associated with the seledcitve COX-2 enzyme inhibitor, rofecoxib. Lancet 2001;357:1946-1947.
88b. Henao, J, Hisamuddin J, Nzerue CM, Vasandaui G, Hewan-Lowe K: Celecoxib-induced acute interstitial nephritis. Am J Kid Dis 2002:39:1313-1317.
88c. Alper AB, Meleg-Smith S, Krane NK: Nephrotic syndrome and interstitial nephritis associated with celecoxib. Am J Kid Dis 2002;40:1086-1090.
89. Abraham PA, Keane WF. Glomerular and interstitial disease induced by nonsteroidal anti-inflammatory drugs. Am J Nephrol 1984; 4: 1-6.
90. Levin ML. Patterns of tubulo-interstitial damage associated with nonsteroidal anti-inflammatory drugs. Sem Nephrol 1988; 8: 55-61.
91. Schwarz A, Krause PH, Kunzendorf U, Keller F, Distler A. The outcome of acute interstitial nephritis: risk factors for the transition from acute to chronic interstitial nephritis. Clin Nephrol 2000; 54: 179-190.
92. Ravnskov U. Glomerular, tubular and interstitial nephritis associated with non-steroidal anti-inflammatory drugs. Evidence for a common mechanism. Br J Clin Pharmacol 1999; 47: 203-210.
93. Bender WL, Whelton A, Beschorner WE, Darwish MO, Hall-Craggs M, Solez K. Interstitial nephritis, proteinuria, and renal failure caused by nonsteroidal anti-inflammatory drugs: immunologic characterization of the inflammatory infiltrate. Am J Med 1984; 76: 1006-1012.
94. Stachura I, Jayakumar S, Bourke E. T + B lymphocyte subsets in fenoprofen nephropathy. Am J Med 1983; 75: 9-16.
95. Shibasaki T, Ishimoto F, Sakai O, Joh K, Aizawa S. Clinical characterization of drug-induced allergic nephritis. Am J Nephrol 1991;11:174-180.
96. Radford MGJr, Holley KE, Grande JP, Larson TS, Wagoner RD, Donadio JV, McCarthy JT: reversible membranous nephropathy associated with the used of nonsteroidal anti-inflammatory drugs. JAMA 1996; 276: 466-469.
97. Perneger TV, Whelton PK, Klag MJ. Risk of kidney failure associated with the used of acetaminophen, aspirin, and nonsteroidal anti-inflammatory drugs. N Engl J Med 1994; 331: 1675-1679.
98. Sandler DP, Burr FR, Weinberg CR. Nonsteroidal anti-inflammatory drugs and the risk of chronic renal disease. Ann Intern Med 1991; 115: 165-172.
99. Field TS, Gurwitz JH, Glynn RJ, Salive ME, Gaziano JM, Taylor JO, Hennekens CH. The renal effects of nonsteroidal anit-inflammatory drugs in older people: findings from the established populations for epidemiologic studies of the elderly. J Am Soc Geriatr Soc 1999; 47: 507-511.

100. Sturmer T, Elseviers MM, De Broe ME. Nonsteroidal anti-inflammatory drugs and the kidney. Curr Opin Nephrol Hypert 2001; 10: 161-163.
101. Sturmer T, Erb A, Keller F, Gunther KP, Brenner H. Determinants of impaired renal function with use of non-steroidal anti-inflammatory drugs: The importance of half-life and other medications. Am J Med 2001; 111: 521-527.
102. Calvo-Alen J, De Cos MA, Rodriguez-Valverde V, Escallada R, Florez J, Arias M. Subclinical renal toxicity in rheumatic patients receiving longterm treatment with nonsteroidal anti-inflammatory drugs. J Rheum 1994; 21: 1742-1747.
103. Segasothy M, Samad SA, Zulfigar A, Bennett WM. Chronic renal disease and papillary necrosis associated with long-term use of nonsteroidal anti-inflammatory drugs as the sole or predominate analgesic. Am J Kidney Dis 1996; 24: 17-24.
104. Shah GM, Muhalwas KK, Winer RL. Renal papillary necrosis due to ibuprofen. Arthritis Rheum 1981; 24: 1208-1210.
105. Robertson CE, Ford MJ, Van Somerem V, Dlugolecka M, Prescott LF. Mefenamic acid nephropathy. Lancet 1980; 2: 232-233.
106. Husserl FE, Lange RK, Kantorw CMJ. Renal papillary necrosis and pyelonephritis accompanying fenoprofen therapy. JAMA 1979; 242: 1896-1898.
107. Atta MG, Whelton A. Acute renal papillary necrosis induced by ibuprofen. Am J Ther 1996; 4: 55-60
108. Kincaid-Smith P. Effects of non-narcotic analgesics on the kidney. Drugs 1986; 32(Suppl 4): 109-128.
109. Kirschenbaum MA, White N, Stein JH, Ferris TF. Redistribution of renal cortical blood flow during inhibition of prostaglandin synthesis. Am J Med 1974; 227: 801-805.
110. Stein JH, Fadem SZ. The renal circulation. JAMA 1978; 239: 1308-1312.
111. Shah GM, Muhalwas KK, Winer RL. Renal papillary necrosis due to ibuprofen. Arthritis Rheum 1981; 24: 1208-1210.
112. Lourie SH, Denman SJ, Schroeder ET. Association of renal papillary necrosis and ankylosing spondylitis. Arthritis Rheum 1977; 20: 917-921.
113. Morales A, Steyn J. Papillary necrosis following phenylbutazone ingestion. Arch Surg 1971; 103: 420-421.
114. Husserl FE, Lange RK, Kantrow CM Jr. Renal papillary necrosis and pyelonephritis accompanying fenoprofen therapy. JAMA 1979; 242: 1896-1898.
115. Robertson CE, Ford MJ, Van Someren V, Dlugolecka M, Prescott LF. Mefenamic acid nephropathy. Lancet 1980; 2: 232-233.
116. Fored CM, Ejerblad E, Lindblad P, Fryzek JP, Dickman PW, Signorello LB, Lipworth L, Elinder CG, Blot WJ, McLaughlin JK, Zack MM, Nyren O. Acetaminophen, aspirin, and chronic renal failure. N Engl J Med 2001; 345: 1801-1808.
117. Weisman JI, Bloom B. Anuria following phenylbutazone therapy. N Engl J Med 1955; 252: 1086-1087.
118. Lipsett MB, Goldman R. Phenylbutazone toxicity: report of a case of acute renal failure. Ann Intern Med 1954; 41: 1075-1079.
119. Hart D, Ward M, Lifschitz MD. Suprofen-related nephrotoxicity. A distinct clinical syndrome. Ann Intern Med 1987; 106: 235-238.
120. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol 1971; 231: 232-235.
121. Verburg KM, Maziasz TJ, Weiner E, Loose L, Geis GS, Isakson PC. COX-2-specific inhibitors: definition of a new therapeutic concept. Am J Ther 2001; 8: 49-64.
122. Lipsky PE, Abramson SB, Crofford L, Dubois MD, Simon LS, van de Putte LBA: the classification of cyclooxygenase inhibitors. J Rheumatol 1998; 25: 2298-2303.
123. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis. The CLASS study: a randomized controlled trial. JAMA 2000; 284: 1247-1255.
124. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ, for the VIGOR study group: Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med 2000; 343: 1520-1528.
125. Goldstein JL, Silverstein FE, Agrawal NM, Hubbard RC, Kaiser J, Maurath CJ, Verburg KM, Geis GS. Reduced risk of upper gastrointestinal ulcer complications with celecoxib, a novel COX-2 inhibitor. Am J Gastroenterol 2000; 95: 1681-1690.
126. Leese PT, Hubbard RC, Karim A, Isakson PC, Yu SS, Geis GS. Effects of celecoxib, a new cyclooxygenase-2 inhibitor, on platelet function in healthy adults: a randomized controlled trial. J Clin Pharmacol 2000; 40: 124-132.
127. Cryer B, Gottesdiener K, Gertz B, Wong P, Dallob A, Feldman M. *In vivo* effects of rofecoxib, a new cyclooxygenase (COX)-inhibitor, on gastric mucosal prostaglandin (PG) and serum thromboxane (TXB2) synthesis in healthy humans. Gastroenterology 1999; 116: G0611.
128. Langman MJ, Jensen DM, Watson DJ, Harper SE, Zhao PL, Quan H, Bolognese JA, Simon TJ. Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs. JAMA 1999; 282: 1929-1933.
129. O'Neill GP, Ford-Hutchinson AW. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. FEBS Lett 1993; 330: 156-160.
130. Rossat J, Maillard M, Nussberger J, Brunner HR, Burnier M. Renal effects of selective cyclooxygenase-2 inhibition in normotensive salt-depleted subjects. Clin Pharmacol Ther 1999; 66: 76-84.

131. Swan, SK, Rudy DW, Lasseter KC, Ryan CF, Buechel KL, Lambrecht LJ, Pinto MB, Dilzer SC, Obrda O, Sundblad KJ, Gumbs CP, Ebel DL, Quan H, Larson P, Schwartz JK, Musliner TA, Gertz BJ, Brater C, Yao SL. Effects of cyclooxygenase-2 inhibition on renal function in elderly persons receiving a low-salt diet. Ann Intern Med 2000; 133: 1-9.
132. Whelton A, Fort JG, Puma JA, Normandin D, Bello AE, Verburg KM. Cyclooxygenase-2-specific inhibitors and cardiorenal function: a randomized, controlled trial of celecoxib and rofecoxib in older hypertensive osteoarthritis patients. Am J Ther 2001; 8: 85-95.
133. Whelton A, White WB Bello AE, Puma JA, Fort JG: Effects of celecoxib and rofecoxib on blood pressure and edema in patients >or= 65years of age with systemic hypertension and osteoarthritis. Am J Cardiol 2002;90:959-963.
133a. Dedier J, Stampfer MJ, Hankinson SE, Willett WC, Speizer FE, Curhan GC: Nonnarcotic analgesic use and the risk of hypertension in US women. Hyperten 2002;40:604-608.
134. Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, Godwin J, Qizilbash N, Taylor JO, Hennekens CH. Blood pressure, stroke, and coronary heart disease. Part 2. Short-term reductions in blood-pressure: Overview of randomized drug trials in their epidemiological context. Lancet 1990; 335: 827-838.
134a. Kilger K, Herrlinger C, Peters J, Seyberth HW, Schweer H, Klotz U: Effects of celecoxib and diclofenac on blood pressure, renal function, and vasoactive prostanoids in young and elderly subjects. J Clin Pharmacol 2002;42:985-994.
134b. Reitblat T, Zamir D, Estis L, Priluk R, Drogenikov T, Viskoper JR: The different patterns of blood pressure elevation by rofecoxib and nabumetone. J Human Hyperten. 2002;16:431-434.
135. American Heart Association. 2001 Heart and Stroke Statistical Update. Dallas, Texas: American Heart Association, 2000.
136. SHEP Cooperative Research Group: Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). JAMA 1991; 265: 3255-3264.
137. Staessen JA, Fagard R, Thijs L, Celis H, Arabidze GG, Birkenhager WH, Bulpitt CS, de Leeuw PW, Dollery CT, Fletcher AE, Forette F, Leonetti G, Nachev C, O'Brien ET, Rosenfeld J, Rodicio JL, Tuomilehto J, Zanchetti A, for the Systolic Hypertension in Europe (Syst-Eur) Trial Investigators: Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (SYST-Eur) Trial Investigators. Lancet 1997; 350: 757-764.
138. ALLHAT Collaborative Research Group. Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). JAMA 2000; 283: 1967-1975.
139. Staessen JA, Gasowski J, Wang JG, Thijs L, Hond ED, Boissel JP, Coope J, Ekbom T, Gueyffier F, Liu L, Kerlikowske K, Pocock S, Fagard RH. Risks of untreated and treated isolated systolic hypertension in the elderly: meta-analysis of outcome trials. Lancet 2000; 355: 865-872.
140. Houston MC. Nonsteroidal anti-inflammatory drugs and antihypertensives. Am J Med 1991; 90(Suppl 5A): 42-47.
141. Perazella MA, Eras J. Are selective COX-2 inhibitors nephrotoxic ? Am J Kidney Dis 2000; 35: 937-940.
142. Wolf G, Porth H, Stahl RAK. Acute renal failure associated with rofecoxib. Ann Intern Med 2000; 133: 394 [letter].
142a. Ahmad SR, Kortepeter C, Brinker A, Chen M, Beitz J: Renal failure associated with the use of celecoxib and rofecoxib. Drug Safety 2002;25:537-544.
142b. Zhao SZ, Reynolds MW, Lejkowith J, Whelton A, Arellano FM: A comparison of renal-related adverse drug rfeactions between rofecoxib and celecoxib, based on World Health Organization/Uppsala Monitoring Centre safety database. Clin Therap 2001;23:1478-1491.
143. Heerdink ER, Leufkens HG, Herings RMC, Ottervanger JP, Stricker BH, Bakker A. NSAIDs associated with increased risk of congestive heart failure in elderly patients taking diuretics. Arch Intern Med 1998; 158: 1108-1112.
144. Page K, Henry D. Consumption of NSAIDs and the development of congestive heart failure in elderly patients. An unrecognized public health problem. Arch Intern Med 2000; 160: 777-784.
144a. Feenstra J, Heerdink ER, Grobbee DE, Stricker BHC: Association of nonsteroidal anti-inflammatory drugs with first occurrence of heart failure and with relapsing heart failure: the Rotterdam study. Arch Intern Med 2002;162: 265-270. 145. Graham DY, Agrawal MN, Roth SH. Prevention of NSAID-induced gastric ulcer with misoprostol: multicentre, double-blind, placebo-controlled trial. Lancet 1988; 2: 1277-1280.
146. Johnston HH, Herzog JP, Lauler DP. Effect of prostaglandin E_1 on renal hemodynamics, sodium and water excretion. Am J Physiol 1967; 213: 939-946.
147. Fülgraff G, Brandenbusch G. Comparison of the effects of prostaglandins A_1, E_2, and F_{2a} on kidney function in dogs. Pflüger's Archiv 1974; 349: 9-17.
148. Chang LCT, Splawinski JA, Oates JA, Nies AS. Enhanced renal prostaglandin production in the dog. Effects on renal function; effects on intrarenal hemodynamics. Circ Res 1975; 36: 197-207.
149. Strandhoy JW, Ott E, Schneider EG, Willis LR, Beck NP, Davis BB, Knox FG. Effects of prostaglandins E_1 and E_2 on renal sodium reabsorption and Starling forces. Am J Physiol 1974; 226: 1015-1021.

150. Paller M. The prostaglandin E_1 analog misoprostol reverses acute cyclosporine nephrotoxicity. Transplant Proc 1988; 20(Suppl 3): 634-637.
151. Weir MR, Klassen DK, Hall PS, Schubert C. Misoprostol minimizes indomethacin-induced renal dysfunction in healthy women: a preliminary report. In: Abstractbook 22nd Annual Meeting of the American Society of Nephrology. Washington DC; 1989: 309A.
152. Antillon M, Cominelli F, Los S, Somberg K, Moran M, Reynolds TB, Zipser RD. Effect of oral prostaglandin in indomethacin induced renal dysfunction on alcoholic cirrhosis: a double-blind randomized study. Gastroenterology 1988; 94: A522.
153. Boers M, Dijkmans BAC, Breedveld FC, Camps JA, Chang PC, Van Brummelen P, Pauwels EK, Cats A. No effect of misoprostol on renal function of rheumatoid patients treated with diclofenac. Brit J Rheumatol 1991; 30: 56-59.
154. Whelton A, Scott H, Stout RL. Effects of misoprostol on nonsteroidal anti-inflammatory drug induced renal insufficiency in patients with stable chronic renal failure: a double-blind, crossover study. Am J Therap 1995; 2(11): 858-863.
155. Gurkowski L, MacDougal M, Weigmann T. Effects of misoprostol on contrast-induced renal dysfunction. Am J Therap 1995; 2(11): 837-842.
156. Russell MW, Miller JD, Taylor DCA, Huse DM: Projected risks and costs of cardiovascular disease due to increased systolic blood pressure associated with use of rofecoxib. Am J Hypert 2001; 14: 195A.

15

Gold salts, D-penicillamine and allopurinol

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Gold nephropathy

Introduction

Gold salts have been used in the treatment of patients with rheumatoid arthritis since 1927 [1]. After a controlled study, the Empire Rheumatism Council [2], confirmed the effectiveness of gold salts for the treatment of rheumatoid arthritis. Even today, chrysotherapy has remained one of the major therapeutic modalities in the second line treatment of progressive rheumatoid arthritis. Gold salts are also used in the treatment of pemphigus vulgaris [3] and bronchial asthma [4]. Before the introduction of an orally administered gold compound, auranofin (triethylphosphine gold tetra-acetyl glycopyranoside), to clinical use

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[5-7], parenterally administered gold salts, such as sodium aurothiomalate and gold thioglucose comprised chrysotherapy. The frequency and severity of the side effects for patients treated with parenteral gold versus those given oral gold preparations are significantly different [8-10]. The nephrotoxicity of parenteral gold preparation will be reviewed followed by a discussion of oral gold compound.

Parenterally administered gold

Despite the efficiency of injectable gold salts in the treatment of rheumatoid arthritis, they are associated with a variety of adverse effects, such as skin rashes [11-13], thrombocytopenia [14, 15], granulocytopenia [11, 16], aplastic anemia [17, 18], interstitial pneumonitis [19, 20], gastrointestinal side effects [11, 21], chrysiasis of cornea and lens [12], and proteinuria and nephrotic syndrome [11, 22, 23]. One or more of these adverse reactions have been reported in approximately one-third of patients treated with gold salts [12]. Proteinuria, including nephrotic syndrome, is the commonest manifestation of gold-induced nephropathy, occurring in 2% to 10% in patients receiving chrysotherapy [10, 22-24]. However, the decreased frequency of proteinuria has paralleled the reduction in dosage of injectable gold salts, prolonging the interval between injections and the introduction of the disease modifying agent, methotrexate. The risk of proteinuria is increased at higher doses [26] and in the patients with HLA DR3 [27-30]. In one-third to half of the patients, the proteinuria is accompanied by microscopic hematuria [31, 32]. The severity of the proteinuria varies greatly and does not correlate with the duration of treatment or the total dose of gold received [31, 33]. The peak incidence of proteinuria occurs after four to six months of treatment [33], but it may develop at any time from 1 week to 39 months after the start of treatment [33, 34]. Renal function is normal to minimal impairment in these proteinuric patients.

Histopathology of glomerular lesions

Histopathological examinations of the renal biopsy specimens from patients with proteinuria show predominantly membranous glomerulopathy [10, 22, 31-40]. Electron microscopy of renal tissue usually demonstrates subepithelial electron dense deposits (especially when the disease is of short duration) [22, 32-40], intramembranous electron dense deposits [32, 34, 40], and fusion and increased density of foot processes of epithelial cells [22, 32, 35-40]. Light microscopy occasionally discloses varying degrees of uniform thickening of the glomerular basement membrane. Small, fuchsinophilic deposits with associated spike like extensions of the basement membrane may be identified on trichrome-stained sections. Immunofluorescent study of the renal tissues with subepithelial electron dense deposits reveals granular deposition of IgG, IgM and/or complements [10, 33, 34, 37, 39]. In addition to membranous glomerulonephritis, there are reports of minimal change glomerulonephritis [32, 41], focal segmental glomerulonephritis [32], and mesangioproliferative glomerulonephritis with immune complex deposition in mesangial areas [10, 31, 40]. Skrifvars et al. [42] reported a highly unusual fatal renal complication induced by sodium aurothiomalate. This complication was characterized by microhematuria, impaired renal function and by a granulomatous glomerulonephritis.

Histopathology of interstitial lesions

In addition to the glomerular lesions mentioned above, focal tubular atrophy of variable severity is a feature of the majority of biopsy specimens of gold induced nephropathy [32, 37, 39, 43]. Interstitial fibrosis can be recognized in many of the specimens (Figure 1), and the degree of fibrosis tends to parallel the severity and extent of the tubular atrophy. However, interstitial inflammation is not usually prominent [32]. Electron microscopy reveals the existence of characteristic filamentous, electron dense cytoplasmic inclusions in various renal cells at high frequency [22, 37-39, 44, 45]. These filamentous inclusions may be complexes containing gold and other molecules [52, 50, 53]. The inclusions are concentrated in proximal tubular epithelial cells, interstitial macrophages, but rarely occur in mesangial cells and visceral epithelial cells, and spare the basement membrane or subepithelial space. They are much more prominent in patients who have received large doses of gold [22]. There may be a significant association between the degree of histological interstitial changes and the number of gold inclusions. Cramer et al. [43] reported a patient who suffered from chronic interstitial nephritis after receiving large quan-

tities of aurothioglucose for rheumatoid arthritis. Gold deposition was seen by electron microscopy and confirmed by microprobe X-ray analysis within both tubular epithelial cells and interstitial macrophages but not the interstitium. They hypothesized that the administration of massive amounts of gold salts resulted in these depositions and the subsequent interstitial nephritis [43]. Lesato et al. [46] reported a high incidence of subtle renal tubular dysfunction in rheumatoid arthritis patients receiving gold treatment, demonstrating tubular proteinuria and the urinary excretion of large amounts of renal tubular epithelial antigen, tubular basement membrane (TBM) antigen, and β2-microglobulin. However, the amounts of these proteins in urine did not correlate with the total dose of gold [46]. Renal tubular dysfunction has been induced in Hartley guinea pigs by the injection of sodium aurothiomalate, as manifested by the urinary excretion of tubular basement membrane and renal tubular epithelial antigens and tubular proteinuria. Excretion of these proteins tended to be dose dependent [47]. Following the tubular dysfunction, autoimmune tubulointerstitial nephritis with anti-TBM antibodies developed in the animals [47].

Pathogenesis

There are mainly two types of gold-induced nephropathy, one being immune complex type glomerulonephritis and the other limited to tubular lesions. The latter may be induced by the direct toxic action of gold, and this toxicity seems to be dose dependent. The morphological changes in the tubules usually involve gold inclusions [22, 37, 39, 44-46]. Nagi et al. [48] using large doses of sodium aurothiomalate (1 mg/week) produced renal tubular necrosis in rats, characterized by degenerative changes of the cytoplasmic contents of epithelial cells of proximal convoluted tubules. The ultracellular structure changes involved swollen mitochondria that had lost their shape. Eiseman et al. [49] reported morphofunctional and biochemical changes in rat kidneys following a single ip injection of a high dose (75 mg/kg) of gold sodium thiomalate. This included severe coagulative necrosis of the proximal tubular epithelium at one day, followed by epithelial regeneration by day 4 and nearly complete resolution by day 8. Alternations in renal heme biosynthesis and drug metabolism paralleled the morphological changes [49]. Tubular dysfunction has also been reported in rheumatoid arthritis patients receiving gold treatment [46] and in animals being treated with low doses of gold salts [47, 48].

The pathogenesis of immune complex type glomerular lesions associated with chrysotherapy remains

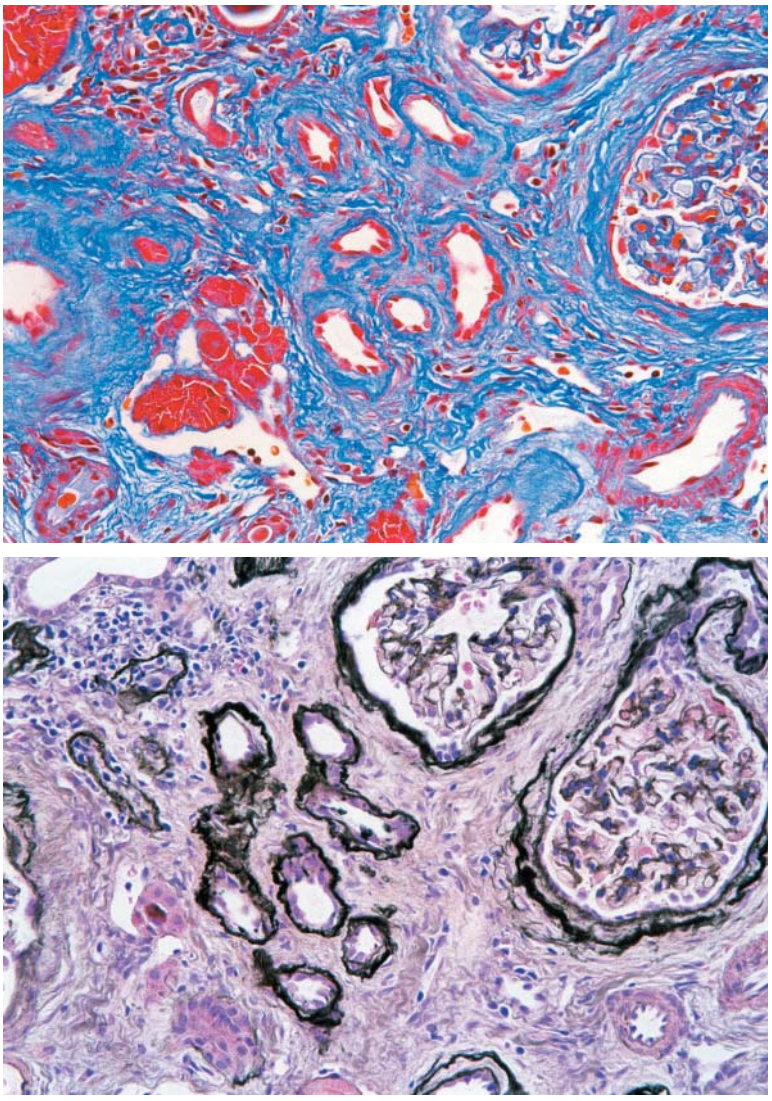

Figure 1. *Photomicrographs of the kidney from a rheumatoid arthritis patient with gold nephropathy, demonstrating prominent interstitial fibrosis and tubular cell degeneration (magn. x340). Above: Masson's trichrome staining; below: PAM staining.*

unclear. To clarify the pathogenesis of this nephropathy, it is necessary to confirm the specificity of the antigens and antibodies responsible for the immune complex of the glomerular lesions. Gold salts may act as a hapten, and specific IgE antibodies against gold salts have been detected in the sera of rheumatoid arthritis patients with mucocutaneous and hematologic adverse reaction to gold salts [50, 51]. A positive lymphocyte transformation test to gold salts has been reported in some rheumatoid arthritis patients with hematologic side effects after chrysotherapy [52]. Derot et al. [53] reported a rare case of fatal acute tubular necrosis due to gold induced nephropathy. Allergic reaction to gold salts might have been responsible for the development of this nephritis; however, such immunological phenomena are rarely seen in the patients with gold-induced nephropathy [50, 51]. To date, no evidence for the presence of gold in renal immune deposits has been reported.

It is difficult to confirm that gold is the causal antigen or hapten in gold-induced immune complex nephropathy. Palosuo et al. [54] demonstrated a circulating antigen in a patient with gold-induced nephropathy before and after the development of nephropathy, which shared immunological determinants with tissue antigens extracted with deoxycholate from microsomal fractions of various organs including human liver, human kidney, and rat liver. Precipitating antibodies against this circulating antigen were found in the serum sample pre-dating diagnosis. This serum reacted with various tissue antigens extracted from human organs, but not with kidney specific antigen [54]. In an experimental rat model, Nagi et al. [48] reported the successful induction of slowly progressive immune complex nephropathy by weekly injections of small doses of sodium aurothiomalate (0.0025 mg/week), suggesting the important pathogenetic role of renal tubular antigen released from damaged tubular epithelial cells (Figure 2). Skrifvars [55] also emphasized the possible role of autoimmunization secondary to released tubular antigens in the pathogenesis of gold-induced glomerular lesions. In the guinea pig model, renal dysfunction was also induced by injections of sodium aurothiomalate, as manifested by the urinary excretion of renal tubular antigens including renal tubular epithelial and tubular basement membrane antigens. Following the tubular dysfunction, immune complex nephropathy with circulating anti-renal tubular epithelial antibody, including deposition of renal tubular epithelial antigen in the glomerular immune complexes, developed in the animals [47]. Thus, shed renal tubular antigens from damaged tubular epithelium may play an important role in the pathogenesis of gold-induced immune complex nephropathy. There are many drugs that injure the renal tubular epithelium, but rarely induce immune complex nephropathy. Thus, in addition to tubular damage, there must be other factors that promote the development of gold nephropathy. Other tissue autoantigens released and/or altered by the effect of gold and heterogeneous antigens may also participate in the pathogenetic mechanisms.

That gold salts possess immunosuppressive effects has been demonstrated by both *in vivo* and *in vitro* studies [56-59]. In addition, they also have an immunoenhancing effect on the immune response of mice, depending on dosage [60]. BALB/c mice are highly susceptible to autoimmune interstitial nephritis, while C57BL/6 mice are genetically resistant to this nephritis when immunized with tubular basement membrane antigen with adjuvant [61]. When both strains of mice are following pretreated with appropriate doses of sodium aurothiomalate immunization with tubular basement membrane antigen with adjuvant, BALB/c mice become resistent to the development of nephritis, but nephritis is induced in the genetically resistant C57BL/6 mice. Thus, gold salts may depress the activity of all T cells, and the phenotypical effect of gold salts on the immune response to some antigens may depend on the character of the dominant T cells [62]. Selective *in vitro* inhibition of T cells has also been shown in patients receiving chrysotherapy [63]. There must be

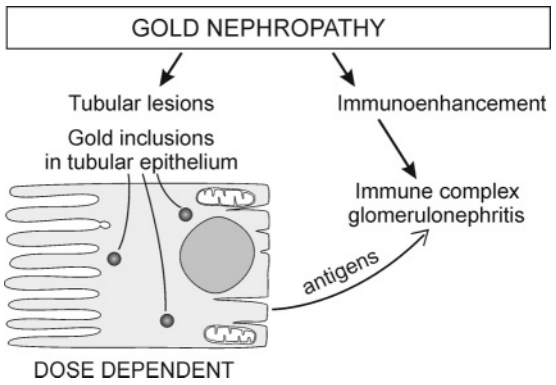

Figure 2. *An illustration of possible mechanisms in the pathogenesis of gold nephropathy.*

other, as yet defined factors that are involved in the development of gold nephropathy.

Therapy and prognosis

Proteinuria is usually slow to resolves after withdrawal of the drug. In 1970, Vaamonde et al. [31] reviewed 19 case reports of nephrotic syndrome associated with chrysotherapy. In 17 patients whose outcomes were known, 13 recovered in 3 months to 7 years. Recently, Hall et al. [33] reported a long-term study of 21 patients with rheumatoid arthritis who developed proteinuria during treatment with sodium aurothiomalate. Ten patients developed proteinuria after 6 months' of treatment, 15 after 12 months, and 18 after 24 months. When chrysotherapy was stopped the proteinuria had reached a median peak of 2.1 g/day (range 0.7-30.7 g/day) at two months (range 1-13 months) before resolving spontaneously, in 8 patients by 6 months, in 13 by 12 months, and in 18 by 24 months. All patients were free of proteinuria after 39 months, the median duration being 11 months after withdrawal. Renal function did not deteriorate, and no patient died from or needed treatment for renal failure. HLA-B8 and/or DR3 alloantigens were identified in seven of the patients [33].

Newton et al. [64] studied 27 patients with gold-induced proteinuria, and provided guidelines as to when gold should be permanently stopped in these patients. They demonstrated that proteinuria of up to 2 g/L is compatible with continued gold therapy, since the low risk of more serious nephropathy developing was low. They concluded: 1) mild proteinuria (less than 0.4 g/L) is common in rheumatoid arthritis patients on gold, and such a level may not even be related to this drug. It usually disappears spontaneously without alteration of therapy, but rarely can proceed to more serious problems. 2) moderate proteinuria (0.4-2.0 g/L) should be treated more seriously. Gold injections should be stopped. If the urine clears within three months, then further treatment with gold may be given without precipitating heavy proteinuria. 3) none of their subjects have sustained permanent renal impairment [66]. The advice of Howard-Lock et al about D-penicillamine therapy may also be suitable for gold therapy. They advocate withholding the drug if there is (1) proteinuria of 2^+ on the dipstick, (2) persistent (longer than 3 weeks) proteinuria of 1^+, (3) if there are red cell casts, white cell casts, or hyaline casts present, or (4) if red cells >10 per high power field are present. For patients whose disease has improved but who developed proteinuria of between 300 to 1,000 mg/day but without other renal abnormality, they suggest continuing the drug cautiously at a reduced dose with close monitoring. If the proteinuria exceeds 2 g/day or the glomerular filtration rate falls, the drug should be discontinued immediately [65]. Manthorpe et al. reported a successful one year treatment with auranofin (6 mg/day) in 7 rheumatoid arthritis patients with previous proteinuria associated with parenterally injected gold salts [66].

Prediction, prevention and monitoring of development of gold nephropathy

To predict the adverse effects of gold, the association with HLA antigen has been studied [27, 28, 67-69]. A genetic predisposition to gold toxicity was first suggested by Panayi et al. [67]. Wooley et al. [68] investigated the possible relation between HLA antigens and toxicity of D-penicillamine and sodium aurothiomalate in rheumatoid arthritis patients. Nineteen of 24 patients in whom proteinuria developed were positive for HLA-B8 and DRw3 antigens. Furthermore, all 13 episodes of proteinuria exceeding 2 g/day occurred in patients with DRw3. Several investigators confirmed the association between gold-induced proteinuria and DR3 [27-30] and B8 [30], but others were unable to confirm it [70]. Conversely, DR3 patients tended to exhibit a better therapeutic response to sodium aurothiomalate than patients with DR4 [28]. DR4 and/or DR2 positive patients may have some degree of protection against gold toxicity [28, 29]. Given the uncertainty about HLA types and toxic reactions, together with the suggestion that patients with DR3 respond better than the more numerous DR4, and taking into account the cost involved, any suggestion of using HLA typing as a guide to therapy seems premature [71]. While Van Riel et al. [72] reported the predictive value of serum IgA for gold toxicity, the study of Ostuni et al., involving a larger population, concluded that the monitoring of serum IgA was not useful in predicting gold toxicity [73]. Recently, Ayesh et al. [74] reported the predictive efficacy of the prior measurement of sulphoxidation capacity. A patient with poor sulphoxidation capacity had a nine-fold greater risk of developing gold-induced adverse reactions including

nephropathy. Hopefully this will be confirmed by prospective studies involving various races and a large population. To date, there is no confirmed method for predicting gold toxicity including nephropathy, thus it is essential to monitor patients closely for any appearance of nephropathy.

The decline in the number of reports of parenterally administered gold-induced nephropathy may indicate that the dose of gold salts used per injection is decreased and intervals between injections are being extended to prevent adverse reactions. Furthermore, introduction of methotrexate therapy for rheumatoid arthritis has contributed to decreased reliance on gold salts.

Auranofin nephropathy

Auranofin, a unique gold compound, has been available for clinical use for 15 years after it proved to be one of the most potent oral antiarthritic compounds among alkylphosphine gold coordination complexes [75]. Initial clinical studies suggested that this compound was therapeutically active when taken by mouth, with no renal adverse effects in any of the 32 patients studied [5-7]. Subsequently, the therapeutic benefits and toxicity of auranofin have been evaluated [24, 76], compared with placebo [9, 77, 78], sodium aurothiomalate [8-10, 79], and D-penicillamine [80-82]. The incidence of proteinuria in a world-wide trial was 3% for auranofin [10, 24]. The risk of developing proteinuria with auranofin therapy is significantly less than with parenteral gold [9, 24], or D-penicillamine [82]. Histopathological findings in renal biopsy specimens from patients with moderate to heavy proteinuria are consistent with the membranous nephropathy similar to injectable gold nephropathy [33, 83, 84]. Heuer et al. [10] reported a total of 3,475 rheumatoid arthritis patients receiving auranofin therapy in 27 countries. Proteinuria developed in 3% of the patients, resulting in drug withdrawal in 0.9%, compared with 4% proteinuria in patients receiving injectable gold, with 0.8% being withdrawn. Katz et al. [24] evaluated proteinuria in 1800 rheumatoid arthritis patients given chrysotherapy. Three percent (41 cases) of 1283 auranofin-treated patients had an abnormal 24-hour urine protein level: 15 had mild (0.15 to 1 g/day), 17 had moderate (1 to 3.5 g/day), and 9 had heavy (>3.5 g/day) proteinuria. Permanent renal impairment did not occur in any patient. In 36 patients with long-term follow-up after drug withdrawal, proteinuria cleared in 31 patients within 1 week to 24 months. Seven of 8 patients who were rechallenged once the proteinuria had cleared were able to continue treatment without recurrent episodes [24].

Pathogenic mechanism of auranofin-induced nephropathy resemble those of parenteral gold-induced nephropathy. The reason for the reduced risk of proteinuria with auranofin compared to parenteral gold salts is not known. However, differences in the pharmacokinetics of the two types of gold preparations may be important. In rats treated with auranofin or sodium aurothiomalate for one year, renal gold concentrations were 33 times higher with the latter formulation [85]. Renal elimination of an orally administered dose of auranofin in human is less than 15%, compared with greater than 70% for parenterally administered sodium aurothiomalate [86].

D-penicillamine

Introduction

D-penicillamine is so named because it was first isolated as an amine, from the degradation products of penicillin by Abraham et al [87]. Later studies showed the characteristic chemical behavior of D-penicillamine which involve three types of reactions, formation of disulphide links, formation of thiazolidine rings, and formation of metal complexes and chelates [67]. It was first used in 1956 in the treatment of Wilson's disease [88]. D-penicillamine has since been used in the treatment of many diseases, such as cystinuria [89], rheumatoid arthritis [90-92], systemic sclerosis [93], primary biliary cirrhosis [94], heavy metal poisoning due to lead [95], cadmium [96], and mercury [97], and hyperviscosity syndrome [99]. In rheumatoid arthritis, D-penicillamine has been widely accepted as an effective second line treatment. Despite of its effectiveness, it causes many adverse effects, such as skin rashes [99, 100], taste abnormalities [100, 101], hepatic dysfunction [102-104], gastrointestinal toxicity [99, 105], proteinuria [100, 106], hematuria [107, 108], thrombocytopenia [92, 109], aplastic anemia [110], lupus-like syndrome [111, 112], Goodpasture's-like pulmonary renal syndrome [113-115], vasculitis [116, 117], myasthenia gravis [118-122], polymyositis [123, 124], and dermatomyositis [125].

One or more of these adverse reactions was recorded in nearly 60% of patients treated with D-penicillamine [100, 126-129]. Among these adverse reactions, nephropathy developed in patients with proteinuria, hematuria, lupus-like syndrome, Goodpasture's-like pulmonary renal syndrome, and vasculitis.

Proteinuria

Proteinuria, including nephritic syndrome, is the commonest manifestation of nephropathy, reported as occurring in between 2 and 32% of patients [100, 101, 109, 124, 126-130]. The risk of proteinuria is increased at higher doses [100, 131-133], in patients with HLA B8 and/or DRW3 antigens [68], and in patients with previous gold toxicity [134, 135]. However, others have not confirmed the relationship to the drug dosage [136], duration of therapy [137], or HLA antigens [70]. In the majority of patients, proteinuria is accompanied by microscopic hematuria [100, 127]. The peak incidence of proteinuria occurs in the second six months of treatment, but it may develop at any time from 6 weeks to 74 months [107, 101, 138]. Proteinuria may be persistent or may slowly progress to nephrotic syndrome if therapy is continued. Up to 1/3 of the patients with significant proteinuria progress to nephrotic syndrome if therapy is continued [106]. Renal function is normal to minimal impairment in patients with isolated proteinuria.

Histopathology

Histopathological examination of renal biopsy specimens from the patients with isolated proteinuria due to D-penicillamine shows predominant membranous glomerulopathy [139-141]. Electron microscopy of renal tissue usually demonstrates subepithelial electron dense deposits and fusion of epithelial foot processes [139-141]. The deposits on the epithelial side of the glomerular basement membrane appear to be slowly covered and later incorporated into the basement membrane. With time the deposits become fainter and move towards the endothelial side of the basement membrane [142]. Immunofluorescent study may demonstrate granular deposits of IgG and C3 in the capillary wall. These changes in glomerular histology can persist for at least a year after the withdrawal of the drug [139]. Sellars et al. [143] reviewed the renal biopsies of 30 patients with rheumatoid arthritis and clinical evidence of renal disease. They reported all 9 patients with membranous glomerulonephritis but only 6 of 13 with mesangial change had received D-penicillamine or gold. Besides membranous glomerulonephritis, there are reports of minimal change glomerulonephritis [144, 145], mild mesangioproliferative glomerulonephritis without crescent [110, 142, 146], or IgM nephropathy [147, 148] associated with D-penicillamine induced proteinuria.

Therapy and Prognosis of Proteinuria

Proteinuria usually resolves slowly after withdrawal of the drug. Hall et al. [149] reported a long-term study of 33 patients with rheumatoid arthritis who developed proteinuria during treatment with D-penicillamine. Of these, fourteen patients developed proteinuria within 6 months after the start of treatment and 27 within 12 months. When treatment was stopped, the proteinuria reached a median peak of 4.2 g/day (range 0.3-15 g/day) at one month (range 0-7 months) before resolving spontaneously by six months in 12 patients, 12 months in 21, and 21 months in all. In all their patients whose nephropathy was due to D-penicillamine the proteinuria resolved completely when the drug was withdrawn; renal function did not deteriorate, and corticosteroids were unnecessary [149]. Jaffe [150] reported that reintroduction of D-penicillamine in patients with drug induced proteinuria, starting with a daily dose of 250 mg, was usually followed by a return of proteinuria at about the same time and at about the same cumulative dose as on the first occasion. However, Hill et al. [133] reported successful reintroduction and continuation for a minimum of 13 months in 5 rheumatoid arthritis patients who developed proteinuria during the first course of the drug. They instituted the "go slow, go low" method of Jaffe [151], starting with a daily dose of 50 mg and increasing by monthly increment of 50 mg to a maintenance dose of 150 mg daily. The dose was held at 150 mg/day for 4 months and thereafter increased by 50 mg at 3-months intervals if disease remained active. Proteinuria did not recur, and improvement of disease was shown in all 5 patients [133]. Howard-lock et al. [65] advocated withholding D-penicillamine if there is (1) proteinuria of 2+ on the dipstick, (2) persistent (longer than 3 weeks) proteinuria of 1+ (3) if there are red cell casts, white

cell casts, or hyaline casts present, or (4) if red cells > 10 per high power field are present. For patients whose disease has improved but who developed proteinuria between 300 to 1, 000 mg/day, but without other renal abnormality, they suggest the continued use of the drug cautiously at a reduced dose with close monitoring. If proteinuria exceeds 2 g/day or the glomerular filtration rate falls, the drug should be discontinued immediately.

Goodpasture's-like syndrome

Besides the benign proteinuria mentioned above, proliferative glomerulonephritis with fulminant renal failure has also occurred with D-penicillamine therapy. One is Goodpasture's-like syndrome, which is characterized by pulmonary hemorrhage and rapidly progressive glomerulonephritis. Goodpasture's -like syndrome associated D-penicillamine treatment has been reported in patients with Wilson's disease [113], rheumatoid arthritis [114, 115, 152, 153], primary biliary cirrhosis [154], and progressive systemic sclerosis [155]. D-penicillamine was given for at least 7 months (range: 7-84 months), and at a daily dose higher than 750 mg (range: 750-2,000 mg) preceding the onset of symptoms. Pulmonary X-rays showed bilateral extensive infiltrates in all 10 cases. Lung hemorrhage was the principle cause of death in 3 cases [113].

The histopathology of renal specimens usually showed proliferative glomerulonephritis with crescent formation in 30 to 100% of the glomeruli. Direct immunofluorescent study failed to show linear IgG deposition along the glomerular basement membrane, but granular deposition of IgG and/or C3 were present along the glomerular capillary walls in 5 of 6 patients. Subepithelial electron dense deposits were observed in 3 of 4 patients tested. Circulating anti-glomerular basement membrane antibody was not detected in any of the cases tested. In Brown Norway rats, the administration of D-penicillamine induced antinuclear antibodies and significantly high concentrations of immune complexes. Iin these animals there was no granular deposition of IgG, but linear deposition of IgG along the glomerular basement membrane. IgG eluted from diseased kidneys bound both *in vitro* and *in vivo* to the kidney basement membrane [156]. HLA-DR2 antigen was absent in the 2 cases where HLA phenotype was determined, whereas there is a strong association between HLA-DR2 and antibody-mediated Goodpasture's syndrome [157]. Anti-nuclear antibodies have been detected both before [115, 156] and after initiation of the drug [152, 115]. Although this syndrome is potentially life-threatening, aggressive treatment with plasmapheresis, steroids, immunosuppressive drugs such as azathioprine and cyclophosphamide, and mechanical ventilation with PEEP may be life saving [113, 152-155].

Renal vasculitis

Extracapillary glomerulonephritis with renal vasculitis is also been reported as a rare complication of D-penicillamine therapy [117, 126, 156]. Necrosis of interlobular arteries with glomerular crescent [117] and necrotic and occluded periglomerular arterioles [156] have been reported. Aggressive treatment with pulse steroid, anticoagulants, and antiplatelet agents may be beneficial. The two patients with renal vasculitis, whose outcome was known, died from bacterial infection within ten months after the onset of the disease [117, 156].

SLE Syndrome

A drug-induced systemic lupus erythematosus with proliferative glomerulonephritis has also been described in patients treated with D-penicillamine [111, 157]. Systemic lupus erythematosus syndrome is induced in approximately 2% of patients treated with D-penicillamine [112, 158]. Unlike other forms of drug-induced systemic lupus erythematosus, anti-double-strand DNA antibodies and/or hypocomplementemia are seen in D-penicillamine-induced systemic lupus erythematosus syndrome [111, 156]. Nephropathy is rare in D-penicillamine-induced systemic lupus erythematosus syndrome [111]. Walshe [112] reported that 8 patients developed the serological change of systemic lupus erythematosus of 120 patients with Wilson's disease treated with D-penicillamine, but none of them showed nephropathy.

Chalmers [111] reported 6 rheumatoid arthritis patients with D-penicillamine-induced systemic lupus erythematosus syndrome. All patients had previous mucocutaneous reactions to chrysotherapy. Manifestations included pleurisy in 5 of 6 patients, rashes in 3, and nephritis in 2. LE cells were present in 5 patients,

anti nuclear antibodies in all 6, anti-double-strand DNA in 3, 3 were Coomb's test positive, and low C4 complement in 5 of the 6 [111]. Results of a renal biopsy from a patient with nephritis showed diffuse endocapillary proliferative glomerulonephritis with focal crescent formation and vasculitis. Electron microscopy showed scattered subendothelial deposits, and immunofluorescent study revealed granular deposition of IgG, IgM, C3 complement and C1q. The patient was successfully treated with prednisolone and azathioprine [112]. Ntoso et al. [156] reported penicillamine-induced rapidly progressive glomerulonephritis in two patients with progressive systemic sclerosis. Anti nuclear antibodies, anti-Sm antibody, and Coomb's antibodies were positive in both patients. Renal biopsies from the two patients demonstrated a diffuse, predominantly extracapillary, proliferative glomerulonephritis with crescents and focal necrosis, and by immunofluorescence, focal areas of IgG, C3, and fibrinogen were observed in areas of glomerular necrosis. Subendothelial and mesangial deposits were observed by electron microscopy. Both patients responded to pulse methylprednisolone and subsequent daily steroids [156].

Pathogenesis of D-penicillamine-induced nephropathy

Deposition of immune complexes in the glomerular basement membrane may play an important role in the pathogenesis of D-penicillamine-induced nephropathy, such as isolated proteinuria, Goodpasture's-like syndrome, and nephritis associated with D-penicillamine-induced systemic lupus erythematosus rheumatoid arthritis syndrome. Immunofluorescent study show predominantly granular deposition of IgG and/or C3, and electron microscopy revealed subepithelial or subendothelial electron dense deposits. In rheumatoid arthritis patients, D-penicillamine alters the circulating immune complexes [159]. D-penicillamine has the capacity to convert large complexes into small ones *in vitro* and there has been speculation that similar mechanisms *in vivo* could explain the deposition of complexes and renal damage [160]. Small immune complexes deposit in the glomeruli easier than big ones. In addition to penicillamine nephropathy, other side effects of the drug may be related to the widespread deposition of immune complexes (Figure 3). Dense, granular immunoglobulin deposits have been identified at the epidermodermal junction in 4 rheumatoid arthritis patients who developed toxic reactions, such as severe rashes, thrombocytopenia, aplastic anemia, and proteinuria. Three of 4 penicillamine-induced systemic lupus erythematosus syndrome patients had similar findings on skin biopsy [161].

Besides immune complex deposition, autoantibodies against several autoantigens are frequently detected in patients treated with D-penicillamine, leading to autoimmune diseases. The exact mechanism by which this drug induces autoimmunity remains to be investigated. It may directly stimulate oligoclonal B cell activity, upset the balance between T cell subsets, or alter antigens by hapten formation. D-penicillamine can bind with various proteins, and may change the antigenicity of these proteins as a hapten. However, to date, no evidence for the presence of penicillamine in renal immune deposits has been reported. Nagata et al. [162] reported that D-penicillamine can act as a hapten for specific T cells when presented on the surface of appropriate stimulator cells, and suggested that the adverse immunological side effects of this drug in patients may have a pathogenesis similar to graft-versus-host reaction.

Prediction and monitoring of development of D-penicillamine nephropathy

To predict D-penicillamine side effects, the asso-

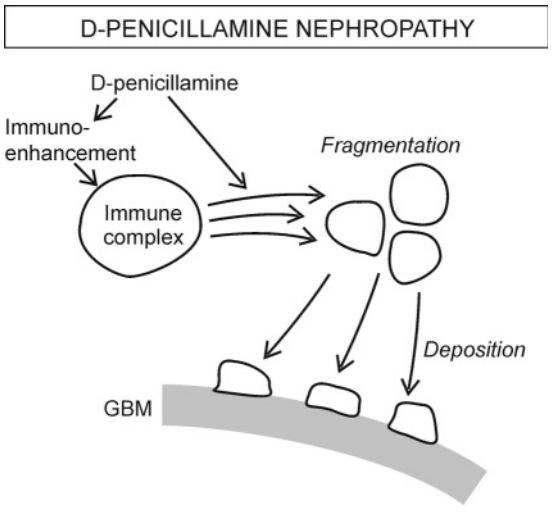

Figure 3. *An illustration of the pathogenesis of D-penicillamine induced nephropathy.*

ciation between side effects and various factors, such as HLA antigens [68, 70, 128, 130, 163, 164], autoantibodies [165, 166], and previous gold toxicity [101, 138, 167, 168] has been studied. Wooley et al. [68] investigated the possible interaction between HLA antigens and toxicity of D-penicillamine and sodium aurothiomalate in rheumatoid arthritis patients. Nineteen of 24 patients in whom proteinuria developed were positive for HLA-B8 and DRw3 antigens. Furthermore, all 13 episodes of proteinuria exceeding 2 g/day occurred in patients with DRw3 [68]. There is also a strong association between idiopathic membrane nephropathy and HLA-DRw3, B8 and B18 [169]. Other investigators have confirmed the association between D-penicillamine-induced proteinuria and DR3 [128, 130, 164] and B8 [70, 128, 130]. However, other investigators could not confirm a significant association between D-penicillamine proteinuria and HLA-DR3 [70, 170]. In addition to HLA antigens, Emery et al. [163] emphasized the sulphoxidation status of patients as a new predictor of outcome of drug toxicity.

Moutsopoulos et al. [165, 166] reported that anti-Ro (SSA) positive Greek rheumatoid arthritis patients experienced a significantly high frequency of side effects from D-penicillamine. Despite their dissimilar chemical structures, the thiol compounds, sodium aurothiomalate and D-penicillamine, have remarkably similar clinical effects, and this similarity extends to the incidence and type of adverse effects [138, 167]. Several investigators have noted the association between prior gold nephropathy and D-penicillamine. Billingsley and Stevens reported the significant correlation of D-penicillamine-induced proteinuria to a previous history of gold nephropathy [134]. Patients with gold-induced proteinuria are at a higher risk for the development of proteinuria during D-penicillamine therapy ($p<0.001$), and this occurs within the first six months of treatment [138]. All six patients who developed systemic lupus erythematosus syndrome while being treated with D-penicillamine had previous mucocutaneous reactions to chrysotherapy [114]. Dood et al. [165] noted that all patients who took D-penicillamine within six months after an adverse reaction to gold developed side effects from D-penicillamine, and recommended an interval exceeding six months between treatment with gold and treatment with D-penicillamine in patients who have developed adverse reactions to gold, to reduce the risk of adverse reactions to D-penicillamine, Kean et al. [101] analyzed the influence of previous sodium aurothiomalate therapy on the toxicity pattern of D-penicillamine, but could not confirm a synergistic effect of D-penicillamine and sodium aurothiomalate leading to increased adverse reaction in patients with rheumatoid disease [101].

Although there are several predictors of adverse reactions, the most useful clinical predictor is urinalysis. Patients on D-penicillamine therapy should be closely monitored, and every visit to the hospital should include a full urinalysis.

Allopurinol

Introduction

Allopurinol (4-hydroxypyrazolo [3,4-d] pyrimidine) is an inhibitor of xanthine oxidase that was successfully introduced in the treatment of primary gout about 35 years ago [171]. Allopurinol is now accepted as standard therapy in the treatment of primary and secondary hyperuricemia. Adverse reactions occur in about 10% of patients treated with allopurinol and are relatively mild and self-limited [171, 172]. A mild maculopapular eruption or gastrointestinal disorders are usually noted, which promptly regress with cessation of therapy. Isolated instances of allopecia [173], bone marrow depression [174], ocular lesions [175], acute cholangitis [176], various types of hepatic injuries [177, 178] temporal arthritis [179], and xanthine stones [180] have been reported.

In 1970, reports began to appear of systemic, severe, prolonged hypersensitivity reactions occurring in patients under treatment with allopurinol [182, 182]. These reactions are characterized by fever, chills, malaise, generalized dermatitis, eosinophilia, abnormalities of liver function tests, and rapidly progressive renal failure [181-188]. Allopurinol-induced nephropathy is usually reported as a part of these reactions. In 1979, Gorge et al. [186] reported 3 cases of such reactions and reviewed 38 patients including their 7 patients. The average dose of the drug in these patients was 300 mg/day. The average time from initiation of the therapy to onset of the reaction was 3.8 weeks. The most common type of dermatitis was a pruritic, diffuse, erythematosus, maculopapular eruption noted in over 60% of the patients. Toxic epidermal necrosis, Stevens-Johnson syndrome, and exfoliative dermatitis

were also noted in some patients. The presence of eosinophilia (4-53%) was noted in all but two patients. Thirty-one of 32 patients (97%) had documented impaired renal function prior to allopurinol therapy. Following the onset of the hypersensitivity reaction, further deterioration of renal function occurred in 30 of 32 patients [186]. In 1986, Singer et al. [188] reported 8 additional patients with such reactions and reviewed an additional 72 patients described in the literature. Forty of 80 patients (50%) had impaired renal function prior to allopurinol therapy. Further deterioration of renal function was found in 48 of 80 patients.

Histopathology

Histopathological examination of renal biopsy or autopsy specimens revealed renal vasculitis [181], focal segmental glomerulonephritis [184], and acute interstitial nephritis [185, 187, 189, 190]. Jarzobski et al. [181] reported a case of the hypersensitivity type of vasculitis with fibrinoid necrosis and eosinophilic reaction, involving multiple organs, especially the kidney, resulting in uremia and death. Boyer et al. [191] also reported 3 cases of the same type including the efficacy of prednisolone in treating this type of disease. Kantor et al. [182] reported a case of glomerulonephritis associated with allopurinol-hypersensitivity. Linear deposition of IgG and complement along the glomerular basement membrane were demonstrated, and a necrotizing, hemorrhagic pneumonitis was also reported. However, no circulating anti-glomerular basement membrane antibody was detected. Acute interstitial nephritis has also been reported associated with by the administration of allopurinol [185, 187, 189, 190]. Gelbart et al. [185] reported a case of allopurinol-induced interstitial nephritis with extensive infiltration of lymphocytes, plasma cells and tubular damage. No immunoglobulins, complement, or fibrin were evident in the tubular basement membrane. This patient also had other typical symptoms of hypersensitivity reactions. Grussendrof et al. [187] also reported a case of acute interstitial nephritis with circulating anti-tubular basement membrane antibody and granular C3 deposition on the tubular basement membrane. The interstitium was diffusely widened, edematous and infiltrated with lymphocytes, plasma cells, histiocytes and numerous eosinophils. The nephritis was induced by controlled re-exposure to allopurinol in a patient who had two successive severe hypersensitivity reactions to this drug.

Pathogenesis

The pathogenesis of nephropathy associated with allopurinol-induced hypersensitivity reactions is unclear. However, pathogenic role of the immune reactions against allopurinol or its metabolites has not been excluded. Emmerson et al. [192] studied the lymphocyte reactivities to allopurinol and its active metabolite, oxypurinol, in 9 patients with previous documented adverse reactions to allopurinol. They suggested that some adverse reactions to allopurinol represented delayed type hypersensitivity to oxypurinol, but not to allopurinol. Allopurinol is oxidized by xanthine oxidase to oxypurinol, which is also an inhibitor of the enzyme (Figure 4). Allopurinol plasma half life is less than 2 hours due to rapid renal clearance and oxidation to oxypurinol [193]. Oxypurinol, because of its reabsorbance by the renal tubules, has a plasma half-life of 18 to 30 hours. The clearance of oxypurinol is diminished in renal insufficiency [194]. In addition, thiazide diuretics might be expected to cause accumulation of oxypurinol since its renal handling is similar to that of uric acid [195]. Hypersensitivity syndrome has been found to occur most frequently when allopurinol is given with thiazides or in patients with renal insufficiency [184, 188]. The immune reactions to oxypurinol may play an important role in the pathogenesis of the syndrome, including being dose dependent. The serum concentration of oxypurinol has been monitored

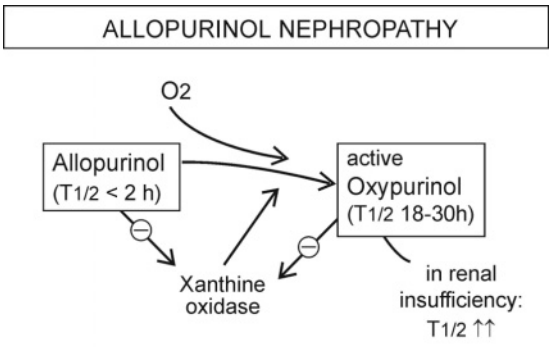

Figure 4. *Suggestion of reactions leading to allopurinol nephropathy.*

to prevent adverse reactions [195, 196]. Recommended plasma oxypurinol concentrations are below 100 μmol/L [196]. Several authors [195, 196] reported that no adverse reactions have occurred in patients with lower plasma oxypurinol levels; however, hypersensitivity syndrome occasionally develops in patients with a therapeutic plasma oxypurinol concentration [197]. In addition to plasma oxypurinol concentration, other factors probably contribute to the development of the syndrome.

Human herpes virus 6 (HHV 6) infection is recently attracted a great deal of attention as a possible cause of drug-induced hypersensitivity. Suzuki et al reported a case of allopurinol-induced hypersensitivity syndrome with dramatically increased anti-HHV 6 IgG antibodies. They also demonstrated the presence of HHV 6 in the skin of this patient using a polymerase chain reaction and *in situ* hybridization [198]. Thus, drug-induced hypersensitivity syndrome may not be a simple allergic reaction to drug. Further investigations regarding the relation of HHV 6 infection and drug-induced hypersensitivity syndrome may provide insight to the pathogenesis of allopurinol-induced hypersensitivity syndrome.

Therapy, prognosis, and prevention

Withdrawal of the drug and the prolonged administration of systemic steroids are beneficial for the hypersensitivity syndrome with renal involvement. Initial dose of steroid should be 1 to 2 mg/kg/day of prednisolone, with careful gradual tapering of steroids required in the majority of patients. The recovery time ranged from 1 week to 11 months. Mortality from this syndrome is high, with twenty-one of 80 patients died as a result of the syndrome [188]. In fulminant cases, such as acute renal failure complicating toxic epidermal necrosis or Stevens-Johnson syndrome, methylprednisolone 'pulse' therapy might be beneficial. Patients with HHV 6 infection also require prednisolone therapy.

To prevent unnecessary morbidity and mortality due to the allopurinol hypersensitivity, Singer et al. [188] recommended the indications for allopurinol as follow: 1) tophaceous gout; 2) major uric acid overproduction (urinary excretion of more than 900 mg of uric acid/day on a diet with rigid purine restriction); 3) frequent gouty attacks unresponsive to prophylactic colchicines, when uricosuric agents cannot be used due to intolerance, lack of efficacy, renal insufficiency, or poor patient compliance; 4) recurrent uric acid renal calculi; 5) recurrent calcium oxalate renal calculi when associated with hyperuricosuria; or 6) prevention of acute urate nephropathy in patients receiving cytotoxic therapy for malignancies. Furthermore, they said that asymptomatic hyperuricemia, uncomplicated gout, and acute gouty attacks are not considered indications for allopurinol therapy [188]. Kelley [199] advised allopurinol therapy for asymptomatic hyperuricemia, but only when it is truly severe (serum uric acid level > 13 mg/dl and 24-hour urine excretion > 1,100 mg). The allopurinol hypersensitivity syndrome occurs most frequently when the drug is given with diuretics or in patients with renal insufficiency. Patients on allopurinol therapy should be closely monitored especially within the first several weeks after initiating administration of the drug. Furthermore, the patients with high risk as mentioned above should start the therapy with lower dose of allopurinol.

References

1. Lande K. Die gunstige Beeinflussung schleichender Dauerinfekte durch Solganal. Munchen Med Wochenshr 1927; 74: 1132-1134.
2. The Research Sub-Committee of the Empire Rheumatism Council. Gold therapy in rheumatoid arthritis. Final report of a multicenter controlled trial. Ann Rheum Dis 1961; 20: 315-333.
3. Penneys NS, Eaglestein WH, Frost P. Management of pemphigus with gold compounds. Arch Dermatol 1976; 112: 185-187.
4. Muranaka M, Miyamoto T, Shida T, Kabe J, Makino S, Okumura H, Takeda K, Suzuki S, Horiuchi Y. Gold salt in the treatment of bronchial asthma - a double blind study. Ann Allergu 1978; 40: 132-137.
5. Finkelstein AE, Walz DT, Batista V, Mizrazi M, Roisman F, Misher A. Auranofin – new oral gold compound for treatment of rheumatoid arthritis. Ann Rheum Dis 1976; 35: 251-257.
6. Berglof FE, Berglof K, Walz DT. Auranofin: an oral chrysotherapeutic agent for the treatment of rheumatoid arthritis. J Rheumatol 1978; 5: 68-74.

7. Weisman MH, Hannifin DH. Management of rheumatoid arthritis with oral gold. Arthritis Rheum 1979; 22: 922-925.
8. Davis P, Menard H, Thompson J, Harth M, Beaudet F. One-year comparative study of gold sodium thiomalate and auranofin in the treatment of rheumatoid arthritis. J Rheumatol 1985; 12: 60-67.
9. Ward JR, Williams HJ, Egger MJ, Reading JC, Boyce E, Altz-Smith M, Samuelson CO, Willkens RF, Solsky MA, Hayes SP, Blocka KL, Weinstein A, Meenan RF, Guttadauria M, Kaplan SB, Klippel J. Comparison of auranofin, gold sodium thiomalate and placebo in the treatment of rheumatoid arthritis: A controlled clinical trial. Arthritis Rheum 1983; 26: 1303-1315.
10. Heuer MA, Pietrusko RG, Morris RW, Scheffler BJ. An analysis of worldwide safety experience with auranofin. J Rheumatol 1985; 12: 695-699.
11. Hartfall SJ, Garland HG, Goldie W. Gold treatment of arthritis. A review of 900 cases. Lancet 1937; 9: 838-842.
12. Gibbons RB. Complication of chrysotherapy. A review of recent studies. Arch Intern Med 1979; 139: 343-346.
13. Penneys NS, Ackerman AB, Gottlieb NL. Gold dermatitis. Arch Dermatol 1979; 109: 372-376.
14. Benson WG, Moore N, Tugwell P, D, Souza M, Singal DP. HLA antigens and toxic reactions to sodium aurothiomalate in patients with rheumatoid arthritis. J Rheumatol 1984; 11: 358-361.
15. Adachi JD, Bensen WG, Singal DP, Powers PJ. Gold-induced thrombocytopenia: platelet associated IgG and HLA typing in three patients. J Rheumatol 1984; 11: 355-357.
16. Gottlieb NL, Gray RG. Diagnosis and management of adverse reaction from gold compounds. J Analytic Toxicol 1978; 2: 173.
17. Baldwin JL, Storb R, Thomas ED, Mannik M. Bone marrow transplantation in patients with gold-induced marrow aplasia. Arthritis Rheum 1977; 20: 1043-1048.
18. Gibson J, McGirr EE, York J, Kronenberg H. Aplastic anemia in association with gold therapy for rheumatoid arthritis. Aust NZ J Med 1983; 13: 130-135.
19. Winterbauer RH, Wilske KR, Wheelis RF. Diffuse pulmonary injury associated with gold treatment. N Engl J Med 1976; 294: 919-921.
20. Partanen J, van Assendelft AHW, Koskimies S, Forsberg S, Hakala M, Ilonen J. Patients with rheumatoid arthritis and fold-induced pneumonitis express two high-risk major histocompatibility complex patterns. Chest 1987; 92: 277-281.
21. Fam AG, Paton TW, Shamess CJ, Lewis AJ. Fulminant colitis complicating gold therapy. J Rheumatol 1980; 7: 479-485.
22. Silverberg DS, Kidd EG, Shnitka TK, Ulan RA. Gold nephropathy. A clinical and pathological study. Arthritis Rheum 1970; 13: 812-825.
23. Kean WF, Anastassiades TP. Long-term chrysotherapy. Incidence of toxicity and efficacy during sequential time periods. Arthritis Rheum 1979; 22: 495-501.
24. Katz WA, Blodgett RC Jr, Pietrusko RG. Proteinuria in gold treated rheumatoid arthritis. Ann Intern Med 1984; 101: 176-179.
25. Brock H, Han MT. Gold nephrosis. N Engl J Med 1966; 274: 210-211.
26. Furst DE, Levine S, Srinivasan R, Metzger L, Bangert R, Paulus E. A double-blind trial of high versus conventional dosages of gold salts for rheumatoid arthritis. Arthritis Rheum 1977; 20: 1473-1480.
27. Gran JT, Husby G, Thorsby E. HLA DR antigens and gold toxicity. Ann Rheum Dis 1983; 42: 63-66.
28. Bensen WG, Moore N, Tugwell P, D Souza M, Singal DP. HLA antigens and toxic reactions to sodium aurothiomalate in patients with rheumatoid arthritis. J Rheumatol 1984; 11: 358-361.
29. Barger BO, Acton RT, Koopman WJ, Alarcon GS. DR antigens and gold toxicity in white rheumatoid patients. Arthritis Rheum 1984; 27: 601-605.
30. Hakala M, van Assendelef AHW, Ilonen J, Jalava S, Tiilikainen A. Association of different HLA antigens with various toxic effects of gold salts in rheumatoid arthritis. Ann Rheum Dis 1986; 45: 177-182.
31. Vaamaonde CA, Hunt FR. The nephritic syndrome as a complication of gold therapy. Arthritis Rheum 1970; 13: 826-834.
32. Francis KL, Jenis EH, Jensen GE, Calcagno PL. God-associated nephropathy. Arch Pathol Lab Med 1984; 108: 234-238.
33. Hall CL, Fothergill NJ, Blackwell MM, Harrison PR, MacKenzie JC, Maclver AG. The natural course of gold nephropathy: long term study of 21 patients. Br Med J 1987; 295: 745-748.
34. Tornroth T, Skrifvars B. Gold nephropathy. Prototype of membranous glomerulonephritis. Am J Med 1974; 75: 573-590.
35. Lee JC, Dushinkin M, Eyring EJ, Engleman EP, Hopper JR. Renal lesions associated with gold therapy. Light and electron microscopic studies. Arthritis Rheum 1965; 8: 1-13.
36. Mattson JC. Glomerular diseases as a complication of gold therapy. Lab Invest 1974; 30: 383.
37. Katz A, Little AH. Gold nephropathy. An immunopathologic study. Arch Pathol 1973; 96: 133-136.
38. Watanabe I, Whitter FC, Moore J, Cuppage FE. Gold nephropathy. Ultrastructural fluorescent and microanalytic study of two human cases. Arch Path Lab Med 1976; 100: 632-635.
39. Davies DJ, Dowling J, Xipell JM. Gold nephropathy. Pathology 1977; 9: 281-288.
40. Skrifvars BV, Tornroth TS, Tallqvist GN. Gold-induced immune complex nephritis in seronegative rheumatoid arthritis. Ann Rheum Dis 1977; 36: 549-556.
41. Wolters J, Frederik P, van Rie H, Zeppenfeldt E. Minimal change nephropathy during gold treatment. A case with unusual histopathological and immunopathological features. Netherland J Med 1987; 31: 234-240.

42. Skirfvars B, Tornroth T, Flalck HM. Granulomatous glomerulonephritis in a patient with rheumatoid arthritis treated with gold salts. Scand J Rheumatol 1979; 8: 234-240.
43. Cramer CR, Hagler HK, Silva FG, Eigenbrodt EH, Meltzer JI, Pirani CL. Chronic interstitial nephritis associated with gold nephropathy. Arch Path Lab Med 1983; 107: 258-263.
44. Tubbs RR, Valenzuela R, McGorman LJ, Pohl MA, Barenberg S. Gold nephropathy. New Eng J Med 1977; 296: 1413-1414.
45. Yarom R, Stein H, Peter PD, Slavin S, Hall TA. Nephrotoxic effect of parenteral and intraarticular gold. Ultrastructural and electron microprobe examination of clinical and experimental materials. Arthritis Rheum 1975; 99: 36-43.
46. Iesato K, Mori Y, Ueda S, Wakashin Y, Wakashin M, Matsui N, Inoue S, Okuda K. Renal tubular dysfunction as a complication of gold therapy in patients with rheumatoid arthritis. Clin Nephrol 1982; 17: 46-52.
47. Ueda S, Wakashin M, Wakashin Y, Yoshida H, Iesato K, Mori T, Mori Y, Akikusa B, Okuda K. Experimental gold nephropathy in guinea pigs: Detection of autoantibodies to renal tubular antigens. Kidney Int 1986; 29: 539-548.
48. Nagi AH, Alexander F, Barabas AZ. Gold nephropathy in rats. Light and electron microscopic studies. Exp Molec Path 1971; 15: 354-362.
49. Eiseman JL, Ribas JL, Knight E, Alvares AP. Acute nephropathy induced by gold sodium thiomalate: Alterations in renal heme metabolism and morphology. Toxicol Appl Pharmacol 1987; 91: 193-203.
50. Bretza J, Wells I, Novey HS. Association of IgE antibodies to sodium aurothiomalate and adverse reactions to chrysotherapy for rheumatoid arthritis. Am J Med 1983; 74: 945-950.
51. Davis P, Ezeoke A, Munro J, Hobbs JR, Hughes GRV. Immunological studies on the mechanism of gold hypersensitivity reactions. Br Med J 1973; 3: 676-678.
52. Denman EJ, Denman AM. The lymphocyte transformation test and gold hypersensitivity. Ann Rheum Dis 1968; 27: 582-588.
53. Derot M, Kahn J, Mazalton A, Peyrafort J. Fatal anuric nephritis with associated chrysocyanosis following gold therapy. Bull Soc Med Hop Paris 1954; 70: 234-239.
54. Palosuo T, Provast TT, Milgrom F. Gold nephropathy. Serologic data suggesting an immune complex disease. Clin Exp Immunol 1976; 25: 311-318.
55. Skirfvars B. Hypothesis for the pathogenesis of sodium aurothiomalate induced immune complex nephritis. Scand J Rheumatol 1979; 8: 113-118.
56. Jessop JD, Vernon-Roberts B, Harris J. Effects of gold salts and prednisolone on inflammatory cells. I. Phagocytic activity of macrophages and polymorphs in inflammatory exudates studied by a 'skin-window' technique in rheumatoid and control patients. Ann Rheum Dis 1973; 32: 294-300.
57. Vernon-Roberts B, Jessop JD, Dore J. Effects of gold salts and prednisolone on inflammatory cells. II. Suppression of inflammation and phagocytosis in the rat. Ann Rheum Dis 1973; 32: 301-307.
58. Panush RS. Effects of certain antirheumatic drugs on normal human peripheral blood lymphocytes. Inhibition of mitogen- and antigen- stimulated incorporation of tritiated thymidine. Arthritis Rheum 1976; 19: 907-917.
59. Harth M, Stiller CR, Sinclair C, Evans J, McGirr D, Zuberi R. Effects of a gold salt on lymphocyte responses. Clin Exp Immunol 1977; 27: 357-364.
60. Measel W. Effects of gold on the immune response of mice. Infect Immunity 1975; 11: 350-354.
61. Ueda S, Wakashin M, Wakashin Y, Yoshida H, Azemoto R, Iesato K, Mori T, Mori Y, Ogawa M, Okuda K. Autoimmune interstitial nephritis in inbred mice. Analysis of mouse tubular basement membrane antigen and genetic control of immune response to it. Am J Pathol 1988; 132: 304-318.
62. Ueda S, Wakashin Y, Yoshida H, Mori T, Mori Y, Azemoto R, Ogawa M, Kato I, Wakashin M. Gold nephropathy - effect of gold on immune response to renal tubular basement membrane (TBM) antigen in mice. In: Nephrotoxicity. *In vitro* to *in vivo*, animals to man. Bach BH, Lock EA, editors. Plenum, New York 1989; p. 139-145.
63. Lorber A, Kunishima D, Simon T. Selective inhibition of T suppressor lymphocytes during chrysotherapy [Abstract]. Clin Pharmacol Ther 1983; 303: 300-302.
64. Newton O, Swinburn WR, Swinson DR. Proteinuria with gold nephropathy: When should gold be permanently stopped? Br J Rheumatol 1983; 22: 11-17.
65. Howard-Lock HE, Lock CJL, Mewa A, Kean WF. D-penicillamine; Chemistry and clinical use in rheumatic disease. Sem Arthritis Rheum 1986; 15: 261-281.
66. Manthorpe R, Bendixen G. Auranofin in rheumatoid arthritis: Use in patients with side-effect or lack of effect to gold sodium thiomalate or gold thioglucose and/or D-penicillamine. Scand J Rheumatol 1988; 17: 401-405.
67. Panayi GS, Wooley P, Batchelor JR. Genetic basis of R.A. HLA antigen, disease manifestations and toxic reactions to drugs. Br Med J 1978; 11: 1326-1328.
68. Wooly PH, Griffen J, Panayi GS, Batchelor JR, Welsh KI, Gibson TJ. HLA-DR antigens and toxic reaction to sodium aurothiomalate and D-penicillamine in patients with rheumatoid arthritis. N Engl J Med 1980; 303: 300-302.
69. Bardin T, Legrand L, Naveau B, Marcelli-Barge A, Debeyre N, Lathrop GM, Poirier JC, Schmid M, Ryckewaert A, Dryll A. HLA antigens and seronegative rheumatoid arthritis. Ann Rheum Dis 1985; 44: 50-53.

70. Dequeker J, Van Wanghe P, Verdickt W. A systematic survey of HLA-A, B, C, and D antigens and drug toxicity in rheumatoid arthritis. J Rheumatol 1984; 11: 282-286.
71. Ford PM. HLA antigens and drug toxicity in rheumatoid arthritis. J Rheumatol 1984; 11: 269-261.
72. Van Riel PL, Van de Putte LB, Gribnau FW, de Waal RM. Serum IgA and gold induced toxic effects in patients with rheumatoid arthritis. Arch Intern Med 1984; 144: 1401-1403.
73. Ostuni PA, Simioni M, Marson P, Travaglia P, Volante D, Gambari PF. Serum IgA and gold toxicity in rheumatoid arthritis: lack of predicting value. Clin Exp Rheumatol 1986; 4: 359-362.
74. Ayesh R, Mitchell SC, Waring RH, Withrington RH, Seifert MH, Smith RL. Sodium aurothiomalate toxicity and sulphoxidation capacity in rheumatoid arthritis patients. Br J Rheumatol 1987; 26: 197-201.
75. Blodgett RC Jr, Heuer MA, Pietrusko RG. Auranofin: a unique oral chrysotherapeutic agent. Semin Arthritis Rheum 1984; 13: 255-273.
76. Bandilla K, Gross D, Gross W, Herrlinger J-D, Kriegel W, Muller W, Siegmeth W, Tausch G, Thumb N, Wagenhauser J. Oral gold therapy with auranofin (SK&F39162). J Rheumatol 1982; 9 (Suppl 8): 154-159.
77. Katz W, Alexander S, Bland J, Blechman W, Bluhm GB, Bonebrake Ra, Falbo A, Greenwald RA, Hartman S, Hobbs T, Indenbaum S, Lergier JE, Lanier BG, Lightfoot RW, Phelps P, Sheon RP, Torretti D, Wenger ME, Wilske K. The efficacy and safety of auranofin compared to placebo in rheumatoid arthritis. J Rheumatol 1982; 9 (Suppl 8): 173-178.
78. Wenger ME, Alexander S, Bland JH, Blechman WJ. Auranofin versus placebo in the treatment of rheumatoid arthritis. Am J Med 1983; 75: 123-127.
79. Menard HA, Beaudet F, Davis O, Harth M, Percy JS, Russell AS, Thompson M. Gold therapy in rheumatoid arthritis. Interim report of the Canadian multicentre prospective trial comparing sodium aurothiomalate and auranofin. J Rheumatol 1982; 9: 179-183.
80. Felix-Davies DD, Stewart AM, Wilkinson BR, Bateman JR, Delamere JP. A 12-month comparative trial of auranofin and D-penicillamine in rheumatoid arthritis. Am J Med 1983; 75(6A): 138-141.
81. Manthrope R, Horbov S, Sylvest J, Vinterberg H. Auranofin versus penicillamine in rheumatoid arthritis. One-year results from a prospective clinical investigation. Scand J Rheumatol 1986; 15: 13-22.
82. Hochberg MC. Auranofin or D-penicillamine in the treatment of rheumatoid arthritis. Ann Intern Med 1986; 105: 528-535.
83. Revach M, Freed RL, Ehrlich GE. Reversible proteinuria as a complication of oral gold therapy [Letter]. Arthritis Rheum 1979; 22: 1417-1418.
84. Plaza JJ, Herrero G, Barat A, Loutaif JJ, Hernando L, Vallado P, Oliva H. Membranous glomerulonephritis as a complication of oral gold therapy [Letter]. Ann Intern Med 1982; 97: 563-564.
85. Gottlieb NL. Comparative pharmacokinetics of parenteral and oral gold compounds. J Rheumatol 1982; 9 (Suppl 8): 99-109.
86. Blocka K. Auranofin versus injectable gold: Comparison of pharmacokinetic properties. Am J Med 1983; 75(6A): 114-122.
87. Abraham EP, Chain E, Baker W, Robinson R. Penicillamine, a characteristic degradiation product of penicillin. Nature 1943; 151: 107.
88. Walshe JM: Penicillamine. A new oral therapy for Wilson's disease. Am J Med 1956; 2: 487-495.
89. Drawhall JC, Scowen EF, Watts RWE. Effects of penicillamine on cystinuria. Br Med J 1963; 1: 588-590.
90. Jaffe IA. Intra-articular dissociation of the rheumatoid factor. J Lab Clin Med 1962; 60: 409-421.
91. Jaffe IA. The effect of penicillamine in the laboratory parameters in rheumatoid arthritis. Arthritis Rheum 1965; 8: 1064-1078.
92. Multicenter trial group. Controlled trial of D-penicillamine in severe rheumatoid arthritis. Lancet 1973; 1: 275-280.
93. Steen VD, Medsger TA, Rodnan GP. D-penicillamine therapy in progressive systemic sclerosis (scleroderma). A retrospective analysis. Ann Intern Med 1982; 97: 652-659.
94. Jain S, Scherer PJ, Samourian S. A controlled trial of D-penicillamine therapy in primary biliary cirrhosis. Lancet 1977; 1: 831-834.
95. Goldberg Am Smith JA, Lochhead AC. Treatment of lead poisoning with oral penicillamine. Br Med J 1963; 1: 1270-1275.
96. Freeman HC, Huq F, Stevens GN: Metal binding by D-penicillamine: Crystal structure of D-penicillamine cadmium (II) hydrate. J Chem Soc Chem Commun 1976; 90-91.
97. Tamir M, Boustein B, Behar M, Chwat M. Mercury poisoning from an unsuspected source. Br J Ind Med 1968; 21: 299-303.
98. Deutsch MF, Morton JI. Dissociation of human serum macroglobulins. Science 1957; 125: 600-601.
99. Kean WF, Dwosh JL, Anastassiades TP, Ford PM, Kelly HG. The toxicity pattern of D-penicillamine therapy. A guide to the use in RA. Arthritis Rheum 1980; 23: 158-165.
100. Stein HB, Patterson AC, Offer RC, Atkins CJ, Teufel A, Robinson HS. Adverse effects of D-penicillamine in rheumatoid arthritis. Ann Intern Med 1980; 92: 24-29.
101. Kean WF, Lock CJL, Howard-Lock HE, Buchanan WW. Prior gold therapy does not influence the adverse effects of D-penicillamine in rheumatoid arthritis. Arthritis Rheum 1982; 25: 917-922.
102. Wollheim FA, Lindstrom CG. Liver abnormalities in penicillamine treated patients with rheumatoid arthritis. Scand J Rheumatol 1979; 28 (Suppl):100-107.

103. Barzilai D, Dickstein G, Enat R, Bassan H, Lichtig C, Gellei B. Cholestatic jaundice caused by D-penicillamine. Ann Rheum Dis 1978; 37: 98-100.
104. Multz CV. Cholestatic hepatitis caused by penicillamine. JAMA 1981; 246: 674-675.
105. Hickling P, Fuller J. Penicillamine causing acute colitis. Br Med J 1979; 2: 367.
106. Crawhall JC. Proteinuria in D-penicillamine-treated rheumatoid arthritis. J Rheumatol 1981; 8: 161-163.
107. Davison AM, Day AT, Golding JR, Thomson D. Effect of penicillamine on the kidney. Proc R Soc Med 1977; 70: 109-113.
108. Barraclough D, Cunningham TJ, Muirden KD. Microscopic haematuria in patients with rheumatoid arthritis on D-penicillamine. Aust NZ J Med 1981; 11: 706-708.
109. Singh G, Fries JF, Williams CA, Zatarain E, Spitz P, Bloch DA. Toxicity profiles of disease modifying antirheumatic drugs in rheumatoid arthritis. J Rheumatol 1991;18: 188-194.
110. Weiss AS, Markenson JA, Weiss MS, Kammerer WH. Toxicity of D-penicillamine in rheumatoid arthritis. A report of 63 patients including two with aplastic anemia and one with the nephritic syndrome. Am J Med 1978; 64: 114-120.
111. Chalmers A, Thompson D, Stein HE, Reid G, Patterson HC. Systemic Lupus Erythematosus during penicillamine therapy for rheumatoid arthritis. Ann Intern Med 1982; 97: 659-663.
112. Walshe JM. Penicillamine and the SLE syndrome. J Rheumatol 1981; 8 (Suppl 7): 155-160.
113. Sternlieb I, Bennet B, Scheinberg IH. D-penicillamine induced Goodpasture's syndrome in Wilson's disease. Ann Intern Med 1975; 82: 673.
114. Gibson T, Barry HC, Ogg C. Goodpastures' syndrome and D-penicillamine. Ann Intern Med 1976; 84: 100.
115. Gavaghan TE, McNaught PJ, Ralston M, Hayes JM. Penicillamine-induced 'Goodpasture's syndrome'; successful treatment of a fulminant case. Aust NZ J Med 1981; 11: 261-265.
116. Jaffe IA. The treatment of rheumatoid arthritis and necrotizing vasculitis with penicillamine. Arthritis Rheum 1970; 13: 436-443.
117. Banfi G, Imbasciati E, Guerra L, Mihatsch MJ, Ponticelli C. Extracapillary glomerulonephritis with necrotizing vasculitis in D-penicillamine treated patients with rheumatoid arthritis. Nephron 1983; 33: 56-60.
118. Torres CF, Griggs RC, Baum J, Penn AS. Penicillamine induced myasthenia gravis in progressive sclerosis. Arthritis Rheum 1980; 23: 505-508.
119. Dawkins RL, Garlepp MJ, McDonald BL, Williamson J. Myasthenia gravis and D-penicillamine. J Rheumatol 1981; 8 (Suppl 7): 169-174.
120. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody characteristic in myasthenia gravis. Patients with penicillamine-induced myasthenia or idiopathic myasthenia of recent onset. Clin Exp Immunol 1982; 49: 266-272.
121. Bever CT, Chang HW, Penn AS, Jaffe IA, Bock E. Penicillamine induced myasthenia gravis. Effects of penicillamine on acetylcholine receptor. Neurology 1982; 32: 1077-1082.
122. Bocanegra T, Espinoza LR, Vassey FB, Germain BF. Myasthenia gravis in penicillamine treatment of RA. JAMA 1980; 244: 1822-1823.
123. Cucher BG, Goldman AL. D-penicillamine induced polymyositis in RA. Ann Intern Med 1976; 85: 615-618.
124. Schreider PL, Peters HA, Dahl DS. Polymyositis and penicillamine. Arch Neurol 1972; 27: 456-457.
125. Wojnarowska F. Dermatomyositis induced by penicillamine. J R Soc Med 1980; 73: 884.
126. Halverson PB, Kozin F, Bernhard GC, Goldman AL. Toxicity of penicillamine. A serious limitation to therapy in rheumatoid arthritis. JAMA 1978; 240: 1870-1871.
127. Steen VD, Blair S, Medsger TA. The toxicity of D-penicillamine in systemic sclerosis. Ann Intern Med 1986; 104: 699-705.
128. Moens HJB, Ament BJW, Feltkamp BW, Korst JK. Longterm followup of treatment with D-penicillamine to rheumatoid arthritis: effectivity and toxicity in relation to HLA antigens. J Rheumatol 1987; 14: 1115-1119.
129. Shiokawa Y, Horiuchi Y, Honma M, Kageyama T, Okada T, Azuma T. Clinical evaluation of D-penicillamine by multicentric double blind comparative study in chronic rheumatoid arthritis. Arthritis Rheum 1977; 20: 1464-1472.
130. Stockman A, Zilko PJ, Major GAC, Tait BD, Property DN, Mathews JD, Hannah MC, McCluskey J, Muirden KD. Genetic markers in rheumatoid arthritis-Relationship of toxicity from D-penicillamine. J Rheumatol 1986; 13: 269-273.
131. Hill HFH, Hill AGS, Day AT, Brown RM, Golding JR, Lyle WH. Maintenance dose of penicillamine in rheumatoid arthritis: A comparison between standard and a response-related flexible regimen. Ann Rheum Dis 1979; 38: 429-433.
132. Williams HJ, Ward JR, Reading JC, Egger MJ, Grandone JT, Samuelson CO, Furst DE, Sullivan JM, Watson MA, Guttadauria M, Cathcart ES, Kaplan SB, Halla JT, Weinstein A, Plotz PH. Low dose D-penicillamine treatment of R.A. A controlled double blind clinical trial. Arthritis Rheum 1983; 26: 581-592.
133. Hill H, Hill A, Davison M. Resumption of treatment with penicillamine after proteinuria. Ann Rheum Dis 1979; 38: 229-231.
134. Billingsley LM, Stevens MB. The relationship between D-penicillamine-induced proteinuria and prior gold nephropathy. Johns Hopkins Med J 1981; 148: 64-67.
135. Halla JT, Cassidy J, Hardin JG. Sequential gold and penicillamine therapy in rheumatoid arthritis. Am J Med 1982; 72: 423-426.
136. Ross JH, McGinty F, Brewer DG. Penicillamine nephropathy. Nephron 1980; 26: 184-186.

137. Dische FE, Swinson DR, Hamilton EBD, Parsons V. Immunopathology of penicillamine-induced glomerular disease. J Rheumatol 1976; 3: 145-154.
138. Halla JT, Cassidy J, Hardin JG. Sequential gold and penicillamine therapy in rheumatoid arthritis. Am J Med 1982; 72: 423-426.
139. Bacon PA, Tribe CR, MacKenzie JC, Jones JV, Cumming RH, Amer B. Penicillamine nephropathy in rheumatoid arthritis. Q J Med 1976; 45: 661-684.
140. Jaffe IA, Treser G, Suzuki Y, Ehrenreich T. Nephropathy induced by D-penicillamine. Ann Intern Med 1968; 69: 549-556.
141. Swinson DR, Hamilton EBD, Dische FE. Penicillamine nephropathy [letter]. Ann Rheum Dis 1975; 34: 544.
142. Neild GH, Gartner HV, Bohle A. Penicillamine induced membranous nephritis. Scand J Rheumatol 1979; 28: 79-90.
143. Sellars L, Siamopoulos K, Wilkinson R, Leohapand T, Morley AR. Renal biopsy appearances in rheumatoid disease. Clin Nephrol 1983; 20: 114-120.
144. Herve JP, Leguy P, Cledes J, Leroy JP. Nephrotic syndrome with minimal glomerular lesions during treatment with D-penicillamine. Nouv Press Med 1980; 9: 2847.
145. Kitazawa K, Ohseto Y, Kawazumi H, Saito K, Shibata T, Uchida J, Yonekura M, Schiwachi S, Ito S, Sugisaki T. D-penicillamine induced lipoid nephrosis in a patient with scleroderma. Ryumachi 1982; 22: 397-403.
146. Hayslett JP, Bensch KG, Kashgarian MZ, Rosenberg LE. Focal glomerulitis due to penicillamine. Lab Invest 1968; 19; 376-381.
147. Rehan A, Johnson K. IgM Nephropathy associated with penicillamine. Am J Nephrol 1986; 6: 71-74.
148. Liao K, Huang CC, Ho HH, Wu CH, Hsueh S. IgM nephropathy after D-penicillamine therapy: Report of a case. J Formosan Med Assoc 1987; 86: 906-909.
149. Hall CL. Jawad S, Harrison PR, MacKenzie JC, Bacon PA, Klouda PT, Maclver AG. Natural course of penicillamine nephropathy: A long-term study of 33 patients. Br Med J 1988; 296: 1083-1086.
150. Jaffe IA. D-penicillamine. Bull Rheum Dis 1977-8; 28: 948-952.
151. Jaffe IA. Penicillamine in rheumatoid disease with particular reference to the rheumatic factor. Postgrad Med J 1968 (suppl): 34-40.
152. Swainson CP, Thompson D, Short AIK, Winney RJ. Plasma exchange in the successful treatment of drug-induced renal disease. Nephron 1982; 30: 244-249.
153. Peces R, Rivera JR, Arboleya LR, Lopez-Larrea C, Alvarez J. Goodpasture's syndrome in a patient receiving penicillamine and carbimazole. Nephron 1985; 45: 316-320.
154. Matloff DS, Kaplan MM. D-penicillamine-induced Goodpasture's-like syndrome in primary biliary cirrhosis: successful treatment with plasmapheresis and immunosuppressives. Gastroenterology 1980; 78: 1046-1049.
155. Devogelaer J-P, Pirson Y, Vandenbroucke J-M, Cosyns J-P, Brichard S, Deuxchaisnes CN. D-penicillamine induced crescentic glomerulonephritis: Report and review of the literature. J Rheumatol 1987; 14: 1036-1041.
156. Falck HM, Tornooth T, Kock B, Wegelius O. Fatal renal vasculitis with minimal change glomerulonephritis complicating treatment with penicillamine. Acta Med Scand 1979; 205: 133-138.
157. Ntoso KA, Tomaszewski JE, Jimenez SA, Neilson DG. Penicillamine-induced rapidly progressive glomerulonephritis in patients with progressive systemic sclerosis: Successful treatment of two patients and review of the literature. Am J Kidney Dis 1986; 8: 159-163.
158. Camus J-P, Homberg JC, Crouzet J, Mery C, Delrieu F, Massias P, Abuaf N. Autoantibody formation in D-penicillamine treated rheumatoid arthritis. J Rheumatol 1981; 7 (suppl): 80-83.
159. Mohammed I, Barraclough D, Holborrow EJ, Ansell BM. Effect of D-penicillamine therapy on circulating immune complexes in rheumatoid arthritis. Ann Rheum Dis 1976; 35: 458-462.
160. Aarden LA. Pathogencity of immune complexes. In: Non-articular forms of rheumatoid arthritis. Feltkamp TEW, editor. Stafleu's Scientific Publishing Co, Leyden 1977; 15.
161. Kirby JD, Dieppe PA, Huskisson EC, Smith B. D-penicillamine and immune complex deposition. Ann Rheum Dis 1979; 38: 344-346.
162. Nagata N, Hurtenbach U, Gleichmann E. Specific sensitization of Lyt-1^{+}2^{-}stereoisomer. J Immunol 1986; 136: 136-142.
163. Emery P, Panayi GS, Huston G, Welsh KI, Mitchell SC, Shah RR, Idle JR, Smith RL, Waring RH. E-penicillamine induced toxicity in rheumatoid arthritis: the role of sulphoxidation status and HLA-DR3. J Rheumatol 1984; 11: 626-632.
164. Speerstra F, Reekers P, van de Putte LBA, Vandenbroucke JP, Rasker JJ, de Rooij DJRAM. HLA-DR antigens and proteinuria induced by aurothioglucose and D-penicillamine in patients with rheumatoid arthritis. J Rheumatol 1983; 10: 948-953.
165. Moutsopoulos HM, Skopouli FN, Sarras AK, Tsampoulas C, Mavridis AK, Constantopoulos SH, Maddison PJ. Anti-Ro(SSA) positive rheumatoid arthritis: a clinicoserological group of patients with high incidence of D-penicillamine side effects. Ann Rheum Dis 1985; 44: 215-219.
166. Moutsopoulos HM, Giotaki H, Maddison PJ, Mavridis AK, Drosos AA, Skopouli FN. Antibodies to cellular antigens in Greek patients with autoimmune diseases: anti-Ro(SSA) antibody a possible marker of D-penicillamine intolerance. Ann Rheum Dis 1984; 43: 285-287.
167. Dodd MJ, Griffiths ID, Thomson M. Adverse reaction to D-penicillamine after gold toxicity. Br Med J 1980; 26: 1498-1500.

168. Webley M, Coomes EN. Is penicillamine therapy in RA. influenced by previous gold ? Br Med J 1978; 2: 91.
169. Klouda PT, Manos J, Acheson EJ, Dyer PA, Goldby FS, Harris R, Lawler W, Mallick NP, Williams G. Strong association between idiopathic membranous nephropathy and HLA-DRW3. Lancet 1979; 2: 770-771.
170. Swiss Federal Commission for the rheumatic diseases, Subcommission for Research. HLA-DR antigens in rheumatoid arthritis –A Swiss collaborative study; final report. Rheumatol Int 1986; 6: 89-92.
171. Rundles RW, Wyngaarden JB, Hitchings GH, Elison B, Silberman HR. Effects of a xanthine oxidase inhibitor on thiopurine metabolism, hyperuricemia, and gout. Trans Assoc Am Physicians 1963; 76: 126-140.
172. Rundles RW, Metz EN, Silberman HR. Allopurinol in the treatment of gout. Ann Intern Med 1966; 64: 229-258.
173. Auerbach R, Orentrich N. Alopecia and ichthyosis secondary to allopurinol. Arch Dermatol 1968; 98: 104.
174. Boston Collaborative Drug Surveillance Program. Allopurinol and cytotoxic drugs. JAMA 1974; 227: 1036-1040.
175. Pinnas G. Possible association between macular lesions and allopurinol. Arch Ophthalmol 1968; 79: 786-787.
176. Korting HC, Lesch R. Acute cholangitis after allopurinol treatment. Lancet 1978; 1: 275-276.
117. Boyer TD, Sun N, Reynolds TB. Allopurinol-hypersensitivity. Vasculitis and liver damage. West J Med 1977; 126: 143-147.
178. Raper R, Barnes P, Ibels L, Lunzer M, Lauer C. Fulminant hepatic failure due to allopurinol. Aust NZ J Med 1984; 14: 63-65.
179. Bailey RR, Neale TJ, Lynn KL. Allopurinol-associated arteritis. Lancet 1976; 2: 907.
180. Greene ML, Fujimoto WY, Seegmiller JE. Urinary xanthine stones, a rare complication of allopurinol therapy. N Eng J Med 1969; 280: 426-427.
181. Jarzobski J, Ferry J, Womboldt D, Fitch DM, Egan JD: Vasculitis with allopurinol therapy. Am Heart J 1970; 79: 116-121.
182. Kantor GC. Toxic epidermal necrolysis, azotemia, and death after allopurinol therapy. JAMA 1970; 212: 478-479.
183. Mills RM. Severe hypersensitivity reactions associated with allopurinol. JAMA 1971; 216: 799-802.
184. Young JC, Boswell RB, Niles AS: Severe allopurinol hypersensitivity. Arch Intern Med 1974; 134: 553-558.
185. Gelbart DC, Weinstein AB, Fajardo CF. Allopurinol-induced interstitial nephritis. Ann Intern Med 1977; 86: 196-198.
186. Lupton GP, Odom RB. The allopurinol hypersensitivity syndrome. J Am Acad Dermatol 1979; 1(4): 365-374.
187. Grussendorf M, Andrassy K, Waldherr R, Ritz E. Systemic hypersensitivity to allopurinol with acute interstitial nephritis. Am J Nephrol 1981; 1: 195-109.
188. Singer JZ, Wallace SL. The allopurinol hypersensitivity syndrome. Unnecessary morbidity and mortality. Arthritis Rheum 1986; 29: 82-87.
189. McMenamie RA, Davies LM, Crasswell OW. Drug induced interstitial nephritis, hepatitis and exfoliative dermatitis. Aust NZ J Med 1976; 6: 583-587.
190. McKendrick MW, Geddes AM. Allopurinol hypersensitivity. Br J Med 1979; i: 988.
191. Boyer TD, Sun N, Reynolds TB. Allopurinol-hypersensitivity. Vasculitis and liver damage. West J Med 1977; 126: 143-147.
192. Emmerson BT, Hazelton RA, Frazer IH. Some adverse reactions to allopurinol may be mediated by lymphocyte reactivity to oxypurinol. Arthritis Rheum 1988; 31: 436-440.
193. Elion GB, Kovensky A, Hitchings GH. Metabolic studies of allopurinol, an inhibitory of xanthine oxidase. Biochem Pharmacol 1965; 15: 863-880.
194. Elion GB, Yu TF, Gutman AB, Hitchings GH. Renal clearance of oxypurinol, the chief metabolite of allopurinol. Am J Med 1968; 45: 69-77.
195. Hande KR, Noone RM, Stone WJ. Severe allopurinol toxicity. Description and guidelines for prevention in patients with renal insufficiency. Am J Med 1984; 76: 47-56.
196. Simmonds HA, Cameron JS, Morris GS, Davies PM. Allopurinol in renal failure and the tumor lysis syndrome. Clin Chim Acta 1986; 31: 189-195.
197. Puig JP, Casas EA, Ramos TH, Michan AA, Mateos FA. Plasma oxypurinol concentration in a patient with allopurinol hypersensitivity. J Rheumatol 1989; 16: 842-844.
198. Suzuki Y, Inagi R, Aono T, Yamanishi K, Shihara T. Human herpesvirus 6 infrction a risk factor for the development of severe drug-induced hypersensitivity syndrome. Arch Dermatol 1998; 134: 1108-1112.
199. Kelley WN. Gout and related disorders of purine metabolism. In: Textbook of Rheumatology (1st edition). Kelly WN, Harris Jr ED, Sledge CB, editors. WB Saunders, Philadelphia 1981; p. 1397-1437.

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Angiotensin I converting enzyme inhibitors and angiotensin II receptor antagonists

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Introduction

Over the last decade, the treatment of hypertension has changed dramatically from the concept of stepped care, advocated in the 1970's, to the more individualized care preferred nowadays. This phenomenon was largely due to the recent development of new classes of antihypertensives, which made it possible to adequately lower blood pressure in most patients with only one or two antihypertensive drugs, thus avoiding the need for a combination of multiple drugs. One of these new drug classes, the angiotensin I converting enzyme inhibitors (ACEI), drew a lot of attention since these were aimed at inhibiting the formation of angiotensin II, a hormone thought to be involved in the origin of systemic hypertension.

However, some major concerns appeared to restrict the widespread use of these drugs, including both renal histological changes such as a membranous glomerulopathy and an acute interstitial nephritis associated with ACEI, and functional changes such as an ACEI-induced fall in glomerular filtration rate (GFR) in some specified risk groups. Interestingly, although this fall in GFR was initially a reason for concern, after further studies that increased our understanding of the causes of this fall, some possible clinical uses of this phenomenon were recognized. Among these was the use of ACEI to improve the diagnostic armamentarium for renovascular hypertension, to treat urinary protein leakage in patients with the nephrotic syndrome, and most importantly, to preserve renal function in patients with progressively declining renal function.

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In this chapter we first will discuss the undesirable aspects of these effects of ACEI and will show how most of these effects may be prevented by cautious use of the agents. Since the mechanisms of the ACEI-induced membranous glomerulopathy and interstitial nephritis are different from those causing the fall in GFR, we will discuss each separately.

Captopril-associated membranous glomerulopathy

Proteinuria in association with membranous glomerulopathy has been described during the use of captopril [1-3]. Because of the similar pattern of these side effects to that of other agents containing a sulfhydryl group, like penicillamine, it was suspected that the sulfhydryl moiety of the captopril molecule was involved in the genesis of these effects, either by a direct toxic action or by an immunological mechanism [4]. It was feared that this would seriously limit the use of captopril and future sulfhydryl-containing angiotensin I converting enzyme inhibitors [5]. However, Lewis et al. reported that only 1.1% of 4878 patients treated with captopril exhibited increased proteinuria, with an incidence of nephrotic range proteinuria (more than 3 grams per 24 hours) of 0.8%. Analysis of these cases revealed that more than half of these patients had pre-existing renal disease, and many were taking doses in excess of 450 mg per day [6]. Furthermore, in studies in which doses of captopril of 37.5 to 150 mg per day were used, no increased incidence of proteinuria was detected as compared to placebo [7]. Lewis even questioned whether the occurrence of proteinuria during ACEI is related to the sulfhydryl moiety of captopril, since proteinuria has also been demonstrated during treatment with the non-sulfhydryl containing enalapril [8]. However, no data are available on biopsy-documented membranous glomerulopathy in relation to enalapril or other ACEI.

The causal role of captopril in the pathogenesis of membranous glomerulopathy has been questioned by the finding of glomerular abnormalities suggestive for membranous glomerulopathy in biopsies of hypertensive patients that had not received the ACEI. In both captopril- and non-captopril-treated patients, spherical dense bodies were found within the glomerular capillary wall with vascular and mesangial deposits of immunoglobulins and C3 that in the early reports were suspected to represent a captopril-induced membranous glomerulopathy [6, 9, 10]. Taken together, data lead us to conclude that proteinuria due to membranous glomerulopathy during captopril treatment seems to be restricted to patients with pre-existing renal disease who use high doses of the drug.

Angiotensin I converting enzyme inhibitor-induced acute interstitial nephritis

Acute interstitial nephritis during treatment with an ACEI has been observed in very few instances. Luderer et al. described a patient with skin rash, Coombs positive hemolytic anemia, eosinophilia, and acute renal failure with eosinophiluria seven weeks after the start of captopril (300 mg per day). An allergic interstitial nephritis was suspected, but unfortunately no renal biopsy was performed and the patient moreover also received furosemide and aspirin [11]. Renal function improved after discontinuation of captopril. Cahan described two patients, one with a biopsy-proven acute eosinophilic interstitial nephritis (together with a membranous glomerulopathy) and the other with chronic interstitial nephritis during treatment with captopril [12]. Since again, both of these patients were also receiving furosemide, the development of interstitial nephritis could not definitely be attributed to captopril. In both patients the nephrotic range proteinuria persisted despite discontinuation of captopril and treatment with prednisolone [12]. Four other cases of acute interstitial nephritis with eosinophils have been described, mostly after usual doses of captopril (50-125 mg) given for a few days or weeks [13-16]. In one patient renal interstitial granulomas were also found [15]. In these cases renal function improved promptly after discontinuation of the drug. Another case report described a hypertensive patient presenting with a generalized maculopapular rash after three weeks of captopril therapy [17]. Eosinophilia was present without eosinophiluria. The renal biopsy showed acute tubular necrosis, however without evidence of allergic interstitial nephritis. Renal function improved promptly after discontinuation of captopril. Although a rash and eosinophilia have also been described during enalapril treatment, no data are available on the occurrence of acute interstitial nephritis in patients on enalapril. Moreover, in one of the abovementioned case reports, captopril rechallenge, but not

enalapril, caused renal functional deterioration [14].

Finally, functional tubular changes have also been described. Renal glycosuria, either with [18] or without [19] a fall in GFR has been found during treatment with captopril. In both cases the abnormality disappeared after withdrawal of the drug.

Thus far, no reports have been published on membranous glomerulopathy or acute interstitial nephritis in relation to the use of angiotensin II receptor antagonists. Whether this is due to the relatively short experience with these agents, or the fact that these ACEI-induced side effects are specific for ACEI and thus not related to the interference in the renin angiotensin system in general, cannot be concluded as yet.

Angiotensin I converting enzyme inhibitor-induced fall in GFR

In order to understand the ACEI-induced fall in GFR, it is important to begin with a basic understanding of the physiological role of the renin-angiotensin system in the regulation of renal hemodynamics (Figure 1). When renal perfusion pressure drops, renin is released into the plasma and lymph by the juxtaglomerular cells of the kidney. This enzyme cleaves angiotensinogen to form angiotensin I, which is further cleaved by converting enzyme to form angiotensin II, the primary effector molecule in this system. Angiotensin II participates in GFR regulation in at least two ways. First, angiotensin II increases arterial pressure, directly and acutely by causing vasoconstriction, and indirectly and more chronically by increasing body fluid volumes through stimulation of renal sodium retention (both indirectly via aldosterone and through a direct effect on the tubules), as well as by stimulating thirst. Second, angiotensin II preferentially constricts the efferent arteriole, thus helping to preserve glomerular capillary hydrostatic pressure and, consequently, GFR. Although the renin-angiotensin system is now known to be much more complicated than originally thought, including the likelihood that it serves paracrine and autocrine functions as well as endocrine functions, the simplified description above still holds true. As shown in figure 1, angiotensin I converting enzyme or kininase II also interferes in the breakdown of bradykinins, which may contribute to the vasodilation of ACE-inhibitors.

Under conditions in which arterial pressure or body fluid volumes are sensed as subnormal, the renin-angiotensin system will be activated and plasma renin activity and angiotensin II levels will be elevated. These conditions include dietary sodium restriction or sodium depletion (such as during diuretic therapy), renal artery stenosis, and congestive heart failure. In each case, fluid and sodium will be retained until the pressure and volume are again sensed as normal. Note that

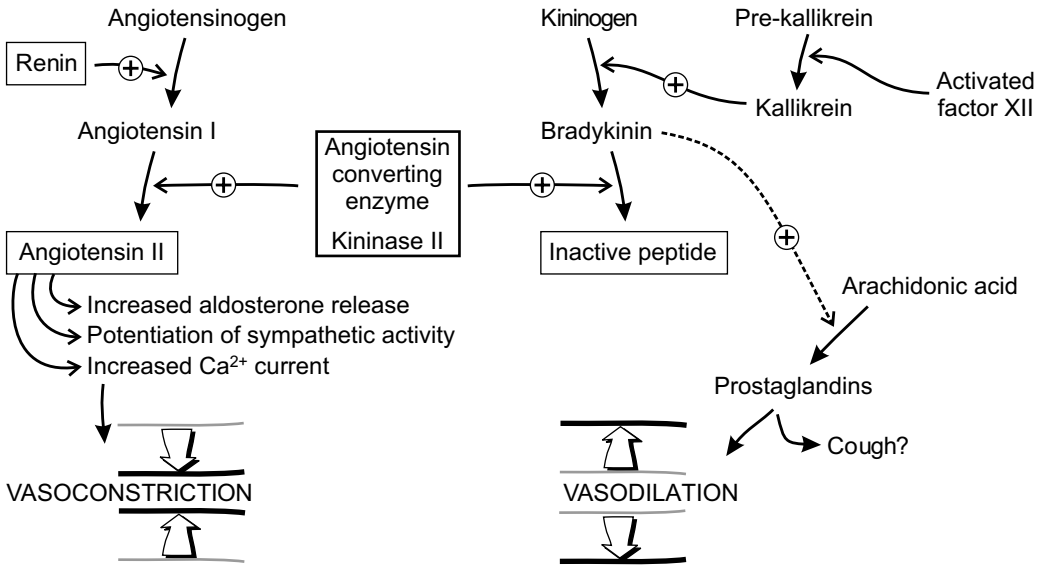

Figure 1. *Inhibition of the angiotensin converting enzyme, or kininase II.*

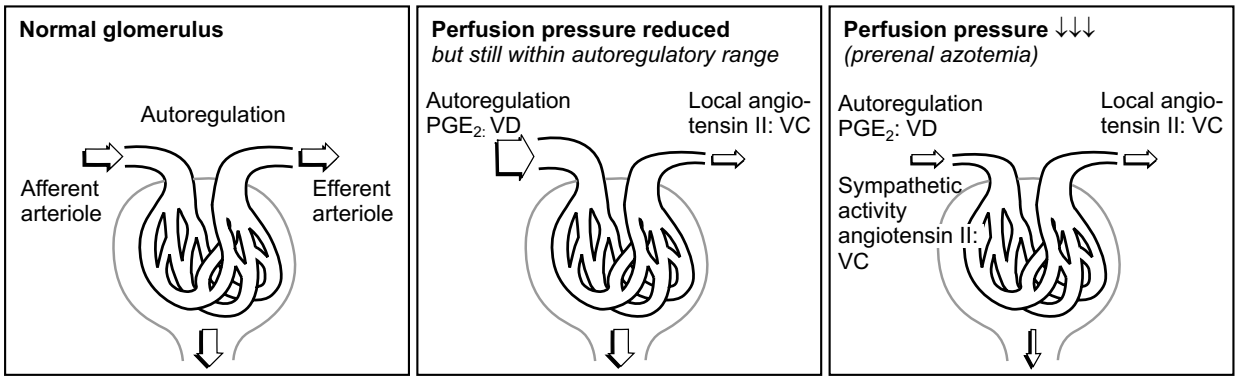

Figure 2. *Renal hemodynamics in normal and hypoperfusion conditions. PGE_2= prostaglandin E_2; VD= vasodilatation; VC= vasoconstriction.*

it is possible for the pressure and/or volume to actually be greater than normal but sensed as normal or subnormal, as in the case of congestive heart failure or renovascular hypertension. In conditions in which the renin-angiotensin system is activated, this system becomes especially important in maintenance of GFR, and the kidney becomes more sensitive to the effects of blockade with an ACEI or angiotensin II receptor antagonist.

The phenomenon of constant GFR and plasma flow during changes in renal arterial pressure is known as autoregulation, which under normal conditions is not renin angiotensin dependent (Figure 2). In case of renal arterial pressures below a certain value (about 80 mmHg), the renin-angiotensin system becomes involved. Its importance in this response has been demonstrated by Hall et al., who showed that intrarenal infusion of an angiotensin II antagonist impaired GFR autoregulation but not renal blood flow autoregulation, and that the impairment was more pronounced in sodium-depleted dogs [20]. During angiotensin II receptor antagonism, filtration fraction and efferent arteriolar resistance progressively fell at all renal arterial pressures below control. These investigators also showed that administration of captopril to sodium-depleted dogs impaired autoregulation of GFR but not of renal blood flow when renal perfusion pressure was reduced [21]. Both GFR and renal blood flow were returned to control values when angiotensin II was infused during captopril administration and aortic constriction. Calculated afferent and efferent resistances suggested that an angiotensin-stimulated increase in efferent resistance is important for efficient autoregulation of GFR when renal arterial pressure is clearly reduced. Thus, these investigators have provided strong evidence that an intact renin-angiotensin system is required for maintenance of GFR when renal perfusion pressure falls, and that angiotensin II participates in this GFR autoregulation by preferentially constricting the efferent arteriole.

Although generally accepted to be true, it is actually not known whether the renal hemodynamic effects of ACEI are necessarily due to blockade of the renin-angiotensin system. Acute renal failure has not been seen after administration of other antihypertensive agents that do not interfere with the renin-angiotensin system, suggesting that it is blockade of this system, which is responsible for the acute renal failure. However, angiotensin-converting enzyme is identical to kininase II, the enzyme responsible for degradation of kinins, so that administration of ACEI causes a buildup of vasodilator kinins (e.g. bradykinin) as well as depletion of angiotensin II. Thus, an excess of vasodilator kinins could theoretically contribute to the fall in GFR during ACEI. However, the finding of Hall et al. that an angiotensin receptor antagonist has similar effects to captopril or renin depletion on GFR autoregulation [20], and that the effects of captopril can be reversed by an infusion of angiotensin II [21], would suggest that changes in the kinin system play a minor role, if any, in the effects of ACEI on renal hemodynamics.

In patients in whom the renin-angiotensin system is activated, one would expect that efferent arteriolar resistance is maintained at least in part by circulating and/or intrarenal angiotensin II. If angiotensin II pref-

erentially constricts the efferent vessels, then administration of an ACEI should preferentially dilate these vessels, thus causing a fall in glomerular hydrostatic pressure and a fall in GFR. This would be expected to occur even if renal perfusion pressure was unchanged. Moreover, a captopril-induced fall in systemic arterial pressure (and therefore renal artery pressure), together with impairment of GFR autoregulatory capability, would further contribute to a reduction in GFR.

These predictions from a basic understanding of the physiology of the renin-angiotensin system are upheld by clinical findings. Specifically, in some pathophysiological conditions in which maintenance of GFR is highly dependent on an angiotensin II-mediated efferent vasoconstriction, ACEI may result in an acute and pronounced fall in GFR. This is true for patients with bilateral renal artery stenosis or renal artery stenosis in a solitary kidney, for patients with congestive heart failure, and for patients with severe renal failure, especially when they are volume depleted. We will discuss the effect of ACEI in these three patient groups separately.

Renal artery stenosis

Shortly after the introduction of ACEI in clinical practice, attention was given to the acute and severe fall in GFR that may be encountered with these drugs in patients with bilateral renal artery stenosis and artery stenosis of a solitary kidney, the latter for example in patients with a renal allograft. In the first report to document such a GFR decline it was suggested to be due to a direct nephrotoxicity of the ACEI [22]. It soon became clear, however, that the fall in filtration was the consequence of renal ischemia, possibly related to the fall in blood pressure and thus in perfusion pressure in the post-stenotic kidney.

In one of the earliest reports it was shown that not only captopril but also minoxidil caused GFR to decrease in a patient with a transplant renal artery stenosis [23], suggesting that it was the fall in blood pressure itself, which caused the reduced GFR. However, in other studies it was found that GFR decreased only during treatment with captopril and enalapril [24] whereas a fall in blood pressure during sodium nitroprusside [25] or minoxidil [26, 27], which do not directly interfere with the renin-angiotensin system, did not result in a decline in GFR. Furthermore, studies from Anderson et al. indicated that during infusion of an ACEI in the renal artery, in doses low enough not to cause any systemic blood pressure effect, an efferent vasodilation occurs resulting in a lowering of filtration pressure in the post-stenotic kidney [28]. The same authors showed that the recovery of GFR that is normally observed after the induction of a renal artery stenosis in dogs is prevented by enalapril treatment [29]. Thus it appears that it is not the fall in systemic blood pressure *per se* that causes GFR to decrease after captopril in a renal artery stenosis patient.

Whatever the precise mechanism may be, the fall in intraglomerular capillary pressure in the post-stenotic kidney may lead to a severe decrease in GFR, even up to total loss of filtration. This is reflected by an acute rise in serum creatinine in patients with bilateral stenosis or stenosis in a solitary kidney [24, 30]. With the contralateral kidney intact however, changes in overall GFR tend to be small and variable, due to compensation by the non-stenotic kidney. Wenting et al., in their elegant study [31] showed not only that captopril greatly reduced the extraction ratio of sodium iodohippurate and iothalamate in the poststenotic kidney in half of the patients with unilateral renal artery stenosis, but also that such a fall in GFR could easily be detected on renal scintigraphy with 99mTc-diethylenetriaminepenta-acetic acid (DTPA), whereas DTPA-uptake had not diminished in the kidney of patients with essential hypertension (Figure 3). Since in patients with a unilateral renal artery stenosis a fall in filtration in the poststenotic kidney may not be detected by a rise in serum creatinine, they concluded that radioisotope renography thus should be performed after beginning captopril treatment in patients with renal artery stenosis [31]. In case of a fall in tracer uptake after captopril the drug should be withdrawn and the physician should aim at a curative approach if possible.

As predicted by our understanding of the basic physiology, the fall in filtration after ACEI in a patient with renal artery stenosis is dependent upon the prevailing sodium status of the patient [27, 32-34]. The critical role of sodium balance in this fall in GFR during ACEI has been nicely documented in a case report by Hricik [34], who showed that GFR decreased more markedly in a patient with a transplant renal artery stenosis when captopril was given in a sodium depleted as compared to a sodium replete situation (Table

1). Moreover, Andreucci et al. reported that intravenous infusion of saline could reverse the fall in creatinine clearance or rise in serum creatinine during captopril administration [35].

Since the fall in GFR after ACEI is reversible immediately after withdrawal of the drug [27, 30-32, 36], some authors concluded that its use in patients with renal artery stenosis is relatively safe [37, 38]. However, both animal data and human experience suggests that after continued treatment with an ACEI, atrophy of the stenosed kidney (Figure 4) [39] or complete obstruction of the artery with ischemia of the poststenotic kidney may occur [40, 41]. Whether these effects are directly related to the ACEI or to the natural history of the disease has yet to be established. Another argument against using ACEI in patients with renovascular hypertension, is the fact that in a number of these patients therapy (either transluminal angioplasty, stenting or operative procedures) that will directly address the primary problem is frequently available. The fact that renal function does not worsen during treatment with an ACEI should be evaluated against an expected improvement in renal function after correction of the stenosis.

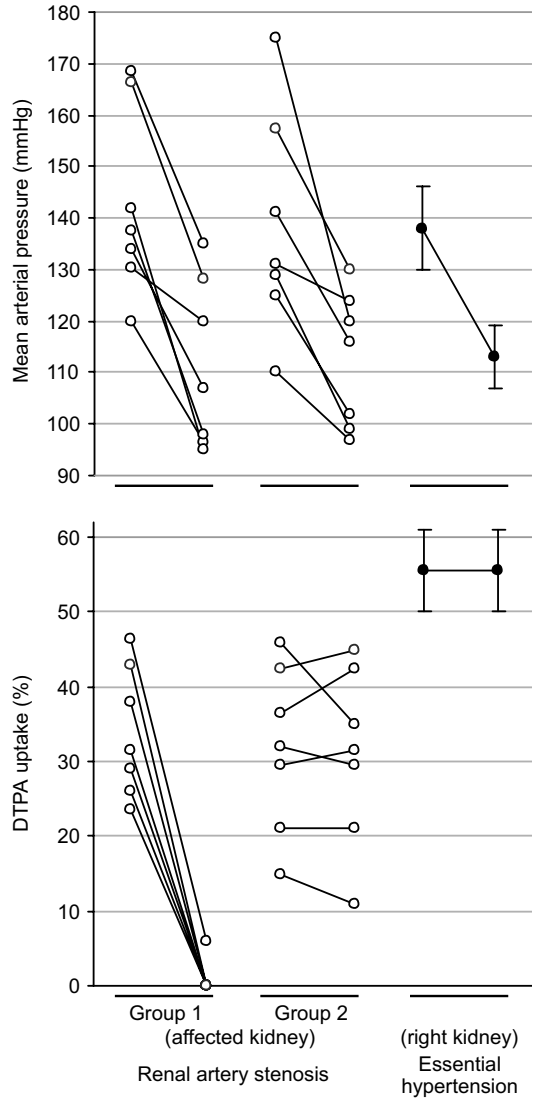

Figure 3. *Effect of long-term captopril 150 mg daily on blood pressure and single kidney uptake of 99mTc-DTPA in 14 patients with unilateral renal artery stenosis and 17 patients with essential hypertension. Note that DTPA uptake diminished impressively in half of the poststenotic kidneys in patients with renovascular hypertension, whereas it did not change in another half of the poststenotic kidneys and in the kidneys of essential hypertensive patients. Reproduced with permission from [31].*

Congestive heart failure

A fall in GFR may also be encountered if ACEI are given to patients with congestive heart failure. In a double blind study, Cleland found GFR to decrease from 53 to 48 ml/min (although not a statistically significant fall) during long-term treatment with captopril in 14 patients with congestive heart failure [42]. This fall in renal function, however, is not observed in all patients [43], and may also be different during the different stages of treatment [44]. Packer et al. showed that creatinine clearance worsened only in one third of the patients with severe chronic heart failure during treatment with captopril or enalapril, whereas creatinine clearance remained stable or improved in the other two thirds of the patients during ACEI [43]. The patients that demonstrated worsening of renal function

Table 1. The effect of sodium intake on the renal response to captopril in a patient with a transplant renal artery stenosis.

	Sodium deplete		Sodium replete	
Captopril:	–	+	–	+
Body weight (kg)	73.6	73.9	75.2	76.8
Blood pressure (mmHg)	150/96	121/71	130/75	131/83
Serum creatinine (mg/dl)	1.5	3.6	1.6	1.6
Inulin clearance (ml/min)	73	37	62	53

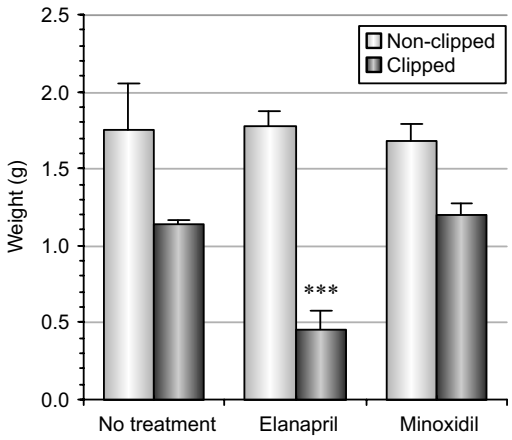

Figure 4. *Weight of the clipped and nonclipped kidneys of two-kidney one-clip rats with Goldblatt´s hypertension after 12 months of no treatment, enalapril, or minoxidil treatment. *** p<0.001 compared with no treatment group. Reproduced with permission from [39].*

had a lower central venous pressure and used more diuretics prior to the start of the ACEI. They exhibited a greater fall in mean arterial pressure and left ventricular filling pressure than the patients in whom renal function remained stable or improved (Table 2). These authors also showed that the drug-induced azotemia resolved after a reduction in the dose of the diuretics, despite unaltered treatment with captopril or enalapril [43]. When instituting a patient with congestive heart failure on an ACEI, renal function should be monitored closely, at least during the first one to two weeks, especially since a decline in renal function may be transient. Mujais et al. showed GFR to decrease the first days after start of the angiotensin I converting enzyme inhibitor, but to improve again during the next days [44]. They interpreted this difference in response during different stages of treatment to reflect the balance between the different mechanisms influencing kidney hemodynamics in heart failure and their alteration by ACEI.

Renal failure

Patients with pre-existing renal failure are also at risk of developing an acute fall in GFR after ACEI [45-48]. Such a fall in GFR again appears to occur especially in situations of a concomitant volume depletion, such as during strict diuretic treatment, diarrhea, or during lithium therapy [49]. Since most ACEI are eliminated via renal excretion it should be emphasized that the dose of the drug has to be adjusted for renal function. In patients with renal failure the ACEI should be started at very low doses and should be titrated only gradually. Since GFR may return to pretreatment levels after withdrawal of the ACEI, it can be concluded that the deleterious effect of ACEI on renal function is the consequence of a functional response, i.e. an efferent renal vasodilation which in the presence of an impaired renal perfusion pressure may result in a severe fall in intraglomerular capillary pressure.

This acute and sometimes severe fall in GFR during ACEI-treatment in patients with renal disease should be considered separately from the expected beneficial effects of ACEI to prevent the progressive renal function decline so commonly observed in patients with renal disease (see later). In this respect the study of Speirs et al. is of interest [50]. They reported on a postmarketing surveillance of enalapril. For that purpose they evaluated the reports of more than 15, 000 patients that had been instituted on enalapril (mostly in a clinical setting) and of whom a prescription event monitoring report had been received. 1098

Table 2. Renal function and hemodynamics during long term angiotensin I converting enzyme inhibition (ACEI) in severe chronic heart failure. The patients are divided in a group with stable renal function (n=70) and a group with worsening renal function (n=34) [from ref. 43].

	Stable renal function		Worsening renal function	
	ACEI –	ACEI +	ACEI –	ACEI +
Serum creatinine (mg/dl)	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	2.2 ± 0.2
Creatinine clearance (ml/min)	42.7 ± 3.7	50.9 ± 2.0	49.6 ± 5.1	31.2 ± 2.8
Mean arterial pressure (mmHg)	83.9 ± 1.7	69.9 ± 2.0	83.1 ± 2.6	62.1 ± 2.8
Left ventricular filling pressure (mmHg)	27.1 ± 0.6	18.7 ± 1.0	26.0 ± 0.9	11.4 ± 1.4

of these patients had died. Reports of these patients were evaluated for the cause of death and the possible role of enalapril in the death. It was found that enalapril appeared to have contributed to a decline in renal function and subsequent death in 10 of these patients. These patients shared some characteristics, i.e. old age, the use of high dose diuretics and/or potassium sparing diuretics, pre-existing renal disease and concomitant use of non-steroidal anti-inflammatory drugs [50].

Risk for combined treatment

In the previous paragraphs it has already been stressed that a decline in GFR during ACEI occurs predominantly in situations that the ACEI is combined with a diuretic regimen. As also mentioned before, the combination of an ACEI with a non-steroidal anti-inflammatory drug should be avoided, especially in patients with a pretreatment impaired GFR [50-52]. In such a situation the combination of afferent vasoconstriction (due to the prostaglandin synthesis inhibition) and efferent vasodilation (due to the ACEI) will result in a further fall in GFR, as has also been shown in patients with nephrotic range proteinuria [53, 54]. During these combined treatment regimens the patient is also at greater risk for the development of hyperkalemia.

Angiotensin I converting enzyme inhibitor-induced fetal nephrotoxicity

Although it is always difficult to prove that a certain drug or drug class induces fetal toxicity, the reports on ACEI-induced fetal renal damage are substantial. The most commonly reported fetal side effect of ACEI is second to third trimester onset of oligohydramnios and growth restriction, followed by delivery of an infant whose neonatal course is complicated by prolonged and often profound hypotension and anuria [55]. Most cases have been described in association with the use of enalapril, captopril and lisinopril (the earliest registered compounds), but there is no reason to expect that the other ACEI should not have the same risk [56]. These side effects have not been described with the use of angiotensin II receptor antagonists. As these drugs have not been used so long to date, it is to early to conclude whether the ACEI fetopathy is limited to the specific characteristics of ACEI or to the interference in the renin angiotensin system in general.

Histological studies of the kidney in ACEI-exposed fetuses show renal tubular dysgenesis [57-59]. Besides, delayed development of the calvaria and persistence of a patent ductus arteriosus has been described [60]. The true incidence of the fetal adverse effects of ACEI is not to determine, but in a series of 31 pregnancies exposed to an ACEI two ended with a miscarriage and three with stillbirth. Preterm delivery occurred in 12, and 6 out of the 26 liveborn babies were small for gestational age. Two infants with patent ductus arteriosus were reported [61]. There is no evidence that the use of ACEI causes harm in the first trimester of pregnancy [60].

The described renal tubular dysgenesis is characterized by dilatation of Bowman's space and of the tubules, diminished to absent differentiation of proximal convoluted tubules, and increased cortical and medullary mesenchyme and later fibrosis (Figure 5) [60]. These abnormalities are compatible with ischemic injury, and it has been argued that the mechanism by which ACEI affect development of the fetal kidney is through decreased renal blood flow [62, 63]. In fact, a similar histological pattern of renal tubular dysgenesis has also been reported in association with exposure to nonsteroidal anti-inflammatory agents [64-68], arguing that these abnormalities are not specific for ACEI exposure.

Based upon the available evidence it is advised not to use ACEI during pregnancy. In the case of an ACEI-exposed fetus, the pediatrician should be notified of the potential for neonatal hypotension and anuria.

Lessons to be learned from these side effects

Interestingly, in contrast to the situation with many other drugs, the documentation of these severe unwanted side effects did not lead to the withdrawal of these agents from the market. It of course is mandatory to constantly be aware of these potential risks. However, once the possible physiologic mechanisms of these side effects had been elucidated, it became increasingly clear that these effects could also be used to extend the diagnostic and therapeutic armamentarium of today's medicine.

Angiotensin I converting enzyme inhibition renography

Although it is a simple screening method for renal artery stenosis, until recently renography alone did not appear to be sufficiently sensitive for this purpose. This is thought to be at least partly due to the fact that the stenotic kidney is able to maintain adequate filtration and blood flow through systemic and local angiotensin II effects, thus obscuring the typical differences in tracer handling between the stenotic and non-stenotic side. The deleterious effects of an ACEI on flow and filtration in the poststenotic kidney in a patient with a renal artery stenosis are currently of use to improve the sensitivity of renography techniques in detecting the presence of a renal artery stenosis. It indeed has been shown, both in animal [69, 70] and human [71, 72] studies that the uptake and/or excretion of the tracer is more impaired in the post-stenotic kidney after ACEI as compared to the situation prior to the administration of the ACEI. This phenomenon appears to contribute to the alleged improvement of renography sensitivity during ACEI for the detection of renal artery stenosis. At present however, ACEI-renography is not so much advocated anymore, due to the high costs of the test in view of the suboptimal sensitivity.

Antiproteinuric effects and renal function preservation

The renal hemodynamic effects of ACEI in patients with renal parenchymal disease and renal function impairment deserves particular attention. Also in these patients renal plasma flow generally will increase dur-

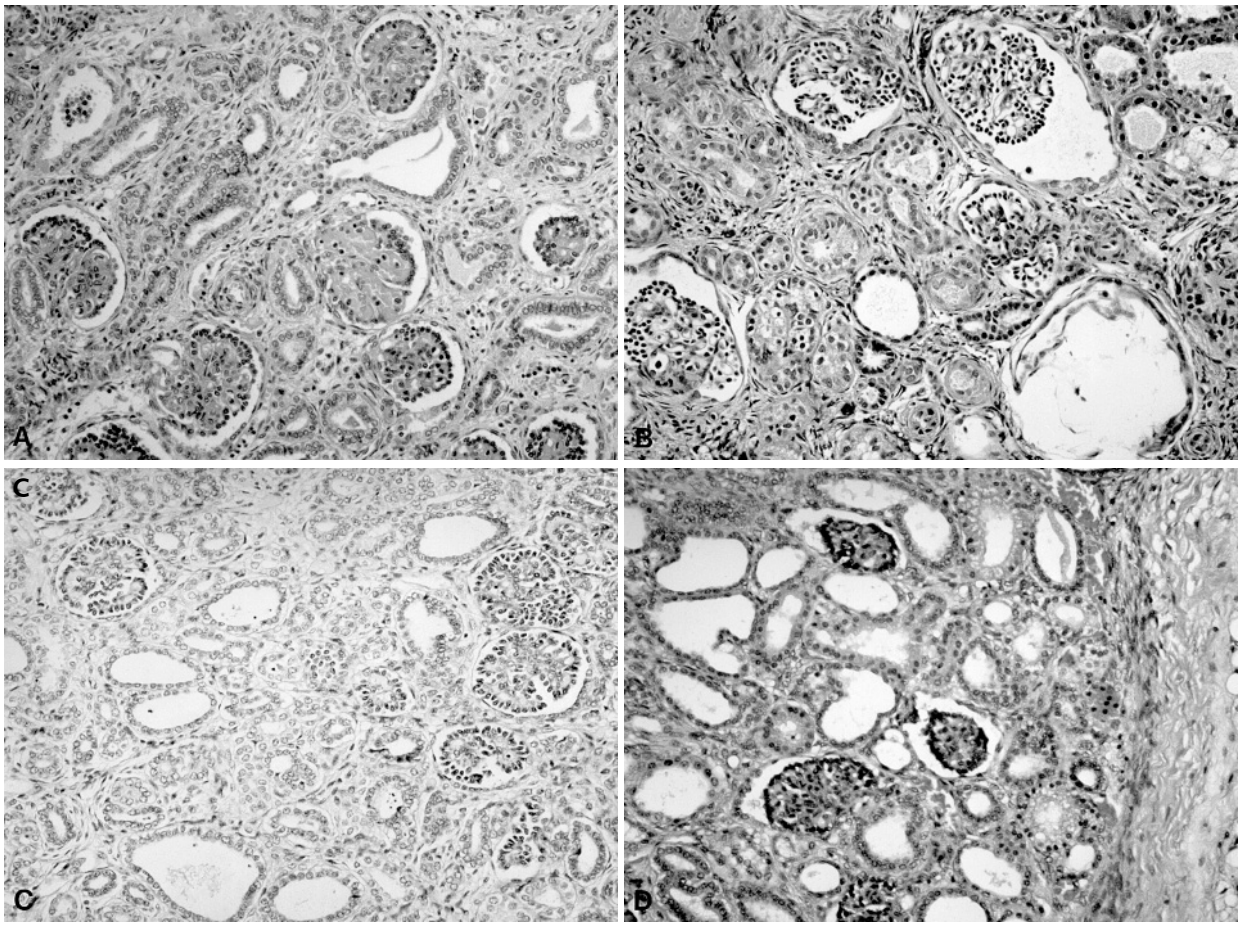

Figure 5. *Renal tubular dysgenesis in four different cases of ACI-induced fetopathy; note particularly the ductular ectasia, dilatation of Bowman's space, and poor to no differentiation of proximal convoluted tubules. Reprinted by permission of M. Barr [60] and Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.*

ing ACEI [73, 74]. The response of GFR again is dependent upon the prevailing sodium balance and the dose of the ACEI (Figure 6) [75, 76]. In contrast to the more acute and severe fall in GFR as discussed before, the fall in filtration in this patient group generally is rather small, and mostly not diagnosed as such if only changes in serum creatinine are used as diagnostic criterium. However, using more accurate measurements of renal function, such as the clearance of inulin or radioisotope labeled tracers, a fall in filtration rate generally can be demonstrated. This fall in filtration reflects a fall in intraglomerular capillary pressure. Since in animal experiments a rise in intraglomerular capillary pressure was found to be associated with a rise in urinary protein loss [77] and a progressive glomerulosclerosis and renal failure [78], it followed that ACEI were used in an attempt to lower proteinuria and to prevent progressive renal function decline. ACEI indeed have been found to lower urinary protein excretion in patients with renal disease of various origins.

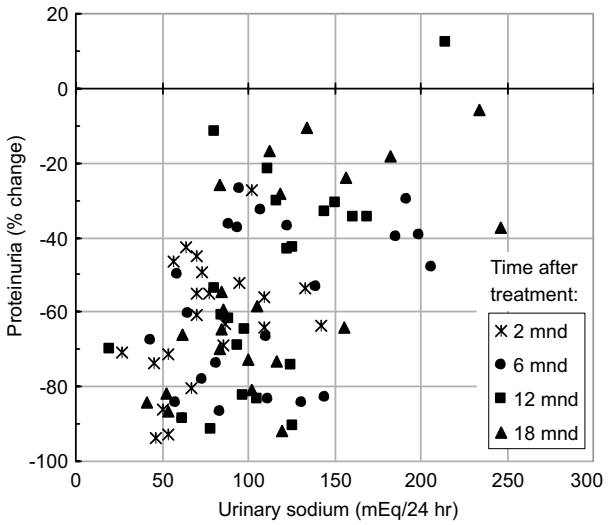

Figure 6. *Correlation between the antiproteinuric response (% change compared to baseline) and the urinary sodium excretion in 22 patients with proteinuria due to non-diabetic renal disease during treatment with the ACE inhibitor lisinopril (r-0.54; p<.001). Each data point represents the antiproteinuric and sodium excretion value of one individual at the time point of 2 months (*), 6 months (l), 12 months (n) or 18 months (p) after start treatment. Reproduced with permission from [76].*

Both in patients with asymptomatic proteinuria and in patients with frank nephrotic syndrome a fall in proteinuria with a rise in serum albumin has been described [73-75, 79, 80]. It has been argued that this improvement in the urinary protein leakage is the consequence of the renal hemodynamic effect of the ACEI, since blood pressure lowering with other antihypertensives does not result in a fall in proteinuria [74, 75]. In addition, the effects of ACEI or angiotensin II receptor antagonism to block the growth-promoting capacity of angiotensin-II adds to the beneficial effects of RAS blockade. In the last years the beneficial effects of both ACEI and angiotensin II receptor antagonists to prevent the progressive renal function decline in both type I [81] and type II [82, 83, 84, 85] diabetic nephropathy as well as in nondiabetic nephropathies [86-89] has been well documented.

Summary

Soon after the introduction of ACEI much attention was given to their renal side effects. This initiated a lot of research, especially because hypertension frequently is present in patients with renal vascular and/or parenchymal disease and the use of ACEI therefore was prompted in such patient groups. In the early eighties nephrotic syndrome due to a membranous glomerulopathy was described in association with the use of captopril. Further detailed studies showed this side effect to be related to the very high doses that at that time were used in patients with renal disease. More common is the acute renal functional deterioration that may occur during ACEI in certain groups, such as in patients with renovascular hypertension, in patients with severe heart failure and in patients with severe renal failure, especially in case of volume depletion. However, this fall in filtration is reversible after withdrawal of the drug or after volume repletion. This finding of a renal hemodynamically mediated fall in intraglomerular capillary pressure prompted studies that provided evidence of an antiproteinuric and renoprotective effect of this class of drugs. Close monitoring of side effects of drugs such as ACEI along with a basic understanding of the role of the renin angiotensin system in patients with renal diseases opens new perspectives for the treatment of such patients.

References

1. Hoorntje SJ, Kallenberg CGM, Weening JJ, Donker AJM, The TH, Hoedemaeker PJ. Immune complex glomerulopathy in patients treated with captopril. Lancet 1980; 1: 1212-1214.
2. Prins EJL, Hoorntje SJ, Weening JJ, Donker AJM. Nephrotic syndrome in a patient on captopril. Lancet 1979; 2: 306.
3. Sturgill BC, Shearlock KT. Membranous glomerulopathy and nephrotic syndrome after captopril therapy. JAMA 1983; 250: 2343-2345.
4. Kallenberg CGM, van der Laan S, de Zeeuw D. Captopril and the immune system. Lancet 1981; 2: 92.
5. Captopril: benefits and risks in severe hypertension. Editorial. Lancet 1980; 2: 129-130.
6. Lewis EJ and the Captopril Collaborative Study Group. Proteinuria and abnormalities of the renal glomerulus in patients with hypertension. Clin Exp Pharmacol Physiol 1982; 7: 105-115.
7. Veterans Administration Cooperative Study Group on Antihypertensive Agents. Low-dose captopril for the treatment of mild to moderate hypertension. Arch Intern Med 1984; 144: 1947-1953.
8. Lewis EJ. Captopril and membranous glomerulopathy. JAMA 1984; 252; 7: 900.
9. Captopril Collaborative Study Group. Does Captopril cause renal damage in hypertensive patients ? Report from the Captopril Collaborative Study Group. Lancet 1982; 1: 988-990.
10. Smit AJ, Hoorntje SJ, Weening JJ, Donker AM, Hoedemaeker PJ. Unilateral membranous glomerulopathy during captopril treatment. Neth J Med 1985; 28: 23-27.
11. Luderer JR, Schoolwerth AC, Sinicopre RA, Ballard JO, Lookingbill DP, Hayes AH. Acute renal failure, hemolytic anemia and skin rash associated with captopril therapy. Am J Med 1981; 71: 493-496.
12. Cahan DH, Ucci AA. Acute renal failure, interstitial nephritis, and nephrotic syndrome associated with captopril. Kidney Int 1984; 25: 160.
13. Chauveau P, Rouveix B, Kleinknecht D. Insuffiscance rénale aiguë d'origine immuno-allergique apres captopril. Néphrologie 1985; 6: 193-194.
14. Hooke D, Walker RG, Walter NMA, D'Apice AJF, Whitworth JA, Kincaid-Smith P. Repeated renal failure with use of captopril in a cystinotic renal allograft recipient. Brit Med J 1982; 285: 1538.
15. Islam S, Dubigeon MP, Guenel J. Néphropathie interstitielle granulomateuse aiguë et réversible apres traitement par le captopril. Rev Méd Interne (Paris) 1990; 11: 231.
16. Smith WR, Neill J, Cusham WC, Butkus DE. Captopril associated acute interstitial nephritis. Am J Nephrol 1989; 9: 230-235.
17. Steinman TI, Silva P. Acute renal failure, skin rash, and eosinophilia associated with captopril therapy. Am J Med 1983; 75: 154-151.
18. Cressman MD, Vidt DG, Acker C. Renal glucosuria and azotaemia after enalapril maleate. Lancet 1982; 2: 440.
19. Jójárt GY, Sonkodi S. Does captopril induce glucosuria ? Brit Med J 1984; 288: 368.
20. Hall JE, Guyton AC, Jackson TE, Coleman TG, Lohmeier TE, Trippodo NC. Control of glomerular filtration rate by renin-angiotensin system. Am J Physiol 1977; 233: F366-F372.
21. Hall JE, Coleman TG, Guyton AC, Kastner PR, Granger JP. Control of glomerular filtration rate by circulating angiotensin II. Am J Physiol 1981; 241: R190-R197.
22. Woodhouse K, Farrow PR, Wilkinson R. Reversible renal failure during treatment with captopril. Brit Med J 1979; 2: 1146-1147.
23. Collste P, Haglund K, Lundgren G, Magnusson G, Östman J. Reversible renal failure during treatment with captopril. Brit Med J 1979; 2: 612-613.
24. Hricik DE, Browning PJ, Kopelman R, Goorno WE, Madias NE, Dzau VJ. Captopril-induced functional renal insufficiency in patients with bilateral renal artery stenoses or renal artery stenosis in a solitary kidney. N Engl J Med 1983; 308: 373-376.
25. Textor SC, Tarazi FM, Novick AC, Bravo EL, Fouad FM. Regulation of renal hemodynamics and glomerular filtration in patients with renovascular hypertension during converting enzyme inhibition with captopril. Am J Med 1984; 76 (suppl): 29-37.
26. Mason JC, Hilton PJ. Reversible renal failure due to captopril in a patient with transplant artery stenosis: case report. Hypertension 1983; 5: 623-627.
27. van Son WJ, Donker AJM, Van der Woude FJ, Meijer S, Slooff MJH, Tegzess AM, Van der Slikke LB. Deterioration of renal function after converting enzyme inhibition in a patient with renal artery stenosis after transplantation. Neth J Med 1984; 27: 10-13.
28. Anderson WP, Korner PI, Johnston CI, Angus JA, Casley DJ. Intrarenal actions of angiotensin II in restoring renal artery pressure after acute renal artery stenosis. Clin Exp Pharmacol Physiol 1978; 5: 529-533.
29. Anderson WP, Denton KM, Woods RL, Alcorn D. Angiotensin II and the maintenance of GFR and renal blood flow during renal artery narrowing. Kidney Int 1990; 38 (suppl 30): 109-113.
30. Curtis JJ, Luke RG, Whelchel JD, Diethelm AG, Jones P, Dustan HP. Inhibition of angiotensin converting enzyme in renal transplant recipients with hypertension. N Engl J Med 1983; 308: 377-381.

31. Wenting GJ, Tan-Tjiong HL, Derckx FHM, de Bruyn JHB, Man in 't Veld AJ, Schalekamp MADH. Split renal function after captopril in unilateral renal artery stenosis. Brit Med J 1984; 288: 886-890.
32. Hollenberg NK. Renal hemodynamics in essential and renovascular hypertension. Influence of captopril. Am J Med 1984; 76 (suppl 5B): 22-28.
33. Watson ML, Bell GM, Muir AL, Buist TAS, Kellett RJ, Padfield PL. Captopril/diuretic combinations in severe renovascular disease: a cautionary note. Lancet 1983; 2: 404-405.
34. Hricik DE. Captopril-induced renal insufficiency and the role of sodium balance. Ann Intern Med 1985; 103: 222-223.
35. Andreucci VE, Conte G, Dal Canton A, Di Minno G, Usberti M. The causal role of salt depletion in acute renal failure due to captopril in hypertensive patients with a single functioning kidney and renal artery stenosis. Renal Failure 1987; 10: 9-20.
36. Jackson B, McGrath BP, Matthews PG, Wong C, Johnston CI. Differential renal function during angiotensin converting enzyme inhibition in renovascular hypertension. Hypertension 1986; 8: 650-654.
37. Reams GP, Bauer JH, Gaddy P. Use of the converting enzyme inhibitor enalapril in renovascular hypertension. Hypertension 1986; 8: 290-297.
38. Franklin SS, Smith RD. A comparison of enalapril plus hydrochlorothiazide with standard triple therapy in renovascular hypertension. Nephron 1986; 44 (suppl 1): 73-82.
39. Jackson B, Franze L, Sumithran E, Johnston CI. Pharmacologic nephrectomy with chronic angiotensin converting enzyme inhibitor treatment in renovascular hypertension in the rat. J Lab Clin Med 1990; 115: 21-27.
40. Hoefnagels WHL, Thien T. Renal artery occlusion in patients with renovascular hypertension treated with captopril. Brit Med J 1986; 292: 24-25.
41. Hodsman GP, Brown JJ, Cumming AMM, Davies DL, East BW, Lever AF, Morton JJ, Murray GD, Robertson JIS. Enalapril in treatment of hypertension with renal artery stenosis. Am J Med 1984; 77 (2A): 52-59.
42. Cleland JGF, Dargie HJ, Gillen G, Robertson I, East BW, Ball SG, Morton JJ, Robertson JIS. Captopril in heart failure: a double blind study of the effects on renal function. J Cardiovasc Pharmacol 1986; 8: 700-706.
43. Packer M, Lee WH, Medina N, Yushak M, Kessler PD. Functional renal insufficiency during long-term therapy with captopril and enalapril in severe chronic heart failure. Ann Intern Med 1987; 106: 346-354.
44. Mujais SK, Fouad FM, Textor SC, Tarazi RC, Bravo EL, Hart N, Gifford RW. Transient renal dysfunction during initial inhibition of converting enzyme in congestive heart failure. Brit Heart J 1984; 52: 63-71.
45. Verbeelen DL, de Boel S. Reversible acute on chronic renal failure during captopril treatment. Brit Med J 1984; 289: 20-21.
46. Murphy BF, Whitworth JA, Kincaid-Smith P. Renal insufficiency with combinations of angiotensin converting enzyme inhibitors and diuretics. Brit Med J 1984; 288: 844-845.
47. Brivet F, Roulot D, Poitrine A, Dormont J. Reversible acute renal failure during enalapril treatment in patient with chronic glomerulonephritis without renal artery stenosis. Lancet 1985; 1: 1512.
48. Chapman AB, Gabow PA, Schrier RW. Reversible renal failure associated with angiotensin converting enzyme inhibitors in polycystic kidney disease. Ann Intern Med 1991; 115: 769-773.
49. Navis GJ, de Jong PE, de Zeeuw D. Volume homeostasis, angiotensin converting enzyme inhibition, and lithium therapy. Am J Med 1989; 86: 621.
50. Speirs CJ, Dollery CT, Inman WHW, Rawson NSB, Wilton LV. Postmarketing surveillance of enalapril. II: Investigation of the potential role of enalapril in deaths with renal failure. Brit Med J 1988; 297: 830-832.
51. Packer M. Why do the kidneys release renin in patients with congestive heart failure ? A nephrotic view of converting enzyme inhibition. Am J Cardiol 1987; 60: 179-184.
52. Seelig CB, Maloley PA, Campbell JR. Nephrotoxicity associated with concomitant ACE inhibitor and NSAID therapy. Sth Med J 1990; 83: 1144-1148.
53. Heeg JE, de Jong PE, Vriesendorp R, de Zeeuw D. Additive antiproteinuric effect of the NSAID indomethacin and the ACE inhibitor lisinopril. Am J Nephrol 1990; 10 suppl 1: 94-97.
54. Heeg JE, de Jong PE, de Zeeuw D. Additive antiproteinuric effect of angiotensin converting enzyme inhibition and non-steroidal anti-inflammatory drug therapy: a clue to the mechanism of action. Clin Science 1991;81:367-372.
55. Rosa FW, Bosco LA, Fossum-Graham C, Milstien JB, Dreis M, Creamer J. Neonatal anuria with maternal angiotensin-converting enzyme inhibition. Obstet Gynecol 1989; 74: 371-374.
56. Brent RL, Beckman DA. Angiotensin-converting enzyme inhibitors, an embryopathic class of drugs with unique properties: information for clinical teratology counsellors. Teratology 1991: 43: 543-546.
57. Knott PD, Thorp SS, Lamont CAR. Congenital renal dysgenesis possibly due to captopril. Lancet 1989; 1: 451.
58. Cunnif C, Jones KL, Philipson K, Short S, Wujek J. Oligohydramnios and renal tubular malformation associated with maternal enalapril use. Am J Obstet Gynecol 1990; 162: 187-189.
59. Pryde PG, Sedman AB, Nugent CE, Barr M. Angiotensin-converting enzyme inhibitor fetopathy. J Am Soc Nephrol 1993; 3: 1575-1582.
60. Barr M Jr. Teratogen Update: Angiotensine-Converting Enzyme Inhibitors. Teratology 1994; 50: 399-409.

61. Kreft-Jais C, Plouin C, Tchobroutsky C, Boutroy MJ. Angiotensin-converting enzyme inhibitors during pregnancy: A survey of 22 patients given captopril and nine give enalapril. Br J Obstet Gynaecol 1988; 95: 420-422.
62. Martin RA, Jones KL, Mendoza A, Barr M, Benirschke K. Effect of ACE inhibition on the fetal kidney: decreased renal blood flow. Teratology 1992; 46: 317-321.
63. Landing BH, Ang SM, Herta N, Larson EF, Turner M. Labeled lectin studies of renal tubular dysgenesis and renal tubular atrophy of postnatal renal ischemia and end-stage kidney disease. Pediatr Pathol 1994; 14: 87-99.
64. Simeoni U, Messer J, Weisburd P, Haddad J, Willard D. Neonatal renal dysfunction and intrauterine exposure to prostaglandin synthesis inhibitors. Eur J Pediatr 1989; 149: 371-373.
65. Restaino I, Kaplan BS, Kaplan P, Rosenberg HK, Witzleben C, Roberts N. Renal dysgenesis in a monozygotic twin: Association with in utero exposure to indomethacin. Am J Med Genet 1991; 39: 252-257.
66. Bavoux F. Toxicite foetal des anti-inflammatoires non steroidiens. Presse Med 1992; 21: 1909-1912.
67. Buderes S, Thomas B, Fahnenstich H, Kowalewski S. Renal failure in two preterm infants: toxic effect of prenatal maternal indomethacin treatment. Br J Obstet Gynaecol 1993; 100: 97-98.
68. Voyer LE, Drut R, Mendez JH. Fetal renal maldevelopment with oligohydramnios following maternal use of piroxicam. Pediatr Nephrol 1994; 8: 592-594.
69. Nally JV Jr, Clarke HS Jr, Grews GP, Saunders M, Gross ML, Potvin WJ, Windham JP. Effect of captopril on 99mTc-diethylenetriaminepentaacetic acid renograms in two-kidney, one clip hypertension. Hypertension 1986; 8: 685-693.
70. Jonker GJ, de Zeeuw D, Huisman RM, Piers DA, Beekhuis H, van der Hem GK. Angiotensin converting enzyme inhibition improves diagnostic procedures for renovascular hypertension in dogs. Hypertension 1988; 12: 411-419.
71. Geyskes GG, Oei HY, Puylaert CBAJ, Dorhout Mees EJ. Renovascular hypertension identified by captopril-induced changes in the renogram. Hypertension 1987; 9: 451-458.
72. Kremer Hovinga TK, de Jong PE, Piers DA, Beekhuis H, van der Hem GK, de Zeeuw D. Diagnostic use of ACE inhibitors in radioisotope evaluation of unilateral renal artery stenosis. J Nucl Med 1989; 30: 605-614.
73. Reams GP, Bauer JH. Effect of enalapril in subjects with hypertension associated with moderate to severe renal dysfunction. Arch Intern Med 1986; 146: 2145-2148.
74. Heeg JE, de Jong PE, van der Hem GK, de Zeeuw D. Reduction of proteinuria by angiotensin converting enzyme inhibition. Kidney Int 1987; 32: 78-83.
75. Heeg JE, de Jong PE, van der Hem GK, de Zeeuw D. Efficacy and variability of the antiproteinuric effect of ACE inhibition by lisinopril. Kidney Int 1989; 36: 272-279.
76. Gansevoort RT, de Zeeuw D, de Jong PE. Long-term benefits of the antiproteinuric effect of angiotensin-converting enzyme inhibition in nondiabetic renal disease. Am J Kidney Dis 1993; 22: 202-206.
77. Pelayo JC, Quan AH, Shanley PF. Angiotensin II control of the renal microcirculation in rats with reduced renal mass. Am J Physiol 1990; 258: F414-F422.
78. Anderson S, Meyer TW, Rennke HG, Brenner BM. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. Clin Invest 1985; 76: 612-619.
79. Taguma Y, Kitamoto Y, Futaki G, Ueda H, Monma H, Ishazaki M, Takahashi H, Sekino H, Sasaki Y. Effect of captopril on heavy proteinuria in azotemic diabetes. N Engl J Med 1985; 313: 1617-1620.
80. Bjorck S, Mulec H, Johnsen SA, Nyberg G, Aurell M. Contrasting effects of enalapril and metoprolol on proteinuria in diabetic nephropathy. Brit Med J 1990; 300: 904-907.
81. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. N Engl J Med 1993; 329: 1456-1462.
82. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S, for the RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001; 345: 861-869.
83. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I, for the Collaborative Study Group. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 2001; 345: 851-859.
84. Parving HH, Lehnert H, Bröchner-Mortensen, Gomis R, Andersen S, Arner P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. N Eng J Med 2001; 345: 870-878.
85. Brenner BM, Cooper MA, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn S, Zhang Z, Shahinfar S. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001; 345: 861-869.
86. Maschio G, Alberti D, Janin G, Locatelli F, Mann JF, Motolese M, Ponticelli C, Ritz E, Zucchelli P. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. N Engl J Med 1996; 334: 939-945.

87. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet 1997; 349: 1857-1863.
88. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, Salvadori M., Scolari F, Schena FP, Remuzzi G. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet 1999; 354: 359-364.
89. Jafar T, Schmid C, Landa M, Giatras I, Toto R, Remuzzi G, Maschio G, Brenner BM, Kamper A, Zucchelli P, Becker G, Himmelmann A, Bannister K, Landais P, Shahinfar S, de Jong PE, de Zeeuw D, Lau J, Levey A, for the ACE-inhibition in progressive renal disease Study Group. Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease: a meta-analysis of patient-level data. Ann Intern Med 2001; 135: 73-87.

17

Diuretics

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Introduction

Diuretics are among the most frequently prescribed drugs for the treatment of both edematous and non-edematous states. With respect to the latter category, they are most often utilized in the therapy of hypertension. They may injure the kidney either reversibly or irremediably, a distinction which often depends upon whether they have induced functional or anatomic damage. Ordinarily, the former type of disorder reverses more rapidly than the latter. However, anatomical lesions, for example those that may be associated with acute renal failure, may also respond to removal of the offending agent.

Functional abnormalities

Effects on renal hemodynamics

Diuretics may cause reductions in glomerular filtration rate either by a direct effect to constrict the renal arterial supply or secondary to their induction of extracellular fluid volume contraction. Listed in Table 1 are the renal hemodynamic alterations induced in the experimental animal or in man by the most commonly employed currently available diuretic agents. Acetazolamide, a proximally active agent and the prototypical carbonic anhydrase inhibitor (Figure 1), consistently reduces renal blood flow by 25 to 37% and

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 339-351

glomerular filtration rate by 10 to 46% [1]. This phenomenon is thought to result from an effect of the drug on the "tubulo-glomerular feedback" mechanism. The latter concept involves the control of glomerular function by some component of flow from the proximal to the distal nephron [2, 3]. The control system for this phenomenon appears to be located in the specialized cells of the distal convoluted tubule represented by the macula densa, and the apparent rationale for this feedback regulation is the maintenance of a relatively constant tubular flow rate. Thus, if diuretics inhibit sodium and fluid transport upstream of the distal convolution leading to increased delivery to the distal nephron, the glomeruli will respond with reduced function, perhaps mediated by alterations in local or regional angiotensin II levels [4]. While the loop of Henle agents such as furosemide would be expected to have similar effects, these drugs appear to inhibit the tubulo-glomerular feedback system, thus preventing a decrease in glomerular filtration rate [5-10]. However, in those studies with loop diuretics in which extracellular fluid volume was substantially contracted, glomerular filtration rate (and single nephron filtration rate, SNGFR), fell [8, 9, 11, 12].

In most studies in which it has been investigated, ethacrynic acid administration results in a marked reduction in renal vascular resistance [7, 13-15]. However, this effect is reversed as in the case of furosemide, by the development of volume depletion [14-16]. Studies of bumetanide in the experimental animal have generally shown no change in glomerular filtration rate [17, 18] or renal blood/plasma flow [17, 18] except for generally transient acute increases in the latter which approximated 27-40%, declining later in the experiments to only modest elevations or to control levels [19, 21]. In man, bumetanide has been found to cause

Table 1. Renal hemodynamic effects of diuretic agents categorized according to their primary nephron sites of action[a,b].

Drug model(s)	Experimental	Effect on RBF or RPF	Effect on GFR	Mechanism(s) of observed alterations in hemodynamics
Proximal tubule				
Acetazolamide	Rat, dog, man	RBF↓d~25-37%	GFR↓d 10-46%	Activation of TGF mechanism.
Benzolamide	Rat	RBF, RPF not determined but nephron plasma ↓d by ~35%	GFR↓d~ 18-21%	Activation of TGF mechanism because of ↓d nephron plasma flow, probably mediated by angiotensin II.
Loop of Henle				
Furosemide	Rat, dog, rabbit, man	↑d (by 25 to 30%)[c], or no change	No change[c]	Inhibits the TGF system preventing a ↓ in SN(GFR) which would otherwise be expected because of an ↑ in distal delivery related to inhibition of transport in the loop.
Ethacrynic acid	Dog, man	↓d (by 28-47%)[c], or no change	No change[c]	Does not alter TGF; renal vasodilatory effects may be reversed by volume depletion.
Bumetanide	Dog, man	↓d (by ≤40%), or no change	No change or ↑d[c]	Effects of TGF similar to those of furosemide.
Early distal convoluted tubule				
Chlorothiazide	Dog, rat, man	No change, or ↓d	No change or ↓d	Decline in SNGFR, when it occurs, may be related to ↑d proximal intratubular pressure, volume contraction or afferent arteriolar vasodilation induced by the drug.
Metolazone	Dog, man	No change	No change	
Indapamide	Dog, man	No change	No change	
Late distal convoluted tubule and collecting duct				
Spironolactone	Man	No change	No change	
Triamterene	Man	No change[d]	No change[d]	
Amiloride	Dog, rat, man	No change	No change	

[a]*Adapted from Puschett JB, Winaver J [1], with permission of the editors.*
[b]*Abbreviations: RBF, RPF= renal blood, plasma flow; GFR= glomerular filtration rate, SNGFR= single nephron filtration rate; TGF= tubuloglomerular feedback.*
[c]*Effects of the drug on renal blood flow and glomerular filtration rate are related to alterations in extracellular fluid volume induced by the agent.*
[d]*Large doses (300 mg/day) have been reported to reduce effective renal plasma flow and glomerular filtration rate.*

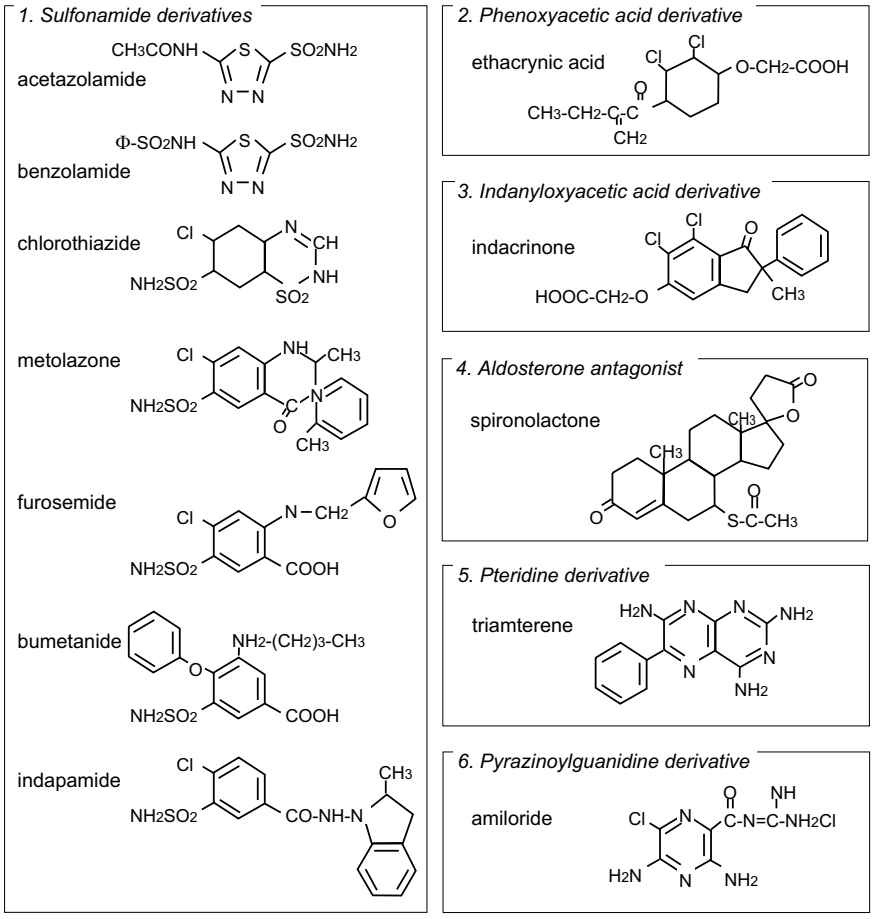

Figure 1. *Chemical structures of the commonly utilized diuretic agents grouped according to drug class.*

either no change [22, 23] or a 12 to 16% increase in effective renal plasma flow and glomerular filtration rate [24].

The thiazides, especially the most extensively studied of this group of agents, chlorothiazide, have been determined either to reduce both RBF and glomerular filtration rate or to cause no change in these parameters [1]. When a reduction does occur, it may be the result of volume contraction [25], as outlined above, an increase in proximal tubular pressure [26], or a vasodilatory effect of the drug on the postglomerular vasculature [27]. Effects of the thiazide-like agent, metolazone, and another sulfonamide derivative, indapamide, have been studied in the experimental animal and in man. In general, these drugs likewise cause no consistent alterations in either RBF or glomerular filtration rate unless volume contraction occurs [1].

Clinically, volume depletion manifests itself in one of two patterns related to alterations in serum chemistry values: either there is an elevation in the blood urea nitrogen with no increase in serum creatinine, or both are elevated but the blood urea nitrogen proportionately more so than the creatinine. This phenomenon, which has been termed "prerenal azotemia", results from reduced flow through the nephron and increased contact time between the tubular contents and the epithelium of the collecting duct. Urea is a small, noncharged moiety, which is transported much more easily than is creatinine (a much larger, usually charged molecule). Ordinarily, prerenal azotemia and reductions in glomerular filtration rate can be reversed with cessation of the diuretic and liberalization of sodium in the diet. In severe cases, however, the infusion of saline may be necessary, assuming the patient's underlying problem permits.

There have been reports of oliguric acute renal failure with high doses (>200 mg/day) of the osmotic diuretic mannitol, which responded to hemodialysis [28-

36]. The rapid and dramatic improvement of renal function with hemodialysis suggests functional abnormalities. However, anatomic changes such as vacuolization and swelling of the renal tubular cells with substantial reduction of proximal tubule lumen (osmotic nephrosis) have been reported with normal renal function [37, 38]. Low dose mannitol has been shown to produce renal vasodilation whereas high doses appear to induce vasoconstriction [35, 39-41]. It has been proposed that the large solute loads that are delivered to the macula densa during high dose mannitol therapy may increase glomerular tubular feedback causing afferent arteriole vasoconstriction producing a marked drop in glomerular filtration rate [30]. Some data also support a direct vasoconstrictive effect of mannitol on the renal artery [41].

The potassium-sparing agents, spironolactone, triamterene and amiloride appear to cause no consistent changes in RBF or glomerular filtration rate. However, when used in large doses, (300 mg per day or more), triamterene has been reported to reduce both effective renal plasma flow and glomerular filtration rate. Reductions in glomerular filtration rate have also been observed with spironolactone, but may have represented artifact related to chemical interference in the determination of serum creatinine.

Renal parenchymal lesions

Interstitial nephritis

The development of an acute interstitial inflammatory reaction in the kidney related to the administration of certain classes of drugs and leading to renal failure has been recognized for almost a century [42]. Antibiotics, in particular the sulfonamides [43] and semisynthetic penicillins [44, 45], were recognized as etiologically associated in many instances. A retrospective review of 1068 kidney biopsies from 1968 to 1997 by Schwarz et al. yielded acute interstitial nephritis in 6.5% of cases. In the majority of instances (85%) acute interstitial nephritis was drug related. Diuretics were implicated in 7.8 % of these cases [46]. Lyons et al. noted that four patients with proliferative glomerulonephritis and nephrotic syndrome treated with sulfonamide-derivative diuretics (furosemide or thiazides) developed severe renal failure, which reversed when the diuretic was withdrawn and prednisone was administered [47]. In each case, renal biopsy demonstrated a diffuse interstitial infiltrate containing many eosinophils, in addition to the expected glomerular lesions. Three of the patients had peripheral eosinophilia and two had rashes, reminiscent of the symptoms and signs associated with the antibiotic-induced renal lesions (fever, eosinophilia, eosinophiluria and rash). Rechallenge with furosemide (as well as azathioprine) resulted in a recurrence of fever, anuria and the development of erythema multiforme in one of these four patients [47]. In a case reported by Fialk et al., eosinophilia and renal failure developed in a patient receiving furosemide, subsided when the drug was stopped, but recurred when ethacrynic acid was substituted [48], improving with the cessation of the latter agent. However, in the patient reported by Lyons et al. [47], removal of furosemide and replacement with ethacrynic acid was successful as was the case in a patient reported by Fuller et al. [49]. Magil and his coworkers noted the development of eosinophilia and fever in a total of three patients receiving a combination of hydrochlorothiazide and triamterene, as well as oliguric acute renal failure in one of them [50]. Biopsies of these three patients demonstrated acute interstitial nephritis. Although the authors suggested the thiazide as the offending agent with triamterene possibly playing a potentiating role, triamterene-induced interstitial lesions have also been reported [51, 52]. Case reports of the development of this renal parenchymal lesion have also been described in association with the administration of chlorthalidone [53, 54], tienilic acid [55], indapamide [56] and other thiazides administered alone [57] or simultaneously with triamterene [58, 59].

It has become clear that interstitial nephritis with progressive renal insufficiency may present in a more subacute or chronic form (Figure 2) without the hallmarks that herald the acute disease process [60]. Furthermore, the renal failure may be nonoliguric [61]. An interesting feature of the patients reported by Kleinknecht et al. [44] and Magil and his coworkers [50, 62] was the presence in the biopsy of noncaseating interstitial granulomas. Furthermore, Magil et al. have reported that mononuclear cells in the renal interstitial tissue stained strongly positive for lysozyme, a finding that was not present in the giant cells. They also obtained evidence of cell-mediated immunity in the granulomas of two cases.

Finally, vasculitis has been reported with the ad-

ministration of the thiazides [63-68] and metolazone [69]. Larsson and his coworkers found high titers of antibodies against myeloperoxidase and cardiolipin in a patient treated with a thiazide. When the drug was stopped, progression of the renal insufficiency also abated and the antibodies disappeared [63].

Nephrolithiasis, nephrocalcinosis, and obstructive uropathy

The administration of acetazolamide has been reported to be associated with hematuria and acute renal failure [70-76]. In each of these patients, intratubular obstruction caused by crystal deposition, stone formation, and the presence of blood clots was either found or suspected. Relief of the obstruction and discontinuation of the drug resulted in the reversal of the renal failure. Volume expansion and mechanical relief of obstruction have been advocated in the treatment of this condition. However, the administration of sodium bicarbonate is controversial, since it may predispose to the development of calcium phosphate stones [77, 78].

Renal calculi have also been reported associated with acetazolamide use [78-82]. A review of patients receiving chronic acetazolamide therapy for glaucoma [83], juvenile myoclonic epilepsy [84], and neuromuscular disease [85] shows an incidence of nephrolithiasis of 15% with a background incidence of 8% in groups not receiving the carbonic anhydrase inhibitor. Acidification of the urine by acetazolamide produces a marked drop in urinary citrate and an elevation in urinary calcium excretion increasing the risk of stone formation [80, 86]. Administration of oral potassium citrate has been shown in some cases to reduce recurrent stone formation possibly by decreasing urinary calcium excretion in these patients [84, 86].

Renal calculi have also been shown as the result of the administration of triamterene [87-93]. Review of the stone analysis from the Stone Research Laboratory at Boston University by Carr et al. showed an annual incidence of 1 triamterene-containing stone for 250 analyses (total 15, 000-18, 000 analyses per year) [92]. Triamterene was commonly found in association with calcium oxalate monohydrate and may act as a source of secondary nucleation for common lithogenic salts [92, 93].

Hyperuricemia is a common accompaniment of diuretic therapy [94]. It results from three factors: (1) the thiazides and other diuretics compete for excretion along a secretory pathway located in the proximal tubule; (2) volume contraction induced by the diuretic compromises renal blood flow, leading to reduced delivery of the diuretic to the secretory site; and (3) as a result of volume contraction, the proximal tubule, also a site of urate reabsorption, is stimulated to increase

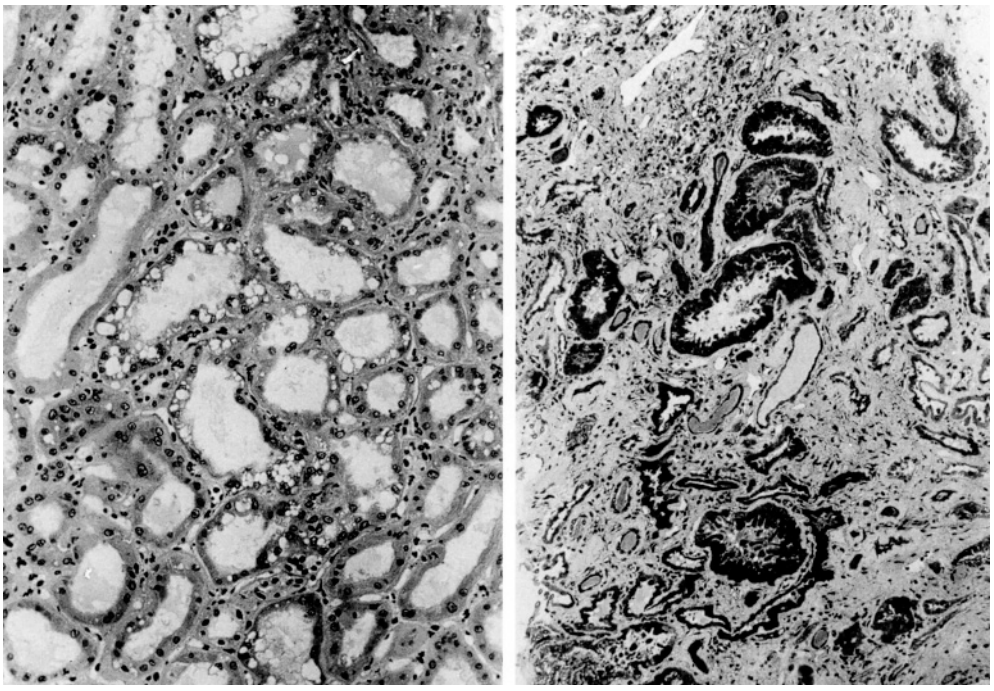

Figure 2. *Sequential renal biopsies, separated by 10 years, in a patient with hypokalemia related to chronic diuretic abuse. Initial biopsy (on the left) shows proximal tubular cell vacuolization and mild interstitial inflammation. The subsequent examination (on the right) demonstrates marked interstitial fibrosis, tubular atrophy and dropout. Reproduced from Bennett, WM [60], with permission.*

its reabsorption of this substance, along with that of sodium [95, 96]. A dose-dependent increase in serum uric acid has been documented with the administration of both bendrofluazide [97] and hydrochlorothiazide [98].

Whether the hyperuricemia leads to the development of uric acid nephrolithiasis and/or urate nephropathy, or even predisposes to the development of these lesions, is unknown [99]. The likelihood that patients with hyperuricemia will undergo silent renal damage related to the development of gouty nephropathy is considered to be small [99]. Hall et al., reporting data from the Framingham study, found that only 12 of 240 patients with a serum uric acid level exceeding 7 mg/dl had renal disease. In 5 of the 12, this was preexistent, and the nature of the renal disease in the other 7 patients was undetermined [100]. Gutman and Yü found that clearances of inulin, creatinine and p-aminohippurate were normal in 13 hyperuricemic but asymptomatic relatives of patients with gout [101]. Fessel et al., could find no statistically significant differences in mean serum creatinine levels in a group of patients before and 4 year after the onset of hyperuricemia [102]. In a subsequent study, Fessel reported that mild azotemia developed in 1.8% (2/113) of patients with asymptomatic hyperuricemia followed for 8 year, but also in 2.1% (4/193) of normouricemic control subjects [103]. In 168 patients with gout followed for 10 year, azotemia was also mild and bore no relationship to serum uric acid level. The risk of uric acid nephrolithiasis was also small in this study. They found one stone episode per 295 patients per year in asymptomatic hyperuricemics, one per 852 patients per year in normouricemic controls, and one per 114 patients per year in patients with gout. They did note, however, that azotemia of clinical importance did occur when serum uric acid exceeded 13 mg/dl in men and 10 mg/dl in women, but suggested that the risk of the development of uric acid stones was so low that hyperuricemia should probably not be treated prophylactically until a patient experienced his/her first stone episode [103]. Furthermore, although the risk of decline in glomerular filtration rate appears to be small in asymptomatic hyperuricemia, evidence of renal tubular dysfunction in this group of patients exists. Thus, Klinenberg et al., found that 5 of 19 subjects had abnormalities in their capacities to maximally concentrate the urine [104] and in five of six patients tested, total acid and titratable acid excretion were reduced by 15-20%.

A different situation might obtain, however, with respect to the prevention of further deterioration of renal function in patients with gouty nephropathy, by treating hyperuricemia [105]. However, as pointed out by Berger and Yü, long-term follow-up of patients with primary gout reveals that hyperuricemia does not cause consistent reductions in renal function [106]. Furthermore, Rosenfeld has demonstrated that normalizing serum uric acid is ineffective in improving glomerular filtration rate in normotensive as well as hypertensive patients both with and without renal dysfunction [107]. Finally, with respect to the production of uric acid nephrolithiasis by diuretics, Steele and his coworkers have pointed out that the hyperuricemia associated with diuretic usage results in a diminution in uric acid excretion, tending to minimize stone formation [108].

Loop diuretic therapy has been implicated in the development of renal calcifications in both preterm and full-term infants [109-115]. According to a study by Jacinto et al., nephrocalcinosis occurred in 20 out of 31 (64%) premature infants with birth weights less than 1500 g [112]. Of those infants, 65% were receiving furosemide. Nephrocalcinosis also was found in 14% of full-term infants with congestive heart failure receiving long-term furosemide therapy [113]. Furosemide may induce high urinary calcium excretion rates, low urinary citrate to creatinine ratios, and an alkaline urinary pH, all of which are considerable risk factors for renal calcifications [116].

Dose and length of therapy with loop diuretics may predict the likelihood of developing calcium deposits in the renal parenchyma. Ten premature infants developed nephrocalcinosis after receiving at least 2 mg/kg per day of furosemide for 12 days [111]. In a study by Saarela and colleagues, infants who developed renal calcifications were receiving higher daily doses of furosemide than infants who had not developed this complication (1.9 ± 0.6 vs. 1.3 ± 0.4 mg/kg per day; p value= 0.01) [113]. Calcifications were diagnosed within a few months of initiating furosemide.

Spontaneous resolution of nephrocalcinosis usually occurs within 6 months after discontinuation of loop diuretics [115]; however, calcifications may persist for greater than 1 year [109, 113]. The one factor that may be predictive of resolution is the calcium-to-creatinine ratio when nephrocalcinosis is first diagnosed [115].

Premature infants with unresolved nephrocalcinosis had mean calcium-to-creatinine ratios upon initial diagnosis that were approximately five-fold greater than ratios in infants with resolved nephrocalcinosis (2.23 ± 0.99 vs 0.34 ± 0.18; $p<0.005$) [115].

Since unresolved nephrocalcinosis may lead to residual abnormalities in the kidney including microscopic hematuria, hypercalciuria, and impaired tubular function [109, 113, 114], renal ultrasonography within a few months of initiating loop diuretics may be warranted [109, 113]. If long-term diuretic therapy is needed, a thiazide diuretic alone or in combination with furosemide may reduce the risk of renal calcifications by decreasing urinary calcium and oxalate excretion [109, 111, 113, 117, 118]. However, two studies of premature infants failed to show a reduction in either urinary oxalate or calcium excretion when thiazides were added to furosemide therapy [116, 119]. The lack of beneficial response may have been due to replacement of the infants' sodium losses with large amounts of supplemental sodium.

Functional and anatomic lesions

Hypokalemic nephropathy

Diuretics commonly cause hypokalemia [120]. Indeed, the more effective these drugs are in natriuresis, the more likely is the development of this side effect [121]. This is the case for the following reasons: (1) with the exception of the potassium-sparing agents, diuretics inhibit transport of sodium upstream of the $Na^+/K^+/H^+$ exchange sites (site 4, Figure 3). Accordingly, they cause the presentation of increased amounts of sodium to these antiporters for exchange with potassium and hydrogen ions [122]. Furthermore, because of the volume depletion that they induce, the diuretics cause the activation of the angiotensin-renin-aldosterone axis. The latter phenomenon stimulates the distal nephron exchange just described, resulting in the excretion of increased amounts of potassium in the urine. (2) Potassium is virtually completely reabsorbed by the time the tubular stream reaches the end of the loop of Henle and enters the distal convoluted tubule [123].

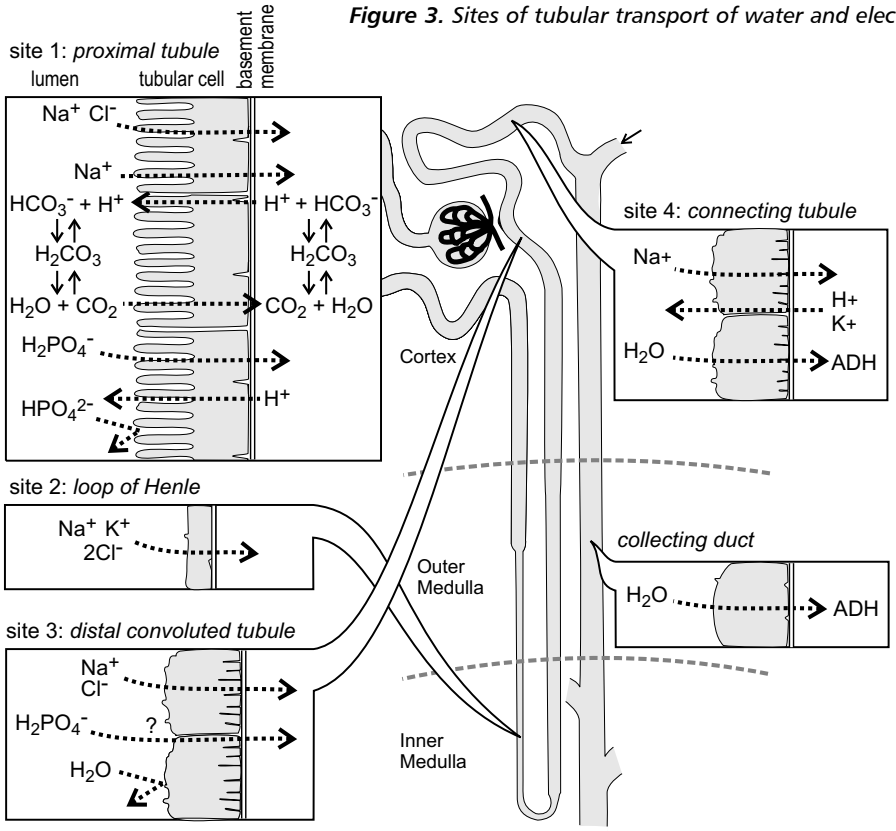

Figure 3. *Sites of tubular transport of water and electrolytes throughout the nephron.*

Accordingly, any drug that impairs transport at sites in the nephron where significant amounts of potassium ion are reabsorbed (the proximal tubule, site 1 and the loop of Henle, site 2, Figure 3) has the potential to also interfere with potassium reabsorption. This results in flooding of the distal nephron with potassium ions that have limited opportunities for reabsorption, so that most of them are excreted in the urine. If potassium losses in the urine exceed ingested amounts of the ion, negative potassium develops over time and hypokalemia as well as potassium depletion occur. This phenomenon is most frequently seen when aggressive diuresis of edematous states has been carried out. On the other hand, the employment of diuretics for the treatment of hypertension, the most common use of these drugs, generally results in only a modest decline in serum potassium levels, which is usually quickly diagnosed and treated. It is only in those cases of chronic potassium depletion that hypokalemic nephropathy occurs. Although there are a few data available on the incidence of this complication following diuretic administration, it appears to be rather small, and seems to require very long-term, marked levels of depletion [124-126]. This is especially true contemporaneously, given the emphasis placed in recent years on potassium replacement, and because of the more recent emphasis on the use of lower doses of diuretics for the treatment of hypertension than were originally employed [127]. Currently, it is generally seen only in patients who abuse diuretics and laxatives [125, 128].

Both functional abnormalities and anatomic damage result from chronic hypokalemia and severe potassium depletion. The chronic tubulointerstitial changes seen have been described as developing slowly over periods of 5 to 10 years [126]. They consist in initial vacuolization in the proximal convoluted tubular cells [129], progressing to marked interstitial inflammatory infiltrate with mononuclear cells and tubular atrophy [126, 130]. The most commonly noted functional abnormalities are polyuria and an impairment in the ability of the patient to concentrate the urine [129, 131]. While the exact mechanism of this functional defect is not known, it appears to relate to vasopressin unresponsiveness at the collecting duct level due either to the release of prostaglandins or some other interference with the generation or action of cyclic adenosine-3',5'-monophosphate [132, 133]. The latter nucleotide serves as the second message that transduces the action of the hormone into permeability of the collecting duct epithelium to tubular water [134]. The metabolic alkalosis that is almost routinely seen in this disturbance, along with the characteristic decrement in ammonium excretion [129], combine to cause the development of a persistently alkaline urine.

Summary

The diuretics cause several direct as well as indirect functional and anatomic lesions in the kidney (Table 2). Reduced RBF and/or glomerular filtration rate associated with the administration of acetazolamide, benzolamide and chlorothiazide relate to their

Table 2. Patterns of renal damage induced by diuretics.

Renal functional abnormalities	Diuretics involved
Reduced renal blood flow and/or glomerular filtration rate[a]	Acetazolamide, Benzolamide, Chlorothiazide, Mannitol (>200 g/day)
Polyuria and abnormal maximal concentrating ability related to chronic hypokalemic nephropathy	All diuretics expect potassium/sparing agents
Anatomical renal damage	
Interstitial nephritis	Thiazides, Furosemide, Triamterene, Ethacrynic acid (?)
Hypokalemic nephropathy[b]	All diuretics except potassium/sparing agents
Vasculitis[b]	Thiazides, Metolazone
Nephrocalcinosis	
	Acetazolamide, Triamterene, Furosemide (and other loop diuretics)
Hyperuricemia[a]; uric acid stones[b]	

[a]Any diuretic which causes a major degree of volume contraction, especially rapidly (a few hours to days) can induce a decrement in glomerular filtration rate, and an increase in serum uric acid. [b]This is a rare complication.

effects on the tubulo-glomerular feedback mechanism, alterations in afferent or efferent arteriolar tone or the development of extracellular fluid volume contraction. Polyuria and failure of the patient to reach maximal levels of urinary concentration are seen with chronic hypokalemic nephropathy. The latter lesion can be seen with any diuretic except for the "potassium-sparing" agents, spironolactone, amiloride and triamterene, which cause potassium retention by the kidney.

The diuretics can result in an acute interstitial nephritis, which may have an immunologic basis and can persist, if undetected, as a chronic lesion leading to renal insufficiency. The acute form may or may not be associated with other hallmarks of an allergic phenomenon such as fever and rash, but eosinophilia and eosinophiluria are often present, if looked for. Thus far, this lesion has been associated with the administration of the thiazides and furosemide, but ethacrynic acid may also be involved. Persistent hypokalemia may also lead to the development of a chronic interstitial nephritis. Vasculitis is, fortunately, a rare complication of diuretic administration having been reported thus far only with the thiazides and metolazone.

Intratubular obstruction due to crystal formation with acetazolamide and stone formation with triamterene has been reported. In addition, uric acid stones, although rare, can result from the administration of those diuretics, which compete with uric acid for secretion, but also from any diuretic that causes severe volume depletion, thus enhancing urate reabsorption and compromising excretion.

Fortunately, the nephrotoxic effects of diuretics are either infrequent or rare.

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References

1. Puschett JB, Winaver J. Effects of diuretics on renal function. In: Handbook of physiology. Section 8 renal physiology. Windhager EE (editor). Oxford Press, New York 1992; p. 2335-406.
2. Schnermann J, Wright FS, Davis JM, Stackerberg WV, Grill G. Regulation of superficial nephron filtration rate by tubuloglomerular feedback. Pflugers Arch 1970; 318: 147-75.
3. Thurau K, Schnermann J, Nagel W, Horster M, Wahl M. Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. Circ Res 1967; 20-21 (Suppl 2): 79-90.
4. Tucker BJ, Steiner RW, Gushwa LC, Blantz RC. Studies on the tubulo-glomerular feedback system in the rat. The mechanism of reduction in filtration rate with benzolamide. J Clin Invest 1978; 62: 993-1004.
5. Puschett JB, Goldberg M. The acute effects of furosemide on acid and electrolyte excretion in man. J Lab Clin Med 1968; 71: 666-677.
6. Burke TJ, Duchin KL. Glomerular filtration during furosemide diuresis in the dog. Kidney Int 1979; 16: 672-680.
7. Hook JB, Blatt AH, Brody MJ, Williamson HE. Effects of several saluretic-diuretic agents on renal hemodynamics. J Pharmacol Exp Ther 1966; 154: 667-673.
8. Vander AJ, Carlson J. Mechanism of the effects of furosemide on renin secretion in anesthetized dogs. Circ Res 1969; 25: 145-152.
9. Tucker BJ, Blantz RC. Effect of furosemide administration on glomerular and tubular dynamics in the rat. Kidney Int 1984; 26: 112-121.
10. Meyer P, Menard J, Papanicolaou N, Alexandre JM, Devaux C, Milliez P. Mechanism of renin release following furosemide diuresis in the rabbit. Am J Physiol 1968; 215: 908-915.
11. Brenner BM, Keimowitz RI, Wright FS, Berliner RW. An inhibitory effect of furosemide on sodium reabsorption by the proximal tubule of the rat nephron. J Clin Invest 1969; 48: 290-300.
12. Burke TJ, Robinson RR, Clapp JR. Determinants of the effect of furosemide on the proximal tubule. Kidney Int 1972; 1:12-18.
13. Birtch AG, Zakheim RM, Jones LG, Barger AC. Redistribution of renal blood flow produced by furosemide and ethacrynic acid. Cir Res 1967; 21: 869-878.
14. Dluhy AG, Wolf GL, Lauler DP. Vasodilator properties of ethacrynic acid in the perfused dog kidney. Clin Sci 1970; 38: 347-357.
15. McNay JL, Kishimoto T. Selective renal vascular effects of ethacrynic acid. J Pharmacol Exp Ther 1970; 174: 159-168.
16. Early LE, Friedler RM. Tubular effects of ethacrynic acid. J Clin Invest 1964; 43: 1495-1506.
17. Olsen UB. Indomethacin inhibition of bumetanide diuresis in dogs. Acta Pharmacol Toxicol 1975; 37: 65-78.

18. Puschett JB, Sylk D, Teredesai PR. Uncoupling of proximal sodium bicarbonate from sodium phosphate transport by bumetanide. Am J Physiol 1978; 235 (Renal Fluid Electrolyte Physiol 4): F403-F408.
19. Olsen UB. Prostaglandin/kinin activity related to changed renal compliance after bumetanide in dogs. Acta Pharmacol Toxicol 1977; 40: 430-438.
20. Olsen UB, Ahnfelt-Ronne I. Bumetanide induced increase of renal blood flow in conscious dogs and its relation to local renal hormones (PGE, kallikrein and renin). Acta Pharmacol Toxicol 1976; 38: 219-228.
21. Olsen UB, Ahnfelt-Ronne I. Renal cortical blood redistribution after bumetanide related to heterogenicity of cortical prostaglandin metabolism in dogs. Acta Physiol Scand 1976; 97: 251-257.
22. Bourke E, Asbury MJA, O'Sullivan S. Gatenby PBB. The sites of action of bumetanide in man. Eur J Pharmacol 1973; 23: 283-289.
23. Karlander S-G, Henning R, Lundvall O. Renal effects of bumetanide, a new saluretic agent. Eur J Clin Pharmacol 1973; 6: 220-233.
24. Jayakumar S, Puschett JB. Study of the sites and mechanism of action of bumetanide in man. J Pharmacol Exp Ther 1977; 201: 251-258.
25. Walter SJ, Laycock JF, Shirley DG. A micropuncture study of proximal tubular function after acute hydrochlorothiazide administration to Brattleboro rats with diabetes insipidus. Clin Sci 1979; 57: 427-434.
26. Krause HH, Dume T, Koch KM, Ochwadt B. Intratubularer druck, glomerularer capillardruck und glomerulumfiltrat nach furosemid und hydrochlorothiazid. Pflugers Arch 1967; 295: 80-89.
27. Cassin S, Vogh B. Effect of hydrochlorothiazide on renal blood flow and clearance of para-aminohippurate and creatinine. Proc Soc Exp Biol Med 1966; 122: 970-973.
28. Gadllah MF, Lynn M, Work J. Case Report: Mannitol nephrotoxicity syndrome: role of hemodialysis and postulate of mechanisms. Am J Med Sci 1995; 309(4): 219-222.
29. Better OS, Rubinstein I, Winaver JM, Knochel JP. Mannitol therapy revisited (1940-1997). Kidney Int 1997; 51: 886-894.
30. Goldwasser P, Fotino S. Acute renal failure following massive mannitol infusion: Appropriate response of tubuloglomerular feedback? Arch Intern Med 1984; 144:2214-2216.
31. Whelan TV, Bacon ME, Madden M, Patel TG, Handy R. Acute renal failure associated with mannitiol intoxication. Arch Intern Med 1984; 144: 2053-2055.
32. Horgan KJ, Ottaviano YL, Watson AJ. Acute renal failure due to mannitol intoxication. Am J Nephrol 1989; 9:106-109.
33. Rello J, Trigner C, Sanchez JM, Net A. Acute renal failure following massive mannitol infusion. Nephron 1989; 53: 377-378.
34. Weaver A, Sica DA. Mannitol-induced acute renal failure. Nephron. 1987; 45: 233-235.
35. Rabetoy GM, Fredericks MR, Hostetteler CF. Where the kidney is concerned, how much mannitol is too much? Ann Pharmacother 1993; 27: 25-28.
36. Dorman HR, Sondheimer JH, Cadnapaphornchai P. Mannitol-induced acute renal failure. Medicine 1990; 69(3): 153-159.
37. Mansbach AB, Madden SC, Latta H. Light and electron microscopic changes in proximal tubules of rats after administration of glucose, mannitol, sucrose, or dextran. Lab Invest 1962; 11: 421-432.
38. Dalgaard OZ, Pederson KJ. Some observations of the fine structure of human kidneys in acute anuria and osimotic diuresis. In: Renal Biopsy. Wolstenholme GEW, Cameron MP (editors). Little Brown, New York, 1962; p. ….
39. Read RC, Johnson JA, Vick JA, Meyre MW. Vascular effects of hypertonic solutions. Circ Research 1960; 8: 538-548.
40. Krishnamurty VSR, Adams HR, Smitherman TC, Templeton GH, Willerson JT. Influence of mannitol on contractile responses of isolated perfused arteries. Am J Physiol 1977; 232(1): H59-66.
41. Temes SP, Lilien OM, Chamberlain W. A direct vasoconstricter effect of mannitol on the renal artery. Surg Gynecol Obstet 1975; 141: 223-226.
42. Councilman WT. Acute interstitial nephritis. J Exp Med 1898; 3: 393-420.
43. Bakken K. The allergic reaction of the Kidney to sulphonamide medication. J Pathol Bacteriol 1947; 59: 501-504.
44. Baldwin DS, Levine BB, McCluskey RT, Gallo GR. Renal failure and interstitial nephritis due to penicillin and methicillin. N Engl J Med 1968; 279: 1245-1252.
45. Simenhoff ML, Guild WR, Dammin GJ. Acute diffuse interstitial nephritis: review of the literature and case report. Am J Med 1968; 44: 618-625.
46. Schwarz A, Krause PH, Kunzendorf U, Keller F, Distier A. The outcome of acute interstitial nephritis: risk factors for the transition from acute to chronic interstitial nephritis. Clin Nephrol 2000; 54(3): 179-190.
47. Lyons H, Pinn VW, Cortell S, Cohen JJ, Harrington JT. Allergic interstitial nephritis causing reversible renal failure in four patients with idiopathic nephrotic syndrome. N Engl J Med 1973; 288: 124-128.
48. Fialk MA, Romankiewicz J, Perrone F, Sherman RL. Allergic interstitial nephritis with diuretics. Ann Intern Med 1974; 81: 403-404.
49. Fuller TJ, Barcenas CG, White MG. Diuretic-induced interstitial nephritis. JAMA 1976; 235: 1998-1999.

50. Magil AB, Ballon HS, Cameron EG, Rae A. Acute interstitial nephritis associated with thiazide diuretics. Am J Med 1980; 69: 939-943.
51. Bailey RR, Lynn KL, Drennan CJ, Turner GAL. Triamterene-induced acute interstitial nephritis. Lancet 1982; 1: 226.
52. Roy LF, Villeneuve J-P, Dumont A, Dufresne LR, Duran MA, Morin C, Jobin J. Irreversible renal failure associated with triamterene. Am J Nephrol 1991; 11(6): 486-488.
53. Peskoe ST, McMillan JH, Lorch A, Sussman H, Ozawa T. Reversible acute renal failure associated with chlorthalidone therapy: possible drug-induced interstitial nephritis. J Med Assoc Ga 1978; 67: 17-18.
54. Ooi BS, Jao W, First MR, Mancilla R, Pollak VE. Acute interstitial nephritis. A clinical and pathologic study based on renal biopsies. Am J Med 1975; 59: 614-28.
55. Walker RG, Whitworth JA, Kincaid-Smith PS. Acute interstitial nephritis in a patient taking tienilic acid. Brit Med J 1980; 280: 1212.
56. Newstead CG, Moore RH, Barnes AJ. Interstitial nephritis associated with indapamide. Brit Med J 1990; 300: 1344.
57. Pusey CD, Saltissi D, Bloodworth L, Rainford DJ, Christie JL. Drug associated acute interstitial nephritis: clinical and pathological features and the response to high dose steroid therapy. Q J Med 1983; 52: 194-211.
58. Ten RM, Torres VE, Milliner DW, Schwab TR, Holley KE, Gleich GJ. Acute interstitial nephritis: immunologic and clinical aspects. Mayo Clin Proc 1988; 63: 921-930.
59. Kleinknecht D, Vanhille Ph, Morel-Maroger L, Kanfer A, Lemaitre V, Mery JP, Laederich J, Callard P. Acute interstitial nephritis due to drug hypersensitivity. An up-to-date review with a report of 19 cases. Adv Nephrol 1983; 12: 277-308.
60. Bennett WM. Diuretic toxicity and drug interactions. In: Diuretics: physiology, pharmacology and clinical use. Dirks JH, Sutton RAL (editors). WB Saunders, Philadelphia 1986; p. 362-373.
61. Grünfeld J-P, Kleinknecht D, Droz D. Acute interstitial nephritis. In: Diseases of the kidney, 4th edition. Schrier RW, Gottschalk CW (editors). Little Brown, Boston 1988; p. 1461-1487.
62. Magil AB. Drug-induced acute interstitial nephritis with granulomas. Hum Pathol 1983; 13: 36-41.
63. Larsson GB, Langer L, Nässberger L. Thiazide-induced kidney damage with circulating antibodies against myeloperoxidase and cardiolipin. J Intern Med 1993; 233: 493-494.
64. Bjornberg A, Gesslen H. Thiazides: a cause of necrotizing vasculitis. Lancet 1965; 2: 982.
65. Grunwald MH, Halevy S, Livni E. Allergic vasculitis induced by hydrochlorothiazide: confirmation by mast cell degranulation test. Isr J Med Sci 1989; 25: 572-574.
66. Reed BR, Huff JC, Jones SK, Orton PW, Lee LA, Norris PA. Subacute cutaneous lupus erythematosis associated with hydrochlorothiazide treatment. Ann Intern Med 1985; 103: 49-51.
67. Fitzgerald EW. Fatal glomerulonephritis complicating allergic purpura due to chlorothiazide. Arch Intern Med 1960; 105: 305-310.
68. Kjellbo H, Stakeberg H, Mellgren J. Possibly thiazide-induced renal necrotizing vasculitis. Lancet 1965; 2: 1035-1035.
69. Cox NH, Hodkin P. Vasculitis due to metolazone. Postgrad Med J 1991; 67: 860.
70. Howlet SA. Renal failure associated with acetazolamide therapy for glaucoma. South Med J 1975; 68: 504-506.
71. West AE. Hematuria in acetazolamide (Diamox) therapy. Crit Care Med 1982; 10: 197.
72. Higenbottam T, Ogg CS, Saxton HM. Acute renal failure from the use of acetazolamide (Diamox). Postgrad Med J 1978; 54: 127-128.
73. Glustien AS, Fisher ER. Renal lesions of sulfonamide type after treatment with acetazolamide (Diamox). JAMA 1956; 106: 204-206.
74. Bertino JR, Rodman T, Myerson RM. Thrombocytopenia and renal lesions associated with acetazolamide (Diamox) therapy. Arch Intern Med 1957; 99: 1006-1008.
75. Orchard RT, Taylor DJE, Parkins RA. Sulphonamide crystalluria with acetazolamide. Brit Med J 1972; 3: 646.
76. Yates-Bell JG. Renal colic and anuria from acetazolamide. Brit Med J 1958; 2: 1392.
77. Persky L, Chambers D, Potts A. Calculus formation and ureteral colic following acetazolamide (Diamox) therapy. JAMA 1956; 161: 1625-1626.
78. Parfitt AM. Acetazolamide and sodium bicarbonate induced nephrocalcinosis and nephrolithiasis. Arch intern Med 1969; 124: 736-740.
79. Davis DW. Acetazolamide therapy with renal complications. Brit Med J 1959; 1: 214-215.
80. Gordon EE, Sheps SG. Effect of acetazolamide on citrate excretion and formation of renal calculi. N Engl J Med 1957; 256: 1215-1219.
81. Mackenzie AR. Acetazolamide-induced renal stone. J Urol 1960; 84: 453-455.
82. Leopold IH. Ureteric calculi due to long-term use of carbonic anhydrase inhibitors. JAMA 1968; 208: 538.
83. Kass MA, Kolker AE, Gordon M, Goldberg I, Gieser DK, Krupin T, Becker B. Acetazolamide and Urolithiasis. Opthalmology 1981; 88:261-265.

84. Resor SR, Resor LD. Chronic acetazolamide monotherapy in the treatment of juvenile epilepsy. Neurology 1990; 40: 1677-1681.
85. Tawil R, Moxley RT, Griggs RC. Acetazolamide-induced nephroliathsis: Implications for the treatment of neuromuscular disorders. Neurology 1993; 43: 1105-1106.
86. Hagashihara E, Nutahara K, Takeuchi T, Nobyukis S, Araie M, Aso Y. Calcium metabolism in acidotic patients induced by carbonic anhydrase inhibitors responses to citrate. J Urol 1991; 145: 942-948.
87. Ettinger B, Weil E, Mandel NS, Darling S. Triamterene-induced nephrolithiasis. Ann Intern Med 1979; 91: 745-746.
88. Ettinger B, Oldroyd NO, Sorgel F. Triamterene nephrolithiasis. JAMA 1980; 244: 2443-2445.
89. Fairley KF, Woo KT, Birch DF, Leaker BR, Ratnaike S. Triamterene induced crystalluria and cylinduria: clinical and experimental studies. Clin Nephrol 1986; 26(4): 169-173.
90. Sica DA, Gehr TWB. Triamterene and the kidney. Nephron 1989; 51: 454-461.
91. Greenburg A. Diuretic complications. Am J Med Sci 2000; 319(1): 10-24.
92. Carr MC, Prien EL, Babayan RK. Triamterene nephrolithiasis: renewed attention is warranted. J Urol 1990; 144: 1339-1340.
93. White DJ, Nancollas GH. Triamterene renal stone formation: the influence of triamterene and triamterene stones on calcium oxalate crystallization. Calcif. Tissue Int 1987; 40: 79.
94. DeMartini FE, Wheaton EA, Healey LA, Larugh JH. Effect of chlorothiazide on renal excretion of uric acid. Am J Med 1962; 32: 572-577.
95. Puschett JB. Clinical uses of diuretics. In: Handbook of experimental pharmacology. Diuretics. Greger R, Knauf H, Mutschler E (editors). Springer-Verlag, Berlin (in press).
96. Steele TH. Importance and pathogenesis of diuretic-induced hyperurcemia. In: Diuretics IV. Chemistry, pharmacology and clinical applications. Puschett JB, Greenberg A, (editors). Elsevier, Amsterdam 1993; p. 231-238.
97. Carlsen JE, Kober L, Torp-Pedersen C, Johansen P. Relation between dose of bendrofluazide, anti-hypertensive effect, and adverse biochemical effects. Br Med J 1990; 300: 975-978.
98. Berglund G, Andersson O. Low doses of hydrochlorothiazide in hypertension. Antihypertensive and metabolic effects. Eur J Pharmacol 1976; 10: 177-182.
99. Liang MH, Fries JF. Asymptomatic hyperuricemia: the case for conservative management. Ann Intern Med 1978; 88: 666-670.
100. Hall AP, Barney PE, Dawber TR, McNamara PM. Epidemiology of gout and hyperuricemia. A long-term population study. Am J Med 1967; 42: 27-37.
101. Gutman AB, Yü TF. Renal function and gout with a commentary on the renal regulation of urate excretion, and the role of the kidney in the pathogenesis of gout. Am J Med 1957; 23: 600-622.
102. Fessel WJ, Siegelaub AB, Johnson ES. Correlation and consequences of asymptomatic hyperuricemia. Arch Intern Med 1973; 132: 44-54.
103. Fessel WJ. Renal outcomes of gout and hyperuricemia. Am J Med 1979; 67: 74-82.
104. Klinenberg JR, Gonick HC, Dornfeld L. Renal function abnormalities in patients with asymptomatic hyperuricemia. Arthritis Rheum 1975; 18 (suppl): 725-730.
105. Briney WG, Ogden D, Bartholomew B, Smythe CJ. The influence of allopurinol on renal function in gout. Arthritis Rheum 1975; 18 (suppl): 877-81.
106. Berger L, Yü TF. Renal function in gout IV. Analysis of 524 gouty subjects including long-term follow-up studies. Am J Med 1975; 59: 605-613.
107. Rosenfeld JB. Effect of long-term allopurinol administration on serial GFR in normotensive and hypertensive hyperuricemic subjects. Adv Exp Med Biol 1974; 41: 581-596.
108. Steele TH, Manuel MA, Boner G. Diuretics, urate excretion and sodium reabsorption: effect of acetazolamide and urinary alkalinization. Nephron 1975; 14: 48-61.
109. Alon US, Scagliotti D, Garola RE. Nephrocalcinosis and nephrolithiasis in infants with congestive heart failure treated with furosemide. J Pediatr 1994; 125: 149-151.
110. Downing GJ, Egelhoff JC, Daily DK, Thomas MK, Alon U. Kidney function in very low birth weight infants with furosemide-related renal calcifications at ages 1 to 2 years. J Pediatr 1992; 120: 599-604.
111. Hufnagle KG, Khan SN, Penn D, Cacciarelli A, Williams P. Renal calcifications: a complication of long-term furosemide therapy in preterm infants. Pediatrics 1982; 70: 360-363.
112. Jacinto JS, Modanlou HD, Crade M, Strauss AA, Bosu SK. Renal calcification incidence in very low birth weight infants. Pediatrics 1988; 81: 31-35.
113. Saarela T, Lanning P, Koivisto M, Paavilainen T. Nephrocalcinosis in full-term infants receiving furosemide treatment for congestive heart failure: a study of the incidence and 2-year follow up. Eur J Pediatr 1999; 158: 668-672.
114. Saarela T, Lanning P, Koivisto M. Prematurity-associated nephrocalcinosis and kidney function in early childhood. Pediatr Nephrol 1999; 13: 886-890.

115. Pope, IV JC, Trusler LA, Klein AM, Walsh WF, Yared A, Brock, III JW. The natural history of nephrocalcinosis in premature infants treated with loop diuretics. J Urol 1996; 156: 709-712.
116. Atkinson SA, Shah JK, McGee C, Steele BT. Mineral excretion in premature infants receiving various diuretic therapies. J Pediatr 1988; 113: 540-545.
117. Cohanim M, Yendt ER. Reduction of urine oxalate during long-term thiazide therapy in patients with calcium urolithiasis. Invest Urol 1980; 18: 170-173.
118. Constanzo LS, Weiner IM. On the hypercalciuric action of chlorothiazide. J Clin Invest 1984; 54: 628.
119. Campfield T, Braden G, Flynn-Valone P, Powell S. Effect of diuretics on urinary oxalate, calcium, and sodium excretion in very low birth weight infants. Pediatrics 1997; 99: 814-818.
120. Puschett JB, O'Donovan R. Renal actions and uses of diuretics. In: Textbook of nephrology. Massry SG, Glassock RJ (editors). Williams & Wilkins, Baltimore (in press).
121. Puschett JB, Rastegar A. Comparative study of the effects of metolazone and other diuretics on potassium excretion. Clin Pharmacol Ther 1973; 15: 397-405.
122. Giebisch G. Effect of diuretics on renal transport of potassium. Methods Pharmacol 1976; 4A: 121-164.
123. Wright FS, Giebisch G. Regulation of potassium excretion. In: The kidney: physiology and pathophysiology. Seldin DW, Giebisch G (editors). Raven Press, New York; 1992: p. 2206-2247.
124. Lawson DH. Allergic reaction to potassium chloride. Q J Med 1974; 43: 443-450.
125. Kassirer JP, Harrington JT. Diuretics and potassium metabolism: a reassessment of the need, effectiveness and safety of potassium therapy. Kidney Int 1977; 11: 505-515.
126. Cremer W, Bock KD. Symptoms and course of chronic hypokalemic nephropathy in man. Clin Nephrol 1977; 7: 112-119.
127. O'Donovan RA, Muhammedi M, Puschett JB. Diuretics in the therapy of hypertension: current status. Am J Med Sci 1992; 304: 312-318.
128. Conn JW, Johnson RD. Kaliopenic nephropathy. Am J Clin Nutr 1956; 4: 523-528.
129. Relman AS, Schwartz WB. The kidney in potassium depletion. Am J Med 1958; 24: 764-773.
130. Cremer W, Blümcke S, Bock KD. Morphologie der kaliopenischen nephropathie beim menschen. Zentrabl Allg Path 1974; 118: 481-482.
131. Healy JJ, McKenna TJ, Canning B St J, Brien TG, Duffy GJ, Muldowney FP. Body composition changes in hypertensive subjects on long-term oral diuretic therapy. Brit Med J 1970; 1: 716-719.
132. Mujais SK, Katz AI. Potassium deficiency. In: Seldin DW, Giebisch G, editors. The kidney: physiology and pathophysiology. Raven Press, New York 1992; p. 2249-2278.
133. Kirk KL, Schaefer JA. Water transport and osmoregulation by antidiuretic hormone in terminal nephron segments. In: The kidney: physiology and pathophysiology. Seldin DW, Giebisch G (editors). Raven Press, New York 1992; p. 1693-1725.
134. Orloff J. Handler JS. The similarity of effects of vasopressin adenosine-3', 5'-monophosphate (cyclic AMP) and theophylline on the toad bladder. J Clin Invest 1962; 41: 702-709.

18

Anticancer drugs

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Introduction

Nephrotoxicity is intrinsic to the pharmacological effect of certain anticancer drugs. Because antineoplastic drugs have a narrow therapeutic index, the amount of drug required to significantly reduce tumor burden usually induces significant nephrotoxicity. Furthermore, the dosage used in clinical trials often represents the maximum tolerated doses determined during phase I drug evaluation. Philosophically, greater toxicity is acceptable for curative therapy as opposed to palliative therapy. However, cancer patients often have reduced renal function as a component of their disease. Dosage adjustment, based on the pharmacokinetics and pharmacodynamics of these drugs in cancer patient, is required to improve tolerance. Conversely, ESRD patients who receive a kidney transplant are at increased risk for developing malignancy.

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 353-372

Clinical syndromes of renal involvement are both diverse and sometimes insidious. This chapter focuses on the major anticancer drugs and their renal consequences. Despite recent advances in understanding the physiopathological mechanism of anticancer drug nephrotoxicity, prevention still relies on adjusting drug dosage, and active screening for renal abnormalities as part of the pre-treatment clinical evaluation (Table 1).

Alkylating agents

Cisplatin

Cis-dichlorodiammine platinum (II), or cisplatin, has emerged as a principal chemotherapeutic agent in the treatment of drug resistant solid tumors and is currently on of the most widely used agents in the cancer chemotherapy [1]. Nephrotoxicity is the primary lim-

Table 1. Potentially nephrotoxic chemotherapeutic agents and options for prevention.

	Type of renal failure	Prevention
Alkylating agents		
Cisplatin*	ATN, dose-related (rare if doses <1600 to 2400 mg/m^2), non-oliguric and reversible Hypomagnesemia usually remits when cisplatin is discontinued	Hydration and vigorous diuresis with saline Daily divided doses for 5 days instead of single infusion Cumulative dose <120 mg/m^2 of body surface area
Carboplatin		
Oxazophosphorines		
Cyclophosphamide	Hemorrhagic cystitis	Aggressive hydration and Mesna® prevents hemorrhagis cystitis induced by both drugs
Ifosfamide	Fanconi syndrome ATN if doses>5 g/m^2	
Nitrosoureas		
Streptozotocin*	Fanconi syndrome (hypophosphatemia++) Glomerular toxicity and renal failure	
Carmustine*	Late development of renal failure (long treatment or over 6 courses with the conventional dose 200 mg/m^2)	
Antimetabolites		
Methotrexate*	ATN or secondary intrarenal obstructive uropathy (precipitation of methotrexate or methotrexate metabolite in the distal tubules if dose >50 mg/m^2) Fall in glomerular filtration rate in a rapid and dose related fashion if dose >1 g/m^2	Urinary alkalinization and hydration Enhanced toxicity if previous treatment with cisplatin, or NSAIDs Charcoal hemoperfusion and sequential hemodialysis if severe renal toxicity (significant clearance of methotrexate can be achieved with high-flux dialyzers)
Antitumor antibiotics		
Mitomycin*	HUS (risk 2 to 10%), late onset (6-17 months following the initiation of treatment)	Cumulative dose should be 40 mg/m^2
Immunotherapy		
Interleukin 2*	Reversible syndrome of hypotension, oliguria, fluid retention, azotemia, and a very low urinary excretion of sodium Capillary leak syndrome Acute interstitial nephritis	Steroids and plasma exchange
Interferon	Proteinuria in up to 5 to 20% patients Rarely nephrotic syndome or/and acute renal failure (acute interstitial nephrotoxicity and minimal change nephropathy)	

*** drugs with asterisk are high-risk drugs, others are low-risk.**

iting factor to achieving greater drug efficacy, and necessitates active hydration during administration and using lower dosages. These techniques have yielded partial success but acute renal failure still occurs, particularly after repeated administration [2, 3]. Other means to protect the kidney against cisplatin nephrotoxicity [4-6] are inconsistent and of uncertain clinical application [7]. At present, significant prevention of the renal toxicity of cisplatin will require a more basic understanding of the mechanism of toxicity.

Pharmacology

The kidney is the principal route of excretion for cisplatin. In the rat, 50% of injected cisplatin is excreted in the urine within 24 hours of administration [8] with the majority appears in the first hour [9]. Platinum is extensively bound to plasma protein. Unbound cisplatin, by virtue of its low molecular weight and neutral charge, undergoes unrestricted filtration at the glomerulus [10]. Rat and human studies suggest that tubular secretion of cisplatin may occur [11, 12]. Microinjection of radiolabeled cisplatin injected into the proximal tubule is almost completely recovered in the urine arguing against any significant tubular reabsorption [13]. Kidney tissue concentrations of platinum are several folds higher than plasma levels and exceed the concentrations in other organs [8]. Almost all of the platinum in the kidney is found in the cortex and is distributed in all subcellular organelles as well as the cytosol [9]. The process by which the kidney accumulates cisplatin is oxygen-dependent [10] and is competitively inhibited by drugs that are transported by the organic base system. Conversely, drugs that compete for the organic anion transport system, such as PAH and pyrazinoic acid, do not inhibit cisplatin uptake. Collectively, these observations indicate that the renal uptake of cisplatin involves transport or binding to components of the base transport system.

Additional evidence linking the kidney's vulnerability to cisplatin transport is provided by autoradiographic studies that show intense uptake of radiolabeled cisplatin in the S3 segments of the proximal nephron [13]. Since the S3 segment of the proximal tubule is the principle site of cisplatin-induced cell toxicity and contains most of the platinum, these studies provide further evidence of the unique vulnerability of this cell type to cisplatin toxicity, depending on its propensity to accumulate and interfere with cisplatin metabolism into toxic cisplatin-conjugates [13a].

Cisplatin is excreted largely unchanged in the urine [10]. However, when cisplatin enters the renal cell it undergoes biotransformation. In addition to binding to cell macromolecules, a large portion [30-50%] of the intracellular platinum exists in a 500 Dalton form with a chromatographic behavior that differs from parent cisplatin. Another characteristic of this platinum metabolite is the absence of mutagenic activity. Whereas excreted platinum is mutagenic, cell platinum is not [14]. Mutagenic compounds either react with or can be converted to compounds that react with DNA to form DNA adducts. The cisplatin DNA adducts cause errors during DNA replication, which lead to mutations, especially G → T transversion [15]. Such mutations may be responsible for second malignancies that arise after cisplatin therapy [16].

Renal tolerance

The clinical use of cisplatin is limited by nephrotoxicity, characterized by a decline in glomerular filtration rate that is in proportion to the number of cycles of cisplatin chemotherapy. Progressive and persistent reductions in glomerular filtration rate and renal blood flow may follow each successive treatment cycle [17]. Renal plasma flow, whole kidney glomerular filtration rate, single nephron glomerular filtration rate, and stop-flow pressure are reduced compared to controls [17]. Since intratubular hydrostatic pressure does not differ significantly from controls in euvolemic and volume expanded animals, it is unlikely that intratubular obstruction plays an important role in early, cisplatin-induced acute renal failure. Following withdrawal of the drug, renal insufficiency stabilizes but remains indefinitely impaired. The cisplatin-induced hypofiltration is usually associated with tubular proteinuria. Severe salt-wasting with orthostatic hypotension has been observed after cisplatin administration in a minority of patients [18].

Polyuria uniformly accompanies cisplatin administration and occurs in two distinct phases. During the first 24-48 hours after administration, urine osmolality falls but glomerular filtration rate remains stable. This early polyuria usually reverses spontaneously. The second phase occurs between 72 and 96 hours after cisplatin and is characterized by an increased urine volume with reduced osmolality. This later phase is accompanied by a persistent reduction in glomerular filtration

rate.

Hypomagnesemia is a well recognized complication of cisplatin administration in humans [19] and persistent magnesium wasting in the presence of severe hypomagnesemia suggests that the hypomagnesemia is the result of a renal defect in magnesium reabsorption [20]. Recent studies in the rat suggest that abnormal magnesium excretion may be due to a defect in magnesium transport in juxtamedullary nephrons or collecting ducts [21], much like the defect in water transport described above. This may be accompanied by secondary hypocalcemia and hypokalemia. By impairing cellular respiration, cisplatin may also induce an incomplete distal tubular acidosis further interfering with the tubular handling of hydrogen, magnesium, potassium and calcium ions [22].

The mediators responsible for the fall in glomerular filtration rate and renal blood flow have not been determined and neither calcium channel blockers [23] or angiotensin converting enzyme inhibitors [24] have been unable to reverse cisplatin-induced acute renal failure. Several suspected targets of pathogenetic importance in cisplatin nephrotoxicity have been identified, including renal tubule energy production and DNA synthesis. Mitochondrial dysfunction has been implicated in the pathogenesis of cisplatin-induced renal failure [25, 26]. When normal tubules are incubated with high concentrations of cisplatin (10^{-3}M), both basal and stimulated rates of oxygen consumption are inhibited. Conversely, transplatin, which also binds to DNA and protein and decreases mitochondrial respiration at lower concentrations (10^{-4}M) is neither antineoplastic nor nephrotoxic [13]. Furthermore, in tubules isolated from rats given a nephrotoxic dose of cisplatin, basal and stimulated rates of respiration are unchanged up to 48 hours after cisplatin administration [13]. In these studies the concentration of platinum in proximal tubules was several hundred fold less when tubules were exposed to cisplatin *in vitro* [13]. The results of these studies suggest that neither the renal cell mitochondria nor the membrane associated Na-K-ATPase are important early pathogenetic targets of cisplatin.

The primary intracellular action induced by cisplatin in cancer cells is inhibition of DNA synthesis [27, 28]. The inhibition of DNA synthesis is persistent and occurs at substantially lower doses compared to those required to inhibit RNA and protein synthesis [29]. Cisplatin binds to two sites in DNA [30] inducing DNA inter- and intrastrand as well as DNA-protein cross-links [30, 31]. However, a relationship between the DNA-binding and renal cytotoxicity of cisplatin has yet to be established. How a decline in renal cell DNA synthesis would explain cell-specific necrosis is problematic although two possibilities have been suggested. First, selective DNA repair by renal cells other than the pars rectus could explain the observation. In support of this proposal is that in cells whose repair processes are deficient, cisplatin is especially toxic [32]. Second, it may be that the levels of the DNA adducts formed in the pars recta cells are lethal while the lower concentrations found in other nephron segments are not. The role of the reduction in DNA synthesis in cisplatin-induced renal cytotoxicity must await additional studies.

Recovery following nephrotoxic acute renal failure involves regeneration of actively dividing cells to replace damaged tubule cells. Recovery from cisplatin-induced acute renal failure is heralded by increased mitosis in renal epithelial cells, a process preceded by increases in nucleic acid synthesis [33].

Prevention of cisplatin-induced nephrotoxicity

Early trials with cisplatin reported more than 70% of patients developing a dose-related acute renal failure [34, 35]. Despite aggressive saline hydration, a commonly used technique to prevent clinical nephrotoxicity [36], renal failure persisted [37-39]. Thus several approaches have been used to reduce nephrotoxicity by either co-administration of other compounds, alternate methods of administration, or by developing analogues with a wider therapeutic index.

Mannitol and furosemide reduce the concentration of platinum in the urine, suggesting that these agents might attenuate cisplatin nephrotoxicity [40, 41]. However, plasma and renal platinum content were unchanged and the degree of cellular necrosis did not improve with use of these diuretics [41]. Platinum does not appear to undergo tubular reabsorption based on the nearly complete urinary recovery of microinjected platinum [13]. Hence, renal platinum content is independent of its luminal concentration.

Prior hydration with hypertonic salt appears effective in reducing the incidence of cisplatin-induced acute renal failure [42]. Since the degree of azotemia produced by cisplatin is highly dependent on the sodium

chloride content of the vehicle [42], it was speculated that the increasing the urinary chloride concentration might reduce the conversion of cisplatin to toxic aquated metabolites, a process known to be sensitive to chloride ion concentration.

Conclusions regarding the renoprotective action of mannitol and furosemide to decrease experimentally induced cisplatin nephrotoxicity are conflicting [36, 41, 43]. However, in humans, a randomized study by Alsarraf et al. [44] comparing hydration + cisplatin to hydration + mannitol + cisplatin concluded that mannitol did not attenuate cisplatin nephrotoxicity.

While protection of kidney function by mannitol was observed after the first cycle, this protection was lost during subsequent cycles. Thus we can not recommend the use of diuretics in prevention of cisplatin-induced nephrotoxicity. Pretreatment hydration beginning at least 12 hours before cisplatin administration targeted to induce a urine flow of at least 100 ml/hr can be recommended but requires continuous electrolyte replacement.

Schilsky et al. [19] first recommended the use of hypertonic saline infusion after noting that when 3% saline was the vehicle for cisplatin, there was no change in either serum creatinine and creatinine clearance despite high dose cisplatin treatments. However, when ^{51}Cr-EDTA was used as a measure of glomerular filtration rate, a significant decrease in GFR was observed despite the use of 3% saline [12, 13]. Recommendations regarding the use of hypertonic saline infusion to prevent high dose cisplatin nephrotoxicity must await a randomized study.

Compared to bolus dose, fractional or continuous infusion of the total dose of cisplatin over 3-5 days is equally effective therapeutically but may reduce renal dysfunction [45].

Infections, a frequent cause of co-morbidity in the immunocompromised cancer patients, require antibiotic therapy. The use of certain antibiotics, many of which are nephrotoxic, will add to the renal toxicity of the anticancer agents. The incidence of nephrotoxicity is greater in patients receiving cisplatin in combination with aminoglycosides as compared to patients receiving only cisplatin [46]. In the majority of cases the degree of renal impairment has usually been mild and not clinically significant [46]. However, acute renal insufficiency has been reported following the combined use of cisplatin with gentamicin-cephalothin [47]. Furthermore animal studies have revealed that a non-nephrotoxic dose of aminoglycosides immediately following a single dose of cisplatin potentates the impairment of renal function caused by cisplatin alone [48, 49]. The administration of nephrotoxic drugs such as aminoglycosides, non-steroidal anti-inflammatory drugs or iodinated contrast media simultaneously with cisplatin should be avoided.

Multiple "renoprotective" compounds have been evaluated in cisplatin nephrotoxicity (ANF, glycine, diethyldithiocarbamate, calcium channel blockers, cimetidine, sodium thiosulphate, glutathione, other sulfhydryl compounds, …). In clinical practice only sodium thiosulphate has been reported to significantly reduce the renal toxicity of cisplatin irrespective of the route of administration, e.g. the intra-arterial, intraperitoneal or intrathoracic routes [50, 51]. However, controversies exists as to the effectiveness of sodium thiosulphate as an antitumor agent. Sodium thiosulphate is most useful in combination with intraperitoneal cisplatin where it confers renal protection without altering local effects of cisplatin [51].

Initiating saline hydration several hours before cisplatin infusion and continuing the saline infusion for several days after cisplatin administration is a routine technique to prevent cisplatin nephrotoxicity [35, 36, 38, 39]. Although several days are required for the severe changes in renal function to develop fully, the critical adverse renal events seem to occur immediately after cisplatin administration. Protective measure should be initiated before, during and immediately after cisplatin treatment. Our prehydration routine involves 100 ml/hr of normal saline solution for the 12 hours prior to cisplatin administration followed by continuous infusion of saline for at least 1 day after cisplatin treatment. Efficacious antiemetic drugs should be given concomitantly to minimize volume depletion. Odansetron is now used to minimize nausea and vomiting which allows early discontinuation of intravenous hydration which shorten the duration of hospitalization. It is noteworthy that odansetron is ineffective as an anti-emetic in 10% of patients undergoing chemotherapy [52]. Since anti-emetic effect cannot be predicted in these patients, intravenous saline hydration may be needed for at least 3 days after stopping cisplatin.

At present, we discourage to administer platinum compounds to volume-depleted patients. Platinum

should be administered slowly in conjunction with a saline solution infusion with a goal of inducing a urine flow of 3 to 4 L/24 hours for the next two to three days.

In summary, the vulnerability of the kidney being the S3 segment renal cells to cisplatin is linked to the principle route of excretion. The mechanism of renal cell death induced by cisplatin is unknown, but mounting evidence points to its genotoxic effect. In cisplatin nephrotoxicity, as with other forms of nephrotoxic renal damage, reduced renal blood flow and diminished renal conservation of water are common physiologic derangements.

The unchanged incidence of nephrotoxicity with inorganic platinum compounds stresses the need for ongoing research to identify platinum complexes with potent antitumor activity that are less nephrotoxic. Until this goal is achieved, it seems prudent to explore combinations of platinum with chemotherapeutic agents that are not associated with significant nephrotoxicity plus avoiding other concomitant nephrotoxic insults, especially volume depletion.

Carboplatin

Carboplatin is a cytotoxic platinum complex structurally related to cisplatin, who's *in vitro* antitumor activity is qualitatively similar to that of cisplatin. Comparative trials with carboplatin alone or in combination with other chemotherapeutic agents suggest comparable efficacy with cisplatin in ovarian cancer. As with cisplatin, nausea and vomiting be delayed for several hours and be mild to moderate in severity. The dose limiting toxicity of carboplatin is myelosuppression made worse by the presence of renal impairment, previous chemotherapy or administration to older patients.

Pharmacology

Tissue distribution is similar to that seen with cisplatin with the highest concentrations of platinum in the kidney, liver, skin and tumors [53]. Tissue platinum concentrations were generally 0.5 to 3 fold greater than those observed after cisplatin. However, once platinum is bound to plasma protein its cytotoxicity is substantially reduced [54]. In contrast to the extensively protein binding of cisplatin, carboplatin is only 15 to 25% bound during the initial hours following administration. Renal clearance appears to be the main route of excretion, and urinary elimination of carboplatin is more rapid than that of cisplatin in both animals [55] and cancer patients [56]. 50 to 80% of the administered dose is excreted in the urine during the first 24 hours [57-59]. It has been speculated that repeated administration of cisplatin may cause decreased carboplatin renal clearance and enhanced toxicity [60]. Renal clearance and total body clearance of carboplatin are reduced in patients with impaired renal function [61-63].

Calculation of dose in renal failure

The administered dose should be adjusted in proportion to the reduction of creatinine clearance for patients with renal impairment to achieve AUCs similar to those in patients with normal renal function. Calvert et al. [64] have proposed the following formula for calculation of dose:

Dose (mg) **= AUC x (GFR + 25)**

where the desired AUC (area under the plasma concentration x time curve) is in the target range of 5 to 7 mg/ml x min for acceptable toxicity in patients receiving carboplatin as a single agent.

Renal Tolerance

Carboplatin was developed to avoid the nephrotoxicity of cisplatin and was initially thought to be less nephrotoxic [65]. Studies of carboplatin in rats found minimal renal effects as judged by serum blood urea nitrogen, creatinine, kidney weight and renal histology [66]. Initial clinical studies established that, at doses of 400 mg/m^2, carboplatin caused virtually no nephrotoxicity, ototoxicity or peripheral neuropathy [67]. Egorin et al. in 1984 [68] reported no reduction in creatinine clearance in 22 patients treated with intravenous carboplatin at 400 mg/m^2 and concluded that no adjustment of dosage was required in patients with diminished renal function. Because the dose-limiting toxicity for carboplatin is myelosuppression, with little renal or neurological toxicity evident, higher doses (800 mg/m^2 body surface area) were combined with granulocyte-macrophage colony-stimulating factor. In these circumstances, without vigorous hydration, a significant decrement in renal function was described [69, 70]. Out of six patients receiving 1200 mg/m^2 carboplatin, a decrease in glomerular filtration rate of 25 to 50% was observed in four [71]. Several studies have also reported the comparative toxicities of carboplatin and cisplatin, all confirming a dose dependent neph-

rotoxicity.

Oxazaphosphorines

Cyclophosphamide

Pharmacology

The oxazophosphorine cyclophosphamide is oxidized to active and inactive metabolites that are secreted by the kidney [72-75]. The 24-hour urinary excretion of intact parent compound and alkylating activity is 1-14% and 7-17% respectively [76]. The fraction of cyclophosphamide and metabolites excreted in the urine is high and unchanged in patients with renal failure [77].

Urothelial toxicity

Direct contact between the catabolites acrolein and 4-hydroxy-cyclophosphamide and the bladder epithelium is responsible for the hemorrhagic cystitis that can occur during therapy with cyclophosphamide [78]. Aggressive hydration provides prophylaxis against this lower urinary tract toxicity [79]. The sulphydryl compound mesna® is reported to have uroprotective properties during therapy with cyclophosphamide [80]. Although hemorrhagic cystitis is a dose-related toxicity, chronic low doses of orally administered cyclophosphamide have also been associated with development of this adverse event [81].

Renal tolerance

Cyclophosphamide can also cause tubular necrosis in experimental animals [82]. No clinical counterpart has been described, despite careful assessment in patients receiving high doses of cyclophosphamide [83, 84]. Although detectable alterations of renal function tests are absent, subtle changes in renal tubular physiology have been noted. Bode et al. [85] studied the mechanism of water retention that occurs following cyclophosphamide. They concluded that cyclophosphamide directly affected the tubules, causing increased water reabsorption and sodium loss. This water retention is self-limited to a day or two and does not present a major clinical problem.

Ifosfamide

Pharmacology

Ifosfamide is becoming more popular for the treatment of pediatric malignancies. It is a prodrug that undergoes biotransformation via the hepatic cytochrome P-450 system before exerting its therapeutic or toxic effects [86, 87]. Ring hydroxylation produces 4-hydroxy-ifosfamide, which is then converted into the active alkylating agent (isophosphoramide mustard) and acrolein (the putative cause of hemorrhagic cystitis and *in vitro* nephrotoxin). Significant molecular degradation occurs by dechloroethylation of ifosfamide [88], subsequent chloroethyl side chain breakdown leading to the production of chloroacetaldehyde which possess neuro- and nephrotoxic effects [89-91].

Urinary excretion accounts for 57-80% of the dose as ifosfamide and metabolites and 27-41% of the dose is recovered in the urine as alkylating activity [92]. Indeed ifosfamide metabolism occurs at low concentrations in the kidney since ifosfamide metabolites were recovered from both urine and renal venous effluent in an isolated perfused rat kidney model after *in vitro* ifosfamide perfusion [93].

Renal Tolerance

Experience with ifosfamide-containing regimens has characterized the presentation of clinical nephrotoxicity. Fanconi syndrome, which involves disturbances of acid, sodium, potassium, and magnesium balance, and the urinary excretion of small molecular weight proteins, occurs in 1-5% of the children receiving repeated treatments of ifosfamide [94, 95]. In fact, renal rickets secondary to Fanconi syndrome have been reported following treatment with ifosfamide [96]. Patients treated with cisplatin or carboplatin, in addition to ifosfamide, may be at greater risk to develop Fanconi syndrome [97]. There is a significant occurrence of hemorrhagic cystitis with ifosfamide administration [98, 99]. However, appropriate hydration combined with the sulfhydryl compound mesna are effective in decreasing the urotoxicity of ifosfamide [100, 101]. Less frequently asymptomatic renal functional abnormalities occur when ifosfamide if given at a dose below 1.5 g/m^2 body surface area [102, 103]. Acute renal failure secondary to tubular necrosis (Figure 2 p. 361) has been described with high–dose therapy (> 5 g/m^2), particularly in patients previously treated with cisplatin [104,

105]. When escalating doses of ifosfamide, given as a 96 hours infusion, renal toxicity is dose-limiting at 18 g/m² [106].

Nitrosoureas

Streptozotocin

Streptozotocin is a naturally occurring nitrosourea useful for the treatment of advanced islet cell carcinomas and carcinoid tumors [107].

Pharmacology

Animal studies confirm the accumulation of streptozotocin in the kidney [108]. Urinary excretion of streptozotocin accounts for 10-20% of the dose, with significant amounts of metabolite detected [109, 110]. Thus, the major excretion pathway of streptozotocin in humans is the kidney.

Renal tolerance

Streptozotocin nephrotoxicity is characterized by renal tubular defects, including the Fanconi syndrome. The onset of streptozotocin-induced nephrotoxicity can be insidious with hypophosphatemia as the presenting sign, soon followed by glycosuria, proteinuria and finally an increase in serum creatinine and blood urea nitrogen [111, 112]. In the first phase I trial reported with this drug, all 18 patients developed renal dysfunction, and 2 of them became anuric [113]. Schein et al. [110] treated 106 patients and noted that renal abnormalities occurred in 28% of the patients and was the most common form of toxicity. Nephrotoxicity contributed to the death of 4 patients in Schein's study. Moertel and colleagues [114] reported evidence of nephrotoxicity in two-third of 38 patients treated with streptozotocin, while it occurred in two-thirds of 52 patients in another series, 5 of whom died of renal failure [115]. The incidence of streptozotocin-induced nephrotoxicity seems to increase with prolonged drug administration.

The site of streptozotocin injury involves both the glomerulus and the tubule, based on histologic changes [115-117]. Although streptozotocin is excreted in the urine, an explanation for the cellular sensitivity at both the glomerulus and tubule is lacking. Streptozotocin markedly suppresses nicotinamide adenine dinucleotide levels in animal liver and islet cells (which is correlated with the diabetogenic effect of streptozotocin) [118, 119]. This effect could explain why streptozotocin has been reported to induce renal tumors in experimental animals, an effect that can be modified by nicotinamide administration [120, 121].

Carmustine, lomustine and semustine

Carmustine (BCNU) and lomustine (CCNU) are antineoplastic drugs that, in combination with other approved drugs, are widely used in the treatment of brain tumors, multiple myeloma, Hodgkin's disease and non-Hodgkin's [122-125].

Carmustine [1, 3bis(2-chloroethyl)1-nitrosourea] can be administered as a single agent at doses of 50-210 mg/m² or in combination chemotherapy regimens at similar or lower doses. Carmustine is also a component of a high-dose chemotherapy regimens used during autologous bone marrow reinfusion [126]. Carmustine may be used with either lomustine [1-(2 chloroethyl)-3-(cyclohexyl)-1-nitrosourea] or methyl-lomustine [1-(2-chloroethyl)-3-(4-methyl-cyclohexyl)-1-nitrosourea].

Pharmacology

Carmustine is highly lipid soluble, rapid metabolism and has a biphasic half-life (1.4 and 20 min). Carmustine is metabolized to an N-nitroso group, which is secreted into the tubular lumen. It has been proposed that this N-nitroso group is pharmacologically active in high concentrations and capable of spontaneously releasing an active methyl group, which may be responsible for its nephrotoxicity [127]. 60 to 70% is excreted in the urine within 96 hours while 6 to 10% is excreted as CO_2 by the lungs. The absence of any parent compound in the urine indicates that nephrotoxicity is most likely due to one of the metabolites. The degree of either alkylating and carbamoylating activity of the nitrosoureas vary a great deal for each compound [128]. Therefore it is difficult to implicate a specific chemical action in the mechanism of nephrotoxicity. Because the toxicity (mainly hematopoietic, hepatic and gastro-intestinal) is cumulative, the Food and Drug Administration recommends that courses should not be given more frequently than every 6 weeks.

Renal tolerance

Renal toxicity of carmustine and lomustine was first

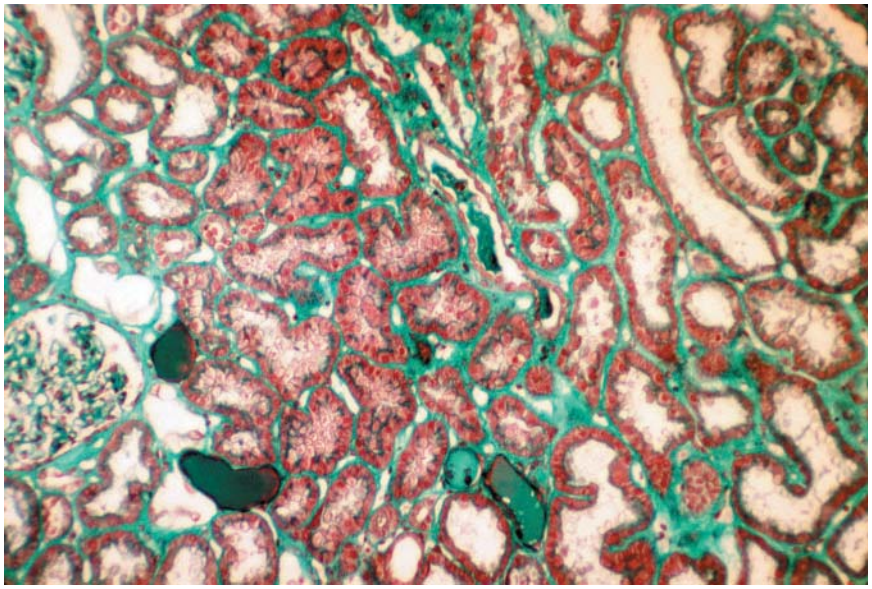

Figure 1. *Focal tubular necrosis with flat epithelial, pauci cellular fibrosis. Patient received cisplatin. Masson's trichrome staining, magn. x125.*

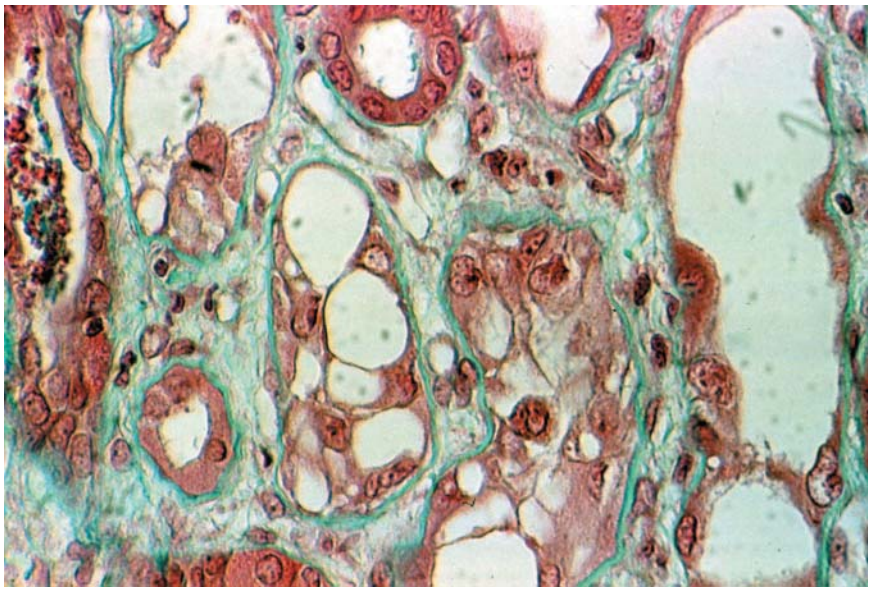

Figure 2. *Tubular necrosis with focal denudation of the basement membrane and pronounced vacuolization, swelling. Some interstitial fibrosis. Patient received ifosfamide. Masson's trichrome staining, magn. x325.*

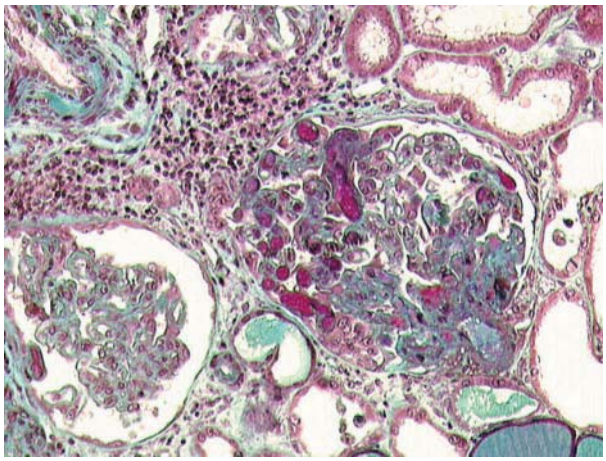

Figure 3. *Mitomycin C induced thrombotic microangiopathy. Glomeruli show swelling and detatchment of endothelial cells and luminal occlusion. The arterioles and arteries show intimal cellular swelling and hyperplasia and fibrin deposition. Masson's trichrome staining, magn. x325.*

Figures 1-3 by courtesy of Dr. Hélène Beaufils.

noted in pre-clinical studies [127]. In the earliest clinical trial of carmustine, 10% of the patients had unexplained elevations of BUN, but there were no instances of severe renal problems [129]. Thus, nephrotoxicity was not considered to be a major concern with any of these drugs. Subsequent reports of semustine- and lomustine-induced renal failure revised this impression [130-135]. Potential renal toxicity is limited to patients receiving unusually long treatment. Indeed, the first 3 cases of nephrotoxicity occurred in patients receiving high cumulative doses of lomustine (2300 to 3360 mg) [131-136]. Schacht et al. [137] reported the delayed development of renal functional impairment following at least one year of therapy when a minimum of 6 courses of these nitrosoureas was administered in conventional dosage (200 mg/m^2 at 8 weeks intervals). Eighteen patients, having received cumulative doses of 1.5 to 7.4 g, developed renal dysfunction characterized by elevations of BUN > 20 mg/dl or decreases in glomerular filtration rate as measured by inulin clearance. Four patients developed end-stage renal disease 9 to 16 months following courses of therapy ranging from 28 to 65 months in duration. The clinical records of these patients failed to reveal any history of hypertension or impaired renal function prior to treatment and no sign of early nephrotoxicity despite bimonthly doses being administered under close supervision. Renal functional impairment developed insidiously, with urinalyses showing either no or trace proteinuria, and no formed element. Twenty-four-hour urinary protein values never exceeded 450 mg. No patient had glycosuria. Renal biopsies were available in 5 patients and tissue from 2 patients was obtained at post mortem histologic examination revealed glomerular sclerosis affecting more than 15% of glomeruli, with focal thickening and reduplication of the basement membranes in many of the remaining glomeruli. Focal areas of tubular atrophy, interstitial fibrosis and infiltration with chronic inflammatory cells were observed in 6 of the 7 patients (Figure 1 p. 361). In patients with severe damage, there was advanced, widespread sclerosis of glomeruli, varying from contraction of tufts with partial obliteration of lumens to complete obliteration of capillary lumens forming cellular contracted tufts. In these cases, tubular atrophy was extensive and severe. Immunofluorescence studies failed to show any localization of immunoglobulin. The histologic abnormalities were similar to that reported by Harmon et al. [132]. The predominance of tubulointerstitial changes in some patients suggested that glomerular sclerosis might have been secondary to a primary interstitial process. However, an independent nephrotoxic effect of the nitrosoureas directed against glomeruli as well as the interstitium could not be excluded. Despite the histologic and clinical evidence of a chronic progressive parenchymal nephropathy, features of acute nephrotoxicity were lacking. That no acute renal insult antidated the chronic progressive disease was supported by the absence of any acute parenchymal lesion in the tissue obtained from the post mortem examination of patients who died from their brain tumors and received only one or two courses of nitrosourea therapy. Renal function decline is insidious, the initial suggestion of renal damage occurring 1 to 6 years after beginning chemotherapy. Having once developed, renal functional impairment may progress rapidly to advanced uremia despite discontinuation of the drug.

The mechanism of BCNU-induced nephrotoxicity is most likely based on a direct nephrotoxic effect but differs from that of streptozotocin which is manifested by proximal tubular dysfunction and acute renal failure, and is usually reversible.

Antimetabolites

Methotrexate

Methotrexate is a folic acid antagonist that inhibits the enzyme dihydrofolate reductase. This agent is mainly used in the treatment of both cancer (trophoblastic neoplasms, leukemias, breast carcinoma, carcinoma of gastric, esophagus, testes, lymphomas) and non-cancer diseases [psoriasis; rheumatoid arthritis]. Recent successful results using high-dose (>1 g/m^2) methotrexate followed by leucovorin in the treatment of head and neck carcinomas and osteosarcoma has led to a more widespread application of this therapy in patients with these and other tumors.

Pharmacology

The kidneys readily filter methotrexate, but renal clearance is determined by the balance between tubular secretion [138-141] and tubular reabsorption [141]. Intravenous administration of methotrexate 140-350 mg/kg in 6 hours or less results in 70-94% of the dose appearing in the urine over 24 hours [142]. In contrast,

when methotrexate is administered as a continuous infusion over 24 hours, 60% of the dose is excreted during the 24 hour infusion [143]. Approximately 10% of the dose is excreted in the urine as 7-hydroxymethotrexate [142, 143]. The 7-hydroxy metabolite is important since it may contribute to the renal toxicity of methotrexate [144] and a significant amount of this metabolite occurs when methotrexate doses are 50 mg/kg or greater [145]. Following oral administration of methotrexate a lesser fraction of the dose is recovered in the urine as compared to intravenous administration [140]. This may reflect the dose-dependent decrease in absorption of methotrexate [140, 146, 147]. Methotrexate is highly bound to plasma proteins.

Renal tolerance

Nephrotoxicity is a recognized adverse effect of methotrexate treatment, particularly at dosages equal or greater than 50 mg/m^2. The most commonly accepted mechanism for this drug-induced toxicity is precipitation of methotrexate or methotrexate metabolite in the distal tubules leading to intrarenal obstructive uropathy and tubular necrosis. Particularly during high-doses methotrexate therapy (> 1 g/m2), the urinary concentration of methotrexate and 7-hydroxymethotrexate can exceed the aqueous solubility of these agents depending on the urinary pH [144]. This hypothesis is supported by ability of combining urinary alkalinization and induced diuresis to decrease the incidence and severity of methotrexate-induced nephrotoxicity [148]. Direct tubular toxicity [144, 149] and decreased tubular filtration [148, 150] may also contribute to methotrexate-induced nephrotoxicity. Toxicity is enhanced when patients have been previously treated with cisplatin, or concomitantly with other nephrotoxic drugs (non-steroidal anti-inflammatory drugs) [151]. Enhanced toxicity is observed when methotrexate is administered concomitantly with another highly protein bound agent such as ketoprofen [151, 151a] but the mechanism of this interaction is unclear. It has been suggested that the decrease in renal clearance of methotrexate observed after concomitant nonsteroidal anti-inflammatory drug treatment could be explained by either a competitive inhibition of methotrexate tubular secretion or inhibition of renal prostaglandin secretion inducing altered glomerular filtration rate in the setting of pre-renal volume contraction [152].

When high-doses of methotrexate are administered, a rapid and dose-related fall in glomerular filtration rate occurs in a majority of patients [153, 154]. To minimize the risk of nephrotoxicity, patients should be euvolemic prior to receiving treatment with methotrexate. In addition, adjunctive hydration and alkalinization of the urine should be initiated for patients receiving dosages equal to or exceeding 50 mg/m^2. Because the kidney is the primary route of methotrexate clearance, and the incidence of toxicity increases as the dose of methotrexate accumulates, dosing of this agent should be adjusted to any reduction in renal function.

If the clinical condition requires acute reduction of serum methotrexate levels, significant clearance of methotrexate can be achieved with high-flux dialyzers. Serum methotrexate levels have be successfully lowered in patients with methotrexate-induced acute renal failure by charcoal hemoperfusion and sequential hemodialysis [155, 156].

Antitumor antibiotics

Mitomycin

Impaired renal function does not require a dosage adjustment for mitomycin C since less than 20% of the dose appears in the urine [157-159].

However, hemolytic-uremic syndrome (HUS), a potentially life-threatening event, has been reported to be associated with mitomycin C therapy [160]. Hematological findings include anemia, thrombocytopenia and detectable schistocytes on peripheral blood smear (Figure 3 p. 361). Acute renal failure of HUS can include proteinuria and microscopic hematuria [161-165]. The onset of signs and symptoms of renal impairment usually occurs 6-17 months following the initiation of mitomycin C treatment [166]. Corticoids and plasma exchanges have been reported to induce drastic reversal of the renal parameters [167]. The mechanism of mitomycin-induced nephrotoxicity is unknown. To prevent the occurrence of this adverse renal effect, the maximum cumulative dose should be restricted to 40 mg/m^2. Hemolytic uremic syndrome has also been reported to occur with other anticancer drugs (5-fluorouracile, vincristine, cisplatin, bleomycin, adriamycin) [168].

Immunotherapy

Interleukin-2

Recombinant interleukin-2 has provided a new approach in the treatment of renal cell carcinoma [169, 170]. High dosage interleukin-2 alone, or combined with interferon α and lymphokine activated killer cells, is being used in patients with advanced melanoma or renal cancer to induce regression of solid tumor and metastasis [171-173]. Renal toxicity, the main limiting adverse effect of interleukin-2 administration, often leads to discontinuation or reduction of the dose, especially in the aged and subjects with reduced renal function. Clinical studies have characterized a reversible syndrome associated with interleukin-2 administration that consists of hypotension, oliguria, fluid retention, azotemia, and a very low urinary excretion of sodium [174, 175]. It has been ascribed to the so-called "vascular leak syndrome". Based on experimental studies, this syndrome is thought due to a primary increase in the vascular permeability with consequent shifting of proteinaceous intravascular fluid into the interstitium of multiple organs, along with hypoalbuminemia and reduction of the intravascular volume [176]. Because of this, the acute renal failure after interleukin-2 administration was initially considered to be secondary to the "vascular leak syndrome". However, subsequent studies in cancer patients with interleukin-2 continuous infusion have reported renal failure occurring in patients with stable hemodynamics [177].

Acute interstitial nephritis characterized by parenchymal infiltration with T lymphocytes has also reported [178]. It has been suggested that acute tubular nephritis could be the result of a cytotoxic lymphocyte-mediated reaction induced by the interleukin-2 treatment [179].

In humans, the pathophysiology of interleukin-2 induced renal dysfunction is poorly understood. Interleukin-2 may act directly on the vascular tonus and endothelial integrity or may stimulate generation of other cytokines, with subsequent increase in vascular permeability. Shalmi et al. [180] suggested that interleukin-2 induced an intrinsic renal lesion since glomerular filtration rate fell in 90% of the patients (mean decrease of 43%) while the renal plasma flow was only slightly altered (mean decrease 5%) in 50% of the patients.

Since interleukin-2 induced response rate in patients with metastatic melanoma or renal cell cancer is schedule and dose dependent, and because renal toxicity is the main cause of treatment discontinuation, continuing studies are needed to elucidate a better definition of the observed nephrotoxicity.

Interferon-alpha

Interferon-α, a 165 amino acid glycoprotein, is effective in the treatment of viral hepatitis C and B, myeloma, melanoma, and renal carcinoma. Little is known about the renal metabolism of interferon-α despite extensive studies in experimental animals. In patients with normal renal function, the serum peak level occurs 8 hours after a subcutaneous injection of $3x10^6$ units of interferon-α. Terminal elimination half-life ranges from 4 to 16 hours and after 24 to 48 hours, the interferon molecule is undetectable in the serum [181]. A-interferon urinary level is undetectable. Some authors have suggested that, despite the lack of urinary excretion, the kidney could play a role in interferon-α metabolism [182]. Indeed, as far as hepatitis C treatment is concerned, dialysis patients often show a better response to therapy than non-dialysis patients. This better efficacy in dialysis patients is associated with an increase of the incidence of adverse effects. This observation raises the question of pharmacokinetic modifications. One study documented that clearance kinetics of interferon-α in patients with chronic renal failure are about half the rate of patients with normal renal function [183]. Indeed interferon is filtered by the glomeruli and largely absorbed and catabolized within tubular cells [184].

Main side effects associated with interferon-α are dose-dependent chills, fatigue and gastrointestinal disturbances. Rarely seizures, encephalopathy and strokes have been reported [185]. Although there has been considerable experience with interferons in clinical trials during the past 20 years, acute renal failure has rarely been reported. In 1976, Gresser et al. [186] described experimental lesions induced in the kidney by interferon in mice. Glomerular nephropathy was observed, either hyalinosis or rapidly progressive glomerulonephritis [187]. In humans, while proteinuria has been noted in up to 15 to 20% of patients treated with interferon [188], acute renal failure syndrome has rarely

been observed [188, 189]. Nephrotic syndrome was present in some cases [191] and the histopathology was described as a combination of acute interstitial nephrotoxicity and minimal change nephropathy. This pattern is similar to that seen with renal injury from nonsteroidal anti-inflammatory drugs and ampicillin [191]. A pathogenic role for cellular immunity being enhanced by interferon therapy has been suspected. The overall incidence of interferon-α acute renal toxicity was recently reported to occur in less than 5% in patients treated for myeloproliferative syndrome [192].

Anecdotal reports of acute renal insufficiency complicating interferon-γ treatment have been reported.

Radiation nephritis

Radiation nephropathy is defined as renal injury caused by ionizing radiation. The number of cases has increases steadily and parallels the increase of bone marrow transplant procedures using total body irradiation [193]. Radiation nephritis is dose dependent [194]. Doses traditionally associated with radiation nephropathy are above 2000 cGy. Fractionation, time and chemotherapy all influence the time course and severity of radiation-induced nephropathy. Tolerance is observed with increasing fractionation, probably because it allows repair of sublethal damage during the time between fractional doses. Therefore, chronic nephropathy can be prevented by kidney shielding or, alternatively, by fractionating doses. Previous cytotoxic chemotherapy, radiocontrast agents, antibiotics potentiate the adverse effects of ionizing radiation [195].

Radiation nephropathy can present in several forms. An acute form is usually seen within a year after radiation and presents with hypertension, anemia and edema. A more insidious, chronic form presents primarily with diminished glomerular filtration, hypertension and occasionally proteinuria. When associated with accelerated hypertension, it can lead to renal failure. Some patients may develop hypertension several years after radiation but no azotemia. In a subset of patients, mild proteinuria may be the only feature of chronic renal disease [196].

Interstitial fibrosis is the common pathologic finding in patients with chronic radiation nephritis.

Morphologic studies of radiation nephropathy have documented injury to blood vessels, glomeruli, tubular epithelium and interstitium. Recent ultrastructural studies indicate that glomerular endothelium is an early site of sustained injury [197] accompanied by endothelial disruption and leukocyte adherence. Later, tubular degeneration and atrophy occur. One pathophysiologic hypothesis places vascular injury as the main initial event [198], which helps understand the hypertension occurring in radiation nephritis but does not account for the glomerular lesions.

Even though radiation nephropathy has been recognized for a long time, only recently therapeutic trials with angiotensin converting enzyme inhibitors (ACE inhibitors) have demonstrated an attenuate of the renal injury in animals [199] and delay in the decline in renal function even after the onset of renal injury [200]. The beneficial effects of angiotensin II blockade was not shared by other antihypertensive agents (hydrochlorothiazide or verapamil). These data point to a role for the renin-angiotensin system in the pathogenesis of radiation nephropathy but clinical confirmation is lacking and the long-term benefit of ACE inhibitors has yet to be established.

References

1. Safirstein RL. Anticancer drugs. In: Clinical Nephrotoxins. De Broe ME, Porter GA, Bennett WM, Verpooten GA (editors). Kluwer Academic Publ, Dordrecht 1998; p. 261-271.
2. Meijer S, Mulder NH, Sleijfer DT, de Jong PE, Sluiter WJ, Schraffordt Koops H, van der Hem GK. Nephrotoxicity of cis-diamminedichloride platinum (CDDP) during remission-induction and maintenance chemotherapy of the testicular carcinoma. Cancer Chemother Pharmacol 1982; 8: 27-30.
3. Brock PR, Koliouska DE, Baratt TM, Yeomans E, Pritchard J. Partial reversibility of cisplatin nephrotox-icity in children. J Pediatric 1991; 118: 531-534.
4. Yuhas JM, Culo F. Selective inhibition of the nephrotoxicity of cis-dichlorodiamine platinum by WR 272l without altering its antitumor properties. Cancer Treat Rep 1980; 64: 57-64.
5. Borch RF, Pleasants ME. Inhibition of cis-platinum nephrotoxicity by diethyldithiocarbamate rescue in a rat renal model. Proc Natl Acad Sci USA 1979; 76: 6611-6614.

6. Berry J-P, Pauwells C, Tlouzeau S, Lespinats G. Effect of Selenium in combination with cis-diamminedichloroplatinum [II] in the treatment of murine fibrosarcoma. Cancer Res 1984; 44: 2864-2868.
7. Citkovic E, Hayes DM, Golbey RB, Krakoff IH. Cisplatin-nephrotoxicity: diethyldithiocarbomate, WR2721 or just water. J Clin Oncol 1991; 9: 707-709.
8. Litterst CL, Torres IJ, Guarino AM. Plasma levels and organ distribution of platinum in the rat, dog, and dog fish following intravenous administration of cis-DDP[II]. J Clin Hemat Oncol 1977; 7: 169-178.
9. Safirstein R, Daye M, Miller P, Guttenplan J. Renal disposition and metabolism of liganded platinum: implications to toxicity. Fed Proc 1980; 40: 651A (abstract).
10. Safirstein R, Miller P, Guttenplan JB. Uptake and metabolism of cisplatin by rat kidney. Kidney Int 1984; 25: 753-758.
11. Jacobs C, Kalman SM, Tretton M, Weiner MW. Renal handling of cis-diammine dichloroplatinum [II]. Cancer Treat Rep 1980; 64: 1223-1226.
12. Levi J, Jacobs C, Kalman S, McTigue M, Weiner MWJ. Mechanism of cis-platinum nephrotoxicity I. Effects on sulfhydryl groups in rat kidneys. Pharmacol Exp Ther 1980; 213: 545-550.
13. Safirstein R, Winston J, Moel D, Dikman S, Guttenplan J. Cisplatin nephrotoxicity insights into mecha-nism. Int J Andrology 1987; 10: 325-346.
13a. Townsend DM, Deng M, Zhang L, Lapus MG, Hanigan MH. Metabolism of cisplatin to a nephrotoxin in proximal tubule cells. J Am Soc Nephrol 2003;14:1-10.
14. Safirstein RL, Daye M, Guttenplan JB. Mutagenic activity and identification of excreted platinum in human and rat urine and rat plasma after administration of cisplatin. Cancer Letters 1983; 18: 329-338.
15. Bradley LJN, Yacema KJ, Lippard SJ, Essigmann JM. Mutagenicity and genotoxicity of the major DNA adduct of the antitumor drug cis-diamminedichloroplatinum [II]. Biochemistry 1993; 32: 982-988.
16. Greene MH. Is cisplatin a human carcinogen ? J Natl Cancer Inst 1992; 84: 306-312.
17. Winston JA, Safirstein R. Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. Am J Physiol 1985; 249: F490-F496.
18. Hutchinson FN, Perez EA, Gandara DR, Lawrence HJ, Kaysen G. Renal salt-wasting in patients treated with cisplatin. Ann Intern Med 1988; 108: 21-25.
19. Schilsky RL, Anderson T. Hypomagnesemia and renal magnesium wasting in patients receiving cis-diamminedichloroplatinum II. Ann Intern Med 1979; 90: 929-931.
20. Schilsky RL, Barlock A, Ozols RF. Persistent hypomagnesemia following cisplatin chemotherapy for testicular cancer. Cancer Treat Reports 1982; 66: 1767-1769.
21. Mavichak v, Wong NLM, Quamme GA, Magil AB, Sutton RAL, Dirks JH. Studies on the pathogenesis of cisplatin-induced hypomagnesemia in rats. Kidney Int 1985; 28: 914-921.
22. Swainson CP, Colls BM, Fitzharris BM. Cisplatinium and distal renal tubule toxicity. NZ Med J 1985; 98: 375-378.
23. Safirstein R, Winston J, Moel D, Dikman S, Guttenplan J. Cisplatin nephrotoxicity insights into mecha-nism. Int J Andrology 1987; 10: 325-346.
24. Schor N, Ichikawa I, Rennke HG, Troy JL, Brenner BM. Pathophysiology of altered glomerular function in aminoglycoside-treated rats. Kidney Int 1981; 19: 288-292.
25. Gordon JA, Gattone VH. Mitochondrial alterations in cisplatin-induced acute renal failure. Am J Physiol 1986; 250: F991-F998.
26. Brady HR, Kone BC, Stroniski ME, Zeidel ML, Giebisch G, Gullans SR. Mitochondrial injury: an early event in cisplatin-toxicity to renal proximal tubules. Am J Physiol 1990; 258: F1181-F1187.
27. Harder HC, Rosenberg B. Inhibitory effects of anti-tumor platinum compounds on DNA, RNA, and pro-tein synthesis in mammalian cells *in vitro*. Int J Cancer 1970; 6: 207-216.
28. Howle JA, Gale GR. Cis-dichlorodiammine platinum II persistent and selective inhibition of deoxyribo-nucleic acid synthesis *in vitro*. Biochem Pharmacol 1970; 19: 2757-2762.
29. Munchausen LL, Rahn RO. Biological and chemical effects of cis-dichlorodiammineplatinum [II] on DNA. Cancer Chemother Rep 1975; 59: 643-646.
30. Roberts JJ, Prascoe JM. Cross linking of complementary strands of DNA in mammalian cells by antitu-mor platinum compounds. Nature 1972; 235: 282-284.
31. Zwelling LA, Anderson T, Kohn KW. DNA-protein and DNA interstrand cross-linking by cis- and trans platinum [II] Diamminedichloride in Ll2lO mouse leukemia cells and relation to cytotoxicity. Cancer Res 1979; 39: 365-369.
32. Fraval HNA, Rawlings CJ, Roberts JJ. Increased sensitivity of UV repair deficient human cells to DNA bound platinum products which unlike thymine dimers are not recognized by an endonuclease extracted from Micrococcus leuteus. Mutat Res 1978; 51: 121-132.
33. Safirstein R, Zelent AZ, Gordon R. Cisplatin-nephrotoxicity: new insights into mechanisms. In: Organ directed toxicities of anticancer drugs. Hacker MP, Lazo JS, Tritton TR (editors). Martinus Nijhof, Bos-ton 1988; p. 172-189.

34. Madias NE, Harrington JT. Platinium nephrotoxicity. Am J Med 1978; 65: 307-314.
35. Goldstein RS, Mayor GH. Minireview: the nephrotoxicity of cisplatin. Life Sci 1983; 32: 685-690.
36. Heidemann HTH, Gerkens JF, Jackson EK, Branch RA. Attenuation of cisplatin-induced nephrotoxicity in the rat by high salt diet, furosemide and acetazolamide. Naunyn-Schmiedeberg's Arch Pharmacol 1985; 329: 201-205.
37. Hayes DM, Cvitkovic E, Golbey RB, Scheiner E, Helson L, Krakoff IA. High dose cisplatinium diam-mine dichloride: amelioration of renal toxicity by mannitol diuresis. Cancer 1977; 39: 1372-1381.
38. Stark JJ, Howell SB. Nephrotoxicity of cisplatinium [II]-dichlorodiammine. Clin Pharmacol Ther 1978; 23: 461-466.
39. Einhorn LH, Donohue J. Cis diammine dichloroplatinium, uniblastine and gleomycine combination che-motherapy in disseminated testicular cancer. Ann Intern Med 1977; 87: 293.
40. Cvitkovic B, Spaulding J, Bethune V, Martin J, Whitemore WF. Improvement of cis-dichlorodiammineplatinum therapeutic index in an animal model. Cancer 1977; 39: 1357-1361.
41. Pera MF Jr, Zook BC, Harder HC. Effects of mannitol or furosemide diuresis on the nephrotoxicity and physiological disposition of cis-dichlorodiammine platinum [II] in rats. Cancer Res 1979; 29: 1269-1278.
42. Litterst CL. Alterations in the toxicity of cis- dichlorodiammine platinum II and in tissue localization of platinum as a function of NaCl concentration in the vehicle of administration. Appl Pharmacol 1981; 61: 99-108.
43. Lehane D, Winston A, Gray R, Daskal Y. The effect of diuretic pretreatment on clinical morphological and ultrastructural cisplatinium induced nephrotoxicity. Int J Radiation Oncology Biol Phys 1979; 5: 1393-1399.
44. All-Sarraf M, Fletcher W, Oishi N, Pugh R, Hewlett JS, Balducci L, McCracken J, Padilla F. Cisplatin hydration with and without mannitol diuresis in refractory disseminated malignant melanoma: a south-west oncology group study. Cancer Treat Rep 1982; 66: 31-35.
45. Salem P, Khalyl M, Jabboury K, Hashimi L. Cis-diamminedichloroplatinium [II] by a 5-day continuous infusion. Cancer 1984; 53: 837-840.
46. Haas A, Anderson L, Lad T. The influence of aminoglycosides on the nephrotoxicity of cis-diammine-chloroplatinium in cancer patients. J Infect Dis 1983; 147: 363.
47. Gonzalez-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS. Acute renal failure after cis-dichlorodiammineplatinum(II) and gentamicin-cephalothin therapies. Cancer Treat Rep 1978; 62(5): 693-698.
48. Jongejan HTM, Provost AP, Molenar JC. Potentiation of cis-diamminedichloroplatinium nephrotoxicity by amikacin in rats. Cancer Chemother Pharmacol 1988; 22: 178-80.
49. Bregman CL, Williams PD. Comparative nephrotoxicity of carboplatin and cisplatin in combination with tobramycin. Cancer Chemother Pharmacol 1986;18(2):117-123.
50. Hirosawa A, Niitani H, Hayashibara K, Tsubo IE. Effects of sodium thiosulfate in combination therapy of cis-dichlorodiammine platinium and vindesine. Cancer Chemother Pharmacol 1989; 23: 255-258.
51. Howell S, Pfeifle C, Wung W, Olshen R, Lucas W, Yon J, Green M. Intraperitoneal cisplatin with sys-temic thiosulfate protection. Ann Intern Med 1982; 97: 845-851.
52. Cubeddu LX, Hoffmann IS, Fuenmayor NT, Finn AL. Efficacity of odansetron and the role of serotonin in cisplatin induced nausea and vomiting. N Engl J Med 1990; 322(12): 810-815.
53. Boven E, Van der Vijgh WJF, Nauta MM, Schlüper HMM, Pinedo HM. Comparative activity and distri-bution studies of five platinium analogues in nude mice bearing human ovarian carcinoma xenografts. Cancer Res 1985; 45: 86-90.
54. Gormley PE, Bull JM, LeRoy AF, Cysyk R. Kinetics of cis-dichlorodiammineplatinum. Clin Pharmacol Ther 1979; 25(3): 351-357.
55. Siddik ZH, Jones M, Boxall FE, Harrap KR. Comparative distribution and excretion of carboplatin and cisplatin in mice Cancer Chemother Pharmacol 1988; 21(1): 19-24.
56. Sharma H, Thatcher N, Baer J, Zaki A, Smith A, McAucliffe CA, Crowther D, Owens S, Fox BW. Blood clearance of radioactively labelled cis-diammine 1, 1-cyclobutane dicarboxylate platinum [II] (CBDCA) in cancer patients. Cancer Chemother Pharmacol 1983; 11(1): 5-7.
57. Elferink F, van der Vijgh WJ, Klein I, Pinedo HM. Interaction of cisplatin and carboplatin with sodium thiosulfate: reaction rates and protein binding. Clin Chem 1986; 32(4): 641-645.
58. Harland SJ, Newell DR, Siddik ZH, Chadwick R, Calvert AH, Harrap KR. Pharmacokinetics of cis-diammine-1, 1-cyclobutane dicarboxylate platinum[II] in patients with normal and impaired renal func-tion. Cancer Res 1984; 44(4): 1693-1697.
59. Koeller JM, Trump DL, Tutsch KD, Earhart RH, Davis TE, Tormey DC. Phase I clinical trial and phar-macokinetics of carboplatin [NSC 241240] by single monthly 30-minute infusion. Cancer 1986; 57(2): 222-225.
60. Trump DL, Elson P, Brodovsky H, Vogl SE. Carboplatin in advanced, refractory germ cell neoplasms: a phase II Eastern Cooperative Oncology Group Study. Cancer Treat Rep 1987; 71(10): 989-990.
61. Egorin MJ, Van Echo DA, Tipping SJ, Olman EA, Whitacre MY, Thompson BW, Aisner J. Pharmacoki-netics and dosage reduction of cis-diammine[1, 1-cyclobutanedicarboxylato]platinum in patients with im-paired renal function. Cancer Res 1984; 44(11): 5432-5438.

62. Gaver RC, George AM, Deeb G. *In vitro* stability, plasma protein binding and blood cell partitioning of 14C-carboplatin. Cancer Chemother Pharmacol 1987; 20(4): 271-276.
63. Harland SJ, Newell DR, Siddik ZH, Chadwick R, Calvert AH, Harrap KR. Pharmacokinetics of cis-diammine-1, 1-cyclobutane dicarboxylate platinum[II] in patients with normal and impaired renal func-tion. Cancer Res 1984; 44(4): 1693-1697.
64. Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, Siddik ZH, Judson IR, Gore ME, Wiltshaw E. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 1989; 7 (11): 1748-1756.
65. Martinez F, Deray G, Dubois M, Beaufils H, Jacquiaud C, Bourbouze R, Benhmida M, Jaudon MC, Ja-cobs C. Comparative nephrotoxicity of carboplatin and cisplatin in euvolemic and dehydrated rats. Anti-cancer Drugs 1993; 4: 85-90.
66. Levine BS, Henry MC, Port CD, Richter WR, Urbanek MA. Nephrotoxic potential of cis-diamminedichloroplatinum and four analogs in male Fischer 344 rats. J Natl Cancer Inst 1981; 67(1): 201-206.
67. Canetta R, Rozencweig M, Carter SK. Carboplatin: the clinical spectrum to date. Cancer Treat Rev 1985; 12(suppl A): 125-136.
68. Egorin MJ, Van Echo DA, Tipping SJ, Olman EA, Whitacre MY, Thompson BW, Aisner J. Pharmacoki-netics and dosage reduction of cis-diammine[1, 1-cyclobutanedicarboxylato]platinum in patients with im-paired renal function. Cancer Res 1984; 44(11): 5432-5438.
69. Reed E, Jacob J. Carboplatin and renal dysfunction. Ann Intern Med 1989; 110(5): 409.
70. Gore ME, Calvert AH, Smith LE. High dose carboplatin in the treatment of lung cancer and meso-thelioma: a phase I dose escalation study. Eur J Cancer Clin Oncol 1987; 23(9): 1391-1397.
71. Sleijfer DT, Smit EF, Meijer S, Mulder NH, Postmus PE. Acute and cumulative effects of carboplatin on renal function. Br J Cancer 1989; 60(1): 116-120.
72. Schuler U, Ehninger G, Wagner T. Repeated high-dose cyclophosphamide administration in bone marrow transplantation: exposure to activated metabolites. Cancer Chemother Pharmacol 1987; 20: 248-252.
73. Bagley CM, Bostick FW and De Wita. Clinical pharmacology of cyclophosphamide. Cancer Res 1978; 88; 226-233.
74. Struck RF, Kirk M, C, Mellet LB, el Dareer S, Hill DL. Urinary metabolites of the antitumor agent cyclo-phosphamide. Mol Pharmacol 1971; 7: 519-529.
75. Dooley JS, James CA, Rogers HJ, Stuart-Harris R. Biliary elimination of cyclophosphamide in man. Cancer Chemother Pharmacol 1982; 9: 26-29.
76. Juma FD, Rogers HJ, Trounce JR. Pharmacokinetics of cyclophosphamide and alkylating activity in man after intravenous and oral administration. Br J Clin Pharmacol 1979; 8: 209-217.
77. Mouridsen HT, Jacobsen E. Pharmacokinetics of cyclophosphamide in renal failure. Acta Pharmacol Toxicol 1975; 86: 409-414.
78. Brock N, Pohl J, Stekar J. Studies of the urotoxicity of oxazophosphorine cytostatics and its prevention. Eur J Cancer 1981; 17: 595-607.
79. Droller MJ, Sral R and Santos G. Prevention of cyclophosphamide-induced hemorrhagic cystitis. Urology 1982; 20: 256-258.
80. Link H, Neef V, Niethammer D and Wilms K. Prophylaxis of haemorrhagic cystatis due to cyclophos-phamide-conditioning for bone marrow transplantation. Blut 1981; 48: 329-330.
81. Balow JE, Austin HA 3rd, Tsokos GC, Antonovych TT, Steinberg AD, Klippel JH. NIH conference. Lupus nephritis. Ann Intern Med 1987; 106(1): 79-94.
82. Philips FS, Sternberg SS, Cronin AP, Vidal PM. Cyclophosphamide and urinary bladder toxicity. Cancer Res 1961; 21: 1577-1589.
83. Bode U, Seif SM, Levin AS. Studies on the antidiuretic effect of cyclophosphamide: vasopressin release and sodium excretion. Med Pediatr Oncol 1980; 8: 295-303.
84. De Fronzo RA, Colvin OM, Braine H, Robertson GL, Davis PJ. Cyclophosphamide and the kidney. Can-cer 1974; 33: 483-491.
85. Bode U, Seif SM, Levin AS. Studies on the antidiuretic effect of cyclophosphamide: vasopressin release and sodium excretion. Med Pediatr Oncol 1980; 8: 295-303.
86. Skinner R, Sharkey IM, Pearson AD, Craft AW. Ifosfamide, mesna, and nephrotoxicity in children (re-view). J Clin Oncol 1993;11(1): 173-190.
87. Kaijser G, Bejnen J, Bult A, Underberg W. Ifosfamide metabolism and pharmacokinetics. Anticancer Res 1994; 14: 517-532.
88. Boos J, Welslau U, Ritter J, Blaschke G, Schellong G. Urinary excretion of the enantiomers of Ifosfamide and its inactive metabolites in children. Cancer Chemother Pharmacol 1991; 28: 455-460.
89. Mohrmann M, Pauli A, Ritzer M, Schonfeld B, Seifert B, Brandis M. Inhibition of sodium-dependent transport systems in LLC-PK1 cells by metabolites of ifosfamide. Renal Physiol Biochem 1992; 15: 289-301.
90. Mohrmann M, Ansorge S, Schmich U, Schonfeld B, and Brandis M. Toxicity of Ifosfamide, cyclophos-phamide and their metabolites in tubular cells in culture. Pediatr Nephrol 1994; 8: 157-163.
91. Zamlauski-Tucker M, Morris M, Springate JE. Ifosfamide metabolite chloroacetaldehyde causes Fanconi syndrome in the perfused rat kidney. Toxicol App Pharmacol 1994; 129: 170-175.

92. Allen LM, Creaven PJ, Nelson RL. Studies on the human pharmacokinetics of Ifosfamide (NSC-109724). Cancer Treat Rep 1976; 60: 451-458.
93. Springate J, Zamlauski-Tucker M, Lu H, Chan KK. Renal clearance of Ifosfamide. Drug Metab Dispos 1997; 29 (9): 1081-1082.
94. Suarez A, McDowell H, Niaudet P, Comoy E, Flamant F. Long term follow up of Ifosfamide renal toxic-ity in children treated for malignant mesenchymal tumors: an international society of pediatric oncology report. J Clin Oncol 1991; 9: 2177-2182.
95. Pratt CB, Meyer WH, Jenkins JJ, Avery L, McKay CP, Wyatt RJ, Hancock ML. Ifosfamide, Fanconi's syndrome and reickets. J Clin Oncol 1991; 9: 1495-1499.
96. Moncrieff M, Foot A. Fanconi syndrome after Ifosfamide. Cancer Chemother Pharmacol 1989; 23: 121-122.
97. Skinner R, Sharkey IM, Pearson ADJ, Craft AW. Ifosamide, mesna and nephrotoxicity in children. J Clin Oncol 1993; 11: 173-190.
98. Brock N, Pohl J, Stekar J. Studies on urotoxicity of oxazophosphorine cytostatics and its prevention. Eur J Cancer 1981; 17: 595-607.
99. Van Dyck JJ, Falkson HC, Van der Merwe AM, Falkson G. Unexpected toxicity in patients treated with ifosfamide. Cancer Res 1972; 32: 921-924.
100. Falkson G, Van Dick JJ, Stapelberg R, Falkson HC. Mesnum as protector against kidney and bladder toxicity with high-dose ifosfamide treatment. Cancer Chemother Pharmacol 1982; 9: 81-84.
101. Stuart-Harris R, Harper PG, Kaye SB, Wiltsaw E. High-dose ifosfamide by infusion with mesna in ad-vanced soft tissue sarcoma. Cancer Treat Rep 1988; 10(suppl A):163-164.
102. Newbury-Ecob RA, Noble VW, Barbor PRH. Ifosfamide-induced Fanconi syndrome. Lancet 1989; 2: 1328.
103. Patterson WP, Khojasteh A. Ifosfamide-induced renal tubular defects. Cancer 1989; 63: 649-651.
104. Stuart-Harris RC, Harper PG, Parsons CA, Kaye SB, Mooney CA, Gowing NF, Wiltshaw E. High-dose alkylation therapy using ifosfamide infusion with mesna in the treatment of adult advanced soft-tissue sarcoma. Cancer Chemother Pharmacol 1983; 11(2): 69-72.
105. Willemse PHB, De Jong PE, Elema JD, Mulder NH. Severe renal failure following high-dose Ifosfamide and Mesna. Cancer Chemother Pharmacol 1989; 23: 329-330.
106. Elias AD, Eder JP, Shea T, Begg C, Frei E III, Antman K. High-dose Ifosfamide with mesna uroprotec-tion: a phase I study. J Clin Oncol 1990; 8: 170-178.
107. Rougier P, Mitry E. Chemotherapy in the treatment of neuroendocrine malignant tumors (review). Diges-tion 2000; 62 (Suppl 1): 73-78.
108. Bhuyan BK, Kuentzel SL, Gray LG, Fraser TJ, Wallach D, Neil GL. Tissue distribution of streptozotocin (NCS-85998). Cancer Chemother Rep 1974; 58: 157-165.
109. Adolphe AB, Glasofer ED, Troetel WM, Ziegenfuss J, Stambaugh JE, Weiss AJ, Manthei RW. Fate of streptozotocin [NSC-85998] in patients with advanced cancer. Cancer Chemother Rep 1975; 59: 547-556.
110. Schein PS, O'Connell MJ, Blom J, Hubbard S, Magrath IT, Bergevin P, Wiernik JL, Ziegler PH, DeVita VT. Clinical antitumor activity and toxicity of streptozotocin (NSC-85998). Cancer 1974; 84: 993-1000.
111. Myerowitz RL, Sartiano GP, Cavallo T. Nephrotoxic and cytoproliferative effects of streptozotocin. Can-cer 1976; 88: 1550-1555.
112. Perry DJ, Weiss RB. Nephrotoxicity of streptozotocin. Ann Int Med 1982; 96: 122.
113. Sadoff L. Nephrotoxicity of streptozotocin (NSC-85998). Cancer Chemother Rep 1970; 54: 457-459.
114. Moertel CG, Reitemeir RJ, Schutt AJ, Hahn RG. Phase II study of streptozotocin (NSC-85998) in the treatment of advanced gastro-intestinal cancer. Cancer Chemother Rep 1971; 55: 303-307.
115. Broder LE, Carter SK. Pancreatic islet cell carcinoma. II Results of therapy with streptozotocin in 52 patients. Ann Intern Med 1973; 79: 108-118.
116. Loftus L, Cuppage FE, Hoogstraten B. Clinical and pathological effects of streptozotocin. J Lab Clin Med 1974; 84: 407-413.
117. Myerowitz RL, Sartiano GP, Cavallo T. Nephrotoxic and cytoproliferative effects of streptozotocin. Re-port of a patient with multiple hormone-secreting islet cell carcinoma. Cancer 1976; 38: 1550-1555.
118. Anderson T, Schein PS, McMenamin MG, Gooney DA. Streptozotocin diabetes. Correlation with extend of depression of pancreatic islet nicotinamide adenine dinucleotide. J Clin Invest 1974; 54: 672-677.
119. Schein PS, Loftus S. Streptozotocin: depression of mouse liver pyridine nucleotides. Cancer Res 1968; 28: 1501-1506.
120. Horton L, Fox C, Corrin B, Sönksen PH. Streptozotocin-induced renal tumors in rat. Br J Cancer 1977; 36: 692-699.
121. Rakieten N, Gordon BS, Cooney DA, Davis RD, Schein PS. Renal tumorigenesis action of streptozotocin (NSC-85998) in rats. Cancer Chemother Rep 1968; 52: 563-567.
122. Dumontet C, Thieblemont C, Espinouse D, Bouafia F, Hequet O, Salles G, Coiffier B. A prospective study of intensive induction therapy with high-dose consolidation in patients with aggressive non-Hodgkin's lymphoma and two or three adverse prognostic factors. Leukemia. 2000; 14(12): 2159-2165.

123. Dillman RO, Soori G, Wiemann MC, Schulof R, Dobbs TW, Ruben RH, DePriest CB, Church C. Phase II trial of subcutaneous interleukin-2, subcutaneous interferon-α, intravenous combination chemo-therapy, and oral tamoxifen in the treatment of metastatic melanoma: final results of cancer biotherapy re-search group 94-11. Cancer Biother Radiopharm 2000;15(5): 487-494.
124. Nieder C, Grosu AL, Molls M. A comparison of treatment results for recurrent malignant gliomas. Can-cer Treat Rev 2000; 26(6): 397-409.
125. Engelhard HH. The role of interstitial BCNU chemotherapy in the treatment of malignant glioma. Surg Neurol 2000; 53(5): 458-464.
126. Spitzer G, Dickie KA, Litam Y, Verma DS, Zander A, Lanzotti V, Valdivieso M, McCredie KB, Samuels ML. High-dose combination of chemotherapy with autologous bone marrow transplantation in adult solid tumors. Cancer 1980; 45: 3075-3085.
127. Olivero VT. Toxicology and pharmacology of the nitrosoureas. Cancer Chemother Rep 1973; 4(3): 13-20.
128. Wheeler GP. A review of studies on the mechanism of action of the nitrosoureas. In: Cancer chemother-apy, vol 30. Sartorelli AC (editor). Am Chem Soc, Washington DC 1976; p. 87-119.
129. De Vita VT, Carbone PP, Owens AH, Gold GL, Krant MJ, Edmonson J. Clinical trials with 1, 3-bis [2-chloroethyl]-1-nitrosourea, NSC 409962. Cancer Res 1965; 25:1876-1881.
130. Bagley CM, Bostick FW, DeVita VT. Clinical pharmacology of cyclophosphamide. Cancer Res 1973; 33(2): 226-233.
131. Goupil A, Baglin A, Clavel B, Verger C, Fritel D. Chronic renal failure after CCNU treatment [author's transl]. Nouv Presse Med 1980; 9(41): 3069-3070.
132. Harmon WE, Cohen HJ, Schneeberger EE, Grupe WE. Chronic renal failure in children treated with methyl CCNU. N Engl J Med 1979; 300(21): 1200-1203.
133. Nichols WC, Moertel CG. Nephrotoxicity of methyl CCNU. N Engl J Med. 1979; 301(21): 1181.
134. Schacht RG, Feiner HD, Gallo GR, Lieberman A, Baldwin DS. Nephrotoxicity of nitrosoureas. Cancer 1981; 48(6): 1328-1334.
135. Silver HKB, Morton DL. CCNU nephrotoxicity following sustained remission in oat cell carcinoma. Cancer Treat Rep 1979; 63: 226-227.
136. Berglund J. Progressive renal insufficiency after CCNU therapy. Läkartidningen 1980; 77: 1760.
137. Schacht RG, Feiner HD, Gallo GR, Liebermann A, Baldwin DS. Nephrotoxicity of Nitrosoureas. Cancer 1981; 48: 1328-1334.
138. Liegler DG, Henderson ES, Hahn MA, Oliverio VT. The effect of organic acids on renal clearance of methotrexate on man. Clin Pharmacol Ther 1989; 10: 849-857.
139. Patel OD, Morgenthaler FR, Khazei AM, Grimaidi R, Watkins E. Methetrexate excretion patterns and renal toxicity. Arch Surg 1969; 98: 305-308.
140. Balis FM, Savich JL, Bleyer WA. Pharmacokinetics of oral methotrexate in children. Cancer Res 1983; 43: 2342-2345.
141. Shen DD, Azarnoff DL. Clinical pharmacokinetics of methetrexate. Clin Pharmacokinet 1978; 8:1-18.
142. Breithaupt H, Kuenzlen E. Pharmacokinetics of meethotrexate and 7-hydroxymethotrexate following infusions of high-dose methetrexate. Cancer Treat Rep 1982; 66: 1733-1741.
143. Winograd B, Lippens RJJ, Oosterbaan MJM, Dirks MJM, Vree TB, van der Kleijn E. Renal excretion and pharmacokinetics of methotrexate and 7-hyfroxymethotrexate following a 24 hours high dose infusion of methotrexate in children. Eur J Clin Pharmacol 1986; 30: 231-238.
144. Rieselbach RF, Garnick MB. 1988. Renal diseases indiced by antineoplastic agents. In: Diseases of the kidney, 4th edition. Schrier RW, Gottschalk CW (editors). Little Brown and Company, Boston 1988; p. 1275-1299.
145. Jacobs SA, Stoller RG, Chabner BA, Johns DG. Dose-dependent metabolism of methotrexate in man and Rhesus monkey. Cancer Treat Rep 1977; 61: 651-658.
146. Pearson ADJ. Mills S, Amineddine HA, Long DR, Craft AW, Chessels JM. Pharmacokinetics of oral and intramuscular methotrexate in children with acute lymphoblastic leukaemia. Cancer Chemother Pharma-col 1987; 20: 243-247.
147. Sonneveld P, Schultz FW, Nooter K, Hahlen K. Pharmacokinetics of methotrexate and 7-hydroxymethotrexate in plasma and bone marrow of children receiving, low-doses oral methotrexate. Cancer Chemother Pharmacol 1986; 18: 111-118.
148. Pittman SW, Frei E. Weekly methotrexate-calcium leucovorin rescue: effect of alkalinization on nephro-toxicity; pharmacokinetics in the CNS, and use in CNS non Hodgkin's lymphoma. Cancer Treat Rep 1977; 61: 695-701.
149. Glode LM, Pitman SW, Ensminger WD. A phase I study of high dose of aminopterin with leucoverin rescue in patients with advanced metastatic tumors. Cancer Res 1979; 39: 3707-3714.
150. Link DA, Fosberg MT, Ingelfinger JR. Renal toxicity of high-dose methotrexate. Pediatr Res 1976; 10: 455 (abstract).
151. Thyss A, Milano G, Kubar J, Namer M, Schneider M. Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. Lancet 1986; 1: 256-258.
151a. Blum R, Seymour JF, Toner G. Significant impairment of high-dose methotrexate clearance following vancomycin administration in the absence of overt renal impairment. Ann. Oncol 2002 Feb;13:327-30.
152. Kremer JM, Hamilton RA. The effects of nonsteroidal antiinflammatory drugs on methotrexate [MTX]pharmacokinetics: impairment of renal clearance of MTX at weekly maintenance doses but not at 7.5 mg. J Rheumatol 1995; 22: 2072-2277.

153. Pitman SW, Parker LM, Tattersall MNH, Jaffe N, Frei E. Clinical trial of high-dose methotrexate with citrovorum factor-toxicologic and therapeutic observations. Cancer Chemother Rep 1975; 6: 43-49.
154. Abelson HT, Fosburg MT, Beardsley GP. Methotrexate-induced renal impairment. J Clin Oncol 1983; 1: 208-216.
155. Molina R, Fabian C, Cowley B. Use of charcoal hemoperfusion wih sequential hemodialysis to reduce serum methotrexate levels in a patient with acute renal insufficiency. Am J Med 1987; 82: 350-352.
156. Relling MV, Stapelton BF, Ochs J, Jones DB, Meyer W, Wainer IW, Crom WR, McKay CP, Evans WE. Removal of methotrexate, leucoverin and their metabolites by combined hemodialysis and hemoperfu-sion. Cancer 1988; 62: 884-888.
157. van Hazel GA, Scott M, Rubin J, Moertel CG, Eagan RT, O'Connell MJ, Kovach JS. Pharmacokinetics of mitomycin C in patients receiving the drug alone or in combination. Cancer Treat Rep 1982; 67: 805-810.
158. Schilder RB, Young JD, Ratanatharathorn V, Karanes C, Baker LH. Clinical pharmacokinetics of high-dose mitomycin C. Cancer Chemother Pharmacol 1984; 13: 186-190.
159. Erlichman C, Rauth AM, Battistella R, Fine S. Mitomycin C pharmacokinetics in patients with recurrent or metastatic colorectal carcinoma. Can J Physiol Pharmacol 1987; 65: 407-411.
160. Lelesne JB, Rothschild N, Erickson B, Koreu S, Sisk R, Keller J, Arbus M, Wooley P, Chiazze L, Schein PS, Neefe JR. Cancer-associated hemolytic-uremic syndrome: analysis of 85 cases from the national reg-istry. J Clin Oncol 1989; 7: 781-789.
161. Cantrell JE, Phillips TM, Schein PS. Carcinoma-associated hemolytic-uremic syndrome: a complication of mitomycin chemotherapy. J Clin Oncol 1985; 3: 723-734.
162. Hanna WT, Krauss S, Regester RF, Murphy WM. Renal disease after Mitomycin C therapy. Cancer 1981; 48: 2583-2588.
163. Gulati SC, Sordillo P, Kempin S, Reich L, Magill GB, Scheiner E and Clarkson B. Microangiopathic hemolytic anemia observed after treatment of epidermoid carcinoma with mitomycin C and 5-fluorouracil. Cancer 1980; 45: 2252-2257.
164. Bayle F, Vialtel P, Bastrenta F, Swiercz P, Dechelette E, Pin I, Carpentier P, Mousseau M, Schaerer R, Couderc P, Cordonnier D. Thrombotic microangiopathy and chronic renal failure caused by mitomycin C in 4 women treated for breast cancer. Nouv Presse Med 1982; 11(30): 2300-2301
165. Hanna WT, Krauss S, Rolland FR, Murphy WM. Renal disease after mitomycin C therapy. Cancer 1981; 48: 2583-2588.
166. LiuK, Mittelmann A, Sproul RR, Elias EG. Renal toxicity in man treated with mitomycin C. Cancer 1971; 28: 1314-1320.
167. Lyman NW, Michaelson R, Viscuso RL, Winn R, Mulgaonakar S, Jacobs MG. Mitomycin-induced hemolytic-uremic syndrome. Successful treatment with corticosteroids and intense plasma Arch Intern Med 1983;143(8):1617-1618.
168. Jackson AM, Rose BD, Graff LG, Jacobs JB, Schwartz JH, Strauss GM, Yang JP, Rudnick MR, Elfen-bein IB, Narins RG. Thrombotic microangiopathy and renal failure associated with antineoplastic chemo-therapy. Ann Intern Med 1984;101(1): 41-44.
169. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghause SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, Seipp CA, Simpson CG, Reichert CM. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Eng J Med 1985; 313: 1485-1491.
170. Law TM, Motzer RJ, Mazumdar M, Sell KW, Walther PJ, O'Connell M, Khan A, Vlamis V, Vogelzang NJ, Bajorin DF. Phase III randomized trial of interleukine-2 with or without lymphokine activated killer cells in the treatment of patients with advanced renal cell carcinoma. Cancer 1995; 76: 824-832.
171. Rozenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, et al. A progress report on the treatment of 157 patients with advanced cancer using lym-phokine activated killer cells and interleukine 2 or high dose interleukine 2 alone. N Eng J Med 1987; 316: 889-897.
172. Thompson JA, Shulman KL, Benyunes MC, Lindgren CG, Collins C, Lange PH, Bush WH Jr, Benz LA, Fefer A. Prolonged continuous intravenous infusion interleukin-2 and lymphokine activated killer cell therapy for metastatic renal cell carcinoma. J Clin Oncol 1992;10: 960-968.
173. Guleria AS, Yang JC, Topalian SL, Weber JS, Parkinson DR, MacFarlane MP, White RL, Steinberg SM, White DE, Einhorn JH, et al. Renal dysfunction associated with the administration of high-dose inter-leukin-2 in 199 consecutive patients with metastatic melanoma or renal cell carcinoma. J Clin Oncol 1994; 12: 2714-2722.
174. Webb DE, Austin HA, Belldegrun A, Vaughan E, Linehan WM, Rosenberg SA. Metabolic and renal effects of Interleukin-2 immunotherapy for metastatic cancer. Clin Nephrol 1988; 30 (3): 141-145.
175. Philip T, Mercatello A, Negrier S, Philip I, Rebattu P, Kaemmerlin P, Gaspard M, Tognier E, Combaret V, Bijman JT, et al. Interleukine-2 with and without LAK cells in renal cell carcinoma: the Lyon first-year experience with 20 patients. Cancer Treat Rev 1989: 13(suppl A): 91-104.
176. Rosenstein M, Ettinghausen SE, Rosenberg SA. Extravasation of intravascular fluid wediated by the systemic administration of recombinant interleukin-2. J Immunol 1986; 137L1735-1742.
177. Ponce P, Cruz J, Travassos J, Moreira P, Oliveira J, Melo-Gomes E, Gouveia J. Renal toxicity mediated by continuous infusion of recombinant interleukin-2. Nephron 1993; 64(1):114-118.
178. Feinfeld DA, D'Agati Y, Dutcher JP, Werfel SB, Lynn RI, Wiernik PH. Interstitial nephritis in a patient receiving adoptive immunotherapy with recombinant interleukin-2 and lymphokine-activated killer cells. Am J Nephrol 1991; 11(6): 489-492.

179. Valsveld LT, van de Wiel-van Kamenade E, de Boer AJ, Sein JJ, Galle MP, Krediet RT, Mellief CJ, Rankin EM, Hekman A, Figdor CG. Possible role for cytotoxic lymphocytes in the pathogenesis of acute intertitial nephritis after recombinant interleukin-2 treatment for renal cell cancer. Cancer Immunol Im-munother 1993; 36(3): 210-213.
180. Shalmi CL, Dutcher JP, Feinfeld DA, Chun KJ, Saleemi KR, Freeman LM, Lynn RI, Wiernik PH. Acute renal dysfunction during interleukin-2 treatment: suggestion of an intrinsic renal lesion. J Clin Oncol 1990; 8(11): 1839-1846.
181. Wills RJ, Clinical pharmacokinetics of interferons. Clin Pharmacokinet 1990; 19: 390-399.
182. Grimbert P, Deray G, Lebon P, Faucher C, Malingre P, Raymond F, Petitclerc T, Jacobs C. Pharmacoki-netics of recombinant leukocyte interferon in a patient on continuous ambulatory peritoneal dialysis. Am J Nephrol 1995; 15(2): 175.
183. Rostaing L, Chatelut E, Payen JL, Izopet J, Thalamas C, Ton-That H, Pascal JP, Durand D, Canal P. Pharmacokinetics of α IFN-2b in chronic hepatitis C virus patients undergoing hemodialysis or with normal renal function. Clinical implications. J Am Soc Nephrol 1998; 9: 2344-2348.
184. Bocci V, Pacini A, Muscettola M, Paulesu L, Pessina GP, santiano M, Viano AI. Renal filtration, absorp-tion and catabolism of human α interferon. J Interferon Res 1981; 3: 347-352
185. Bailly F, Mattei A, SiAhmed SN, Trepo C. Uncommon side-effects of interferon. J Viral Hep 1997; 4(suppl 1): 89-94.
186. Gresser I, Maury M, Tovey M, Morel Maroger L, Pontillon F. Progressive glomerulonephritis in mice treated with interferon preparation at birth. Nature 1976; 263: 420-422.
187. Morel-Maroger L, Sloper JC, Vinter J, Woodrow D, Gresser I. An ultrastructural study of the develop-ment of nephritis in mice treated with interferon in the neonatal period. Lab Invest 1978; 39(5): 513-522.
188. Quesada JR, Talpaz M, Rios A, Kurzrock R, Gutterman JU. Clinical toxicity of interferons in cancer patients: a review. J Clin Oncol 1986; 4(2): 234-243.
189. Miranda-Guardiola F, Fdez-Llama P, Badia J, Botey A, Estruch R, Darnell A, Rozman C and Revert L. Acute renal failure associated with α-interferon therapy for chronic hepatitis B. Nephrol Dial Trans-plant 1995; 10: 1441-1443.
190. Nair S, Ernstoff M, Bahnson R, Arthur S, Johnston J, Downs M, Neuhart J, and Kirkwood J. Interferon-induced reversible acute renal failure with nephrotic syndrome. Urology 1992; 39(2): 169-172.
191. Averbuch SD, Austin HA, Sherwin SA, Antonovych T, Bunn PA, Longo DL. Acute interstitial nephritis with the nephrotic syndrome following recombinant leukocyte a interferon therapy for mycosis fun-goides. N Engl J Med. 1984; 310(1): 32-35.
192. Kurschel E, Metz-Kurschel U, Niederle N, Aulbert E. Investigations on the subclinical and clinical nephrotoxicity of interferon α IIB in patients with myeloproliferative syndromes. Renal Failure 1991; 13: 87-93.
193. Cohen E. Radiation nephropathy after bone marrow transplantation. Kidney Int 2000; 58: 903-918.
194. Redd BL. Radiation nephritis: review, case report and animal study. Roentgenol 1960; 83: 88.
195. Phillips TL, Wharam MD, Margolis LW. Modification of radiation injury to normal tissues by chemo-therapeutic agents. Cancer 1975; 35: 1678-1684.
196. Kelly CJ and Neilson EG. Interstitial diseases. In Brenner & Rector's 5th edition. Brenner BB (editor). WB Saunders Company, Phildelphia 1996; p. 1655-1679
197. Jaenke RS, Robbins MEC, Bywaters T, Whitehouse E, Rezvani M, Hopewell JW. Capillary endothelium: target site of renal radiation injury. Lab Invest 1993; 68: 396-405.
198. Rubin P, Casarett GW. Clinical Radiation pathology. Saunders, Philadelphia 1968; p. 293-333.
199. Robbins MEC, Hopewell JW: Physiological factors effecting renal radiation tolerance: A guide to the treatment of late effects. Br J Cancer 1986; 53: 265-267.
200. Cohen EP, Fish BL, Moulder JE. Treatment of radiation nephropathy with captopril. Radiat Res 1992; 132: 346-350.

19

Anesthetic agents

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Introduction

Renal function impairment remains a common event in connection with anesthesia and surgery. Severe perioperative renal dysfunction accounts for one half of all patients requiring acute dialysis [1] and is associated with a mortality in excess of 50% [2]. Mild to moderate renal function impairment is surprisingly common after surgery. In a group of 278 patients undergoing non-emergency general, vascular or gynecological surgery, 65 of the patients developed an increase of serum creatinine levels ≥ 20% within the first six postoperative days [3]. Thirty-two of the patients had increases that were sustained for more than 48 hours. For half of these patients, creatinine clearance had not returned to baseline levels by the time of discharge. In most cases, the perioperative changes in renal function are not due to the anesthetic agent itself, although some volatile anesthetics have nephrotoxic potential due to direct toxicity of their metabolites. Instead, postoperative renal failure is more commonly multifactorial. Risk factors include: preexisting renal and/or cardiac disease, the type of surgical procedure, occurrence of rhabdomyolysis or hemolysis, adverse hemodynamic events, inappropriate fluid management, and concurrent administration of potentially nephrotoxic substances such as radiographic contrast dyes, aminoglycoside antibiotics and cyclosporine. Such risk factors usually play a more important role than the anesthetic agent in the development of postoperative renal dysfunction [4].

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Comparative renal pharmacology of inhaled and injectable anesthetic agents

Inhaled anesthetic agents

Since Pringle et al [5] described oliguria during ether anesthesia (1905); many investigators have focused on the effects of anesthesia on renal function. All general anesthetics have significant but reversible effects on renal function. These effects are mediated either directly, by changes in renal vascular resistance, renal blood flow, glomerular filtration rate and renal tubular function or indirectly, by changes in cardiovascular function and neuroendocrine activity.

Modern inhaled anesthetic agents; halothane, enflurane, isoflurane, desflurane and sevoflurane all decrease glomerular filtration rate, sodium excretion and urine output [6-10]. Studies of the response of renal blood flow to these agents have yielded conflicting results. Initially investigators using clearance techniques concluded that halothane and enflurane reduce renal blood flow [7, 10]. Later studies employing direct measurement techniques indicate that clinical doses of inhaled agents decrease renal vascular resistance thus maintaining renal blood flow when perfusion pressure decreases during anesthesia [11-15]. These changes are transient and usually return to normal in the immediate postoperative period. Even prolonged hypotension to a mean arterial pressure of 60 mmHg, induced with isoflurane, was, in one study, not associated with any persistent derangement of renal function postoperatively [16].

Injectable anesthetic agents

Sodium thiopental does not alter renal blood flow although glomerular filtration rate and urine output are moderately affected [17]. The same is true for opioids such as morphine [18] and fentanyl [19-20] and the more recently introduced i.v. agent propofol [21]. The effects of these drugs on renal function are transient. There is no evidence that injectable anesthetic agents are associated with direct nephrotoxicity.

In addition to the effects of anesthetic agents themselves, other intraoperative interventions may also influence renal function. The initiation of mechanical ventilation, especially with the application of positive end-expiratory pressure, is associated with decreases in sodium excretion and urine output [22-24]. Decreased cardiac output, increased sympathetic outflow and release of renin and decreased release of atrial natriuretic peptide have all been implicated as being responsible for these changes [25, 26].

In summary, virtually all anesthetic agents and techniques are associated with reductions in glomerular filtration rate and urine output. These changes are usually readily reversed in the immediate postoperative period. They represent the net effect of complex interactions between direct actions of the anesthetics on the kidney and indirect effects mediated through changes in cardiac output, blood pressure and neuroendocrine function.

Metabolism of inhaled anesthetic agents

Anesthetic agents may also directly influence renal function due to toxic effects from biodegradation products. On rare occasions renal failure will result.

Modern inhalation anesthetics are fluorinated to reduce the flammability. They were initially considered to be biochemically inert substances. However, with time came the recognition that not only are inhaled anesthetics metabolized *in vivo* [27] but also that their metabolites are responsible for both acute and chronic toxicities [28, 29]. Information gained from research over the past 30 years has led to changes in anesthesia practice, discontinuing the use of some anesthetics, for example methoxyflurane, due to its nephrotoxicity and more selective use of others, i.e. halothane, due to its rare liver toxicity. It has also provided the impetus for the development of new agents, isoflurane and desflurane, with properties that lower their toxic potential. The result has been improved safety but room remains for further improvement as our insight into toxicological mechanisms expands.

Initial metabolism of inhaled anesthetics involves the cytochrome P-450 enzymes, located in the microsomes of the liver and the kidneys [30, 31], most commonly by oxidation. Some agents, i.e. halothane, may under certain circumstances, also undergo reduction. In addition to their primary metabolism, some agents, for instance sevoflurane, also undergo phase II conjugation reactions prior to excretion.

The cytochrome P-450 enzyme system is comprised of multiple isoenzymes, which are inducible to vary-

ing degrees [32, 33]. These two characteristics are major determinants of metabolic pathways and rates. Induction can be caused by exposure to one or more of a large variety of compounds. Examples include: ethanol, phenobarbital, cimetidine, phenytoin, isoniazid and some volatile anesthetics. Both transcriptional and translational processes are stimulated by the inducer to produce cytochrome P-450 [34]. Expression of the various isoenzymes depends, not only on induction, but also on such factors as sex, obesity, fasting and diabetes. Streptozotocin induced diabetes in rats, which increases (P-4502E) several fold, enhances enflurane and isoflurane metabolism [35].

Most halogenated anesthetics are similar in composition. Despite that, they vary greatly in their rate and pathway of metabolism. Minor alterations in configuration can be associated with major changes in metabolism. Also, their degree of lipid solubility, which governs the drugs access to and duration at metabolizing enzyme sites, is important in determining metabolic rate and the amount of drug that is biotransformed.

Halothane

Halothane [CF_3 CH Br Cl] the first of the modern halogenated volatile anesthetics was introduced into clinical practice in 1956. It is normally metabolized in an oxidative pathway forming bromide ions and trifluoroacetic acid, neither of which has tissue toxic potential [36, 37]. Reductive metabolism of halothane takes place during low tissue oxygen tension states [38]. This pathway has been linked to halothane induced liver necrosis through production of free radicals that bind to cellular macromolecules [39, 40]. Reductive metabolism is also associated with production of fluoride ions [41]. The quantities found are too small to have nephrotoxic importance.

The extent of halothane metabolism has been reported to be 17-20% of an administered dose [36]. The major route of halothane metabolism, oxidation to trifluoroacetic acid, does not release fluoride. Fluoride induced renal toxicity, therefore, is not a concern with halothane.

Enflurane

Enflurane (CHF_2OCF_2CHClF), in clinical use for the last three decades, is metabolized to a much lesser degree than halothane. Approximately 2-3% of a given dose undergoes biodegradation [42]. The chief metabolite is difluoromethoxydifluoroacetic acid but also fluoride ions, in sufficient quantity, to merit some concern regarding renal function [43]. Plasma inorganic fluoride concentrations after clinical enflurane anesthesia are usually in the 15-25 μM range [9, 10, 44]. Longer procedures [45] and obesity [46] are associated with higher postanesthetic fluoride levels. A study of surgical patients with preanesthetic chronic consumption of enzyme inducing drugs such as phenobarbital, phenytoin, diazepam and ethanol did not reveal increased plasma fluoride levels compared to untreated patients [47]. In contrast, about 50% of surgical patients on chronic isoniazid therapy demonstrated significantly elevated plasma fluoride concentrations after enflurane anesthesia [48]. Enflurane is the only modern inhaled anesthetic that has been linked to fluoride induced renal failure in a very limited number of cases [49, 50].

Isoflurane

Isoflurane ($CHF_2OCH_2ClCF_3$), in clinical use for about almost twenty years, is an isomer of enflurane. It has a very low degree of defluorination [51]. Approximately 0.2-0.4% of a given dose is metabolized. Fluoride levels in humans after isoflurane anesthesia peak at 4-6 μM, which represents only a modest rise over basal fluoride levels. Enzyme induction increases defluorination somewhat, but is not associated with plasma fluoride concentrations of clinical significance [52, 53].

Sevoflurane

Sevoflurane (fluoromethyl-1,1,1,3,3,3-hexafluoro-2-propyl ether) first used in Japan, was introduced into American clinical practice in 1995. Sevoflurane is defluorinated to approximately the same extent as enflurane. Initial studies reported plasma levels of fluoride, in connection with sevoflurane anesthesia, comparable to those seen after enflurane administration [54, 55]. More recent studies report that plasma fluoride concentrations often rise above 50 mM [56, 57]. Due to sevoflurane's low blood/gas solubility, only limited stores build up during anesthesia and as a result, fluo-

ride levels fall very quickly after termination of anesthesia. *In vivo*, defluorination in rats is increased by pretreatment with phenobarbital [58].

Numerous studies have addressed the same issues, as with enflurane, regarding fluoride production and nephrotoxic potential. These studies include fluoride levels after prolonged exposure [56, 57, 59], urine concentrating ability [57, 59-61], effect of obesity [60] and the effect of preexisting renal function impairment [62, 63]. The consensus from these studies is that sevoflurane has little potential for fluoride-induced nephrotoxicity.

Sevoflurane undergoes degradation by soda lime and barium hydroxide lime both of which are used in modern anesthesia machines for CO_2 absorption. The chief degradation product is fluoromethyl-2,2-difluoro-1(trifluoromethyl) vinylether, also called compound A [64]. In an anesthesia circle circuit, using the above absorbents, compound A concentrations correlate directly with sevoflurane concentrations and absorbent temperature and inversely with fresh gas inflow rate [65]. Increasing inflow rates decrease compound A concentrations by decreasing rebreathing of gas, that has passed through the absorbent, thereby decreasing the amount of CO_2 that reaches the absorbent. The amount of CO_2 absorbed determines the temperature of the absorbent, since CO_2 absorption is an exothermic reaction [65, 66].

Compound A is nephrotoxic in rats at thresholds estimated at 180 ppm/hour [67, 68]. Renal toxicity is characterized histologically by proximal tubular cell degeneration and necrosis in the corticomedullar region of the kidney and biochemically by proteinuria, glucosuria and enzymuria (NAG and α-GST) with increased serum creatinine and BUN concentrations occurring with severe toxicity [67-70].

In humans there seems to be a dose dependent association between compound A exposure and the appearance of urinary biomarkers, such as albumin, glucose and enzymes (NAG or α-GST). These findings appear in studies where the compound A exposure exceeds 160 ppm/hour [71-74], while they are absent from studies with lower compound A exposure [75-77]. In all studies associated with higher exposure of compound A, the urinary markers have been transient, lasting 3-5 days, with total normalization within one week. There is no correlation between serum creatinine and the urinary markers.

In summary: The available information indicates that sevoflurane anesthesia is nontoxic to the kidney as long as exposure to compound A is kept below 150 ppm/hour. However, there are significant questions regarding the potential for compound A to cause renal injury: are larger doses than 160 ppm/hour harmful? Do they cause histologically detectable tissue damage? Is there a cumulative effect of repeated exposures? Are particular patients more prone to injury?

The concerns and questions around the degradation of sevoflurane by CO_2 absorbents to toxic compounds may become a moot point, by the use of absorbents that minimally degrade sevoflurane [78-80]. Such absorbents exist and they do not contain sodium and/or potassium hydroxide. An example of such an absorbent is Amsorb® (Armstong Medica Ltd., Coleraine, Northern Ireland). This absorbent is widely used in Europe and it is completely inert when brought into contact with sevoflurane [81].

Desflurane

Desflurane ($CHF_2OCHFCF_3$), in clinical use in the U.S. for about a decade, has a very low lipid solubility [82]. Desflurane is highly resistant to metabolism and to degradation in soda lime [83]. Data from studies in rats and humans suggest that desflurane is free from hepatic and renal toxicity [84-86]. Serum inorganic fluoride concentrations do not rise above background levels even after prolonged exposure to desflurane [87, 88]. Due to its boiling point of 23.5°C it requires a special vaporizer to ensure a stable output.

Mechanisms of fluoride toxicity

The exact mechanism(s) responsible for fluoride's nephrotoxicity remain to be defined. The fluoride ion interferes with normal cell function on several levels. Fluoride is an inhibitor of several cellular enzyme systems and diminishes tissue respiration and anaerobic glycolysis [89]. In the kidney, fluoride interferes with transport of sodium in the proximal convoluted tubule. It also inhibits adenylate cyclase in the collecting system and diminishes the action of antidiuretic hormone. Experimental evidence in rats indicates that the chloride dependent pump, in the thick ascending part of Henle's loop, also is inhibited [90]. In human collecting duct cell cultures, exposure to fluoride ions inhib-

its Na-K-ATPase and causes morphologic changes in mitochondria [91].

In 1966 renal failure was reported in 13 of 41 patients receiving methoxyflurane anesthesia for abdominal surgery [92]. Subsequently the causative agent was shown to be the fluoride ion, an end product of the biotransformation of methoxyflurane [93]. The clinical picture consisted of vasopressin resistant polyuria, hypernatremia, hyperosmolality and azotemia. The nephrotoxic threshold in man is believed to be around 50 μM of fluoride. The degree of nephrotoxicity is positively correlated with plasma fluoride levels. A fluoride concentration of 90-120 μM is associated with established renal failure, which becomes severe when fluoride levels reach 150-175 μM [94]. Despite the overall correlation between nephrotoxicity and peak plasma fluoride concentrations, individual patients vary in their nephrotoxic susceptibility. Genetic heterogeneity, drug interactions and preexisting renal disease are some risk factors that may account for this variability. Also, the exposure time to fluoride, which is dependent on production and elimination, is important for the development of nephrotoxicity. Finally, the lack of correlation between peak serum fluoride levels and nephrotoxicity following administration of sevoflurane has led one investigator to suggest that intrarenal production of F^- is more important in the etiology of nephrotoxicity than the blood levels resulting from total body production of F^- [95]. If the intrarenal production of F^- turns out to be a significant factor for toxicity, then the renal cytochrome P-450 isozymes will become very important in design considerations for new fluorinated anesthetics.

Fluoride elimination

Fluoride is removed from plasma by urinary excretion [96] and uptake into calcified tissues [97]. Normally each mechanism represents about 50% of the removal [98]. Renal fluoride excretion is characterized by glomerular filtration followed by variable tubular reabsorption. The tubular reabsorption is influenced by tubular fluid flow rate [99] and urinary pH [100, 101]. Manipulation of urinary pH in patients undergoing a standard enflurane anesthetic resulted in plasma levels of fluoride in patients with alkaline urine that was 50% lower than in patients with acidic urine [102].

Bone uptake may also influence plasma fluoride concentrations. It has been reported that metabolic acidosis increases the rate of bone resorption while metabolic alkalosis increases the rate of osseous accretion in rats [103].

Considerations in pediatric patients

Renal function is markedly diminished in neonates because of low perfusion pressure and immature glomerular and tubular function. Nearly complete maturation of glomerular filtration and tubular function occurs by approximately 20 weeks after birth in term infants, but it is delayed in premature infants. Complete maturation of renal function occurs by approximately two years of age [104,105]. The ability to handle potentially nephrotoxic degradation products in connection with anesthesia may therefore be impaired in neonates and small children.

Halothane and sevoflurane are commonly used for inhaled induction of anesthesia in children, because of their lack of noxious smell. These drugs and isoflurane or desflurane are then used for maintenance of anesthesia according to the preference of the anesthesiologist. Enflurane is rarely used presently because of its airway irritant properties [106]. Therefore, of the currently used inhaled agents in pediatric patients, only sevoflurane has nephrotoxic potential (fluoride ions and compound A).

Mean plasma fluoride levels, in two studies in children undergoing sevoflurane anesthesia were 15.8 μM, ages 1-12 years and 21.5 μM, ages 3 months – 7 years, respectively [107, 108]. The latter study also reported on compound A levels in the breathing system. Maximum inspired concentration was 5.4 ± 4.4 ppm (mean ± S.D.) and maximum expired concentration was 3.7 ± 2.7 ppm. There were no changes in serum creatinine values from samples obtained 24 hours post-anesthesia compared with control. These limited studies give no reason for worry about an increased risk for nephrotoxicity from sevoflurane in the pediatric population.

Clinical implications

Enflurane and sevoflurane are the only volatile anesthetics that have nephrotoxic potential due to their significant release of fluoride ions during metabolism. In sevoflurane's case also due to biodegradation by the currently used CO_2 absorbents in anesthesia circuits.

Both enflurane [109] and sevoflurane [62, 63] have been used in patients with moderate renal function impairment, without any worsening of renal failure. To minimize the risks with compound A formation from sevoflurane, it seems prudent to follow the Food and Drug Administration's (FDA) recommendations regarding sevoflurane. FDO warns against prolonged administration of sevoflurane at relatively low fresh gas flows (< 1 L/min). However, since its introduction to the US in 1995, sevoflurane has been given to tens of millions of patients without a single report of nephrotoxicity [110].

Halothane, isoflurane and desflurane do not have any known nephrotoxic properties and are, from a kidney point of view, excellent choices for anesthetizing patients with preexisting renal disease.

References

1. Kasiske BL, Kjellstrand CM. Perioperative management of patients with chronic renal failure and postoperative acute renal failure. Urol Clin North Am 1983; 10: 35-40.
2. Abreo K, Moorthy AV, Osborne M. Changing patterns and outcome of acute renal failure requiring hemodialysis. Arch Intern Med 1986; 146: 1338-1344.
3. Charlson ME, MacKenzie CR, Gold JP, Shires GT. Postoperative changes in serum creatinine. When do they occur and how much is important ? Ann Surg 1989; 209: 328-332.
4. Coggins CH, Fang LS-T. Acute renal failure associated with antibiotics, anesthetic agents, and radiographic contrast agents. In: Acute renal failure. Brenner BM, Lazarus JM (editors). Churchill Livingstone, New York 1988; p. 295-352.
5. Pringle H, Mannsell RCB, Pringle S. Clinical effects of ether anaesthesia on renal activity. Brit Med J 1905; 2: 542-545.
6. Blackmore WP, Erwin KW, Wiegand OF, Lipsey R. Renal and cardiovascular effects of halothane. Anesthesiology 1960; 21: 489-495.
7. Deutsch S, Goldberg M, Stephen GM, Wu WH. Effects of halothane anesthesia on renal function in normal man. Anesthesiology 1966; 27: 793-803.
8. Mazze RI, Cousins MJ, Barr GA. Renal effects and metabolism of isoflurane in man. Anesthesiology 1974; 40: 536-542.
9. Cousins MJ, Greenstein LR, Hitt BA, Mazze RI. Metabolism and renal effects of enflurane in man. Anesthesiology 1976; 44: 44-53.
10. Jarnberg P-O, Ekstrand J, Irestedt L, Santesson J. Renal function and fluoride formation and excretion during enflurane anaesthesia. Acta Anaesth Scand 1979; 23: 444-452.
11. Bastron RD, Pyne JL, Inagaki M. Halothane-induced renal vasodilation. Anesthesiology 1979; 50: 126-131.
12. Priano LL. Effect of halothane on renal hemodynamics during normovolemia and acute hemorrhagic hypovolemia. Anesthesiology 1985; 63: 357-363.
13. Gelman S, Fowler KC, Smith LR. Regional blood flow during isoflurane and halothane anesthesia. Anesth Analg 1984; 63: 557-565.
14. Jarnberg P-O, Marrone B, Priano LL. Enflurane preserves renal blood flow. Anesthesiology 1990; 73: A572.
15. Hysing ES, Chelly JE, Doursout M-F, Merin RG. Comparative effects of halothane, enflurane, and isoflurane at equihypotensive doses on cardiac performance and coronary and renal blood flows in chronically instrumented dogs. Anesthesiology 1992; 76: 979-984.
16. Lessard MR, Trepanier CA. Renal function and hemodynamics during prolonged isoflurane-induced hypotension in humans. Anesthesiology 1991; 74: 860-865.
17. Kyung WC, Suhn HK, Gou YK. Renal and renin effects of sodium thiopental in rabbits. Renal Physiol 1987; 10: 261-266.
18. Bidwai AV, Stanley TH, Bloomer HA, Blatnick RA. Effects of anesthetic doses of morphine on renal function in the dog. Anesth Analg 1975; 54: 357-360.
19. Hunter JM, Jones RS, Utting JE. Effect of anaesthesia with nitrous oxide in oxygen and fentanyl on renal function in the artificially ventilated dog. Brit J Anaesth 1980; 52: 343-348.
20. Priano LL. Effects of high-dose fentanyl on renal haemodynamics in conscious dogs. Can Anaesth Soc J 1983; 30: 10-18.
21. Jarnberg P-O, Leyden P, Woods L. Renal blood flow is maintained during propofol anesthesia in dogs. Anesthesiology 1992; 77: A655.
22. Baratz RA, Philbin DM, Patterson RW. Plasma antidiuretic hormone and urinary output during continuous positive pressure breathing in dogs. Anesthesiology 1971; 34: 510-513.
23. Hall SV, Johnson EE, Hedley-Whyte J. Renal hemodynamics and function with continuous positive-pressure ventilation in dogs. Anesthesiology 1974; 41: 452-461.
24. Jarnberg P-O, De Villota ED, Eklund J, Granberg P-O. Effects of positive end-expiratory pressure on renal function. Acta Anaesth Scand 1978; 22: 508-514.

25. Kharasch ED, Yeo KT, Kenny MA, Buffington CW. Atrial natriuretic factor may mediate the renal effects of PEEP ventilation. Anesthesiology 1988; 69: 862-869.
26. Andrivet P. Adnot S, Brun-Buisson C. Involvement of ANF in the acute antidiuresis during PEEP ventilation. J Appl Physiol 1988; 65: 1967-1972.
27. Van Dyke R, Chenowith M, Poznak AV. Metabolism of volatile anesthetics: I. Conversion *in vivo* of several anesthetics to $^{14}CO_2$ and chloride. Biochem Pharmacol 1964; 13: 1239-1248.
28. Subcommittee on the National Halothane Study of the Committee on Anesthesia. National Academy of Sciences-National Research Council. Summary of the national halothane study: possible association between halothane anesthesia and postoperative necrosis. JAMA 1966; 197: 775-783.
29. Mazze RI, Trudell JR, Cousins MJ. Methoxyflurane metabolism and renal dysfunction: Clinical correlation in man. Anesthesiology 1971; 35: 247-252.
30. Thummel KE, Kharasch ED, Podoll T, Kunze K. Human liver microsomal enflurane defluorination catalysed by cytochrome P-450 2E1. Drug Metab Dispos Biol Fate Chem 1993; 21: 350-357.
31. Pacifici GM, Viani A, Franchi M, Gervasi PG, Longo V, Di Simplicio P, Temelini A, Romiti P, Santerini S, Vannucci L. Mosca F. Profile of drug-metabolizing enzymes in the cortex and medulla of the human kidney. Pharmacology 1989; 32: 299-308.
32. Nebert DW, Adesnik M. Coon MJ. The P450 gene super-family: recommended nomenclature. DNA 1987; 6: 1-7.
33. Conney AH. Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 1967; 19: 317-348.
34. Waxman DJ. Interactions of hepatic cytochromes P-450 with steroid hormones: regioselectively and stereospecificity of steroid metabolism and hormonal regulation of rat P-450 enzyme expression. Biochem Pharmacol 1988; 37: 71-79.
35. Pantuck EJ, Pantuck CB, Conney AH. Effect of streptozotocin-induced diabetes in the rat on the metabolism of fluorinated volatile anesthetics. Anesthesiology 1987; 66: 24-28.
36. Rehder K, Forbes J, Alter H. Halothane biotransformation in man: A quantitative study. Anesthesiology 1967; 28: 711-715.
37. Cohen EN, Trudell JR, Edmunds HN, Watson E. Urinary metabolites of halothane in man. Anesthesiology 1975; 43: 392-401.
38. Lind RC, Gandolfi AJ, Sipes IG, Brown BR, Waters SJ. Oxygen concentrations required for reductive defluorination of halothane by rate hepatic microsomes. Anesth Analg 1986; 65: 835-839.
39. Wood CL, Gandolfi AJ, Van Dyke RA. Lipid binding of a halothane metabolite: Relationship to lipid peroxidation *in vitro*. Drug Metab Dispos 1976; 4: 305-311.
40. Trudell JR, Bosterling B, Trevor AJ. Reductive metabolism of halothane by human and rabbit cytochrome P-450. Binding of 1-chloro-2,2,2-trifluoroethyl radical to phospholipids. Mol Pharmacol 1982; 21: 710-716.
41. Van Dyke RA, Gandolfi AJ. Anaerobic release of fluoride from halothane. Drug Metab Dispos 1976; 4: 40-45.
42. Chase RE, Holaday DA, Fiserova-Bergerova V. The biotransformation of ethrane in man. Anesthesiology 1971; 35: 262-267.
43. Burke TR Jr, Branchflower RV, Lees DE, Pohl LR. Mechanisms of defluorination of enflurane: Identification of an organic metabolite in rats and man. Drug Metab Dispos 1981; 9: 19-25.
44. Maduska AL. Serum inorganic fluoride levels in patients receiving enflurane anesthesia. Anesth Analg 1974; 53: 351-353.
45. Mazze RL, Calverley RK, Smith TN. Inorganic fluoride nephrotoxicity: prolonged enflurane and halothane anesthesia in volunteers. Anesthesiology 1977; 46: 265-271.
46. Rice SA, Fish KJ. Anesthetic metabolism and renal function in obese and nonobese Fischer 344 rats following enflurane or isoflurane anesthesia. Anesthesiology 1986; 65: 28-34.
47. Dooley JR, Mazze RI, Rice SA, Borel JD. Is enflurane defluorination inducible in man? Anesthesiology 1979; 50: 213-217.
48. Mazze RI, Woodruff RE, Heerdt ME. Isoniazid-induced enflurane defluorination in humans. Anesthesiology 1982; 57: 5-8.
49. Loehning RW, Mazze RI. Possible nephrotoxicity from enflurane in a patient with severe renal disease. Anesthesiology 1974; 40: 203-205.
50. Eichhorn JH, Hedley-Whyte J, Steinman TI. Renal failure following enflurane anesthesia. Anesthesiology 1976; 45: 557-560.
51. Holaday DA, Fiserova-Bergerova V, Latto IP, Zumbiel MA. Resistance of isoflurane to biotransformation in man. Anesthesiology 1975; 43: 325-332.
52. Mazze RI, Hitt BA, Cousins MJ. Effect of enzyme induction with phenobarbital on the in vivo and *in vitro* defluorination of isoflurane and methoxyflurane. J Pharmacol Exp Ther 1974; 190: 523-529.
53. Rice SA, Talcott RE. Effects of isoniazid treatment on selected hepatic mixed function oxidases. Drug Metab Dispos 1979; 7: 260-265.
54. Holaday DA, Smith FR. Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers. Anesthesiology 1981; 54: 100-106.
55. Fujii K, Morio M, Kikuchi H. Pharmacokinetic study in excretion of inorganic fluoride ion, a metabolite of sevoflurane. Hiroshima J Med Sci 1987; 36: 89-94.
56. Kobayashi Y, Ryoichi O, Takeda J, Sekogochi H, Fukushima K. Serum and urinary inorganic fluoride concentrations after prolonged inhalation sevoflurane in humans. Anesth Analg 1992; 74: 753-757.
57. Frink EJ, Malan TP, Isner J, Brown BR. Renal concentrating function with prolonged sevoflurane or enflurane anesthesia in volunteers. Anesthesiology 1994; 80: 1019-1025.

58. Cook TL, Beppu WJ, Hitt BA. A comparison of renal effects and metabolism of sevoflurane and methoxyflurane in enzyme induced rats. Anesth Analg 1975; 54: 829-835.
59. Nishiyama T, Hirasaki A. Effects of sevoflurane anesthesia on renal function: Duration of administration and area under the curve and rate of decrease of serum inorganic fluoride. Eur J Anaesthesiol 1995; 12: 477-482.
60. Frink EJ, Malan TP, Brown EA, Morgan S, Brown BR, Jr. Plasma inorganic fluoride levels with sevoflurane anesthesia in morbidly obese and non obese patients. Anesth Analg 1993; 76: 1333-1337.
61. Higuchi H, Arimura S, Sumikura H Satoh T, Kanno M. Urine concentrating ability after prolonged sevoflurane anesthesia. Br J Anaesth 1994; 73: 239-240.
62. Conzen PF, Nuscheler M, Melotte A, Verhaegen M, Leupolt T, Van Aken H, Peter K. Renal function and serum fluoride concentrations in patients with stable renal insufficiency after anesthesia with sevoflurane or enflurane. Anesth Analg 1995; 81: 569-575.
63. Tsukamoto N, Hirabayashi Y, Shimizu R, Mitsuhata H. The effects of sevoflurane and Isoflurane anesthesia on renal tubular function in patients with moderately impaired renal function. Anesth Analg 1996; 82: 909-913.
64. Hanaki C. Fujii K, Morio M, Tashima T. Decomposition of sevoflurane by sodalime. Hiroshima J Med Sci 1987; 36: 61-65.
65. Fang ZX, Eger EI, II. Factors affecting the concentration of Compound A resulting from the degradation of sevoflurane by soda lime and Baralymeâ in a standard anesthetic circuit. Anesth Analg 1995; 81: 564-568.
66. Bito H, Ikeda K. Effect of total flow rate on the concentration of degradation products generated by reaction between sevoflurane and soda lime. Br J Anaesth 1995;74: 667-966.
67. Gonsowski CT, Laster MJ, Eger EI II, Ferrell LD, Kerschmann RL. Toxicity of compound A in rats: Effect of a 3-hour administration. Anesthesiology 1994: 80: 556-565.
68. Keller KA, Callan C, Prokocimer P, Delgado-Herrera L, Friedman MB, Hoffman GM, Wooding WL, Cusick, PK, Krasula RW. Inhalation toxicology study of a haloalkene degradant of sevoflurane, Compound A (PIFE), in Sprague-Dawley rats. Anesthesiology 1995; 83: 1220-1232.
69. Morio M, Fujii K, Satoh N, Inmai M, Kawakami U, Mizuno T, Kawai, Y, Ogasawara Y, Tamura T, Negishi A, Kumagai Y, Kawai T. Reaction of sevoflurane and its degradation products with soda lime: Toxicity of the by-products. Anesthesiology 1992; 77: 1155-1164.
70. Kharasch ED, Thorning DT, Garton K, Hankins DC, Kilty CG. Role of renal cysteine conjugate ß-lyase in the mechanism of compound A nephrotoxicity in rats. Anesthesiology 1997; 86: 160-171.
71. Goldberg ME, Cantillo J, Gratz I, Deale E, Vekeman D, McDougall R, Kham A, Zaterides A. Dose of Compound A, not sevoflurane, determines changes in the biochemical markers of renal injury in healthy volunteers. Anesth Analg 1999; 88: 437-445.
72. Higuchi H, Sumita S, Wada H, Ura T, Jkemoto T, Kanno M, Satoh T. Effects of sevoflurane and isoflurane on renal function and on possible markers of nephrotoxicity. Anesthesiology 1998; 89: 307-322.
73. Eger EI, II, Koblin DD, Bowland T, Ionescu P, Laster MJ, Fang Z, Gong D, Sommer J, Wesikopf RB. Nephrotoxicity of sevoflurane versus desflurane anesthesia in volunteers. Anesth Analg 1997; 84: 160-168.
74. Eger EI, II, Gong D, Koblin DD, Bowland T, Ionescu P, Laster MJ, Weiskopf RB. Dose-related biochemical markers of renal injury after sevoflurane versus desflurane anesthesia in volunteers. Anesth Analg 1997; 85: 1154-1163.
75. Bito H, Ikeuchi Y, Ikeda K. Effects of low-flow sevoflurane anesthesia on renal function: comparison with high-flow sevoflurane anesthesia and low-flow isoflurane anesthesia. Anesthesiology 1997; 86: 1231-1237.
76. Kharasch ED, Frink EJ, Jr, Zagar R, Bowdle TA, Artu A, Nogami WM. Assessment of low-flow sevoflurane and isoflurane effects on renal function using sensitive markers of tubular toxicity. Anesthesiology 1997; 86: 1238-1253.
77. Ebert TJ, Messana LD, Uhrich TD, Staacke TS. Absence of renal and hepatic toxicity after four hours of 1.25 minimum of alveolar anesthetic concentration sevoflurane anesthesia in volunteers. Anesth Analg 1998; 86: 662-667.
78. Forster H, Dudziak R. Über die ursachen der Reaktion swischen trockenem atemkalk and halgogenierten inhalationsanästhetika. Anaesthesist 1997; 46: 1054-1063.
79. Forster H, Warnken UH, Askali F. Unterschiedliche reaction von sevofluran mit einzelnen komponenten von atemkalk. Anaesthesis 1997; 46: 1071-1075.
80. Neumann MA, Laster MJ, Weiskopf RB, Gong DH, Dudziak R, Forster H, Eger EI. The elimination of sodium and potassium hydroxides from desiccated soda lime diminishes degradation of desfurane to carbon monoxide and sevoflurane to Compound A but does not compromise carbon dioxide absorption. Anesth Analg 1999; 89: 768-773.
81. Murray JM, Renfrew CW, Bedi A, McCrystal CB, Jones DS, Fee JPH. Amsorb. A new carbon dioxide absorbent for use in anaesthetic breathing systems. Anesthesiology 1999; 91: 1342-1348.
82. Koblin DD, Eger EI, Johnson BH. I-653 resists degradation in rats. Anesth analg 1988; 67: 534-538.
83. Eger EI II. Stability of I-653 in soda lime. Anesth Analg 1987; 66: 983-985.
84. Eger EI II, Johnson BH, Strum DP, Ferrell LD. Studies of the toxicity of I-653, halothane, and isoflurane in enzyme-induced, hypoxic rats. Anesth Analg 1987; 66: 1227-1229.

85. Eger EI II, Johnson BH, Ferrell LD. Comparison of the toxicity of I-653 and isoflurane in rats: a test of the effect of repeated anesthesia and use of dry soda lime. Anesth analg 1987; 66: 1230-1233.
86. Jones RM, Koblin DD, Cashman JN, Eger EI, Johnson BH, Damask MC. Biotransformation and hepato-renal function in volunteers after exposure to desflurane (I-653). Brit J Anaesth 1990; 64: 482-487.
87. Koblin DD, Weiskopf RB, Holmes MA, Konopka K, Rampil IJ, Eger EI II, Waskell L. Metabolism of I-653 and isoflurane in swine. Anesth Analg 1989; 68: 147-149.
88. Sutton TS, Koblin DD, Gruenke LD, Weiskopf RB, Rampil IJ, Waskell L, Eger EI II. Fluoride metabolites after prolonged exposure of volunteers and patients to desflurane. Anesth Analg 1991; 73: 180-185.
89. Haynes RC Jr. Agents affecting calcification. In: Goodman and Gilman's. The pharmacological basis of therapeutics. Gilman AF, Rall TW, Nies AS, Taylor P (editors). Pergamon Press, New York 1990; p. 1518.
90. Roman RJ, Carter JR, North WC, Kauker ML. Renal tubular site of action of fluoride in Fischer 344 rats. Anesthesiology 1977; 46: 260-264.
91. Cittanova M-L, Lelongt B, Verpont M-C, Geniteau-Legendre M, Wahbe F, Prie D, Coriat P, Ronco PM. Fluoride Ion Toxicity in Human Kidney Collecting Duct Cells. Anesthesiology 1996; 84: 428-435.
92. Crandell WB, Pappas SG, Macdonald A. Nephrotoxicity associated with methoxyflurane anesthesia. Anesthesiology 1966; 27: 591-607.
93. Mazze RI, Shue GL, Jackson SH. Renal dysfunction associated with methoxyflurane anesthesia. A randomized prospective clinical evaluation. JAMA 1971; 216: 278-288.
94. Cousins, MJ, Mazze RI. Methoxyflurane nephrotoxicity. A study of dose response in man. JAMA 1973; 225: 1611-1616.
95. Kharasch ED, Hankins DC, Thummel KE. Human Kidney Methoxyflurane and Sevoflurane Metabolism. Anesthesiology 1995; 82(3): 689-699.
96. Chen PS Jr, Smith FA, Gardner DE, O'Brien JA, Hodge HC. Renal clearance of fluoride. Proc Soc Exp Biol (NY) 1956; 92: 879-883.
97. Carlson CH, Armstrong WD, Singer L. Distribution and excretion of radiofluoride in the human. Proc Soc Exp Biol (NY) 1960; 104: 235-239.
98. Hosking DJ, Chamberlain MJ. Studies in man with 18F. Clin Sci 1972; 42: 153-61.
99. Ekstrand J, Ehrnebo M, Boreus LO. Fluoride bioavailability after intravenous and oral administration: importance of renal clearance and urine flow. Clin Pharmacol Ther 1978; 23: 329-337.
100. Whitford GM, Pashely DH, Stringer GI. Fluoride renal clearance: a pH-dependent event. Am J Physiol 1976; 230: 527-532.
101. Ekstrand J, Ehrnebo M, Whitford GM, Jarnberg P-O. Fluoride pharmacokinetics during acid-base changes in man. Eur J Clin Pharmacol 1980; 18: 189-194.
102. Jarnberg P-O, Ekstrand J, Irestedt L. Renal fluoride excretion and plasma fluoride levels during and after enflurane anesthesia are dependent on urinary pH. Anesthesiology 1981; 54: 48-52.
103. Barzel US, Jowsey J. The effect of chronic acid and alkali administration on bone turnover in adult rats. Clin Sci 1969; 36: 517-524.
104. Leake RD, Trygstad CW. Glomerular filtration rate during the period of adaptation to extrauterine life. Pediatr Res 1977; 11: 959-962.
105. Jose PA, Fildes RD, Gomez RA, Chevalier RL, Robillard JE. Neonatal renal function and physiology. Curr Opin Pediatr 1994; 6: 172-177.
106. Fisher DM, Robinson S, Brett CM, Perin G, Gregory GA. Comparison of enflurane, halothane, and isoflurane for diagnostic and therapeutic procedures in children with malignancies. Anesthesiology 1985; 63: 647-650.
107. Levine MF, Sarner J, Lerman J, Davis P, Sikich N, Maloney K, Motoyama E, Cook D. Plasma Inorganic Fluoride Concentrations after Sevoflurane Anesthesia in Children. Anesthesiology 1994; 80:1019-1025.
108. Frink EJ, Green WB, Brown EA, Malcomson M, Hammond LC, Valencia FG, Brown BR. Compound concentrations during sevoflurane anesthesia in Children. Anesthesiology 1996; 84: 566-571.
109. Mazze RI, Sievenpiper TS, Stevenson J. Renal effects of enflurane and halothane in patients with abnormal renal function. Anesthesiology 1984; 60:161-163.
110. Bedford RF, Ives HE. The renal safety of Sevoflurane. Anesth Analg 2000; 90: 505-508.

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Illicit drug abuse

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Introduction

The illicit use of drugs involves millions of people worldwide. Drug abuse is associated with a variety of medical complications. In recent years the abuse of both heroin and cocaine have been major problems worldwide. Other illicit agents, however, which continue to induce medical complications, include barbiturates, ethyl alcohol, amphetamines, and phencyclidine, as well as drug combinations. There are a number of renal disorders associated with drug abuse. Some may be related to nephrotoxicity associated with the drugs themselves, e.g. heroin nephropathy, while others are caused by complications related to drug abuse, e.g. post-infectious glomerulonephritis, HIV nephropathy and hepatitis related glomerulonephritis. Some nephrotoxic problems are relatively specific for abuse of one class of drugs (e.g. heroin nephropathy with opiate abuse), while other renal manifestations are found with a variety of abused drugs linked through similar pathogenetic mechanisms (e.g. acute renal failure related to non-traumatic rhabdomyolysis). This chapter will review the various renal manifestations of illicit drug abuse. It will focus on the clinical and pathologic presentation, the course, the treatment, and the pathogenesis of these lesions. Secondary renal infectious complications will not be discussed, except for the interrelationship of HIV nephropathy and heroin nephropathy.

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Table 1. Nephrotoxicity associated with illicit drug abuse.

Opiate abuse
• heroin nephropathy
• AA amyloidosis
• rhabdomyolysis - acute tubular necrosis
• tubulointerstitial lesions
• glomerulonephritis (membranoproliferative)
• HIV associated nephropathy
Cocaine abuse
• rhabdomyolysis - acute tubular necrosis
• malignant hypertension
• chronic renal failure
Phencyclidine abuse
• rhabdomyolysis - acute tubular necrosis
Amphetamine abuse
• necrotizing angiitis
• rhabdomyolysis - acute tubular necrosis
• acute interstitial nephritis
Other drugs
• ethyl alcohol abuse
- rhabdomyolysis - acute tubular necrosis
- tubular defects
• barbiturates, benzodiazepines, glutethimide
- rhabdomyolysis - acute tubular necrosis
- diazepam - ? acute interstitial nephritis

Opioids

The opioid drugs are responsible for more reported cases of renal damage than any other class of abused drug. While opioids include morphine, codeine, methadone, meperidine, and other agents, most cases of renal damage are related to heroin abuse. Heroin is derived from the acetylation of morphine at two-sites. It is rapidly absorbed from all mucous membranes and the lungs. There are also other non-opioid drugs producing a similar pattern of addiction that may cause renal disease (e.g. pentazocine).

In the early 1970's intravenous drug addiction became a contributor to renal disease and chronic renal failure in urban centers throughout the U.S. [1-9]. Almost all patients injected heroin, although it was often mixed with other drugs. Many patients developed albuminuria and the nephrotic syndrome and the glomerular histology described in these patients has been pleomorphic. They include focal segmental and global sclerosis, membranous glomerulopathy, membranoproliferative glomerulonephritis, mesangial proliferative glomerulonephritis, minimal change disease, and amyloidosis [2-5, 7, 9, 10-14]. By far the most frequent lesion has been focal segmental glomerulosclerosis (FSGS) progressing to global sclerosis. This lesion has been classically referred to as "heroin nephropathy". Ninety percent of all nephrotic black male addicts biopsied in the original report of heroin nephropathy from the Kings County Hospital in Brooklyn, New York were found to have this histologic lesion [7].

In the late 1970's and early 1980's there was a change in the spectrum of the glomerular lesion noted in heroin addicts [15-21]. Secondary amyloidosis emerged as a common biopsy finding in intravenous drug abusers with the nephrotic syndrome. After prolonged intravenous drug abuse, addicts exhausted their venous accesses and resorted to subcutaneous injections of drugs, so-called "skin-popping". The persistent subcutaneous injections led to chronic ulcerations and suppurative skin infections and appeared to be the stimulus for the development of secondary amyloidosis [17, 18, 22, 23].

In the mid and late 1980's as the prevalence of HIV infection increased dramatically among intravenous drug abusers, a new more aggressive form of focal segmental glomerulosclerosis appeared. This collapsing variant of focal segmental glomerulosclerosis appears clinically and pathologically distinct from the lesion formerly described as heroin nephropathy [24-26]. Because of the overwhelming prevalence of HIV positivity among intravenous drug abusers in urban centers, such as New York City (50-80% HIV positivity), HIV-associated nephropathy has now emerged as the major glomerulopathy related to illicit drug abuse and the lesion of classic "heroin nephropathy" has essentially disappeared in this population [27]. Perhaps the speed with which HIV nephropathy develops among addicts may not allow for the longer time interval required to express the lesions of heroin nephropathy. Moreover, the lesions of heroin nephropathy may be underdiagnosed in the HIV infected addict population since, by definition, heroin nephropathy must exclude the presence of the HIV virus. Regardless, the reported incidence of both heroin nephropathy and amyloidosis has decreased recently in the addict population as the incidence of HIV seropositivity and HIV nephropathy has increased [27].

Classic heroin nephropathy

There are over three hundred cases of classic focal

segmental glomerulosclerosis associated with intravenous drug abuse described in the literature, including 30 cases from the Columbia Presbyterian Medical Center and Harlem Hospital in New York City [1, 5, 7, 11, 13, 16, 22, 28-35]. The preponderance of patients are young Black males (95% Black, 92% male, mean age 29 years). In three of the larger series, including our own study, all patients were Black [7, 22, 28]. Duration of drug abuse varied from 6 months to 30 years prior to the onset of renal disease with a mean duration of 6 years. Two thirds of patients presented with the nephrotic syndrome and an average 24 hour urinary protein excretion between 9-10 g. Over 40% of patients had greater than 10 g of proteinuria daily. Mean serum albumin was 2.6 g/dl and cholesterol was 321 mg/dl. Despite the presence of substantial proteinuria, the mean serum cholesterol concentrations were not extremely elevated, probably due to chronic illness and/or malnutrition in this population. Urinalyses demonstrated pyuria in 50% of cases and microhematuria in over 30%. Occasional patients presented with gross hematuria.

At initial presentation 3/4 of the patients had renal insufficiency with an average serum creatinine concentration of 3.6 mg/dl. Ten percent of patients presented with serum creatinine concentrations greater than 9 mg/dl. Hypertension correlated best with the presence of renal insufficiency [22, 28, 35]. In one large series serum creatinine averaged 4.7 mg/dl in hypertensive patients and only 1.4 mg/dl in normotensive patients [35].

Our own study compared 30 patients with focal segmental glomerulosclerosis due to heroin nephropathy to patients with the idiopathic form of focal segmental glomerulosclerosis [22]. The mean serum creatinine at presentation was 4.5 mg/dl in those patients with heroin nephropathy and 1.2 mg/dl in those with idiopathic focal segmental glomerulosclerosis, despite similar degrees of proteinuria, hypoalbuminemia, and glomerulosclerosis. Hypertension and hypercholesterolemia were more prevalent in the idiopathic form of focal segmental glomerulosclerosis despite greater renal dysfunction in the drug abusers.

The pathologic lesions of heroin nephropathy can be either focal or diffuse with sclerosis involving glomeruli segmentally or globally. This variability may relate to the stage of the disease at biopsy since some patients present with preserved renal function and others with renal failure. The glomeruli show collapse, thickening, and wrinkling of the glomerular basement membrane, sometimes with an increase in mesangial matrix (Figure 1). In early stages, there is often swelling and proliferation of visceral epithelial cells with foam cells in the capillary lumina. Hyalinosis develops similar to the hyaline deposits in many sclerosing glomerular lesions. The immunofluorescent findings of granular IgM and C3 deposition in the areas of sclerosis are thought to represent nonspecific trapping, similar to that seen in other sclerotic processes [7, 22]. Linear staining for IgG along the glomerular basement membrane without evidence for anti-glomerular basement membrane antibodies has been described and probably also represents nonspecific trapping of plasma proteins [5, 28, 35]. The electron microscopic findings are similar to those seen in idiopathic focal segmental glomerulosclerosis with glomerular basement membrane thickening and new basement membrane formation without electron dense deposits. Occasionally deposits have been described which again most likely represent nonspecific trapping and not true immune complexes.

More severe interstitial mononuclear cell infiltrates, greater tubular atrophy, and more interstitial fibrosis have been described in heroin nephropathy as compared to idiopathic focal segmental glomerulosclerosis in most reported series [5, 22, 25, 29, 30]. Although several investigators believe that the severity of the tubulointerstitial changes are consistent with the degree of glomerular damage, other workers have found the degree of interstitial inflammation to be out of proportion to the degree of glomerular disease [5, 22, 28, 33, 35].

The pathogenesis of the glomerular lesions seen in classic heroin nephropathy remains unclear. Since intravenous drug abusers develop many infections and other complications from their addiction, it was initially debated whether heroin nephropathy was a unique lesion. One group of investigators found a high incidence of hepatitis C infection (HCV) in Black intravenous drug abusers with renal biopsies demonstrating heroin nephropathy and proposed that HCV infection may play a role in the development of FSGS in this predisposed population [36]. Moreover, since Black patients have a greater tendency to develop idiopathic focal segmental glomerulosclerosis, the use of intravenous drugs might merely potentiate this pre-

disposition. A study of renal specimens from 179 autopsies of European intravenous drug abusers in Germany (almost all Caucasian) over a ten year period (1987-1997) demonstrated membranoproliferative glomerulonephritis and no cases of FSGS, as seen in the American Black population; there was only a weak association of this membranoproliferative glomerulonephritis with hepatitis B or C infection in this autopsy study [37]. One well-performed epidemiologic study evaluated all patients 18 to 45 years of age with sclerosing glomerulonephritis who developed end stage renal disease over 4½ years in the Buffalo Standard Metropolitan Statistical Area (SMSA) [28]. The annual incidence of glomerulosclerosis was 41 times greater in addicts than in controls and 29 times greater in Black male addicts than in nonaddicted Black men. End stage renal disease developed 18 times more frequently in addicts than nonaddicts. Thus, this study suggested that the entity of focal segmental glomerulosclerosis related to substance abuse truly exists and appears to be more common in the Black population [28, 38]. Moreover, a recent case-controlled study examined the association between drug use and end stage renal disease (ESRD). 716 patients who reached ESRD in 1991 were compared to age matched controls and examined for the lifetime use of heroin, cocaine, and other illicit drugs. After adjustment for age, sex, race, socio-economic status, history of hypertension and diabetes, persons who had ever used heroin or opiates were at increased risk for developing ESRD (odds ratio of 19:1) [39]. Likewise, use of crack and other drugs were associated with ESRD but the effect could not be separated from the effects of heroin [39]. A genetic basis for this predisposition has been suggested by the demonstration of an increased incidence of HLA-Bw53 genotype among Black drug addicts who have developed heroin nephropathy [38, 40].

The actual mechanism whereby intravenous drug abuse produces glomerular disease is unclear. In the clinical setting there is still some debate as to whether it is the drug itself or a contaminant that mediates the glomerular damage. Addicts typically use street heroin mixed with a number of adulterants, such as quinine or lactose, and they not infrequently "shoot up" with combinations of illicit drugs. Three patients who developed the clinical and morphologic picture of heroin nephropathy claimed to have used only intravenous pentazocine and tripelennamine [41]. One study suggested that the contaminants rather than the narcotic itself might be the inciting factor through the mechanism of mesangial overload [42].

It has been hypothesized that an abnormal immune response plays a role in the development of focal sclerosis in intravenous drug abusers since abnormalities in humoral and cellular immunity have been described in addicts [43]. The repeated injection of heroin could induce an immunologic response to the narcotic as a tissue haptene. Morphine binding activity in the serum of rabbits has been demonstrated with repeated injections of the opiate [44]. While some researchers have found that the γ globulin fraction of serum from heroin addicts also has morphine binding activity [45], this has not been a uniform finding [46]. A recent report of an increased incidence of antinuclear and anticardiolipin antibodies in ten heroin addicts suggested that these immune responses may play a role in heroin-related systemic complications, including renal disease [68a]. Chronic administration of morphine to rats has produced both biochemical as well as marked morphologic changes in the kidney by electron microscopy [47]. An electron microscopic study in rats injected with morphine demonstrated glomerular abnormalities consisting of microprojections on the podocytes [48]. These findings suggest that morphine itself may directly affect the kidney perhaps via altered intracellular cyclic AMP levels [48].

The effects of morphine on cultured mesangial cell proliferation and matrix formation suggest that the drug itself may induce cell proliferation and mesangial sclerosis [49]. More recent laboratory investigation has suggested that morphine intensifies the accumulation of macromolecules in the mesangium and that it stimulates TNF-α production by lipopolysaccharide activated mesangial cells in culture, which in turn, amplifies mesangial cell nitrite production [50, 51]. The latter effect appears to be morphine receptor mediated since opiate receptor antagonists abolish this effect of morphine; in addition, anti-TNF-α antibody diminishes morphine-induced nitrite generation [51].

In vitro laboratory studies have suggested that morphine may also have direct effects on renal interstitial fibrosis. Morphine has been shown to enhance proliferation of cultured rat renal medullary interstitial cells as well as their mRNA expression for c-jun and c-myc [52]. Morphine also increased the accumulation of types 1 and 3 collagen in the renal interstitium [52]. Mor-

phine has been shown to enhance the proliferation of kidney fibroblasts, and at higher doses induces apoptosis as well as synthesis of p53 by the kidney fibroblasts [53]. This data suggests that opiates may play a role in the development of "heroin nephropathy" via an effect on the renal interstitium.

Most patients with heroin nephropathy develop end stage renal disease from several months to five years following diagnosis. There appears to be a spectrum in terms of rapidity of progression from the idiopathic form of focal sclerosis to heroin nephropathy and then to the collapsing form of focal sclerosis and HIV nephropathy. Idiopathic focal segmental glomerulosclerosis typically progresses to end stage renal disease over a 5 to 10 year period, collapsing focal segmental glomerulosclerosis and HIV nephropathy progress over several weeks to months to end stage, and heroin nephropathy appears to be between these

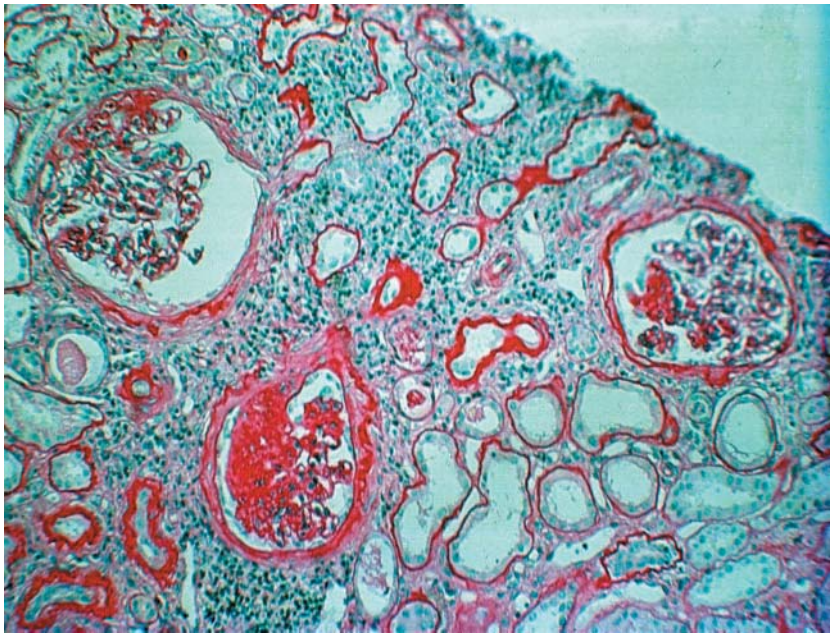

Figure 1. *Renal biopsy of patient with heroin nephropathy showing focal glomerulosclerosis plus severe tubulointerstitial damage. H&E staining, orig. magn. x300.*

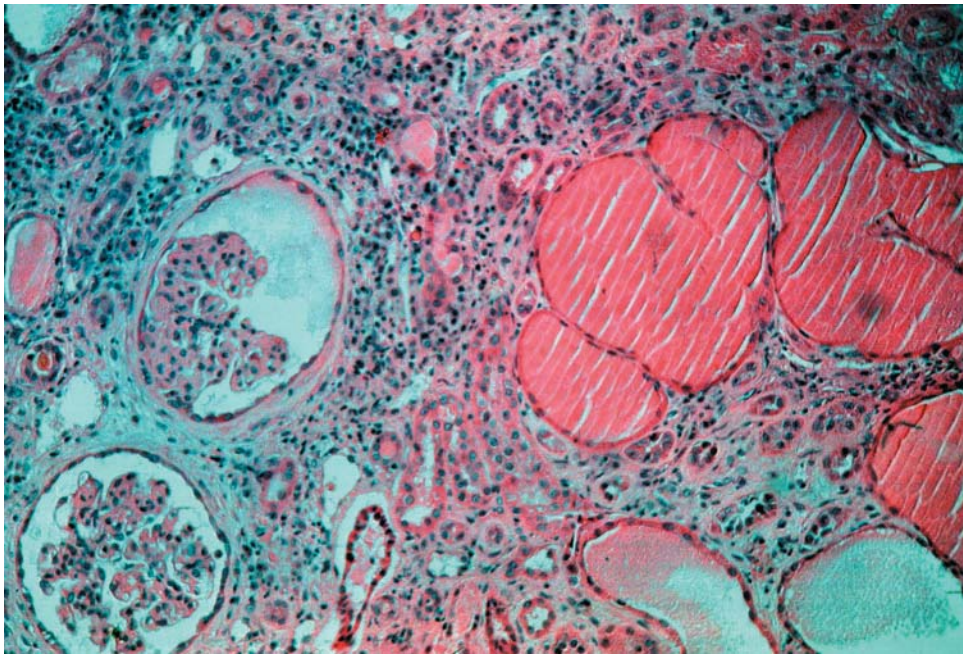

Figure 2. *Renal biopsy of patient with HIV nephropathy showing pronounced tubular dilatation. H&E staining, orig. magn. x300.*

two extremes in its rapidity of progression.

One study demonstrated that the mean time to end stage renal disease for heroin abusing patients with an initial glomerular filtration rate greater than 60 cc/min was 43 months compared to 3.6 months for patients with HIV nephropathy who had a similar glomerular filtration rate [54]. By stratifying the patients with heroin nephropathy, those with a glomerular filtration rate from 20-60 cc/min took a mean of 20 months to reach uremia, while those with an initial clearance of less than 20 cc/min progressed to uremia in a mean of 7 months [54]. Isolated reports have suggested that abstinence from substance abuse may allow for improvement and/or stabilization of renal function [11, 22, 34], but this data has not been confirmed by a systematic study.

There is no confirmation that any form of immunosuppressive therapy will induce significant remission of the nephrotic syndrome or prevent progression to renal failure in heroin nephropathy. More recent data in patients with idiopathic focal segmental glomerulosclerosis suggests that longer courses of immunosuppressive therapy may induce a higher incidence of remission of the nephrotic syndrome [55-60]. Cyclosporine A has been shown to be effective in inducing partial and complete remissions in patients with idiopathic FSGS who are steroid resistant [61]. While several patients with HIV nephropathy have been treated with cyclosporine A and some series suggest benefit from short courses of steroid therapy, neither the use of intensive immunosuppressive therapy nor cyclosporine A have been reportedly used in heroin nephropathy [62, 63]. In those few patients who have been transplanted and remained completely drug free, results have been favorable without recurrence [64, 65].

Amyloidosis associated with heroin abuse

Following the description of focal segmental glomerulosclerosis as the classic lesion of heroin nephropathy, a second pattern of glomerular damage due to amyloidosis was described in intravenous drug abusers. As this form of amyloidosis has become more prevalent, the spectrum of glomerular damage seen with drug abuse has clearly expanded [15]. Between 1978 and 1992, almost seventy cases of heroin-related renal amyloidosis were reported [15-19, 22, 35, 66-68]. The epidemiology of this lesion resembles that of heroin related focal segmental glomerulosclerosis as nearly all patients are Black and most are male. Interestingly, the age of the patients with amyloid (mean age 41 years) was almost ten years greater than those with FSGS and the duration of drug abuse was significantly longer. Table 2 compares the clinical data in our patients with heroin related amyloidosis (n=24) to our cases of heroin nephropathy (n=30) [22]. Most had exhausted their intravenous access for drug abuse and resorted to subcutaneous "skin-popping". Almost all patients used heroin, some mixed with cocaine, and two patients reportedly abused only pentazocine and tripelennamine [18]. All patients had chronic dermal ulcerations and suppurative skin infections.

Almost all patients had nephrotic range proteinuria with an average daily urinary protein excretion of 13 g, marked hypoalbuminemia (mean serum albumin 2.1 g/dl), and an elevated serum cholesterol (mean 247 mg/dl). Hypertension has been variable, occurring in less than 20% of most reported series, but was as high as 40% in one series [16, 22, 35]. Most patients present with renal insufficiency, the average serum creatinine concentration being 2.5 mg/dl. In our patients the initial serum creatinine concentration was significantly higher (6.2 mg/dl) [22].

When tested, the amyloid is of the secondary type, amyloid A protein. Serum amyloid A, an acute phase reactant produced by hepatocytes, circulates complexes to high density lipoprotein and is cleaved into smaller fragments which subsequently polymerize into the β pleated sheet configuration of amyloid [23, 35]. In heroin related amyloidosis, the amyloid is heavily distributed in the tubular basement membranes, vessel walls, and interstitium as well as the glomeruli. There is greater tubular basement membrane and interstitial amyloid deposition in drug related renal amyloid than in secondary renal amyloid unrelated to heroin abuse [31]. Amyloid was not found in the skin biopsies of several of our patients with heroin related secondary amyloidosis, although interestingly, multiple pulmonary nodules due to AA amyloid have been described in an HIV positive intravenous drug abuser [31a].

As with heroin-induced focal segmental glomerulosclerosis, the interstitial cell inflammation is more prominent in the biopsies of patients with substance abuse amyloidosis than in other forms of secondary amyloidosis [22]. Accompanying the tubulointerstitial involvement are a number of physiologic and clinical

Table 2. Heroin nephropathy - clinical features

	FGS	Amyloid	
Number of patients	30	24	
Age (years)	35 (range 23-51)	40 (range 27-56)	$p < 0.05$
Drug abuse (years)	14.5 (range 4-30)	18.6 (range 5-33)	NS
Proteinuria (g/24 hrs)	7.7 ± 1.18*	6.1 ± 1.37*	NS
Nephrotic syndrome (%)	85%	66%	NS
Plasma albumin (g/dl)	2.3 ± 0.18*	2.0 ± 0.18*	NS
Plasma cholesterol (mg-/dl)	234 ± 31.8*	199 ± 33.6*	NS
Plasma creatinine (mg/dl)	4.5 ± 0.97*	6.2 ± 1.45*	NS
Hypertension (%)	27%	15%	NS
Skin ulcers/abcessess (%)	13%	100%	$p < 0.01$

** Mean ± S.E.*

tubular abnormalities including renal tubular acidosis, glycosuria, phosphaturia, and symptoms of nephrogenic diabetes insipidus [69].

Our patients with drug related secondary amyloidosis progressed to end stage renal disease more rapidly than patients with other forms of secondary renal amyloidosis [22]. This more rapid progression correlated with the presence of marked interstitial inflammation. In contrast, the control patients with amyloidosis often died of their underlying disease before developing end stage renal disease. However, by two years following the diagnosis both groups experienced a high mortality rate (64 and 66%) [22]. Two patients with heroin related renal amyloidosis who subsequently abstained from subcutaneous drug abuse experienced remissions of proteinuria and improvement or stabilization of renal function [66, 67]. However, most patients have developed progressive renal dysfunction despite abstinence from drug abuse and/or improvement in their suppurative skin infections. Colchicine has been reported to markedly lower proteinuria to near normal levels and improve renal function in one patient with drug-related renal amyloidosis despite no demonstrable change in the degree of amyloid on a repeat renal biopsy [70]. Transplantation has generally not been recommended for these patients because of the severe chronic skin infections, aside from the risk of their continuing and/or returning to substance abuse post transplantation.

HIV nephropathy and its relationship to heroin nephropathy

As of the year 2000 there were between 650,000 and 900,000 HIV-1 seropositive people in the USA. In 1996 there were 57,000 new AIDS cases reported in the United States. While the incidence is decreasing in Caucasians, it is increasing in African Americans. Almost 40,000 persons died from AIDS in 1995. In some cities intravenous drug abusers have a 60-85% carriage rate for HIV [71]. While infection with this virus has been associated with a number of patterns of renal disease, the most frequent form of glomerular damage has been designated HIV nephropathy [24-26, 54, 71-80]. It is characterized by heavy proteinuria, large echogenic kidneys on ultrasonography, and rapid progression to renal failure with characteristic renal biopsy findings [72-80]. HIV associated nephropathy is a better term than AIDS nephropathy since this glomerulopathy may occur in patients with AIDS, early manifestations of HIV infection, as well as in asymptomatic HIV carriers [73]. While HIV nephropathy has an incidence of only 3-7% of unselected autopsy series in HIV infected patients, it is by far the most common lesion found in HIV infected patients undergoing renal biopsy. Of 104 biopsies in HIV positive patients with glomerular disease at the Columbia Presbyterian Medical Center, 73 had classic HIV nephropathy [25]. In both intravenous drug abusers and nondrug users who are HIV positive, the incidence of HIV nephropathy has been much greater in African Americans. In San Francisco where

most AIDS patients are White and homosexual, HIV nephropathy has been extremely uncommon [76-78]. Akin to the predilection in African Americans for idiopathic focal segmental glomerular sclerosis and heroin nephropathy, the Black race is probably the single most important predisposing epidemiologic factor for classic HIV nephropathy in HIV infected persons. The typical patient has renal insufficiency and signs of the nephrotic syndrome at presentation. In our patients with HIV nephropathy the initial serum creatinine was markedly elevated (mean 5.4 mg/dl), the serum albumin low (mean 2.2 g/dl), and edema frequent (62%) [73]. The kidneys of patients with HIV nephropathy when evaluated by ultrasonography were large for the degree of renal failure (mean size 12.3 cm) and echogenic. This echogenicity correlates with dilated cystic renal tubules rather than glomerular changes.

The pathology of HIV nephropathy has several distinct features, which differ from classic idiopathic focal segmental glomerulosclerosis and heroin nephropathy [25-27, 76-81]. On light microscopy diffuse global glomerular sclerosis and collapse is common with striking visceral epithelial cell hypertrophy accompanied by large cytoplasmic vacuoles and resorption droplets. There are also severe tubulointerstitial changes with interstitial inflammation, edema, microcystic dilatation of tubules (Figure 2), and severe tubular degenerative changes. On electron microscopy tubulo-reticular inclusions are prevalent in the glomerular endothelium. While these tubulo-reticular inclusions may only be markers for the presence of severe viral infection, their presence in a patient with classic light microscopic changes of HIV nephropathy confirms the diagnosis. Children with AIDS often have other glomerular lesions such as mesangial changes or minimal change disease.

The course of HIV nephropathy is usually a rapid progression to renal failure. An early study by Rao et al. [54] found that the average time from diagnosis to uremia was 3-4 months for AIDS nephropathy patients with creatinine clearances > 60 cc/min. By contrast, it was almost 44 months for patients with heroin nephropathy with a similar creatinine clearance; even with a creatinine clearance < 20 cc/min at diagnosis, the time to uremia for heroin nephropathy was still 7 months. In another study, Rao et al. followed 55 patients with HIV nephropathy in which 43 progressed rapidly to uremia, 2 died without uremia and ten were lost to follow-up [72]. In our study of 26 patients with HIV nephropathy, life table analysis confirmed the rapid development of end stage renal disease in this population [73]. Patient survival most closely correlated with the stage of HIV infection. Patients with AIDS and end stage renal disease had a mean survival of only 1.9 months; patients with early symptoms of HIV infection survived a mean of 3.6 months, while patients with asymptomatic HIV infection survived a mean of 9.7 months [73]. In our experience all patients with classic HIV nephropathy eventually developed AIDS.

Data suggests that HIV nephropathy is a late consequence of HIV infection associated with a high viral load [80], although the nephrotic syndrome and renal failure can be the initial presentation of HIV infection. The kidney appears to be an important long-term reservoir for the HIV-1 virus [82]. A recent report documented the presence of viral transcripts in renal epithelial cells even in the presence of effective therapy [82]. In addition to ACE inhibition, the use of antiretroviral therapy, especially when begun early in the course of HIV nephropathy, has markedly improved the prognosis for progression to end stage renal disease and discontinuing such therapy could lead to rapid viral replication [82-84]. Some patients with asymptomatic HIV infection can survive many years on dialysis and recent statistics demonstrate a much improved prognosis for HIV-infected patients maintained on hemodialysis [85].

The pathogenesis of HIV nephropathy is unknown. Speculations include acute renal hemodynamic alterations, toxicity to specific glomerular cell populations, and possibly toxicity to the tubules as well [76, 78]. The HIV genome is commonly found in renal tissue in HIV seropositive patients by the DNA polymerase chain reaction. HIV core protein (p-24) and pro-viral HIV DNA have been localized to the tubular cells and visceral epithelial cells, but their role in the pathogenesis of HIV nephropathy is unclear [81, 86]. One group of investigators found that HIV virus was readily able to infect 90% of cultured human glomerular endothelial cells and 5% of mesangial cells, but glomerular epithelial cells could not be infected [87]. Experiments with mice made transgenic for HIV-1 proviral DNA show a characteristic course of proteinuria and progressive azotemia [88]. Histology reveals focal segmental sclerosing lesions and microcyst formation with interstitial infiltrates similar to the histologic pattern seen in

human HIV nephropathy. These findings implicate HIV gene constructs directly in the pathogenesis of HIV nephropathy. Laboratory data has recently demonstrated a role for the HIV-1 gp120 envelope protein on tubular cell interaction products in promoting renal fibroblast proliferation and apoptosis; in addition, this effect was enhanced by morphine [89]. These effects may play a role in the renal interstitial disease, which is so prominent in HIV nephropathy.

An important question is whether HIV nephropathy is truly different from the older entity of heroin nephropathy. Clinical and histologic data would suggest they are distinct. While heroin nephropathy by definition occurs only in heroin addicts, the classical clinical and histologic picture of HIV nephropathy has been seen in patients who acquired the virus through homosexual contact, blood transfusions, and maternal transmission as well as intravenous drug abuse. The progression to renal failure as described above is much more rapid in patients with HIV nephropathy. The pathology of HIV nephropathy has unique features of the collapsing form of glomerulosclerosis, marked tubular microcyst formation, and electron microscopic tubulo-reticular inclusions. Moreover, the transgenic animal models with features typical of HIV nephropathy also suggest a unique pathogenesis for this form of collapsing FSGS. Since dysregulation of VEGF (vascular endothelial growth factor) expression within the glomerulus has been demonstrated in a variety of renal diseases, recent animal data has shown that podocyte-specific overexpression of the VEGF-164 isoform can produce a collapsing glomerulopathy, similar to that seen in HIV-associated nephropathy [89a]. In addition, recent studies of chemokines have demonstrated higher levels of MCP-1, RANTES, and IL-8 interstitial and glomerular tissue levels in HIV infected patients, regardless of renal disease, but MHC Class II, interferon α and γ receptor protein expression was greater in HIV patients with nephropathy [89b]. This suggests that upregulation of these proteins may be important in the pathogenesis of HIV associated nephropathy [89b].

Opiate induced acute renal failure (rhabdomyolysis)

In addition to chronic renal failure, the abuse of narcotics is associated with a characteristic pattern of acute renal failure. Overdose can cause non-traumatic rhabdomyolysis associated with myoglobinuric acute renal failure [90-96]. It has been reported with other narcotics including methadone, codeine, and drug combinations as well as heroin (90, 91, 97]. In a number of centers rhabdomyolysis is the etiology for 5-7% of all cases of acute renal failure [90, 92, 98]. While in one large series, drug-related cases comprised only 11 of 157 of rhabdomyolysis cases, drug ingestion had a very high likelihood of being complicated by acute renal failure.

The typical patient is a young male addict who presents with coma or stupor after intravenous use of heroin. Decreased levels of consciousness can develop rapidly since such patients are not infrequently found comatose with the injection needle still inserted in their veins [97]. This occurs commonly in inexperienced addicts who misjudge the dose of drug administered or when there is a sudden change in the potency of available street heroin. Most of the patients with acute renal failure have had a prolonged period of coma prior to presentation [97]. Less frequently an addict will present with rhabdomyolysis and acute renal failure and deny prior coma or stupor. In some of these cases seizures, trauma, or excessive exertion prior to the use of the drug may explain the rhabdomyolysis. At presentation many patients will be hypotensive, hypoxic, acidotic, and dehydrated [90, 91]. Patients may or may not have clinical evidence of muscle damage with myalgias and tender swollen muscle groups. Evidence for muscle damage, however, is readily documented by very elevated levels of muscle enzymes (CPK and aldolase) and myoglobinuria.

The acute renal failure is typical for acute tubular necrosis and is characterized by a urine sediment with granular pigmented casts, and benzidine positive urine often in the absence of significant hematuria. With rhabdomyolytic acute tubular necrosis the urinary sodium concentration and fractional excretion of sodium are not always increased as in classic acute tubular necrosis [99]. One half to two-thirds of patients have oliguria, which may last from hours to many weeks. During this phase of the acute renal failure, there is a very rapid rise in the serum creatinine (often > 2.0 mg/dl/day), and profound increases in the serum levels of a variety of solutes normally found in muscle or produced from muscle derived precursors. Thus, the levels of potassium, phosphate, and uric acid all rise dra-

matically. Associated with the oliguria many patients develop severe hypocalcemia [90, 92, 97]. This may be due to deposition of calcium salts in the damaged muscle, tissue deposition of calcium salts elsewhere due to the high circulating levels of phosphate, decreased parathyroid hormone levels, or altered vitamin D metabolism [100, 101]. During the polyuric recovery phase of acute renal failure, a rebound hypercalcemia occurs in many patients due to reversal of the processes that led to hypocalcemia [90, 92, 100, 101].

Almost half of the reported patients required some dialytic support during their episode of acute renal failure. Nevertheless, the majority of patients regain significant renal function. Perhaps because the addicted population at risk is young and without prior multisystem disease, there has been a very low mortality associated with this form of acute renal failure despite the common occurrence of intercurrent infection.

The mechanism of muscle damage is most likely related to profound and prolonged compression of muscle with compromise of the regional vascular supply [90, 92, 96, 101-103]. The presence of hypovolemia and hypotension may further contribute to the ischemic damage. There is a direct correlation between the duration of altered consciousness and the severity of the rhabdomyolysis. Moreover, there is no evidence for any major direct toxic effect of narcotics on muscle in the vast majority of addicts who present without coma or stupor. Trauma, exertional stress, and seizures may contribute to the muscle damage in some patients.

The mechanism of the acute renal failure is thought to be multifactorial and similar to other cases of myoglobinuric renal failure [101, 104-109]. These factors include obstruction of tubules, toxic effects of the pigment or iron on renal tubular cells, and altered hemodynamics in association with inhibition of the vasodilator nitric oxide by myoglobin. Experimental animals exposed to heme pigment have increases in the renal synthesis of both heme oxidase and ferritin (108]. This allows for more rapid heme degradation and greater sequestration of potentially toxic iron by the tubular cells [108]. Whether narcotics or the hypotensive, hypoxic environment associated with rhabdomyolysis interfere with these protective effects of the kidney is unknown.

Initial treatment of the acute renal failure consists of intravascular volume repletion and restoration of the blood pressure. Treatment with mannitol, alkalinization of the urine, and diuretics have all been tried with variable success [93, 94, 105, 110]. Clearly, supportive care and dialytic intervention when necessary are crucial to allow adequate recovery from the renal failure. Hemodialysis may be more effective than peritoneal dialysis on highly catabolic patients with rhabdomyolysis-induced renal failure.

Other opiate-related renal lesions

In addition to glomerular lesions inducing progressive renal failure and acute renal failure due to narcotics, a number of patients have been described with chronic renal insufficiency associated with chronic tubulointerstitial changes on biopsy [111, 112]. Some have evidence of granulomatous changes in the interstitium with foreign body giant cells and particulate matter noted in the granulomas and interstitial areas [112]. There have been 5 recent reports of granulomatous glomerulonephritis with 2 described as non-immunoglobulin-associated fibrillary glomerulonephritis with oxycodone suppository addiction, usually used intravenously [112a]. There has also been a case report of the hemolytic-uremic syndrome in a heroin addict as well as a number of cases of membranoproliferative glomerulonephritis [113, 37].

Cocaine

Cocaine has been used by the Indians of South America for at least 2500 years. Its central nervous system effects have been long known and ironically in 1884 Freud wrote one of the first reports on the mental effects of cocaine. In the mid 1980's widespread abuse of various forms of cocaine led to major medical and social problems [114]. This coincides with a decrease in the price of the drug "on the street" and more widespread availability. The use of cocaine has changed from that of "social and recreational" use by the wealthy to a common addiction and affliction that affects all segments of the population, as many millions of Americans use cocaine.

Cocaine HCl is an alkaloid derived from the leaves of the South American coca plant. The free base alkaloid, made by extraction from cocaine HCl, is relatively insoluble in water, but dissolves in a variety of organic solvents. There has been a dramatic increase in the use of cocaine free base, which is most commonly known

by its street name "crack". Since free base is not destroyed by heating, but rather vaporizes, it can be smoked and inhaled [115]. This provides speedy absorption from the respiratory tract inducing a short-lived but rapid euphoria. The free base is also well absorbed by nasal, vaginal, and sublingual mucous membranes as well as by the gastrointestinal tract. Cocaine can also be injected intravenously, intramuscularly, or subcutaneously. Crack is often combined with heroin or other drugs of abuse and taken intravenously [114]. Cocaine is detoxified by cholinesterases and cocaine or its metabolites may be present in the urine for one to two days after use.

Cocaine is a central nervous system stimulant that inhibits the peripheral re-uptake of catecholamines, leading to increased sympathomimetic activity [115]. Its abuse is associated with a variety of medical problems. These include acute myocardial infarction, cardiac arrhythmias, cerebrovascular accidents, hyperpyrexia and stimulated sympathetic activity, seizures and coma, obstetrical complications, intestinal ischemia, and a variety of psychiatric complications [114-117]. The most prominent renal complication of cocaine abuse is acute renal failure associated with rhabdomyolysis.

A number of reports in the mid to late 1980's described patients who developed rhabdomyolysis while using cocaine [118-120]. Some of these patients experienced acute renal failure [121-125]. While the exact incidence of acute renal failure secondary to cocaine rhabdomyolysis is unknown, in one reported series it occurred in only three of 211 admissions for cocaine related complications [114]. On the other hand, in another series of nearly 40 patients the incidence of cocaine related acute rhabdomyolysis increased over the period of enrollment from 2 patients in 1985 to 22 patients in 1987 [126]. Several reports of patients with cocaine-induced rhabdomyolysis have clearly defined both the clinical syndrome and the risk factors for the development of acute renal failure and an adverse outcome [123, 126, 127]. Most patients have been previously healthy young males (mean age 30-35 years old and 80-85% male). The cocaine has been smoked, used intravenously, snorted, or taken orally implying that route of administration was not relevant [122, 123, 126, 127]. In contrast to narcotic related rhabdomyolysis, a history of prolonged coma or stupor is absent. On presentation, the majority of patients are combative and agitated although some are frankly comatose. Only one-half of the patients had evidence of muscle tenderness or myalgias. The creatinine phosphokinase was more than 10 times normal in all patients developing acute renal failure. Between 30 and 50% of patients with cocaine associated rhabdomyolysis develop acute renal failure.

Several features identify patients at risk for developing acute renal failure [126]. While hypertension (blood pressure greater than 140/90 mmHg) was present in about 20 to 30% of the patients, severe hypotension (blood pressure less than 100 mmHg) on presentation occurred in 46% of patients with acute renal failure but only 4% of those who maintained renal function [126]. In this same series patients developing acute renal failure were also more likely to have severe hyperpyrexia (70% versus 15%), and documented seizure activity (30% versus 8%). Patients with acute renal failure have also had higher creatinine phosphokinase levels than those without renal failure [126, 127]. The mean creatinine phosphokinase level for patients developing renal failure has been greater than 20,000 U/L. As might be expected serum uric acid values have been higher and serum calcium values lower in patients with acute renal failure. The mean hematocrit has also been higher in the renal failure group implying more severe volume depletion on admission. Admission serum creatinine ranged from 1.9 mg/dl to greater than 12 mg/dl with peaks as high as 24 mg/dl [126, 127]. About 50% of the acute renal failure patients were oliguric. The urinalysis was positive for heme pigment in 70% and microscopic hematuria and proteinuria were variable.

A bleeding tendency was reported in many of the patients, and in one series 7 of 9 patients with acute renal failure had abnormal coagulation tests with increased fibrin degradation products, decreased fibrinogen levels, prolonged prothrombin times and thrombocytopenia [126]. These 7 patients were felt to have disseminated intravascular coagulation and six of them died despite treatment with plasma infusion and heparin. The associated disseminated intravascular coagulation has been noted by other authors [127]. In one large study, 85% of the acute renal failure patients had evidence of severe liver abnormalities with markedly elevated levels of serum aspartate aminotransferase (at least 40 times above normal for the laboratory) as opposed to only 8% of the patients without acute renal

failure [126].

Almost all patients with cocaine rhabdomyolysis without renal failure survive and are discharged after an average hospital stay of 5 days. The patients with acute renal failure require hemodialysis, and have both a lower survival rate and a more prolonged hospitalization. Of patients with acute renal failure who die, most did so between 2 to 15 days after admission with associated disseminated intravascular coagulation and severe liver dysfunction. Autopsies on these patients showed no evidence of pre-existing renal disease or underlying glomerulopathy.

The exact pathogenesis of cocaine associated rhabdomyolysis remains to be defined [121, 124, 126]. The route of cocaine administration does not predispose to rhabdomyolysis. Moreover hypotension, hyperpyrexia, coma, muscle crush injury, and associated nephrotoxins do not appear to be crucial to the muscle toxicity. Whether there is any direct role of cocaine induced muscle necrosis or a role in combination with sympathetic discharge causing severe arterial vasoconstriction and subsequent ischemia remains to be clarified [114]. The factors predisposing to acute renal failure are similar to other forms of non-traumatic rhabdomyolysis and include volume depletion, hypotension, and increased severity of muscle damage [126, 127]. Rhabdomyolysis may release tissue thromboplastin and other factors inciting disseminated intravascular coagulation and the resulting thrombotic process might accentuate the renal ischemia. The mechanism(s) by which cocaine rhabdomyolysis and myohemoglobinuria produce acute renal failure are probably similar to other forms of myohemoglobinuric acute renal failure.

While acute renal failure due to rhabdomyolysis is by far the most common form of renal damage associated with cocaine, several patients have developed acute renal failure secondary to acute malignant hypertension [128]. With resolution of the malignant hypertension, some patients regain sufficient renal function to terminate dialysis. The etiology of this form of renal failure may be secondary to drug-induced acute vasoconstriction resembling the hypertensive crises seen in patients with scleroderma. Scleroderma with a scleroderma renal crisis has actually been reported as a complication of cocaine abuse (129). Several patients have presented with angiographic evidence of renal infarction in the setting of active intravenous cocaine use [130, 131]. The hypertension abated and the patients were left with no long-term clinical morbidity. A number of other case reports of renal disease associated with cocaine abuse include acute interstitial nephritis [132], antiglomerular basement membrane antibody-mediated glomerulonephritis (133, 134), Henoch-Schonlein purpura with necrotizing vasculitis [135], and a syndrome resembling thrombotic thrombocytopenic purpura [136, 137]. There has been a report of urinary tract infection in infants exposed to cocaine in utero [138]. HIV nephropathy is also occurring with increased frequency in cocaine abusers [139].

The association of cocaine abuse with progressive chronic renal failure has received increased attention in recent years [140-142]. Ward and co-workers have reported the possibility of a progressive nephropathy with features of hypertension, azotemia and proteinuria in 50 African American cocaine abusers [143]. The renal presentation is often nonspecific with low-grade proteinuria and lack of specific findings on urinalysis. Some patients displayed nephrotic range proteinuria similar to heroin nephropathy. Renal biopsy was obtained in 20 of these patients and revealed the presence of a variety of glomerular and vascular abnormalities including focal glomerulosclerosis, collapsing focal sclerosis, immune complex glomerulonephritis and ischemic arteriolitis. On average, the patients were predominantly African American with 1 to 10 years of abusing cocaine at least once weekly. However, an earlier study by the same group noted that Caucasian cocaine users also displayed an increased risk for renal disease [140]. A discharge diagnosis of hypertensive renal disease was associated with cocaine use in over one third of cases in that study. Finally, cocaine has been implicated as a risk factor for the development of ESRD in young dialysis patients with a shorter duration of hypertension by history [142]. The relative risk of developing ESRD with cocaine abuse was nearly 10 times higher than that of race and blood pressure matched controls. In summary, evidence for a progressive nephropathy associated with cocaine abuse is accumulating and could constitute a significant cause for ESRD in the United States.

Phencyclidine

Phencyclidine is an anesthetic, analgesic, hallucinogenic drug, which was widely abused in the 1970's.

As a street drug it was known as "peace pill", "crystal", "hog", and most commonly "PCP" or "angel dust" [144]. It is often used in combination with other illicit drugs, and may be smoked, inhaled, snorted, or taken by injection. The abuse of phencyclidine has been associated with respiratory depression, convulsions, hyperpyrexia, hypertensive crisis, and schizoid psychoses. It has also caused rhabdomyolysis in many reported cases, often in association with acute renal failure [144-149]. In one group of 1000 patients admitted with a diagnosis of phencyclidine abuse, 25 patients (2.5%) experienced rhabdomyolysis, and 10 developed acute renal failure [145]. Thus, 40% of the patients with phencyclidine-associated rhabdomyolysis develop acute renal failure, while others may develop mild, rapidly reversible renal insufficiency probably related to volume depletion. As with cocaine and heroin induced acute renal failure most patients have been young males [145, 148]. About 50% are comatose on admission while others display a variety of organic brain syndromes and mental dysfunctions. Complaints of myalgias are common. Hyperpyrexia, tachycardia, hypertension, exaggerated muscle activity and acute dystonic motor reactions are all common findings on admission [144, 145, 148). Markedly elevated levels of serum creatinine phosphokinase and leukocytosis are also common. Of the patients with acute renal failure, the serum creatinine is usually elevated on admission (range 1.2 mg/dl to 12.7 mg/dl with a mean of 4.1 mg/dl in one large series). The urine is typically orthotoluidine positive in the absence of significant hematuria, and granular casts and a positive test for myoglobin are common. The serum creatinine rapidly peaks and then returns toward normal. Even though some patients will require dialytic support, the majority of patients recover significant renal function. During the period of acute renal failure, only 50% of patients are oliguric, but most manifest hyperuricemia, hyperphosphatemia, and hypocalcemia. Rebound hypercalcemia may occur during the recovery phase of renal failure.

In some patients the etiology of the acute renal failure may relate to isometric tension in restrained limbs, and in others to ischemic damage to muscle in the presence of hyperthermia and/or limb compression [144, 149]. While it is possible that the drug itself may possess direct myopathic toxicity when abused in certain settings, it does not induce rhabdomyolysis in unrestrained animals [150]. Animals restrained in immobilizing cages, however, develop rhabdomyolysis, which correlates with isometric muscle tension during the restrained period, and this can be prevented by prior denervation [150].

Treatment with avoidance of restraints, intravascular volume repletion, and perhaps muscle paralyzing drugs has been advocated. Although urinary acidification had been advocated to promote phencyclidine excretion, this may be deleterious in patients with rhabdomyolysis, hyperuricemia, and myoglobinuria and should be avoided [149].

Amphetamines

Amphetamines are sympathomimeticamines with central nervous system stimulatory activity. They may induce a number of patterns of renal damage including rhabdomyolysis related acute renal failure, acute interstitial nephritis, and an angiitis resembling polyarteritis nodosa.

Methamphetamine alone or in combination with heroin or d-lysergic acid diethylamide has been associated with a necrotizing angiitis similar to that seen in idiopathic polyarteritis nodosa [151]. Although most of these patients have been intravenous abusers of multiple drugs, the common denominator in most cases and the sole drug in others has been methamphetamine [151, 152]. One study described 14 patients with drug (and presumably methamphetamine) related vasculitis seen in a short time period [152]. While others cite the rarity of this lesion with no case in over 1000 consecutive autopsies in addicts, the diligence with which the lesions were sought in this population has been questioned [153, 154]. The lesions have occurred in both male and female intravenous drug abusers who usually present with a prodromal illness of fever, weight loss, malaise, and weakness. The angiitis may involve any body organ and patients may experience central nervous system symptoms, abdominal pain, arthralgias, myalgias, and other systemic findings akin to idiopathic polyarteritis [152]. Renal involvement is characterized by mild proteinuria, hematuria, hypertension, and often progressive renal failure. On angiographic examination and at autopsy the lesions in the kidneys are similar to those found in classic polyarteritis with involvement of middle size vessels especially at bifurcations with aneurysms, lumenal irregularities, and sacculations [151]. The lesions are

noted to be in different stages of development with some showing active inflammation of the vessel wall, other neighboring lesions showing more chronic healing lesions, and still others demonstrating occluded vessels with evidence of distal infarction [151].

The relationship between amphetamine abuse and the presence of hepatitis B antigenemia and an immune complex vasculitis remains unclear [155-157]. While similar lesions have been described in non-drug abusing patients who are serologically positive for the hepatitis B antigen, in the largest series of amphetamine abusers only 30% were positive for the hepatitis B antigen [154]. Nevertheless, the method and sensitivity of these earlier screening tests for hepatitis B have been questioned. The situation may be even less clear now that hepatitis C has been shown to be associated with a polyarteritis like syndrome and vasculitis [158]. Nevertheless, it is equally possible that in some cases either the direct effects of amphetamines or immune complexes formed by drug-induced release of tissue antigens can produce a vasculitis similar to polyarteritis nodosa [151].

Amphetamines have also been associated with a syndrome of acute renal failure and rhabdomyolysis. Several series describe patients following intravenous injection of methamphetamine or phenmetrazine who presented with hyperactivity, fever, chills, sweats, abdominal cramps, diarrhea, and hypotension [88, 89, 159, 160]. The patients have developed acute renal failure, which is usually oliguric and associated with classic rhabdomyolysis. Several patients have had a picture of disseminated intravascular coagulation and liver function abnormalities as well. The patients' course was typical for oliguric rhabdomyolytic acute tubular necrosis with recovery of renal function over time. The syndrome greatly resembles recent cases of cocaine-associated rhabdomyolysis.

Ecstacy (MDMA; methylenedioxymethamphetamine) use is the fastest growing new form of drug abuse in the United States and has already been implicated as a cause of rhabdomyolysis and acute renal failure [161]. Ironically, efforts to pre-empt overheating and dehydration by drinking large fluid volumes ("chill out" rooms at "rave" parties) has led to cases of life-threatening hyponatremia [162]. Accelerated hypertension and unexplained chronic renal failure has also been observed. Methamphetamine abuse is associated with acute lead poisoning since a common reagent used in its production utilizes lead acetate.

At least one case of acute interstitial nephritis has been attributed to amphetamine use [163]. This amphetamine abuser presented with acute nonoliguric renal failure, large kidneys by ultrasonography, and microhematuria. The biopsy revealed interstitial edema and focal infiltrates of mononuclear cells and eosinophils with only patchy tubular degeneration. There was no evidence for rhabdomyolysis and the urine was negative for myoglobin. Although he required temporary hemodialytic support, renal function returned to normal after treatment with intravenous corticosteroids. The mechanism of this reaction remains unclear and its true relation to amphetamine abuse remains unproven.

Other drugs

Rhabdomyolysis and acute renal failure have been reported with a variety of other drugs and potentially abused medications. Many cases of this syndrome have been attributed to ethyl alcohol abuse [90-92, 103]. There are many potential etiologies for rhabdomyolysis in these patients including trauma to muscles, alcohol related hypokalemia and metabolic disturbances, sustained seizure activity, and a direct toxic effect of the alcohol [102, 103]. Alcohol has been shown to produce a rise in muscle enzymes and electron microscopic morphologic changes in muscles even without trauma, seizures, or ischemia to a limb. Nevertheless, the vast majority of patients present with coma or stupor, limb compression and a picture similar to that seen with other drugs. Indeed, many patients have a combined overdose of alcohol and a second drug as the etiology of their altered mental state [90, 103].

While alcohol abuse may be associated with a variety of electrolyte and acid-base disorders, the role of the kidneys in this process has only recently been fully defined [164]. Renal functional abnormalities have now been related to chronic alcoholism in patients without liver disease and these defects have reverted to normal with abstinence from alcohol abuse. These abnormalities include decreases in the maximal reabsorptive ability and threshold for glucose, a decrease in the threshold for phosphate excretion, and increases in the fractional excretion of β2-microglobulin, uric acid, calcium, magnesium, and amino acids. Defective tubular acidification and impaired renal concentrating ability

are also commonly found. Thus, defects at multiple sites along the nephron are common in patients with chronic alcohol abuse [164].

Acute renal failure has been associated with a variety of sedatives and hypnotics including barbiturates, benzodiazepines, glutethimide, and chlorpromazine [90, 91, 94, 102]. The acute renal failure is usually related to rhabdomyolysis but the classical clinical picture of acute interstitial nephritis has been reported in one patient with the use of diazepam, although no renal biopsy was performed [165]. In those patients with rhabdomyolysis, multiple seizures often develop prior to the rhabdomyolysis and others are febrile at the time. However, the most common presentation is that of a young person without prior major medical history who presents with coma-stupor of one to several days duration, variable signs of volume depletion, and limb compression [90-92]. Some ingestions are secondary to accidental over-doses and some are due to suicide attempts or gestures. The acute renal failure is oliguric in 1/3 to 1/2 of patients and follows the typical course of acute tubular necrosis with a high recovery rate [90, 91].

References

1. Avram MM, Iancu M, Weiss S. Heroin usage nephropathy - subclinical to end stage nephrotic syndrome. J Am Soc Nephrol 1971; 5A.
2. Eknoyan G, Gyorkey F, Dichoso C, Hyde SE, Gyorkey P, Suki WN, Matinez-Maldonado M. Renal involvement in drug abuse. Arch Intern Med 1973; 132: 801-806.
3. Friedman EA, Rao TKS, Nicastri AD. Heroin-associated nephropathy. Nephron 1974; 13: 421-426.
4. Kilcoyne MM, Gocke DJ, Meltzer JI, Daly JJ, Thomason GE, Hsu KC, Tannenbaum M. Nephrotic syndrome in heroin addicts. Lancet 1972; 1: 17-20.
5. Matalon R, Katz L, Gallo G. Glomerular sclerosis in adults with nephrotic syndrome. Ann Int Med 1974; 80: 488-495.
6. McGinn JT, McGinn TG, Cherubin CE, Hoffman RS. Nephrotic syndrome in drug addicts. NY State J Med 1974; 74: 92-95.
7. Rao TKS, Nicastri AD, Friedman EA. A natural history of heroin associated nephropathy. N Engl J Med 1974; 290: 19-23.
8. Sapira JD, Ball JC, Penn H. Causes of death among institutional narcotic addicts. J Chronic Dis 1970; 22: 733-742.
9. Salomon MI, Poon TP, Goldblatt M, Tchertkoff V. Renal lesions in heroin addicts: a study based on kidney biopsies. Nephron 1972; 9: 356-363.
10. Arruda JAL, Kurtzman NA, Pillay VKG. Prevalence of renal disease in asymptomatic heroin addicts. Arch Intern Med 1975; 135: 535-537.
11. Llach F, Descoeudres C, Massry SG. Heroin associated nephropathy: clinical and histological studies in 19 patients. Clin Nephrol 1979; 11: 7-12.
12. Thompson AM, Anthonovych T, Lin R. Focal membranoproliferative glomerulonephritis in heroin users. J Am Soc Nephrol 1973; 105A.
13. Treser G, Cherubin C, Lonergan ET, Yoshizawa N, Viswanathan V, Tannenberg AM, Pompa D, Lange K. Renal lesions in narcotic addicts. Am J Med 1974; 57: 687-694.
14. Gallo G, Neugarten J, Buxbaum J, Katz L. Renal amyloidosis in subcutaneous heroin abusers. ASN (New Orleans) 1985; 38A.
15. Brus I, Steiner G, Maceda A, Lejano R. Amyloid fibrils in urinary sediment. Heroin addiction with renal amyloidosis. NY State J Med 1979; 79: 768-771.
16. Dubrow A, Mittman N, Ghali V, Ghali V, Flamenbaum W. The changing spectrum of heroin-associated nephropathy. Am J Kidney Dis 1985; 5: 36-41.
17. Jacob H, Charytan C, Rascoff JH, Golden R, Janis R. Amyloidosis secondary to drug abuse and chronic skin suppuration. Arch Intern Med 1978; 138: 1150-1151.
18. Meador KH, Sharon Z, Lewis EJ. Renal amyloidosis and subcutaneous drug abuse. Ann Intern Med 1979; 91: 565-567.
19. Novick DM, Yancovitz SR, Weinberg PG. Amyloidosis in parenteral drug abusers. Mt Sinai J Med 1979; 46: 163-167.
20. Scholes JV, Derosena R, Appel GB. Amyloidosis and the nephrotic syndrome in chronic heroin addicts. Proc 7th Int Congr Nephrol 1978; L9.
21. Scholes J, Derosena R, Appel GB, Ao W, Boyd MT, Pirani CL. Amyloidosis in chronic heroin addicts with the nephrotic syndrome. Ann Intern Med 1979; 91: 26-29.
22. Kunis C, Olesnicky M, Nurse H. Heroin nephropathy-clinical pathologic correlations. Proc 9th Int Congr Nephrol 1984; 102A.
23. Menchel S, Cohen D, Gross E, Frangione B, Gallo G. AA protein-related renal amyloidosis in drug addicts. Am J Pathol 1983; 112: 195-199.
24. D'Agati V, Cheng JI, Carbone L, Cheng JT, Appel G. The pathology of HIV-nephropathy: a detailed morphologic and comparative study. Kidney Int 1989; 35: 1358-1370.
25. D'Agati V, Appel GB. HIV infection and the kidney. J Am Soc Nephrol 1997; 8: 138-152.

26. D'Agati V, Appel GB. Renal pathology of human immunodeficiency virus infection. Sem Nephrol 1998; 18: 406-421.
27. Friedman EA, Tao TK. Disappearance of uremia due to heroin-associated nephropathy. Am J Kidney Dis 1995: 5: 689-693.
28. Cunningham EE, Brentjens JR, Zielezny MA, Andres GA, Venuto RC. Heroin nephropathy - a clinicopathologic and epidemiologic study. Am J Med 1980; 68: 47-53.
29. Cunningham EE, Zielezny MA, Venuto RC. Heroin-associated nephropathy - a nationwide problem. JAMA 1983; 250: 2935-2936.
30. Davis JS, Lie JT. Extracellular glomerular microparticles in nephrotic syndrome of heroin users. Arch Pathol 1975; 99: 278-282.
31. Gardiner H, Mahajan S, Briggs W. Renal disease in heroin addicts. J Am Soc Nephrol 1977; 15A.
32. Grishman E, Churg J. Focal glomerular sclerosis in nephrotic patients. An electron microscope study of glomerular podocytes. Kidney Int 1975; 7: 111-122.
33. Grishman E, Churg J, Porush JG. Glomerular morphology in nephrotic heroin addicts. Lab Invest 1976; 35: 415-424.
34. Rao TKS, Nicastri AD, Friedman EA. Renal consequences of narcotic abuse. Adv Nephrol 1979; 7: 261-290.
35. Baldwin DS, Gallo GR, Neugarten J. Drug abuse with narcotics and other agents in diseases of the kidney. In: Diseases of the kidney, 5th ed. Schrier RW, Gottschalk CW (editors). Little Brown and Co, Boston 1992; p 1219-1236.
36. Stehman-Breen C, Alpers CE, Fleet WP, Johnson RJ. Focal segmental glomerular sclerosis among patients infected with hepatitis C virus. Nephron 1999; 81: 37-40.
37. Dettmeyer R, Wessling B, Madea B. Heroin-associated nephropathy – a post-mortem study. Forensic Sci Int 1998; 95: 109-116.
38. Glicklich D, Haskell L, Senitzer D, Weiss RA. Possible genetic predisposition to idiopathic focal segmental glomerulosclerosis. Am J Kidney Dis 1988; 12: 26-30.
39. Perneger TV, Klag MJ, Whelton PK. Recreational drug use: a neglected risk factor for ESRD. Am J Kidney Dis 2001; 38: 49-56.
40. Haskell LP, Glicklich D, Senitzer D. HLA associations in heroin-associated nephropathy. Am J Kidney Dis 1988; 12: 45-50.
41. May DC, Helderman JH, Eigenbrodt EH, Silva FJ. Chronic sclerosing glomerulopathy (heroin-associated nephropathy) in intravenous T's and blues abusers. Am J Kidney Dis 1986; 8: 404-409.
42. Moody C, Kaufman R, McGuire D, Grossman S. The role of adulterants in heroin nephropathy. NKF (New Orleans) 1985; 32.
43. Brown SM, Stimmel B, Taub RN, Kochwa S, Rosenfield RE. Immunologic dysfunction in heroin addicts. Arch Intern Med 1974; 134: 1001-1006.
44. Ringle DA, Herndon BL. In-vitro morphine binding by sera from morphine-treated rabbits. J Immunol 1972; 109: 174-175.
45. Ryan JJ, Parker CW, Williams RC. Gamma-globulin binding of morphine in heroin addicts. J Lab Clin Med 1972; 80: 155-164.
46. Weksler ME, Cherubin C, Kilcoyne M, Koppel G, Yoel M. Absence of morphine-binding activity in serum from heroin addicts. Clin Exp Immunol 1973; 13: 613-617.
46a. Nikolova M., Liubomirova M, Iliev A, Krasteva R, Andreev E, Radenkova J, Minkova V, Djerassi R, Kiperova B, Vlahov ID. Clinical significance of antinuclear antibodies, anti-neutrophil cytoplasmic antibodies and anticardiolipin antibodies in heroin abusers. Isr Med Assoc J 2002 ;4 (11 Suppl):908-910.
47. Marchand C, Cantin M, Cote M. Evidence for the nephrotoxicity of morphine sulfate in rats. Can J Physiol Pharmacol 1969; 47: 649-655.
48. Johnson JE, White JJ, Walovitch RC, London ED. Effects of morphine on rat kidney glomerular podocytes. A scanning electron microscope study. Drug Alcohol Depend 1987; 19: 249-257.
49. Singhal PC, Gibbons N, Abromovici M. Long term effects of morphine on mesangial cell proliferation and matrix synthesis. Kidney Int 1992; 41: 1560-1570.
50. Singhal PC, Pan CQ, Sagar S, Valderrama E, Stahl RA. Morphine modulates mesangial immunoglobulin G uptake in rats with antithymocyte serum-induced mesangial cell injury. Nephron 1996; 74: 197-203.
51. Kapasi AA, Gibbons N, Mattana J, Singhal PC. Morphine stimulates mesangial cell TNF-α and nitrite production. Inflammation 2000; 24: 463-476.
52. Singhal PC, Sharma P, Gibbons N, Franki N, Kapasi A, Wagner JD. Effect of morphine on renomedullary interstitial cell proliferation and matrix accumulation. Nephron 1997; 77: 225-234.
53. Singhal PC, Sharma P, Sanwal V, Prasad A, Kapasi A, Ranjan R, Franki N, Reddy K, Gibbons N. Morphine modulates proliferation of kidney fibroblasts. Kidney Int 1998; 53: 350-357.
54. Rao TK, Filippone EJ, Nicastri AD, Landesman SH, Frank E, Chen CK, Friedman EA. Associated focal and segmental glomerulosclerosis in the acquired immunodeficiency syndrome. N Engl J Med 1984; 310: 669-673.
55. Mendoza SA, Reznik VM, Griswold WR, Krensky AM, Yorgin PD, Tune BM. Treatment of steroid-resistant focal segmental glomerulosclerosis with pulse methylprednisolone and alkylating agents. Ped Nephrol 1990; 4: 303-307.
56. Banfi G, Moriggi M, Sabadini E, Felling G, D'Amico G, Ponticelli G. The impact of prolonged immunosuppression of the outcome of idiopathic focal segmental glomerulosclerosis with nephrotic syndrome in adults: a collaborative retrospective study. Clin Nephrol 1991; 36: 53-59.

57. Pei Y, Cattran D, Delmore T, Katz A, Lang A, Rance P. Evidence suggesting under-treatment in adults with idiopathic focal segmental glomerulosclerosis. Regional glomerulonephritis registry study. Am J Med 1987; 82: 938-944.
58. Korbet SM, Schwartz MM. Primary focal segmental glomerulosclerosis: a treatable lesion with variable outcomes. Nephrology 2001; 6: 47-56.
59. Matalon A, Valeri A, Appel GB. Treatment of focal segmental glomerulosclerosis. Sem Nephrol 2000; 20: 309-317.
60. Appel GB, Valeri A. Focal segmental glomerulosclerosis in primer on Kidney Diseases (3rd edition). Greenberg A (editor). Academic Press, San Diego 2001.
61. Cattran DC, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE, Maxwell DR, Kunis CL. A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. Kidney Int 1999; 56: 2220-2226.
62. Ingalli E, Tejani A, Fikrig S, Nicastri A, Chen CK, Pomrantz A. Nephrotic syndrome associated with acquired immunodeficiency syndrome in children. J Ped 1991; 119: 710-716.
63. Eustace JA, Wuermberger K, Choi M, Scheel P, Moore R, Briggs W. Cohort study of the treatment of severe HIV-associated nephropathy with steroids. Kidney Int 2000; 58: 1253-1260.
64. Ross G, Weinstein S, Dutton S, Whittier FC. Renal transplantation in end stage renal disease of drug abuse. J Urol 1983; 129: 14-15.
65. Gordon MJ, White R, Matas A, Tellis VA, Glicklich D, Quinn T, Soberman R, Veith FJ. Renal transplantation in patients with history of heroin abuse. Transplantation 1986; 42: 556-557.
66. Amigo JS, Orriols J, Modol J. Resolution of nephrotic syndrome secondary to heroin-associated renal amyloidosis. Nephrol Dial Transplant 1990; 158.
67. Crowley S, Feinfeld DA, Janis R. Resolution of nephrotic syndrome and lack of progression of heroin associated renal amyloidosis. Am J Kidney Dis 1989; 13: 333-335.
68. Derosena R, Koss MN, Pirani CL. Demonstration of amyloid fibrils in urinary sediment. N Engl J Med 1975; 293: 1131-1133.
68a. Shah SP, Khine M, Anigbogu J, Miller A. Nodular amyloidosis of the lung from intravenous drug abuse: an uncommon cause of multiple pulmonary nodules. South Med J 1998 ;91 (4):402-404.
69. Neugarten J, Gallo GR, Buxbaum J, Katz LA, Rubenstein J, Baldwin DS. Amyloidosis in subcutaneous heroin abusers. Am J Med 1986; 81: 635-640.
70. Tan AU Jr, Cohen AH, Levine BS. Renal amyloidosis in a drug abuser. J Am Soc Nephrol 1995; 5: 1653-1658.
71. Appel GB, Nicolaides M. HIV-AIDS nephropathy in the inner city. New York State J Med 1991; 91: 207-210.
72. Rao TK, Friedman EA, Nicastri AD. The types of renal disease in the acquired immunodeficiency syndrome. N Engl J Med 1987; 316: 1062-1068.
73. Carbone L, D'Agati V, Cheng J-T, Appel GB. Course and prognosis of human immunodeficiency virus-associated nephropathy. Am J Med 1989; 87: 389-395.
74. Pardo V, Aldana M, Colton RM, Fischl MA, Jaffe D, Moskowitz L, Hensley GT, Bourgoignie JJ. Glomerular lesions in the acquired immunodeficiency syndrome. Ann Intern Med 1984; 101: 429-434.
75. Strauss J, Abibol C, Zilleruelo G, Scott G, Paredes A, Malaga S, Montane B, Mitchell C. Renal disease in children with the acquired immunodeficiency syndrome. N Engl J Med 1989; 321: 625-630.
76. Bourgoigne JJ. Renal complications of human immuodeficiency virus type I. Kidney Int 1990; 37: 1571-1584.
77. Seney FD, Burns DK, Silva FG. Acquired immunodeficiency syndrome and the kidney. Am J Kidney Dis 1990; 16: 1-13.
78. Glassock RJ, Cohen AH, Danovitch G, Parsa P. Human immunodeficiency virus infection and the kidney. Ann Int Med 1990; 112: 35-49.
79. Pardo V, Meneses R, Ossa L, Jaffe DJ, Strauss J, Roth D, Bourgoignie JJ. AIDS-related glomerulopathy: occurrence in specific risk groups. Kidney Int 1987; 31: 1167-1173.
80. Winston JA, Burns GC, Klotman PE. The human immunodeficiency virus epidemic and HIV associated nephropathy. Sem Nephol 1998; 18: 373-377.
81. Cohen AH, Sun NCJ, Shapshak P, Imagawa DT. Demonstration of HIV in renal epithelium in HIV associated nephropathy. Modern Path 1989; 2: 125-128.
82. Winston JA, Bruggeman LA, Ross MD, Jacobson J, Ross L, D'Agati V, Klotman PE, Klotman ME. Nephropathy and establishment of a renal reservoir of HIV type 1 during primary infection. N Engl J Med 2001; 344: 1979-1984.
83. Wali RK, Drachenberg CI, Papadimitriou JC, Keay S, Ramos E. HIV-1-associated nephropathy and respose to highly-active antiretroviral therapy. Lancet 1998; 352: 783-784.
84. Ho DD, Zhang L. HIV-1 rebound after abti-retroviral therapy. Nat Med 2000; 6: 736-737.
85. Monahan M, Tanji N, Klotman PE. HIV-associated nephropathy: An urban epidemic. Semin Nephrol 2001; 21: 394-402.
86. Kimmel PL, Ferreira-Centeno A, Farkas-Szallasi T, Abraham AA, Garrett CT. Viral DNA in microdissected renal biopsy material from HIV infected patients with the nephrotic syndrome. Kidney Int 1993; 43: 1347-1352.
87. Green DF, Resnick L, Bourgoignie J. HIV infects glomerular endothelial and mesangial cells but not epithelial cells *in vitro*. Kidney Int 1992; 41: 956-960.

88. Kopp JB, Klotman ME, Adler SH, Bruggeman LA, Dickie P, Marinos NJ, Eckhaus M, Bryant JL, Notkins AL, Klotman PE. Progressive glomerulosclerosis and enhanced renal accumulation of basement membrane components in mice transgenic for HIV type 1 genes. Proc Nat Acad Sci 1992; 89: 1577-1581.
89. Singhal PC, Sagar S, Reddy K, Sharma P, Ranjan R, Franki N. HIV-1 gp120 envelope protein and morphine-tubular cell interaction products modulate kidney fibroblast proliferation. J Invest Med 1998; 46: 243-248.
89a. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest 2003; 111 (5):707-716.
89b. Kimmel PL, Cohen DJ, Abraham AA, Bodi I, Schwartz AM, Philips TM. Upregulation of MHC class II, interferon-α and interferon-γ receptor protein expression in HIV-associated nephropathy. Nephrol Dial Transplant 2003; 18 (2):285-292.
90. Koffler A, Friedler RM, Massry SG. Acute renal failure due to nontraumatic rhabdomyolysis. Ann Intern Med 1976; 85: 23-28.
91. Penn AS, Rowland LP, Fraser DW. Drugs, coma, and myoglobinuria. Arch Neurology 1972; 26: 336-344.
92. Grossman RA, Hamilton RW, Morse BM, Penn AS, Goldberg M. Nontraumatic rhabdomyolysis and acute renal failure. N Engl J Med 1974; 291: 807-811.
93. Ward MM. Factors predictive of acute renal failure in rhabdomyolysis. Arch Intern Med 1988; 148: 1553-1557.
94. Eneas JF, Schoenfeld PY, Humphreys MH. The effect of infusion of mannitol-sodium bicarbonate on the clinical course of myoglobinuria. Arch Intern Med 1979; 139: 801-805.
95. Robinson SF, Woods AH. Heroin induced rhabdomyolysis and acute renal failure: a case report. Arizona Med 1974; 31: 246-251.
96. Richter RW, Challenor YB, Pearson J, Kagen LJ, Hamilton LL, Ramsey WH. Acute myoglobinuria associated with heroin addiction. JAMA 1971; 216: 1172-1176.
97. Rao TK, Nicastri AD, Friedman EA. Natural history of heroin associated nephropathy. In: Nephrology. Hamburger J, Crosnier J, Maxwell MH (editors). Wiley, New York 1979; p 843-856.
98. Rice EK, Isbel NM, Becker GJ, Atkins RC, McMahon LP. Heroin overdose and myoglobinuric acute renal failure. Clin Nephol 2000; 54:449-454.
99. Corwin HL, Schreiber MJ, Fang LS. Low fractional excretion of sodium. Occurrence with hemoglobinuric and myoglobinuric-induced acute renal failure. Arch Intern Med 1984; 144: 981-982.
100. Llach F, Felsenfeld AJ, Haussler MR. The pathophysiology of altered calcium metabolism in rhabdomyolysis-induced acute renal failure. N Engl J Med 1981; 305: 117-123.
101. Gabow PA, Kaehny WD, Kelleher SP. The spectrum of rhabdomyolysis. Medicine 1982; 61: 141-152.
102. Penn AS. Myoglobin and myoglobinuria. In: The handbook of clinical neurology - disease of muscle. Vinken PJ, Bruyn GW (editors). North-Holland Publishing Co, Amsterdam, New York, Oxford 1979; 41: 259-285.
103. Rowland LP, Penn AS. Myoglobinuria. Med Clin N Am 1972; 56: 1233-1256.
104. Zager RA. Studies of mechanisms and protective maneuvers in myoglobinuric acute renal failure. Lab Invest 1989; 60: 619-629.
105. Better OS. Early management of shock and prophylaxis of acute renal failure in traumatic rhabdomyolysis. N Engl J Med 1990; 322: 825-828.
106. Venuto RC. Pigment-associated acute renal failure: is the water clearer 50 years later ? J Lab Clin Med 1992; 119: 452-454.
107. Odeh M. The role of reperfusion-induced injury in the pathogenesis of the crush syndrome. N Engl J Med 1991; 324: 1417-1422.
108. Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, Levitt, Rosenberg ME. Induction of heme oxidase is a rapid protective response in rhabdomyolysis in the rat. J Clin Invest 1992; 90: 267-270.
109. Zager RA. Combined mannitol and desferoxamine therapy for myohemoglobinuric renal injury and oxidant tubular stress. Mechanistic and therapeutic implications. J Clin Invest 1992; 90: 711-719.
110. Ron D, Taitelman U, Michaelson M, Bar-Joseph G, Bursztein S, Better OS. Prevention of acute renal failure in traumatic rhabdomyolysis. Arch Intern Med 1984; 144: 277-280.
111. Steinmuller DR, Bolton WK, Stillman MM, Couser WG. Chronic interstitial nephritis and mixed cryoglobulin associated with drug abuse. Arch Pathol Lab Med 1979; 103: 63-66.
112. McAllister CJ, Horn R, Havron A, Abramson JH. Granulomatous interstitial nephritis: a complication of heroin abuse. S Med J 1979; 72: 162-165.
112a. Hill P, Dwyer K, Kay T, Murphy B. Severe chronic renal failure in association with oxycodone addiction: a new form of fibrillary glomerulopathy. Hum Pathol 2002; 33(8): 783-787.
113. Peces R, Diaz-Corte C, Baltar J, Seco M, Alvarez-Grande J. Haemolytic-uraemic syndrome in a heroin addict. Nephrol Dial Transplant. 1998; 13: 3197-3199.
114. Rubin RB, Neugarten J. Medical complications of cocaine: changes in pattern of use and spectrum of complications. Clin Toxicology 1992; 30: 1-12.
115. Adverse effects of cocaine abuse. Med Letter 1984; 26: 51-52.

116. Cregler LI, Mark H. Medical complications of cocaine abuse. N Engl J Med 1986; 315: 1495-1500.
117. Isner JM, Estes NA, Thompson PD, Costanzo-Nordin MR, Subramanian R, Miller G, Katsas G, Sweeney K, Sturner WQ. Acute cardiac events temporally related to cocaine abuse. N Engl J Med 1986; 315: 1438-1443.
118. Zamoa-Quezada JC, Dinerman H, Stadecker MJ, Kelly JJ. Muscle and skin infarction after free-basing cocaine (Crack). Ann Intern Med 1988; 108: 564-566.
119. Krohn KD, Slowman-Kovacs S, Leapman SB. Cocaine and rhabdomyolysis. Ann Intern Med 1988; 108: 639-640.
120. Rubin RB, Neugarten J. Cocaine-induced rhabdomyolysis masquerading as myocardial ischemia. Am J Med 1989; 86: 551-553.
121. Lombard J, Wong B, Young JH. Acute renal failure due to rhabdomyolysis associated with cocaine toxicity. West J Med 1988; 148: 466-468.
122. Herzlich BC, Arsura EL, Pagala M, Grob D. Rhabdomyolysis related to cocaine abuse. Ann Intern Med 1988; 109: 335-336.
123. Pogue VA, Nurse HM. Cocaine-associated acute myoglobinuric renal failure. Am J Med 1989; 86: 183-186.
124. Merigian KS, Roberts JR. Cocaine intoxication: hyperpyrexia, rhabdomyolysis and acute renal failure. Clin Toxicol 1987; 25: 135-148.
125. Faulkner M, Singhal P, Peters A. Rhabdomyolysis and acute renal failure following cocaine abuse. Kidney Int 1989; 35: 225A.
126. Roth D, Alarcon FJ, Fernandez JA, Preston RA, Bourgoignie JJ. Acute rhabdomyolysis associated with cocaine intoxication. N Engl J Med 1988; 319: 673-677.
127. Singhal PC, Rubin RB, Peters A, Santiago A, Neugarten J. Rhabdomyolysis and acute renal failure associated with cocaine abuse. Clin Toxicology 1990; 28: 321-330.
128. Barrido DT, Joseph AJ, Rao TK, Friedman EA. Renal disease associated with acute and chronic "Crack" abuse. Kidney Int 1988; 33: 181A.
129. Attoussi S, Faulkner ML, Oso A, Umoru B. Cocaine-induced scleroderma and scleroderma renal crisis. South Med J 1998; 91: 961-963.
130. Sharff JA. Renal infarction associated with intravenous cocaine use. Ann Emerg Med 1984; 13: 1145-1147.
131. Goodman PE, Rennie WP. Renal infarction secondary to nasal insufflation of cocaine. Am. J Emerg Med 1995; 13: 421-423.
132. Alvarez D, Nzerue CM, Daniel JF, Faruque S, Hewan-Lowe K. Acute interstitial nephritis induced by crack cocaine binge. Nephrol Dial Transplant 1999; 14: 1260-1262.
133. Peces R, Navascues RA, Baltar J, Seco M, Alvarez J. Antiglomerular basement membrane antibody-mediated glomerulonephritis after intranasal cocaine use. Nephron 1999; 81: 434-438.
134. Garcia-Rostan GM, Garcia-Bragado F, Puras-Gil AM. Pulmonary hemorrhage and antiglomerular membrane antibody-mediated glomerulonephritis after exposure to smoked (crack) cocaine: A case report and review of the literature. Pathol Int 1997: 47: 692-697.
135. Chevalier X, Rostoker G, Larget-Piet B, Gherardi R. Schoenlein-Henoch pupura with necrotizing vasculitis after cocaine snorting. Clin Nephrol 1995; 43: 348-349.
136. Volcy J, Nzerue CM, Oderinde A, Hewan-Lowe K. Cocaine-induced acute renal failure, hemolysis, and thrombocytopenia mimicking thrombotic thrombocytopenic purpura. Am J Kidney Dis 2000; 35: E3.
137. Tumlin JA, Sands JM, Someren A. Hemolytic uremic syndrome following "crack" cocaine inhalation. Am J Med Sci 1990; 299: 366-371.
138. Gottbrath-Flaherty EK, Agrawal R, Thaker V, Patel D, Ghai K. Urinary tract infections in cocaine-exposed infants. J Perinatol 1995; 15: 203-207.
139. Klotman PE. HIV-associated nephropathy. Kidney Int 1999; 56: 1161-1176.
140. Ward HJ, San Diego R, Pan DK. Substance abuse as a risk factor for hypertensive renal disease. J Am Soc Nephrol 1998; 162 A (abstract).
141. Norris K, Thornhill-Joynes M, Robinson C, Strickland T, Alperson B, Witana S, Ward HJ. Cocaine use, hypertension and end stage renal disease. Am J Kidney Dis 2001; 38: 523-528.
142. Dunea G, Arruda JL, Bakin-Share DS, Smith C. Role of cocaine in end-stage renal disease in some hypertensive African-Americans. Am J Nephrol 1995; 15: 5-9.
143. Ward HJ, Pickett R, Tovar L. Cocaine associated nephropathy: a new clinicopathologic entity. J Am Soc Nephrol 2000; 11; 79A (abstract).
144. Cogen FC, Rigg G, Simmons JL, Domino EF. Phencyclidine-associated acute rhabdomyolysis. Ann Intern Med 1978; 88: 210-212.
145. Akmal M, Valdin JR, McCarron MM, Massry SG. Rhabdomyolysis with and without acute renal failure in patients with phencyclidine intoxication. Am J Nephrol 1981; 1: 91-96.
146. Hoogwerf B, Kern J, Bullock M, Comty CM. Phencyclidine-induced rhabdomyolysis and acute renal failure. Clin Toxicol 1979; 14: 47-53.

147. Patel R, Ansari A, Hughes JL. Myoglobinuric acute renal failure associated with phencyclidine abuse. West J Med 1979; 131: 244-247.
148. Patel R, Das M, Patazzolo M, Ansari A, Balasubramaniam J. Myoglobinuric acute renal failure in phencyclidine overdose. Report of observations in eight cases. Ann Emerg Med 1980; 9: 549-553.
149. Barton CH, Sterling ML, Vaziri ND. Rhabdomyolysis and acute renal failure associated with phencyclidine intoxication. Arch Intern Med 1980; 140: 568-569.
150. Kuncl RW, Meltzer HY. Pathologic effect of phencyclidine and restraint on rat skeletal muscle: prevention by prior denervation. Exp Neurology 1974; 45: 3887-3402.
151. Appel GB, Radhakrishnan J, D'Agati V, Secondary glomerular disease. In: The Kidney (chapter 30), 6th edition. BH Brenner (editor). WB Saunders Company, Philadelphia 2000; p 1350-1449.
152. Citron BP, Halpern M, McCarron M, Lundberg GD, McCormick R, Pincus IJ, Tatter D, Haverback BJ. Necrotizing angiitis associated with drug abuse. N Engl J Med 1970; 283: 1003-1011.
153. Baden MM. Angiitis in drug abuse. N Engl J Med 1971; 284: 111-112.
154. Citron BP, Peters RL. Angiitis in drug abuse. N Engl J Med 1971; 284: 112.
155. Gocke DJ, Hsu K, Morgan C, Bombardieri S, Lockshin M, Christian CL. Association between polyarteritis and Australia antigen. Lancet 1970; 2: 1149-1153.
156. Koff RS, Widrich WC, Robbins AH. Necrotizing angiitis in a methamphetamine user with hepatitis B. N Engl J Med 1973; 288: 946-947.
157. Lignelli GJ, Buchheit WA. Angiitis in drug abuse. N Engl J Med 1971; 284: 112-123.
158. Cacoub P, Lunel-Fabiani F, Huong Dup LT. Polyarteritis nodosa and hepatitis C virus infection. Ann Intern Med 1992; 116: 605-606.
159. Ginsberg MD, Hetrzman M, Schmidt-Nowara WW. Amphetamine intoxication with coagulopathy, hyperthermia, and reversible renal failure. Ann Intern Med 1970; 73: 81-85.
160. Kendrick WC, Hull AR, Knochel JP. Rhabdomyolysis and shock after intravenous amphetamine administration. Ann Intern Med 1977; 86: 381-387.
161. Crowe AV, Howse M, Bell GM, Henry JA. Substance abuse and the kidney. Quart J Med 2000; 93: 147-152.
162. Verebey K, Alraze J, Depace A. The complications of "Ecstacy" (MDMA). JAMA 1988; 259: 1649-1658.
163. Foley RJ, Kapatkin K, Verani R, Weinman E. Amphetamine-induced acute renal failure. Southern Med J 1984; 77: 258-260.
164. De Marchi S, Cecchin E, Basile A. Renal tubular dysfunction in chronic alcohol abuse - effects of abstinence. N Engl J Med 1993; 329: 1927-1934.
165. Sadjadi SA, McLaughlin K, Shah RM. Allergic interstitial nephritis due to Diazepam. Arch Intern Med 1987; 141: 579-580.

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Calcineurin inhibitors and sirolimus

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INTRODUCTION

Approximately 20 years ago the first calcineurin inhibitor, cyclosporine A (CsA), was launched and it rapidly became clear that the new immunosuppressive drug was a breakthrough in the field of transplantation. The incidence of acute rejection in renal transplantation decreased dramatically, making this event a very unusual cause of graft failure nowadays. Early (1 to 2 years) graft and patient survival in solid organ transplantation increased to unparalleled levels and soon the drug showed its efficacy in bone marrow transplant immunosuppression and in the treatment of autoimmune diseases refractory to conventional therapy [1-6]. The development of self-emulsifying formulations of CsA improved bioavailability and decreased inter- and intra-patient variability of the drug [3, 7]. To have an idea of the magnitude of the importance cyclosporine has acquired since its discovery one can search the rate of publication of papers related to the drug in Pubmed: it increased from around one hundred in the 70s to 80s to a mean of 1500/year in the last decade.

In 1989 tacrolimus (FK 506), a second calcineurin inhibitor started its clinical journey [8]. Tacrolimus has an immunosuppressive effect approximately 100 times more potent than CsA and early clinical trials demonstrate that FK 506 was effective in reversing refractory acute rejection in renal, liver and heart transplantation. Subsequently, this drug showed to be at least as effective as CsA in the primary immunosuppression schedules for solid organ and bone marrow transplantation and, similarly to CsA, proved to be a valuable alternative in the treatment of autoimmune diseases [3, 9-11]. At the moment, FK 506 is considered the only drug that can substitute CsA in primary immunosuppression schedules and it is currently used in almost 60% of liver transplantation immunosuppressive prescriptions.

Both drugs inhibit interleukin-2 gene transcription and the transition of T-lymphocytes from the G0 to G1 phase of the cell cycle. They bind to cytoplasmic immunophilin, cyclophilin for CsA and FK-binding protein (FKBP12) for FK 506. The complex immunosuppressive drug-immunophilin reduces calcium signaling, blocking a calcium dependent enzyme, calcineurin, responsible for the nuclear translocation and dephosphorylation of the cytosolic activating nuclear factor of T lymphocytes (NF-AT-c). NF-AT-c regulates the transcription of genes responsible for several cytokines, including interleukin 2 [3].

Ironically, these two drugs used intensely in kidney transplantation have important renal related side effects: acute and chronic renal dysfunction, hemolytic-uremic syndrome, hypertension, electrolyte disturbances (hyperkalemia, hypomagnesemia and hypocalcemia), tubular acidosis and defects in urinary concentrating ability. Indeed, nephrotoxicity is the most significant and limiting adverse effect caused by the calcineurin inhibitors CsA and FK 506. Interestingly, the new immunosuppressive drug sirolimus, which reduce interleukin 2 production without blocking calcineurin, it is not nephrotoxic [12]. When calcineurin is inhibited many genes, besides interleukin 2, have their transcription impaired, including other interleukins, interleukin 2 receptor, nitric oxide synthase, transforming growth factor β (TGF-β), endothelin, collagen I and IV and bcl-2, responsible for protein Bcl-2, which is likely implicated in cellular protection against apoptosis [13]. It is possible that calcineurin inhibition blocks immune cell-mediated reaction against the transplanted tissue and at the same time it triggers a sequence of undesirable events that will eventually lead to renal toxicity [13]. The recent development of selective calcineurin inhibitors that disrupt particular genes transcription without affecting the others may clarify this issue [14].

The term calcineurin inhibitors nephrotoxicity has sometimes been used loosely, and it is crucial to be aware that this term comprises two particular and very distinct forms of renal injury. Acute nephrotoxicity induced by CsA and FK 506 is a hemodynamically mediated phenomenon, characterized by the absence of permanent structural changes and by the reversibility with decrease or discontinuation of the offending drug. Conversely, chronic nephrotoxicity produced by these drugs is an insidious lesion, whose characteristic is an irreversible and progressive renal interstitial fibrosis, followed in its late stages by important decrease in renal function.

In this chapter we will review some of the most relevant clinical aspects of CsA and FK 506 acute and chronic nephrotoxicity and discuss the available information on the mechanisms involved in the pathogenesis of the observed lesions.

CYCLOSPORINE A NEPHROTOXICITY

Acute nephrotoxicity

Acute CsA-induced nephrotoxicity is essentially a functional abnormality related to an imbalance of vasoconstrictor and vasodilator mediators. The stamp of this form of nephrotoxicity is an intense intrarenal vasoconstriction that induces decreases in renal blood flow and reciprocally increases in RVR, accompanied by variable degrees of glomerular filtration rate impairment. This vasoconstriction occurs preferentially in the afferent arteriole but also in adjacent small arteries, including the glomerular tuff [13, 15, 16]. The experimental model for acute nephrotoxicity is very consistent and the described hemodynamics changes have been demonstrated after different doses and route of administration in animals. In the same way acute changes in renal hemodynamics and function have been observed after CsA administration in patients and healthy human volunteers. The phenomenon is reversible with dosage decrease or drug withdrawal. Histological changes found in experimental animals or patients are minimal and nonspecific or absent, even when renal dysfunction is striking [15, 17].

There is a long roll of possible mediators for acute CsA nephrotoxicity (Table 1), with the majority of the studies being done in experimental models and using pharmacological blockade of the candidate systems. It should be stressed that so far, individual blockade of those systems resulted in improvement but not complete recovery of renal hemodynamics, strongly suggesting that CsA-induced vasoconstriction is complex and caused by the interaction of different mechanisms [18, 19].

Table 1. Some mediators and mechanisms possibly involved in the pathogenesis of acute *CsA* nephrotoxicity.

Angiotensin II
Endothelin
Nitric oxide
Prostaglandins
Leukotrienes
Sympathetic system
Free radicals
Adenosine
Vasopressin
Platelet activating factor
Atrial natriuretic factor
Kallikrein-kinin system
Cholesterol
Hypomagnesemia
Extracellular volume depletion
Cremophor
Direct contraction – mesangial and smooth vascular cells
Direct tubular epithelial cell toxicity

Mechanisms of injury: *Vascular/hemodynamic*

The interaction of cyclosporine and the plasma and tissue renin-angiotensin-aldosterone system has been extensively studied with excellent reviews available [20, 21]. Sodium depletion, a condition that stimulates renin release, enhances acute CsA nephrotoxicity [22, 23]. In rats, CsA treatment enhances plasma renin activity [20, 24, 25], increases renal renin content [20], promotes juxtaglomerular hypertrophy and hyperplasia [26, 27], increases renin staining cells in juxtaglomerular apparatus and renin containing cells in the afferent arterioles [28], increases the number of renal angiotensin II AT_1 receptors [29] but promotes a down regulation of AT_1 receptor in renal tissue [28]. *In vitro* studies showed that CsA induces renin release in renal cortical slices and culture of juxtaglomerular cells of rats, stimulates renin synthesis in juxtaglomerular cells and up-regulates angiotensin II receptors in cultured human smooth muscle cells [30-32]. In humans, CsA has been reported not to change or even to decrease plasma renin activity [33]. Conversely, increased levels of prorenin and total renin and juxtaglomerular apparatus hyperplasia have been found in CsA-treated heart and liver transplant recipients [34]. Clinical evidences of CsA-induced activation of local renal tissue renin-angiotensin-aldosterone systems have been offered by Gardiner et al, who showed that conversion from CsA to azathioprine decreased the number of renin-containing cells in renal allograft biopsies [35]. If there is little doubt that CsA has an important modulator effect on renin-angiotensin-aldosterone systems, the actual role of this system in CsA-induced renal vascular changes is much less clear. Blocking of the renin-angiotensin-

aldosterone system in experimental acute CsA nephrotoxicity produced conflicting results. Saralasin prevented renal blood flow decrease and intrarenal vasoconstriction in a model of isolated hydronephrotic rat kidney [36], losartan attenuated the increase in RVR [18] and angiotensin converting enzyme blocker minimized CsA-induced decreases in glomerular filtration rate and renal blood flow [37-38]. On the other hand, many authors failed to demonstrate amelioration of renal function and/or hemodynamics when angiotensin converting enzyme inhibitors were given concomitantly with CsA [39-42]. In humans, attempts of prevention or attenuation of CsA-induced renal functional changes by pharmacological blockade of renin-angiotensin-aldosterone system have been mostly disappointing, with some studies finding improvement in renal blood flow and RVR but not in glomerular filtration rate when angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists were administered to patients under CsA therapy [43-45].

In 1987 O'Brien et al reported that cultured endothelial cells were able to produce a vasoconstrictor substance [46]. In 1988, Yanagisawa et al described the substance as endothelin, a 21-amino acid peptide that is considered the most powerful mammalian vasoconstrictor agent known [47]. Next, three distinct genes for endothelin were identified, each encoding a particular peptide, named endothelin-1, endothelin-2 and endothelin-3 [48]. Different renal resident and infiltrating cells are able to produce endothelin-1 like vascular smooth muscle cells, endothelial cells, epithelial cells, mesangial cells, tubular cells, macrophages and monocytes. Moreover, endothelin-converting enzyme-1, the enzyme responsible for production of endothelin-1 from big endothelin-1, has an ubiquitous intrarenal distribution and recently endothelin-converting enzyme-1 mRNA was described in glomeruli and in whole nephron segments including proximal tubules, medullary and cortical thick ascending limbs, cortical collecting ducts, outer and inner medullary collecting ducts. The renal hemodynamics effects of endothelin include constriction of mesangial cells, intrarenal vessels and afferent and efferent arterioles, increasing RVR and decreasing renal blood flow and glomerular filtration rate [49, 50]. In 1990, the first evidences linking endothelin and acute CsA nephrotoxicity came out with the demonstration that CsA stimulated endothelin release from a cultured renal epithelial cells line (LLC-PK1) and that renal endothelin receptors were up-regulated in rats with CsA-induced nephrotoxicity [51, 52]. In the same year, Kon et al and Perico et al carried out experimental studies in rats showing that CsA elevated circulating levels of endothelin-1 and that anti-endothelin antibodies partially prevented the renal and glomerular vasoconstrictive pattern and glomerular filtration rate decrease induced by CsA and, in 1992, Takeda et al showed that CsA was able to promote *in vitro* mesangial cell contraction [53-55]. Subsequently, several authors reported increased urinary and/or plasma levels of endothelin after CsA treatment in animals and solid organ transplant recipients [56-61]. Additional evidences of CsA-induced stimulation of endothelin production come from molecular biology studies disclosing up-regulation of endothelin$_A$ receptor mRNA in the aorta and mesenteric artery and increased mRNA expression of pre-pro-endothelin-1 and up-regulation of endothelin-converting enzyme 1 mRNA expression in renal cortex in CsA-treated rats [62, 63]. Nakayama et al, showed that a single IV injection of CsA in rats caused a rapid increase in glomerular pre-pro endothelin-1 mRNA and plasma endothelin-1 followed by a late glomerular and tubular decrease of endothelin-converting enzyme-1, endothelin$_A$ and endothelin$_B$ receptor mRNA and protein levels, suggesting that CsA-induced endothelin-1 synthesis induced down-regulation of endothelin-converting enzyme-1 expression [64]. Marsen et al demonstrated that CsA induces a calcium-dependent pre-pro endothelin gene transcription and endothelin-1 mRNA production in human umbilical vein endothelial cells in culture [65]. Experimental use of endothelin$_A$ or endothelin$_A$/ endothelin$_B$ receptor antagonists attenuated CsA-induced decreases in renal blood flow and glomerular filtration rate and increase in RVR [66], vasoconstriction of large pre-glomerular arteries and reduction in glomerular blood flow [67], afferent arteriole vasoconstriction [68], myosin light chain phosphorylation (a parameter of mesangial cell contraction) in glomerular mesangial cells [55] and calcium rise in smooth muscle cells [69]. When endothelin$_A$ and endothelin$_A$/ endothelin$_B$ receptors antagonists were compared in the same study, additional endothelin$_B$ blockade did not attenuate CsA effects further [67]. However, blockade of endothelin receptors also provided some conflicting results. Fogo et al found that an endothelin$_A$ receptor antagonist attenuated CsA-induced fall in

glomerular filtration rate and renal blood flow only when it was infused in the renal artery before CsA administration. When the endothelin$_A$ receptor antagonist was infused systemically or after CsA there was no protection [66]. Davis et al showed that use of a selective endothelin$_A$ receptor antagonist or a combination of an endothelin$_A$ and endothelin$_B$ receptor antagonists did not prevent CsA-induced renal vasoconstriction in rats [70]. Binet et al recently reported that bosentan, a non-peptide mixed endothelin$_A$ and endothelin$_B$ receptor antagonist, attenuated renal blood flow decrease, but not glomerular filtration rate fall caused by CsA in healthy human volunteers [71].

There are several evidences demonstrating that CsA causes endothelial cells injury. CsA was found to have a direct cytotoxic effect on cultured endothelial cells and to inhibit human umbilical endothelial cell proliferation [72, 73]. CsA increases the plasma level of many markers of endothelial damage like von Willebrand factor, endothelin tissue factor pathway inhibitor, P-selectin and thrombin-antithrombin complexes in renal and heart transplant patients [74-77]. Similarly, the release of von Willebrand factor, endothelin tissue factor pathway inhibitor and thrombomodulin was enhanced by CsA in the supernatant of endothelial cells in culture [77, 78]. The vascular endothelium produces nitric oxide, which modulates relaxation of adjacent smooth muscle cells by a cyclic GMP-dependent mechanism. Nitric oxide is produced from L-arginine by the action of nitric oxide synthase family. Three nitric oxide synthase isoforms have been identified: neuronal nitric oxide synthase, markedly expressed in brain, inducible nitric oxide synthase expressed in macrophages and renal tubular cells and endothelial nitric oxide synthase mainly expressed in endothelial cells. The three isoforms exist in different renal structures and have a major function in the management of glomerular and vascular tone and tubular function [79, 80]. Considering the pattern of hemodynamic changes caused by CsA it is not surprising that a growing body of data emerged linking acute CsA nephrotoxicity to disturbances in L-arginine-nitric oxide pathway. Numerous authors have consistently shown that CsA impairs endothelium-dependent vasodilatation mediated by nitric oxide of *in vitro* human subcutaneous vessels and *in vivo* forearm vessel of heart transplant recipients [81, 82] and in a range of *in vitro*, *ex vivo* and *in vivo* experimental studies evaluating mesenteric arteries, aortic rings, renal arteries, afferent and efferent arterioles, femoral arteries and thoracic aorta of rats [83-90]. On the other hand, studies assessing CsA influence on nitric oxide tissue, plasma and urinary levels and on tissue expression of nitric oxide synthase isoforms have provided contradictory results. *In vivo* experiments in rats shown that CsA treatment did no change, increase or decrease urinary nitric oxide metabolites [91-93]. *Ex vivo* and *in vitro* experiments using murine macrophage cell line or thoracic aorta of rats and VSMC of rats found CsA-induced decrease in tissue nitric oxide metabolites [86] or nitric oxide production [94]. Studies performed in healthy volunteers found that CsA increases nitric oxide synthase activity [95] whereas studies in renal transplant recipients found CsA-induced impaired basal and stimulated nitric oxide production [96] and a biphasic pattern of decrease and then increase in plasma nitric oxide accompanied by a non significant decrease in urinary excretion of nitric oxide metabolites after the first dose of CsA [97]. The evaluation of CsA effects on nitric oxide synthase genes showed increase of mRNA for endothelial nitric oxide synthase in renal cortex and increased induction of endothelial nitric oxide synthase gene in bovine aortic endothelial cells [79, 98, 99] or no change in vascular endothelial nitric oxide synthase [86]. CsA-induced decreases of inducible nitric oxide synthase and neuronal nitric oxide synthase mRNA and inducible nitric oxide synthase protein have been found in renal medulla, renal tissue, aorta, macrophages and VSMC [79, 86, 93, 94, 99]. Enhancement of nitric oxide production by administration of L-arginine improved and blockade of nitric oxide production by administration of L-NAME worsened the changes in endothelium-dependent vasodilation, glomerular filtration rate and in renal and glomerular hemodynamics induced by CsA in experimental animals [79, 84, 86, 91, 92, 100-103]. Recently, it was reported that nifedipine prevented the changes induced by CsA in renal nitric oxide synthase mRNA in rats [99] and that enalapril and valsartan restored acetylcholine-dependent relaxation in the renal arteries of CsA-treated spontaneously hypertensive rats [104]. Interestingly, Asberg et al found better long-term microvascular function in CsA-treated renal transplant recipients receiving lisinopril as compared to patients receiving nifedipine [105]. Clinical trials assessing the effects of L-arginine supplementation in CsA-treated renal or heart

transplant patients were generally negative, showing no improvement in renal function and/or hemodynamics [106-108], with the exception of Andrés et al that found increases in RPF, glomerular filtration rate and natriuresis after administration of L-arginine to stable renal transplant recipients [109].

Eicosanoids, i.e. arachidonic acid metabolites, have an important role in the local control of renal blood flow, mainly in the setting of systemic or intrarenal hemodynamic disorder. They are produced by renal resident cells (endothelial, mesangial, tubular and interstitial cells) as well as by infiltrating cells (macrophages, lymphocytes platelets and neutrophils). The cyclooxygenase pathway produces the vasodilators prostaglandins PGI_2 or prostacyclin (that undergoes spontaneous hydrolysis to 6-keto-PGF1α) and PGE_2 and the vasoconstrictor thromboxane A_2 whereas the lipoxygenase pathway produces the vasoconstrictor leukotrienes. CsA-induced unbalance in the vasodilator/vasoconstrictor rate of these metabolites favors vasoconstriction and is likely related to the development of the functional changes after acute CsA nephrotoxicity [3, 110]. CsA administration to rodents consistently resulted in increased urinary excretion of thromboxane A_2 metabolites: thromboxane B_2, 2, 3 dinor-thromboxane B_2 and 11-dehydro-thromboxane B_2, reflecting enhancement of renal and systemic thromboxane production [111-119]. Many authors disclosed that this activation of thromboxane synthesis was paralleled by glomerular filtration rate and renal blood flow decreases and RVR increase [112-114, 116-122]. In fact, Perico et al and Parra et al found a strong and significant negative correlation between glomerular filtration rate decrease and urinary thromboxane B_2 levels in rats receiving CsA [117, 119]. Experimental CsA administration increased *ex-vivo* renal production of thromboxane B_2 [111, 112], renal tissue thromboxane levels [120, 122, 123] and production of thromboxane B_2 by isolated glomeruli and peritoneal macrophages [111, 123]. Supporting these experimental findings, clinical studies have also showed CsA-related urinary thromboxane B_2 and 11-dehydro-thromboxane B_2 increases in renal and liver transplant recipients and healthy volunteers [74, 124-126]. The enhancement of thromboxane production by CsA has been related to activated infiltrating platelets and macrophages in renal tissue, increased renal lipid peroxidation and reactive oxygen species production and endothelial injury and systemic platelet activation [74, 111, 119]. Experimental administration of thromboxane synthase inhibitors, thromboxane receptor antagonists or fish oil (that reduces the production of thromboxane) resulted in decrease or normalization in urinary thromboxane metabolites levels and variable degrees of improvement in renal function and hemodynamics [114, 116-118, 120-123, 127, 128]. Contrasting with the experimental studies, the clinical use of selective thromboxane synthase inhibitors did not prevent CsA-induced renal dysfunction despite decreased urinary and systemic levels of thromboxane metabolites [124, 129, 130]. In the same way, the use of fish oil in CsA-treated patients resulted in contradictory results, with some authors finding improvement in renal function and decrease in urinary thromboxane B_2, and others no effect at all [125, 131-133]. A role for the 5-lipoxygenase pathway in CsA acute nephrotoxicity has also been suggested by the demonstration that a leukotriene receptor antagonist partially prevented the decrease in glomerular filtration rate and RPF after IV CsA. In the same paper the simultaneous administration of a leukotriene receptor antagonist and a thromboxane receptor antagonist completely abolished CsA-induced changes in renal function [128]. Butterly et al recently showed that CsA increased urinary excretion of leukotriene metabolites in a rat model of renal transplantation. In this study the use of a leukotriene receptor antagonist totally prevented the glomerular filtration rate impairment caused by CsA [134]. The precise role of vasodilative prostaglandins in CsA-induced acute nephrotoxicity has been more elusive. CsA has been shown to increase, decrease or did not change the levels of 6-keto-PGF1α and PGE_2 in urine, blood, renal venous effluent of *ex-vivo* preparations, renal tissue and supernatant of cultured mesangial cells or isolated glomeruli of rodents and humans [61, 97, 112-114, 117, 121-124, 126, 130, 135, 136]. The experimental manipulation of vasodilative prostaglandins by prostacyclin analogues or PGE precursors resulted in protection against the functional changes caused by CsA [137, 138]. However, data derived from clinical studies or human tissue is conflicting. Moran et al reported that misoprostol, a synthetic PGE_2 analogue, improved renal function in CsA-treated renal recipients [139] and Le Guen et al showed that iloprost, a prostacyclin analogue, prevented CsA-induced glomerular constriction in human isolated glomeruli [140]. In contrast, several authors failed to

demonstrate any beneficial effect after the administration of PGE_2 or prostacyclin analogues to patients receiving CsA [141-145].

CsA-stimulated activation of the sympathetic system has been demonstrated in animals and humans and linked to hypertension and to systemic and renal hemodynamic abnormalities caused by this drug [146-148]. Some of the mechanisms implicated in adrenergic stimulation by CsA in rats are augmented norepinephrine release from terminal nerves, blockade of neuronal calcineurin, activation of excitatory neural reflexes in the subdiaphragmatic area and elevation of plasma and platelets catecholamines [149-152]. Zhang et al recently showed that knockout mice lacking synapsin (synaptic vesicle proteins that regulated neurotransmitter release at synapses) were protected against efferent sympathetic nerve activation and blood pressure increase after CsA administration [153]. Adrenergic pharmacological blockade, renal denervation, chemical sympathectomy, administration of glycine (an inhibitory neurotransmitter) and catecholamine stores depletion by reserpine prevented or significantly reduced CsA-induced hemodynamics changes and hypertension in experimental studies [154-158]. However, other authors did not find protection against experimental CsA-induced renal functional impairment after renal denervation in transplanted or *in situ* kidneys [159-162] and a number of clinical studies were unable to correlate the renal and systemic hemodynamics changes found in solid organs transplant recipients or autoimmune diseases patients treated with CsA to increased sympathetic activity [163-167].

There is a growing body of evidence suggesting the participation of free radicals in CsA acute nephrotoxicity. Experimental *in vivo* and *in vitro* studies found that functional derangements caused by this drug were accompanied by increased renal tissues content of malondialdehyde, lipid hydroperoxides and conjugated dienes, increased glomerular synthesis of hydrogen peroxide, superoxide anion and malondialdehyde, increased production of malondialdehyde by cultured human endothelial cells, increased formation of malondialdehyde and hydrogen peroxide by renal mitochondria, increased urinary excretion of free radicals and increased levels of plasma malondialdehyde [158, 168-175]. Results regarding CsA effect on glutathione renal contents showed decreased glutathione levels or increased tissue concentrations of oxidized and reduced glutathione [171, 176]. If we consider the pivotal role of glutathione in cellular protection from free radicals damage these results can be reconciled. It is possible that the increases in oxidized and reduced glutathione were caused by accelerated glutathione peroxidase activity and adaptation of glutathione pathway in order to counterbalance excessive free radicals production and that reduced glutathione levels indicate an exhaustion of the system. This excessive free radical production has been attributed to renal underperfusion and hypoxia-reoxygenation injury or direct cellular membrane lesion caused by CsA [158] and related to increased thromboxane production, increased mRNA production for cyclooxygenase I and decreased mRNA production for cyclooxygenase II, up-regulation of Bcl-2 protein expression and increased expression of endothelial nitric oxide synthase mRNA indicating an important interrelation of these systems and events [169, 170, 177, 178]. Inhibition of free radical production by antioxidants like lazaroid, vitamin E, melatonin and N-acetylcysteine, administration of the xanthine oxidase inhibitor allopurinol, blockade of renal sympathetic system by glycine or renal denervation and viral delivery of superoxide dismutase genes consistently resulted in renal function improvement in experimental models of acute CsA nephrotoxicity [91, 158, 170-175, 177, 179, 180]. Conversely, administration of vitamin E and selenium deficient diet to rats enhanced acute CsA nephrotoxicity [173]. The few clinical studies that addressed the role of free radicals in acute CsA nephrotoxicity found negative results [181, 182].

Other mediators have also been related to CsA-induced functional nephrotoxicity. Increased plasma level of adenosine due to reduced uptake by red blood cells was observed in CsA-treated renal transplant recipients [183]. In the same way, rats receiving CsA showed increased concentration of adenosine in renal artery paralleled by a decrease in mRNA expression for A_1 and A_{2a} renal adenosine receptors [184]. Experimental use of selective A_1 adenosine receptors antagonists or theophylline resulted in contradictory results with some authors finding renal hemodynamic and functional protection whereas other did not [38, 185-187]. Moderate increases in plasma vasopressin were observed in CsA-treated renal transplant recipients [188] and CsA enhanced vasopressin-induced rise in intracellular calcium in cultured glomerular mesangial

cells, human coronary myocytes and vascular smooth muscle cells [189-191]. In addition, incubation of vascular smooth muscle cells with CsA increased the expression of AVP receptors and vasopressin V1A receptors mRNA [191, 192]. Use of platelet activating factor antagonists improved CsA-induced changes in glomerular filtration rate, renal blood flow and glomerular hemodynamics as well as CsA toxicity in cultured tubular LLC-PK1 cells [193-196]. A recent study showed that cyclosporine impaired atrial natriuretic factor-induced glomerular guanylyl cyclase activation in rats. Indeed, experimental or clinical administration of atrial natriuretic factor, use of neutral endopeptidase (atrial natriuretic factor degrading enzyme) inhibitors or simultaneous utilization of both maneuvers prevented the adverse effects of CsA on renal function and hemodynamics, smooth muscle cells and blood vessels [197-201]. Reduced urinary excretion of kallikrein was demonstrated in CsA-treated patients [202-204]. Studies in rats showed that 3 days of CsA administration decreased cortical mRNA expression for kallikrein and bradykinin 2 receptors [205] whereas CsA administration for 28 days increased renal tissue kallikrein mRNA and kallikrein content, increased urinary excretion of kallikrein, increased hepatic expression of kininogen mRNA and increased renal bradykinin B_2 receptors mRNA, suggesting an enhancement in the activity of the kallikrein-kinin system trying to compensate for CsA nephrotoxicity [206]. A potential role for cholesterol, a known vasoconstrictor substance, in CsA-acute nephrotoxicity is suggested by the finding of renal dysfunction aggravation in hereditary hypertriglyceridemic rats receiving CsA or dietary cholesterol supplementation in CsA-treated rats [207, 208]. CsA-induced hypomagnesemia has been proposed as a possible factor in the pathogenesis of the functional changes induced by the drug. Effects of magnesium dietary supplementation on CsA nephrotoxicity are conflicting, with lack of functional protection in normotensive rats on low salt diet versus renal function improvement in SHR rats on high sodium diet [209, 210]. Extracellular fluid volume depletion due to increased vascular permeability and loss of renal autoregulatory capacity have also been linked to acute CsA nephrotoxicity [211-213]. Recent studies showed that systemic administration of insulin-like growth factor I and recombinant human relaxin ameliorated acute CsA nephrotoxicity in rats [214, 215].

CsA demonstrated to have an intrinsic capacity to stimulate direct contraction of animal and human mesangial cells, smooth vascular cells and resistance vessels with obvious consequence for renal function and hemodynamics. These CsA effects are associated to augmented intracellular influx of calcium, impaired relaxation response of vascular wall to vasodilatory stimuli and endothelin-1 [16, 19, 89, 197, 216-221].

The vehicle used for CsA solubilization may take part in acute nephrotoxicity genesis after using the intravenous formulation of the drug. Cyclosporine is a very lipophilic and hydrophobic compound, making mandatory the use of lipid vehicles in order to obtain stable preparations for experimental or clinical use. The commercial intravenous formulation of CsA uses as vehicle Cremophor-EL, a polyethylated castor oil, which possesses important hemodynamics effects already demonstrated in experimental animals and humans [222-224]. This intravenous formulation has been incriminated in episodes of acute renal failure in transplant recipients and patients with autoimmune diseases and caused abrupt glomerular filtration rate decrease after a single dose in healthy volunteers [225-228]. Substitution of Cremophor by a soybean lipid used for parenteral nutrition (Intralipid) in an experimental model of CsA-induced acute renal failure resulted in preservation of glomerular filtration rate with maintenance of CsA immunosuppressive activity measured by decrease in interleukin 2 production and inhibition of lymphocyte activation. This favorable profile was related to increased CsA clearance and lower trough level but similar tissue amount of CsA, what probably reduced the vascular exposition to the drug [229].

Mechanisms of injury: *Tubular*

The seminal study by English et al clearly showed that major proximal tubular functions were preserved in acute CsA nephrotoxicity [15] and in fact, documented ATN is rarely seen in CsA-treated patients. On the other hand, experimental and clinical studies provided evidences of more subtle CsA-induced tubular cell injury like increased urinary excretion of tubular enzymes, increased fractional excretion of magnesium in the presence of hypomagnesemia, impaired urinary concentrating ability and hyperkalemia consequent to impaired tubular excretion of potassium [209, 230, 231]. Experiments using cultured LLC-PK1 and MDCK re-

nal tubular cell line showed a dose dependent CsA direct cell toxicity manifest as reducing of cell proliferation, reduction of Na-K-ATPase and Na-K-2Cl cotransporter activity, apoptosis or necrosis, increased LDH release, DNA damage and cell cycle arrest and decreased cell viability [232-236]. This toxicity has been related to lipid peroxidation, p53b protein expression elevation, increased intracellular calcium and reduced nitric oxide production [234-237]. Other indirect evidence of CsA-induced tubular cell damage is the induction of heat shock proteins in renal tubular cells after CsA addition to cultured cells or CsA administration to rats [238, 239] and CsA-induced inhibition of potassium channels in cortical collecting tubules cells of rabbits [240]. Using fresh isolated rats proximal tubules da Costa et al showed that only very high concentrations of CsA caused direct tubular injury, which was prevented by low calcium or high magnesium concentrations in the medium [241].

Clinical aspects

There are four possible relevant clinical presentations for acute CsA nephrotoxicity: asymptomatic increases in serum creatinine without overt renal dysfunction, acute renal failure, delayed graft function after renal transplantation and recurrent or *de novo* hemolytic uremic syndrome (Table 2).

The most frequent presentation of acute CsA nephrotoxicity is a dose-related, clinically asymptomatic increase in serum creatinine, which can occur even when drug whole blood trough levels are in the so-called "therapeutic range" [3, 242]. This situation may be difficult to distinguish from kidney rejection in renal transplant recipients or from primary renal disease progression in glomerulonephritis patients treated with the drug, and CsA nephrotoxicity and rejection or worsening of primary renal disease can coexist. Otherwise, in extrarenal organ transplantation and non-renal autoimmune diseases patients these serum creatinine elevations are very likely caused by CsA. Actually, this form of renal impairment is relatively frequent in cardiac, hepatic and pulmonary transplantation [243-245]. Renal histology of these patients is usually normal or shows only nonspecific changes like vacuolization or presence of giant mitochondria in tubular cells [17, 246, 247]. The defining parameter for diagnosis will be improvement in serum creatinine in about one week after dosage manipulation or drug discontinuation [3]. There are also several important clinical studies showing that even patients under CsA therapy who were apparently doing well in terms of renal function suffer a significant renal hemodynamic impact from the immunosuppressive drug. Curtis el al showed that CsA withdrawal for economical reasons in renal transplant recipients with stable and normal serum creatinine was followed by a 30% increase in renal blood flow with a parallel drop in renal vascular resistance and blood pressure [248]. A particularly important aspect of this paper was that this improvement in renal function occurred after a long period of CsA treatment. More recently, Hilbrands et al studied patients without clinical evidence of acute nephrotoxicity who discontinued CsA at 3 months after renal transplantation. One week after the withdrawal there was a significant increase in glomerular filtration rate and decrease in serum creatinine [249]. In another carefully conducted series of studies it was demonstrated that a single daily dose of CsA in steady chronically CsA-treated renal transplant patients caused a daily significant and transitory decrease in glomerular filtration rate [60] which could be prevented by the use of a calcium channel blocker [250]. More recently, various studies showed enhancement in renal function when transplant patients on "classic" dosages of CsA had their immunosuppressive regimes changed to low dosage CsA plus mofetil mycophenolate. It is possible that the price to be paid for effective immunosuppression with full doses of CsA is inexorably some degree of renal hemodynamic impairment [251].

Table 2. Clinical presentations of acute cyclosporine A nephrotoxicity

Asymptomatic increases in serum creatinine
Acute renal failure
Delayed recovery of renal graft function
Hemolytic-uremic syndrome

Clinically important acute renal failure associated with CsA use can occur in a significant number (ranging from 10 to more than 50%) of patients in the postoperative period of heart, liver and bone marrow transplantation [226, 242-244, 252-254]. Acute renal failure in these patients is generally multifactorial and very seldom related exclusively to CsA. More than 40% of

heart transplant recipients are rehospitalized in the first year post-transplantation and roughly 30% of them require intensive care unit admission. Moreover, cardiac transplant patients can have impaired pre-transplant renal function due to chronic heart failure, suffer the insult of cardiopulmonary bypass during surgery, require doses of CsA 30% higher than in other solid organs transplantation and may have activated renin-angiotensin-aldosterone system due to chronic renal ischemia [244, 255-257]. Liver transplant recipients rarely had previous renal impairment, but liver transplantation is an important surgical trauma that induces significant cytokine activation, and not infrequently is associated to intravascular volume depletion, hypotension and coagulopathy. Additionally, the early liver transplant postoperative period, when CsA administration is normally initiated, may be complicated by sepsis, liver graft dysfunction, use of nephrotoxic drugs and multi-organ failure syndrome [242, 243]. In the same way, the early period after bone marrow transplantation might be affected by graft-versus-host-disease, veno-occlusive liver disease, infection, hemodynamic instability, volume depletion and use of nephrotoxic antibiotics, [253, 258-260]. Another possible scenario for the development of acute renal failure in CsA-treated patients is when the profound effects of this drug on renal hemodynamics are combined with the administration of nephrotoxic drugs like aminoglycoside antibiotics, amphotericin B, foscarnet or iodinated contrast media or with other drugs with important action on intrarenal vascular tonus regulation, like non steroidal anti-inflammatory drugs or angiotensin converting enzyme inhibitors [261-270]. In rare occasions, CsA can induce acute renal failure in patients with autoimmune diseases [225, 271] or acute tubular necrosis related to extremely high dosage [272, 273].

Delayed recovery of post-transplant renal allograft dysfunction was seen more often in the past when elevated doses of cyclosporine were used, principally with the concomitant occurrence of prolonged ischemic times. This event has been largely diminished by the delay of onset of CsA administration until good renal graft function is present or by the use of alternative schedules for induction of immunosuppression that spare calcineurin blockers [2, 3]. Withdrawal of CsA in renal transplant patients with delayed graft function has been associated with less severe and shorter renal dysfunction [274].

CsA can rarely cause fulminant acute renal failure due to endothelial cell injury, promoting a syndrome evocative of hemolytic-uremic syndrome. This entity occurs primarily in bone marrow transplantation but has also been reported in kidney transplantation, occasion when may be very difficult to distinguish from acute vascular rejection. CsA-related hemolytic-uremic syndrome is associated with an extremely poor prognosis of renal allograft. A recent case-control study showed that early therapy with isradipine, aspirin and pentoxiphylline resulted in increased transplant survival as compared to previous reports [275]. The clinical presentation of CsA-related hemolytic-uremic syndrome can be exuberant with severe anemia, low platelet count, increased rate of schizocytes and increased serum levels of lactic dehydrogenase or be ambiguous, with inconsistent laboratory findings. In the same way, renal biopsy may show the typical findings of thrombotic microangiopathy, eventually associated to afferent arteriolar thrombosis, or be misleading in the early phases of the disease [3, 251, 275-278].

Management of acute CsA nephrotoxicity

Regular monitoring of CsA blood levels in order to keep drug concentration within its narrow therapeutic window is an apparent potential maneuver to prevent CsA nephrotoxicity [211, 242, 254]. Nevertheless, the clinical reality is quite disappointing, since many patients may develop renal toxicity with CsA trough blood levels in the therapeutical range [211, 279, 280]. In reality, area under the curve but not trough levels represents real exposure and may predict CsA efficacy and nephrotoxicity. Lower area under the curve is associated with acute rejection and higher area under the curve with acute nephrotoxicity. Unfortunately, the correlation between CsA trough levels and area under the curve is very poor, even with the new microemulsion formulation [281, 282]. Furthermore, a prospective study, which tried to adjust CsA daily intake by optimized area under the curve, found that was very difficult to control CsA exposure by examining area under the curve and modifying CsA dose in accord [281]. Other pharmacokinetic-related factor that can affect CsA acute nephrotoxicity is the possibility that once daily versus more frequent dosing has different impacts on the drug renal toxicity [283]. Since hepatic metabolism and biliary excretion are the major route

for elimination of CsA and its metabolites, downstream adjustments in CsA doses are needed in patients with liver disease in order to avoid episodes of acute nephrotoxicity [3, 251].

Special attention should be paid when new medications are prescribed to CsA-treated patients. CsA is extensively metabolized by the cytochrome P-450 liver microsomal enzyme system [2, 3], and consequently drugs that interfere with this pathway can cause important changes in CsA blood levels (Table 3). Compounds that inhibit P-450 enzymes like ketoconazole, erythromycin, verapamil, and diltiazem increase concentration of parent CsA and may cause acute nephrotoxicity. On the other hand, drugs that induce these enzymes like phenobarbital, carbamazepine and rifampicin, can lower CsA blood levels and impair immunosuppression [251]. As mentioned early, potentially nephrotoxic drugs (aminoglycosides, amphotericin B, foscarnet, vancomycin, contrast media, NSAID, anesthetics, etc.) or drugs that induce efferent arteriole vasodilatation (angiotensin converting enzyme inhibitors and angiotensin II AT_1 receptors antagonists) should be used with extreme prudence in CsA-treated patients, since then can act synergistically with CsA-induced preglomerular vasoconstriction in order to promote renal injury.

The ability of calcium channel antagonists to induce afferent arteriole vasodilation makes this class of drugs as a very suitable pharmacological antidote against CsA acute vascular effects [242]. Indeed, several studies showed improvement in renal hemodynamics and/or function when different calcium antagonists like verapamil, diltiazem, nifedipine, lacidipine, isradipine, amlodipine, etc. were given for CsA-treated patients [44, 250, 284-290]. Some studies also suggest that perioperative administration of calcium antagonists to donators or receptors may prevent or diminish the time and intensity of delayed graft function and improve long-term kidney outcome [211, 291, 292]. A further potential advantage of add those drugs to CsA therapy is an adjunctive immunosuppressive effect, since calcium antagonist have some intrinsic immunomodulatory activity [293, 294].

Table 3. Some drugs that interfere with cytochrome P450 enzymes and change *CsA* blood concentrations.

Increase Level	Decrease Level
Verapamil	Rifampicin
Diltiazem	Isoniazid
Nicardipine	Phenytoin
Amlodipine	Carbamazepine
Erythromycin	Barbiturates
Clarithromycin	
Ketoconazole	
Fluconazole	
Itraconazole	

The antiinflammatory, antithrombotic, hypolipidemic, vasodilatory and immunomodulatory properties of fish oil (omega-3 fatty acids) make then an attractive treatment for CsA nephrotoxicity [110, 295]. Omega-3 fatty acids decrease the formation of prostaglandin E_2 metabolites, inhibit the production of thromboxane A_2, reduce production of biologically active leukotrienes and enhance prostacyclin release. In consequence, they inhibit platelet aggregation, promote vasodilation, low blood pressure and are antiatherogenic [295]. Daily dietary supplementation of 6 to 18 g of fish oil to CsA-treated patients has been performed in different clinical trials with conflicting results. Psoriasis patients receiving CsA plus 6 g of fish oil/day for 3 months did not change their renal blood flow and RVR and had less glomerular filtration rate impairment that the control group that received only CsA [296]. In a series of placebo-controlled, randomized, prospective studies, Homan van der Heide et al found that the supplementation of 6 g of fish oil to CsA-treated renal transplant recipients increased glomerular filtration rate and RPF and decreased blood pressure and RVR, influenced favorably the recovery of renal function after early acute rejection and, at one year post-transplant, promoted higher glomerular filtration rate, lower blood pressure, fewer rejection episodes and a non statistically significant trend to better graft survival [133, 297, 298]. In contrast, three placebo-controlled, randomized, double-blind, prospective studies failed to find significant improvement of renal function in omega-3 fatty acid supplemented, CsA-treated, renal transplant recipients [299-301]. Liver transplant recipients under CsA immunosuppression and supplemented with 12 g of fish oil for 2 months increased significantly renal blood flow and effective RPF, decreased significantly RVR, and had a marginally statistically significant increase in glomerular filtration

rate, whereas corn-oil treated controls did not change any of these parameters [125]. Finally, a recent study in hypertensive heart transplant patients showed that one year of omega-3 fatty acids supplementation resulted in stable blood pressure, systemic vascular resistance, serum creatinine and glomerular filtration rate, in opposite to the findings of the placebo group, where a significant increase in blood pressure, systemic vascular resistance, serum creatinine and a significant decrease in glomerular filtration rate were observed [302].

There are few other potential pharmacological interventions for treatment of functional CsA nephrotoxicity already tried clinically with positive results. Atrial natriuretic factor has opposite effects of CsA in renal hemodynamics. Candoxatrilat inhibit neutral endopeptidase, an enzyme which is found most abundantly in the kidney, and which degrades atrial natriuretic factor. Acute intravenous infusion of candoxatrilat in stable renal transplant recipients resulted in significant increases in glomerular filtration rate, renal blood flow, diuresis, fractional excretion of sodium, plasma atrial natriuretic factor and fall in RVR [201]. Dehydropeptidase-I is a glutathione-processing enzyme, found on both the brush border and the basolateral membranes of proximal tubular cells. Administration of cilastatin, an inhibitor of dehydropeptidase, prevented elevations of serum creatinine in the early postoperative phase of CsA-treated heart transplant patients, decreased the rate of acute renal failure after allogeneic bone marrow transplantation and reduced serum creatinine levels in the first 2 weeks after kidney transplantation [303-306]. The mechanisms of cilastatin prevention of CsA acute nephrotoxicity are still unclear. Activation of the endothelin system has been consistently correlated to the adverse renal vascular effects os CsA. Administration of bosentan, an endothelin$_A$ and endothelin$_B$ receptor antagonist, blunted CsA-induced fall in RPF but not glomerular filtration rate decrease in healthy human volunteers [71].

CsA has more than 700 analogues, but few have immunosuppressive capacity. The finding of an analogue with similar immunosuppressive effect but with lesser nephrotoxicity than CsA has been an obvious target for pharmaceutical research laboratories. Cyclosporine G (CSG), a natural occurring single norvaline substituted analogue of CsA with potent immunosuppressive activity, disclosed a significantly lower acute and chronic nephrotoxic profile when experimentally administered to rats [307]. Clinical phase II and phase III studies in renal transplant and uveitis patients showed comparable efficacy and less functional nephrotoxicity when CSG was compared to CsA [308, 309]. However, the short-term differences between CsA and CSG in renal function were not remarkable, what may hamper the market introduction of the new drug. Likewise, SDZ IMM 125, a new derivative of cyclosporine, showed substantial immunosuppressive activity and less renal toxicity than CsA in experimental studies. The limited clinical information about this drug did not demonstrate significant differences in renal effects but more hepatotoxicity when it was compared to CsA [310, 311].

CsA standard oral formulation has an erratic and unpredictable gastrointestinal absorption that causes marked inter- and intra-patient variability. To overcome this problem a microemulsion pre-concentrate was developed. When used in healthy volunteers and transplant recipients this new galenic formulation reached faster time to peak concentration, higher peak concentration, greater area under the curve and was not affected by the physiological state of the gastrointestinal tract [3, 312, 313]. Undoubtedly, the microemulsion formulation provides a more regular and reliable pharmacokinetic profile and improves bioavailability, allowing reduction in the daily dosage of CsA [7, 314-316]. The new microemulsion formula was also used as an alternative for the intravenous CsA formulation in the induction of immunosuppression in liver transplantation, resulting in a non statistically significant reduction in nephrotoxicity episodes from 45 to 25% [317]. To date, the microemulsion formulation has showed similar immunosuppressive efficacy when compared to the standard presentation, although some studies have suggested a reduction in the rate of acute rejection with the new formula [7, 315, 317-319]. However, the high peak levels reached by the microemulsion administration might be associated with an increase in acute nephrotoxicity [320]. Like the standard formulation, the microemulsion reduced glomerular filtration rate and renal blood flow after a single daily dose in healthy subjects [181]. The nadir of renal blood flow impairment occurs 5 hours after drug ingestion [71]. Conversion from standard formula to microemulsion apparently did not cause long-term nephrotoxicity when dosages were adjusted for the same blood

level, but there are a number of studies showing transitory impairment of renal function in the early phase of microemulsion treatment [314, 316, 318]. When standard to microemulsion switch was done in a 1:0.8 ratio instead of a 1:1 ratio there was an alleviation of this short-term nephrotoxicity, suggesting that this conversion should be done in an individualized manner [321].

A simple way to prevent or reverse functional CsA nephrotoxicity is to reduce its dosage or to withdraw the drug. The rational for this maneuver is that the most noteworthy benefit of CsA use in transplantation is a remarkable decrease in early acute rejection, and so the drug might be decreased or withdrawal after the initial immunosuppression phase [242, 322]. However, this approach is still controversial, with conflicting published data [323, 324]. This is particularly true in the field of transplantation, where lack of or inappropriate immunosuppression could imply in morbidity and mortality. Reduced doses of CsA at one year post-transplant have been associated with major risk for development of acute rejection and chronic rejection whereas higher CsA doses correlated with better long-term graft survival [325, 326]. Accordingly, many authors found high rates of acute rejection when cyclosporine was electively discontinued in stable renal transplant recipients [327-329], or development of rejection in heart or liver transplant patients who had CsA withdrawal because impaired renal function was present [330, 331]. On the other hand, several studies, including a recent large meta-analysis by Kasiske et al [322], reported that CsA withdrawal, 3 to 12 months after renal transplantation, did not result in worst graft or patient survival, independently of increased rate of acute rejection, as compared to controls that continued on CsA therapy [324, 332, 333]. Some of them even found better long-term renal function (more than 5 years post-transplant), measured by serum creatinine or creatinine clearance, in the groups where CsA was withdrawn [332, 334, 335]. The combination available data suggests that long-term CsA treatment might be not obligatory in a significant number of renal transplant patients, and that CsA withdrawal is a potential valid maneuver to control functional nephrotoxicity in selected patients.

The advent of potent non-nephrotoxic immunosuppressive agents might make the replacement of CsA or the decrease to it exposure in drugs protocols more feasible. At the moment, mofetil mycophenolate and sirolimus are the only commercially available new drugs that meet these characteristics [336].

Mofetil mycophenolate is a noncompetitive, reversible, inhibitor of inosine monophosphate dehydrogenase that inhibits lymphocyte proliferation. The use of mofetil mycophenolate with concomitant CsA dose reduction or withdrawal has showed to be safe in terms of acute rejection and in many times to induce rapid and significant improvements in renal function and hemodynamics in renal transplant recipients with stable or impaired renal function [337-340] and stable liver or heart transplant recipients with renal dysfunction [341-343]. David-Neto et al retrospectively analyzed a group of 13 renal transplanted children with biopsy showing chronic transplant nephropathy whom had CsA dose reduced or withdrawn and azathioprine switched to mofetil mycophenolate. Six months after the introduction of mofetil mycophenolate median serum creatinine decreased from values of 2.2 mg/dl to 1.5 mg/dl, and no acute rejection occurred [344]. In an interesting study, Gregoor et al randomized 64 stable renal transplant recipients for conversion of CsA to azathioprine (30 patients) or mofetil mycophenolate (34 patients). Both groups had a significant decrease in serum creatinine, but the azathioprine group presented significantly more acute rejections (11/30) than the mofetil mycophenolate group (4/34) [345]. Another population where mofetil mycophenolate use may be extremely useful is in patients receiving grafts from marginal donors, like donors with advanced age, where the issue of acute CsA nephrotoxicity is even more sensitive [340, 346]. Mofetil mycophenolate has also shown to be a safe and effective therapeutic option when CsA has to be discontinued due to CsA-associated hemolytic-uremic syndrome [347]. A recent study showed that stepwise replacement of CsA by mofetil mycophenolate induced a dose-related decrease in von Willebrand Factor and sP-selectin in renal transplant recipients suggesting that CsA causes a reversible endothelial dysfunction [76]. Although the vast majority of the studies regarding mofetil mycophenolate introduction as a sparing regime for CsA showed positive outcomes, there have also been a few unfavorable results. A tentative to change cyclosporine A to mofetil mycophenolate in 8 patients with severe psoriasis resulted in renal function improvement in 90% of them, but all subjects had worsening of the disease control, that was significant in 5 and small in 3 [348]. A word of caution was also raised by the study of Thervet et

al. In this multicenter French study, 20-cadaveric kidney recipients had CsA gradually discontinued and azathioprine switched to mofetil mycophenolate. All patients had a baseline renal biopsy in the previous 12 months. Worsening of the histological injury was observed in 50% of the patients after a mean follow-up of 9 months after mofetil mycophenolate conversion and 5.4 months after CsA withdrawal. These patients did not change their serum creatinine or glomerular filtration rate, in contrast with the 9 patients without structural deterioration, which had a significant improvement in renal function. Although the histological worsening might be already present when the patient switched to mofetil mycophenolate and started CsA withdrawal (some of the baseline biopsies were done up to 6 months before study initiation) the trial was discontinued [349].

Sirolimus (rapamycin) is a macrolide compound related to erythromycin and tacrolimus. It binds to the same immunophilin that tacrolimus, but it does not inhibit calcineurin. Sirolimus blocks T-cell activation at a posterior stage, interfering with the signal from IL2 receptors and receptors for other cytokines and growth factors, and so blocking the cytokine or growth factor-induced activation of the proliferation cell cycle response [350]. This powerful immunosuppressive drug has demonstrated excellent efficacy in the prevention of acute rejection and did not show direct nephrotoxic effects in experimental or clinical studies [12, 351-353]. However, when used together CsA, sirolimus may intensify CsA-induced functional nephrotoxicity [354-356], probably due to a pharmacokinetic interaction between the two drugs, increasing CsA concentrations in whole blood and renal tissue [357]. These results, and the already known synergistic immunosuppressive interaction between CsA and sirolimus, indicate that if the two drugs are used simultaneously, CsA dosages should be reduced [281, 356, 358]. Recent clinical results showed that conversion from cyclosporine to rapamycin in renal and liver transplant patients with chronic or acute CsA nephrotoxicity resulted in an immediate and significantly serum creatinine decrease [281, 359].

In conclusion, several studies using mofetil mycophenolate and sirolimus provided promising results, suggesting that the use of these drugs in immunosuppressive schedules can allow CsA reduction or withdrawal, preventing or significantly improving its acute toxicity. However, almost all of these initial studies involve a small number of patients and long-term follow up outcomes are not available yet. Certainly more clinical data and research are required before these drugs are used as concomitant or alternate agents in immunosuppressive schedules in a wider way.

Chronic nephrotoxicity

Chronic CsA-induced nephropathy is best defined as "a clinicopathologic entity produced by exposure of the patient to cyclosporine, characterized by tubulointerstitial fibrosis in a striped pattern beginning in the medulla and progressing to the medullary rays of the cortex. Usually, but not inevitably, this pathologic finding is associated with some degree of renal dysfunction" [360]. Unfortunately, many authors have used inadequately the term chronic CsA nephrotoxicity when describing renal functional changes without histological evaluation after variable times of CsA administration in humans and animals. This expression must be reserved for the description of CsA-induced structural damage, namely irreversible interstitial fibrosis. This injury has been classically associated with degenerative hyaline changes in the afferent arteriole walls, consisting of endothelial swelling, nodular hyaline protein deposition and areas of smooth muscle cell lesion and necrosis [17, 361]. In kidney transplant recipients, this arteriolar lesion is the key for the discrimination between CsA chronic nephropathy from rejection [246, 361]. Recently, clinical and experimental studies have shown that this arteriopathy, which was considered irreversible, can remit after CsA discontinuation, whereas the tubulointerstitial changes did not regress [17, 361-364]. CsA-induced chronic nephrotoxicity was described in renal and non-renal transplant recipients and in patients with autoimmune diseases receiving the drug for periods of 6 months or more [365, 366].

The lack of a suitable animal model has hampered the study of the mechanisms of chronic CsA nephrotoxicity for a long time. Using the observation that sodium depletion exacerbates CsA nephrotoxicity [367, 368], Rosen et al and Elzinga et al developed a reproducible animal model of chronic CsA nephrotoxicity [369, 370]. In this model, CsA treatment in rats on low salt diet produced histological changes similar to those described in patients on long-term CsA therapy accom-

panied by a profound decrease in glomerular filtration rate [369-371]. When the drug was discontinued glomerular filtration rate improved, returning to baseline values, but the tubulointerstitial injury was progressive, even in the absence of CsA [369].

Mechanism of injury

The well-documented effects of CsA on afferent arterioles led to the logical hypothesis that chronic afferent arteriolopathy would ultimately result in vascular occlusion causing downstream renal tissue ischemia with consequent fibrosis, nephron dropout and tubular atrophy in the affected areas [361, 372, 373]. In fact, experimental renal ischemia induced by unilateral clamping of renal artery for 28 days can induce significant chronic interstitial fibrosis [374]. However, several experimental studies done in the last years found strong clues of dissociation between the mechanisms promoting the interstitial scarring and the hemodynamics changes in CsA nephropathy [369, 375]. Dieperink et al found that felodipine reduced functional nephrotoxicity but did not prevent interstitial damage after long-term cotreatment with CsA [376]. Wolf and Nielson showed increased collagen mRNA in a murine model of CsA nephrotoxicity while serum creatinine was normal [377]. In the same way, Kon et al and Hunley et al showed that endothelin receptor blockers normalized renal hemodynamics but had no effect on structural lesions produced in the low salt chronic CsA model [378, 379]. Conversely, blockade of the renin-angiotensin-aldosterone system by enalapril and losartan strikingly reduce the progression of CsA-induced tubulointerstitial fibrosis despite failure to normalize glomerular filtration rate [380]. More recently, Vieira Jr et al found that salt-depleted rats receiving low and clinically relevant dosages of CsA (5 mg/kg) for 8 weeks developed significant interstitial fibrosis without any decrease in renal blood flow or structural afferent arteriole injury, clearly showing that the interstitial injury can occur in a totally independent way of the preglomerular vasoconstriction [381].

Angiotensin II seems to play a major role in CsA-induced chronic nephrotoxicity. As already pointed, there are several evidences of intrarenal renin-angiotensin-aldosterone system activation by CsA [35, 382]. The salt depletion maneuver used for achievement of the chronic model enhances systemic and intrarenal renin-angiotensin-aldosterone system, and consequently angiotensin II generation [383, 384]. Angiotensin II can act as a potent growth factor inducing fibroblasts activation, extracellular matrix deposition and tissue scarring [385-387]. Chronic infusion of angiotensin II in rats induced tubulointerstitial injury similar to that following CsA chronic nephropathy [388]. A high concentration of angiotensin II AT_1 receptors is present in the inner zone of the outer medulla, particularly in longitudinal bands paralleling the vasa recta bundles, that is the preferential area of CsA damage [389, 390]. Likewise, renal outer medulla type 1 interstitial cells have a high density of angiotensin II receptors. These interstitial cells have extensive cytoplasmic processes, which are closed related to the basement membrane of the vasa recta [391]. If taken together these evidences strongly suggest that the regional regulation of medullary blood flow is regulated by angiotensin II. Blockade of the renin-angiotensin-aldosterone system by an ACE inhibitor (enalapril) and/or an AT_1 angiotensin II receptor antagonist (losartan) in salt-depleted CsA-treated rats reduced blood pressure, promoted afferent arteriole vasodilation and significantly attenuated interstitial fibrosis generation without improving renal hemodynamics. Losartan and losartan plus enalapril, but not enalapril alone decreased renal cortical α1 (I) procollagen mRNA. Treatment with hydralazine plus furosemide reduced blood pressure in the same extent as enalapril and/or losartan but did not prevent tubulointerstitial injury [380]. Lafayette et al compared the effects of enalapril and the combination of minoxidil/hydrochlorothiazide/reserpine in rats on normal salt diet treated with CsA for 12 months. Enalapril and the three drugs in combination reduced blood pressure similarly, but whereas the ACE inhibitor reduced interstitial fibrosis the combination therapy worsened it [392]. In a different model of CsA nephrotoxicity, using spontaneously hypertensive rats on high salt diet, enalapril and valsartan cotreatment prevented CsA-induced renal dysfunction and interstitial fibrosis [393]. In an interesting study Johnson et al showed that enalaprilat, the active metabolite of enalapril, completely reversed the stimulatory effect of CsA on collagen synthesis by cultured renal cortical fibroblasts, stressing that renin-angiotensin-aldosterone system blockade can prevent CsA chronic nephrotoxicity independently of hemodynamic or systemic angiotensin II effects [394]. Angiotensin

blockade by losartan has also been shown to prevent CsA-induced epidermal growth factor decrease in salt-depleted rats. Epidermal growth factor promotes kidney tubular regeneration after injury and so, may be important for the prevention of apoptosis and subsequent fibrosis in this model [395].

CsA stimulates *in vitro* and *in vivo* renal and systemic production of TGF-β [381, 396-400]. The potential sources for renal interstitial TGF-β include interstitial macrophages, interstitial fibroblasts and tubular epithelial cells. Using a double immunolabeling technique Pichler et al suggested that the majority of CsA-induced TGF-β-expressing cells are likely fibroblasts [398]. This cytokine plays a major role in the generation of renal fibrosis by directly stimulating extracellular matrix components and reducing collagenase production, ultimately leading to renal scarring [401]. Data from experimental and clinical studies suggest that TGF-β overexpression may be an important factor in the development of CsA chronic nephrotoxicity. Shihab et al demonstrated that CsA induced a progressive increase in mRNA TGF-β 1 expression preceding the later development of tubulointerstitial fibrosis in the salt-depleted rat model [402]. Vieira Jr et al, showed a progressive TGF-β immunostaining in renal tissue of CsA-treated rats on low salt diet, which was more prominent at the juxtaglomerular arterioles [381]. Cuhaci et al found that 72% of the renal biopsies from CsA-treated transplant patients with chronic allograft fibrosis expressed high levels of TGF-β. These patients had a rate of renal function decline approximately 3 times higher (-19.5 ml/min/year) than patients with minimal or no TGF-β renal expression (-6.2 ml/min/year) [403]. In heart transplant recipients the presence of TGF-β 1 codon 10-gene polymorphism was associated to a higher prevalence of renal dysfunction seven years after transplantation [404]. There is a well-defined link between renin-angiotensin-aldosterone system and TGF-β, traduced by angiotensin II-induced stimulation of TGF-β expression in the kidney [405]. In fact, renal mRNA TGF-β expression was enhanced only in salt depleted rats in contrast to normal salt diet rats treated with CsA [406]. Enalaprilat prevented the CsA-induced TGF-β secretion by cultured human proximal tubular cells as well as losartan and enalapril decreased mRNA TGF-β and extracellular matrix proteins expression and reduced interstitial fibrosis in the salt-depleted rat model [394, 407]. Similarly, losartan decreased plasma levels of TGF-β in renal transplant recipients treated with CsA [44]. Use of anti-TGF-β antibodies in CsA-treated salt-depleted rats reduced renal TGF-β expression, normalized α (I) collagen mRNA expression, partially prevented the decrease in renal tissue levels of metalloproteinase-9 and tissue increase of metalloproteinase-1 inhibitor, prevented glomerular filtration rate impairment and attenuated arteriolar hyalinosis but surprisingly did not change the extent of tubulointerstitial fibrosis [396]. These results suggest that TGF-β, although clearly related to CsA-induced fibrosis, is not responsible for all the effects of this drug on the mechanisms of exaggerated extracellular matrix deposition and renal scarring.

Experimental data indicate the involvement of renal infiltrating and resident cells in the induction of chronic CsA nephrotoxicity. The presence of infiltrating mononuclear cells has been previously shown in the interstitial area of the cortex and outer medulla of salt-depleted rats treated with CsA [368, 408]. Subsequently, Young et al and Vieira Jr et al demonstrated that significant cortical and medullar macrophage infiltration occurs very early in the salt-depletion model of chronic nephropathy, preceding glomerular filtration rate decrease and development of interstitial fibrosis [381, 409]. This infiltration was accompanied by an impressive interstitial and tubular cell proliferation that started in the medulla and progressed to areas of cortical fibrosis [368, 409]. An upregulation of the macrophage chemoattractant osteopontin was observed in proximal tubular cells of CsA-treated rats, and was closely correlated with macrophage infiltrate degree and fibrosis development [398, 409]. Likewise, Benigni et al found an intense staining for monocyte chemoattractant protein 1 (MCP-1) in renal biopsies with CsA nephrotoxicity from kidney transplant recipients [410]. Recently, Hudkins el al confirmed the presence of osteopontin in human biopsies with CsA nephrotoxicity, but there was no significant inflammatory cells infiltration, suggesting that this molecule might be important in the early but not in the established phase of chronic CsA nephrotoxicity [411]. Macrophages are known sources of cytokines and other mediators of inflammation and play a key factor in several processes that lead to progressive renal fibrosis [412, 413]. Activated infiltrating macrophages amplify and retro-activate the inflammatory and pro-fibrogenic response by recruiting of more immunocompetent cells, stimula-

tion of fibroblast proliferation and migration and increasing of collagen synthesis [414]. Over again, there is an intense and close relationship between angiotensin II and macrophage function. Angiotensin II stimulates the production of monocyte chemoattractant protein 1 (MCP-1) and osteopontin and induces the expression of adhesion molecules, responsible for the rolling, adhesion and penetration of monocytes into the interstitial spaces [413]. Additionally, rat and human macrophages express functional components of the renin-angiotensin-aldosterone system [415, 416]. Angiotensin not only recruited but also activated macrophages [413], and in fact human cells presented strikingly renin-angiotensin-aldosterone system activation during human monocyte/macrophage differentiation [416]. If there is no doubt about the relevance of macrophage as an important participant in CsA chronic nephrotoxicity, a crucial question remains unanswered: what is (or are) the stimulus for renal macrophage infiltration and activation? Clearly, preglomerular ischemia has a role, but as already pointed CsA can cause significant interstitial fibrosis with normal renal blood flow. Conceivable candidates are post-glomerular ischemia due to vasa recta constriction, sublethal tubular epithelial cells injury, and endothelial cells lesion allowing plasma leakage into renal interstitial area or activation of resident renal interstitial cells. It is patent that further research is urgently necessary in this field.

Several experimental studies showed that CsA could act directly on resident renal cells inducing changes favoring the development of interstitial fibrosis. Wolf, Killeen and Neilson described that CsA stimulated procollagen production by cultured murine tubulointerstitial fibroblasts and proximal tubule cells [417]. Subsequently, Ghiggeri et al demonstrated that even very low concentrations of CsA induced collagen synthesis in a variety of cultured human and rat renal fibroblasts, mesangial and tubular epithelial cells [418]. Addition of CsA to primary culture of human renal cortical fibroblasts and proximal tubule cells resulted in direct toxicity, release of pro-fibrotic cytokines and increased collagen synthesis. CsA stimulated insulin-like growth factor secretion and inhibited secretion of IGF-1 binding protein by fibroblasts. In tubular cells CsA enhanced the secretion of TGF-β and platelet-derived growth factor [419]. Recent results showed that CsA may have different effects on different types of cells, namely cultured human epithelial and endothelial cells and fibroblasts. Collagen production was enhanced in endothelial and epithelial cells, whereas mRNA for tissue inhibitors of metalloproteinase was up regulated in fibroblasts. Toxicity occurred only in endothelial and epithelial cells and was associated with apoptosis induction. It should be noted, however, that just the epithelial cells had a renal origin, since the fibroblasts and endothelial cells came from human skin, what may limit the generalization of these findings [420]. Impairment of the proteolytic system responsible for renal matrix degradation is also an important element of CsA chronic nephrotoxicity. CsA significantly increased *in vivo* expression of tissue inhibitor of matrix metalloproteinase type 1 by renal interstitial and epithelial cells and promoted intense staining for plasminogen activator inhibitor type 1 in atrophic cortical proximal tubules [421], up-regulated mRNA for tissue inhibitors of metalloproteinase in human skin fibroblasts [420] and inhibited metalloproteinase production by cultured human renal fibroblasts [419]. It has been recently shown that only mesangial cells from mice susceptible to glomerulosclerosis increased collagen content and inhibited matrix metalloproteinase activity and mRNA after exposure to low CsA doses in contrast with mesangial cells from a strain of glomerulosclerosis resistant mice, suggesting that the genetic background may influence CsA-induced pro-fibrotic cellular response [422].

Injured tubular epithelial cells may be another cause for CsA-induced fibrosis. As previously discussed there are many evidences that CsA causes sublethal tubular cell lesion. Moreover, use of the salt depletion model of chronic CsA nephrotoxicity showed that this drug induced an early activation of apoptosis genes, preceding the appearance of apoptotic cells and fibrosis [423]. Once again, angiotensin II seems to partially mediate this phenomenon, since cotreatment with losartan significantly reduced the number of tubular and interstitial apoptotic cells in CsA-treated animals [424]. There is also evidence of nitric oxide participation in apoptosis induced by CsA. Thomas et al found that L-arginine administration decreased significantly the magnitude of tubulointerstitial apoptosis in salt-depleted rats treated with CsA [424]. Amore et al showed that CsA stimulated apoptosis in various renal cell lines, including human tubular cells, was related to increased inducible nitric oxide synthase mRNA via activated p53 proteins [425]. A possible mechanism for

CsA-caused tubular injury is impairment of the P-glycoprotein system (P-GP). This transporter expels hydrophobic substances from the cell, acting as a detoxification system. CsA is a known inhibitor of P-GP, and so it can potentially promote intracytoplasmic accumulation of its own metabolites and toxic cell catabolism metabolites. Supporting this hypothesis, tubular cells overexpression of P-GP in salt depleted rats receiving CsA was inversely related to interstitial fibrosis and intrarenal angiotensin II deposits [426]. In renal transplant recipients, increased P-GP expression was found in infiltrating and resident cells of biopsies showing ATN, acute rejection and chronic rejection but not in chronic CsA nephrotoxicity, suggesting that failure to up-regulated P-GP is associated with CsA-induced apoptosis and fibrosis [427].

Nitric oxide pathway manipulation reflected in CsA chronic nephrotoxicity. Supplementation of the nitric oxide substrate, L-arginine, ameliorated whereas use of the nitric oxide synthase inhibitor, L-NAME, aggravated tubulointerstitial fibrosis [100]. Likewise, CsA-induced upregulation of TGF-β1, plasminogen activator inhibitor 1 and deposition of extracellular matrix components were aggravated by nitric oxide blockade and ameliorated by nitric oxide enhancement [428].

There are abundant evidences that CsA markedly increases endothelin production. Endothelin up-regulates TGF-β expression, that is clearly involved in CsA chronic nephrotoxicity. The existence of an endothelin-transforming growth factor β factor pathway in CsA-induced fibrosis was proposed [429]. Supporting this hypothesis, increased tubular cells endothelin mRNA expression was found in human biopsies with chronic CsA nephrotoxicity; [410] and Ramirez et al found dramatic elevations in endothelin system components in CsA-treated rats that strongly correlate with renal structural lesions [382]. However, experimental use of endothelin receptor antagonist did not prevent interstitial fibrosis in the salt-depleted chronic nephrotoxicity model [378, 379].

Recent studies raised the possibility of vascular endothelial growth factor participation in CsA structural nephrotoxicity. VEGF is a potent endothelial cell mitogen that mediates endothelial cell proliferation and survival, induces angiogenesis, participates in vascular remodeling and repair and causes vasodilation and increased vascular permeability through increase in nitric oxide production. Shihab et al found an upregulation of vascular endothelial growth factor in salt-depleted CsA-treated rats but not in normal salt diet animals. As previously pointed, in this model only salt-depleted animals developed renal structural injury resembling the human picture of chronic CsA nephrotoxicity [430]. In an interesting study, Kang et al found that VEGF administration to rats with established chronic CsA nephropathy resulted in improvement of interstitial fibrosis, decreasing in osteopontin expression, macrophage infiltration and collagen III deposition and blood pressure reduction [431].

Other mechanisms and mediators have been experimentally implicated in the genesis and prevention of chronic CsA nephrotoxicity. Reactive oxygen species, besides its functional effects on renal function, are also mediators of tissue injury favoring fibrosis. Use of the antioxidant vitamin E inhibited increases in TGF-β and osteopontin mRNA and development of renal fibrosis in CsA-treated rats [177]. Mazzali et al showed that hyperuricemia exacerbates experimental chronic CsA nephrotoxicity, apparently due to activation of the renin-angiotensin-aldosterone system and inhibition of renal nitric oxide production [432]. Use of a thromboxane A_2 receptor antagonist in a renal isograft renal model treated with CsA strikingly prevented development of interstitial fibrosis [433]. Preliminary evidence showed that prednisone altered the structural changes induced by CsA in salt depleted rats, enhancing tubular hypertrophy in medullary area and inducing a tendency to lower tubulointerstitial fibrosis [434]. Colchicine administration to CsA-treated rats significantly decreased medullary interstitial fibrosis, but did not change the inflammatory cells infiltrate or prevented serum creatinine increase [435]. Johnson el al demonstrated that simvastatin, a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor, completely prevented CsA-stimulated cultured human renal cortical fibroblasts collagen synthesis and IGF-1 secretion [436].

A working hypothesis for chronic CsA nephrotoxicity

At this point it is clear that chronic CsA nephrotoxicity can be caused by preglomerular vasoconstriction dependent mechanisms and/or by a way totally independent of afferent arteriole functional and structural changes. In the clinical setting and in most of the ex-

perimental models used it is likely that interstitial fibrosis occurs through a combination of both processes. The mechanisms dissociated from preglomerular ischemia seem to be closely dependent on CsA-induced intrarenal angiotensin II enhancement, macrophage infiltration and fibroblasts and interstitial cell activation. The signals causing macrophage migration may come from interstitial ischemia due to vasa recta constriction, activation of interstitial resident cells, leakage of plasma into interstitial area due to endothelial cells damage or inflammatory stimulus originated from injured or apoptotic tubular cells. Obviously, more than one factor may be occurring at the same time. The final pathway is an inflammatory interstitial microenvironment where a cross-talk of angiotensin II, macrophage, fibroblasts and resident cells will result in overexpression of pro-fibrotic substances (cytokines, growth factors, ROS) inhibition of antifibrotic components (metalloproteinase etc.) in a self-perpetuating cycle of activation of renin-angiotensin-aldosterone system macrophage recruitment and activation and enhancement of extracellular matrix deposition (Figure 1).

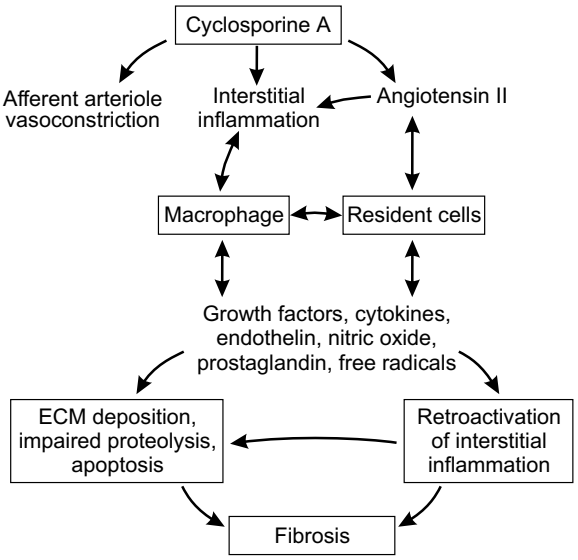

Figure 1. *A working hypothesis for chronic CsA nephrotoxicity.*

Clinical aspects: *Renal transplantation*

To identify the specific role of chronic CsA nephrotoxicity in renal allograft durability is an almost impossible task to complete. From the very beginning, the transplanted kidney will suffer from mechanic manipulation, ischemic injury and immunologic attack. Later on acute rejection, recurrent or *de novo* renal disease, hypertension, chronic viral infection, metabolic derangements (dyslipidemia, diabetes, hyperuricemia), chronic rejection and aging may work in diverse combinations causing progressive structural damage and functional impairment.

In this complex situation the histological diagnosis of chronic CsA nephrotoxicity should rely in the finding of the typical afferent arteriolar lesion with nodular focal or circular protein deposits in the *tunica media* [242]. However, when renal biopsies are performed tardily, after months or years of continuous renal injury, the morphological picture may be "dirty" and very difficult to characterize. Moreover, vascular or chronic rejection may coexist with CsA chronic nephrotoxicity. Finally, as previously pointed, recent studies showed that CsA-induced fibrosis can occur without afferent arteriole injury and that CsA-induced afferent arteriole hyalinosis can remit without parallel improvement in renal fibrosis, strongly suggesting that the vascular lesion and renal fibrosis may occur in an independent way [362, 364, 381]. Potential clues for differentiate chronic CsA nephrotoxicity from rejection came from an interesting study by Abrass el al. These authors showed that extracellular matrix composition of renal fibrosis found in human renal allograft biopsies was different depending on the pathological diagnosis made. Fibrosis associated with CsA chronic nephrotoxicity had a widespread interstitial accumulation of collagens I and III, whereas biopsies with rejection showed an increased expression of proximal tubular basement membrane collagen IV α chain 3 and laminin-β2, suggesting that interstitial fibrosis in these patients can result from different pathogenic mechanisms [437].

Impairing further the correct understanding of the problem, few prospective studies assessed concomitantly functional and histological changes in kidney transplant recipients. Actually, studies supporting the absence of negative chronic effects for CsA on renal allografts were largely based on retrospective analysis of functional data, which frequently were only serum creatinine measurements [438, 439]. When histology was made, most of the evidences indicating an expressive role for CsA-induced fibrosis in the chronic failure of transplanted kidneys were indirect. Different au-

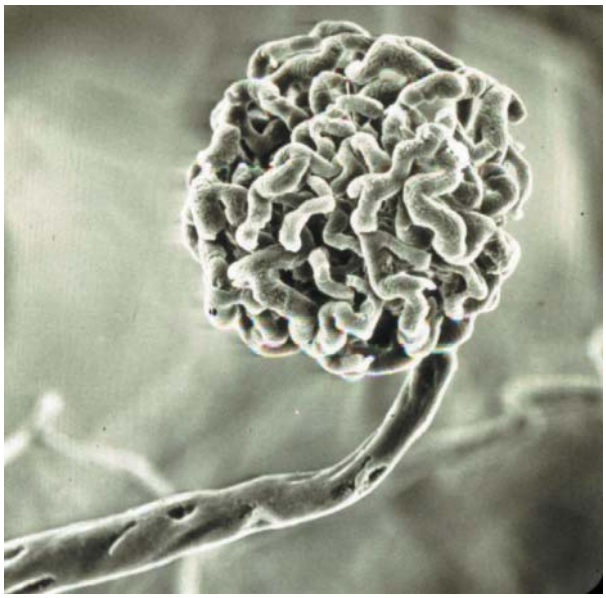

Figure 2. *Striking afferent arteriole vasoconstriction in a Sprague-Dawley rat on low salt-diet treated for 28 days with CsA 15 mg/kg/day. The narrowest point of the arteriole measured 11.02 mm, whereas control animals presented a mean diameter of 12.67 mm. Vascular cast, scanning electron micrograph, orig. magn. x450. From [380], with permission.*

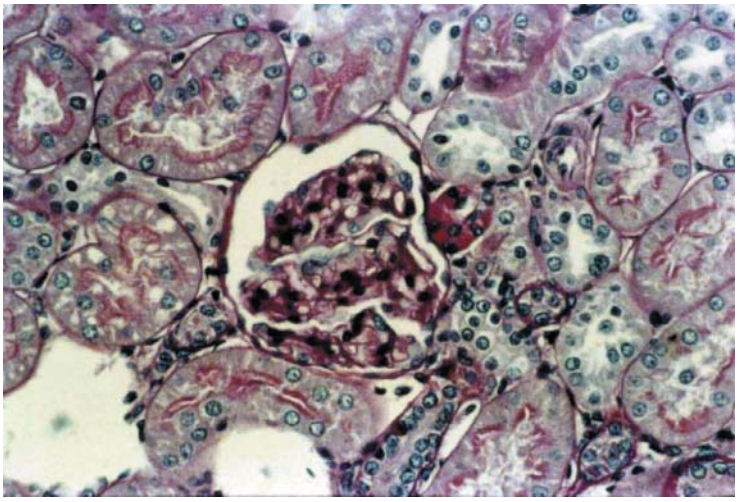

Figure 3. *Afferent arteriole hyalinosis in a Munich-Wistar rat on low salt diet treated with CsA 15 mg/kg for 4 weeks. H&E staining, orig. magn. x400.*

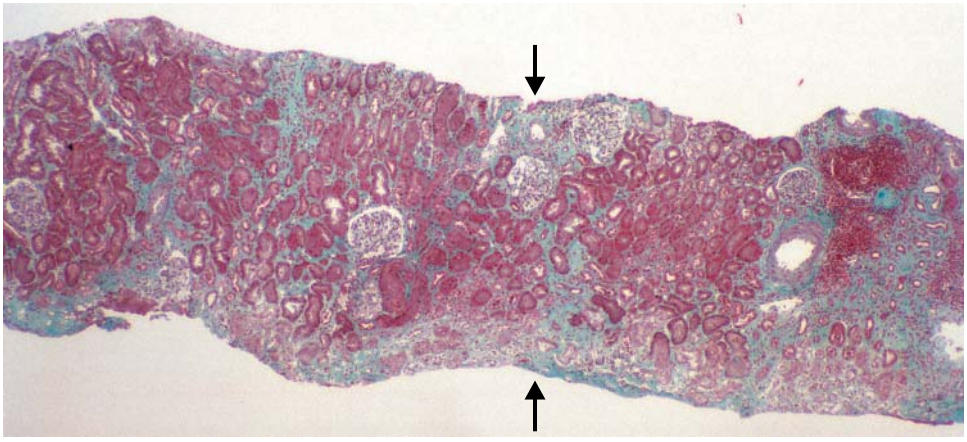

Figure 4. *Striped interstitial fibrosis (arrows) in a CsA treated recipient. Masson's trichrome staining, orig. magn. x40.*
By courtesy of Dr. JL Bosmans.

thors found significant development of interstitial fibrosis occurring generally after 6 months or more of CsA treatment [440, 441]. When CsA-treated renal and azathioprine-treated renal recipients were compared, the first had significantly worst interstitial fibrosis [442, 443]. An aspect to be considered is that the contribution of CsA-induced interstitial fibrosis for progressive renal dysfunction in kidney transplant recipient is probably not an "evil or god" situation. Genetic background, concomitant diseases and many other factors may modulate different degrees of interstitial fibrosis, inducing individual and unequal patients response. Indeed, Mourad et al found that only 6% of 23 patients with biopsy-proven chronic CsA nephropathy improved renal function after CsA reduction or withdrawn [444].

While CsA remarkably improved short-term graft and patient survival as compared to azathioprine-based immunosuppression, the same outcome has not been consistently demonstrated on long-term survival. A recent study found a substantial increase in short and long-term survival of renal grafts in the 93, 934 patients transplanted in the United States between 1988 and 1996, but the immunological schedules were not detailed [445]. Conversely, Marcén et al studied 128 CsA-treated cadaveric first renal transplant recipients followed for at least 10 years comparing them with 185 azathioprine-treated patients. In the first three years post-transplant actuarial graft survival was significantly higher in CsA-treated patients. Afterward, this difference disappeared and azathioprine-treated patients showed a non-statistically significant tendency to better graft survival at 5 and 10 years. The leading cause of graft failure in CsA-treated patients was chronic allograft nephropathy [446]. In fact, chronic allograft nephropathy, an entity with obscure pathogenesis, is considered the most important cause of progressive renal failure in renal transplant patients nowadays [447]. A potential CsA participation in chronic allograft nephropathy was uncovered by the finding of a significant reduction in the rate of the loss of renal function in patients with histologically confirmed disease when CsA dosage was reduced or suspended [447].

Clinical aspects: *Other solid organs transplantation*

While the individual weight of CsA chronic nephrotoxicity in renal transplant longevity has not been precisely determined, the use of CsA in patients with healthy native kidneys showed clearly the real potential for significant renal injury owned by this drug, that ultimately may lead to end stage renal failure requiring dialysis and/or renal transplantation [243, 244, 360, 448, 449].

Myers et al were the first to describe that CsA use in heart transplantation was associated with chronic renal failure development. In a careful series of observations they showed that CsA used for more than 12 months caused a progressive glomerular filtration rate decline accompanied by a concomitant renal blood flow drop and RVR increase, obliterative arteriolopathy, striped interstitial fibrosis, tubular atrophy and glomerulosclerosis in heart transplant recipients [365]. In a subset of patients followed over 48 m, CsA was reduced or withdrawn, but glomerular filtration rate did not improve and repeated biopsies disclosed further histological deterioration [373]. The starting doses of CsA (17 mg/kg) and target trough levels used in the first studies were substantially higher than dosages adopted latter. However, when patients on "old high-doses" were compared to patients on "modern low-doses" by the same group of investigators, reduction in CsA dosage did not alleviate the intensity and progression of renal injury [372]. In the last paper of this seminal series the authors reported a 10% cumulative incidence of end stage renal failure in a 10-year period of observation [450]. A number of subsequent studies substantiated these findings, confirming that prolonged CsA administration to heart transplant recipients causes a progressive functional impairment, with a sharp renal function decline in the first year followed by a slower rate of deterioration thereafter, and a prominent structural injury represented by afferent arteriole arteriolopathy, tubulointerstitial fibrosis and glomerulosclerosis [252, 451, 452]. Variable rates of end stage renal failure were reported by different groups, ranging from 1 to 10% [252, 449, 450, 453-459], with an apparent trend for increased rate of end stage renal failure with longer follow-up time (Table 4). The mean time elapsed from cardiac transplantation to initiation of dialysis was around 6 to 7 years [453, 455, 456, 458] and the devel-

Table 4. The frequency of end stage renal failure after heart transplantation apparently increases in conjunction with longer follow-ups, with few exceptions.

Authors	Number of patients	Follow-up time (years)	End stage renal failure frequency (%)
Lewis et al (ref. 457)	100	4	1.0
Gonwa et al (ref. 454)	69	4	1.3
Zietse et al (ref. 459)	187	5	3.2
Goral et al (ref. 455)	39	6	4.0
Greenberg et al (ref. 252)	228	7	2.2
Herlitz and Lindelöw (ref. 449)	151	9	4.0
Goldstein et al (ref. 453)	293	9	6.5
van Gelder (ref. 458)	304	10	8.0
Myers and Newton (ref. 450)	200	10	10.0
Kuo et al (ref. 456)	430	13	3.3

opment of end stage renal failure requiring renal replacement seems to be associated with higher mortality in these patients [244, 453, 458]. Attempts of identification of risk factors for end stage renal failure development originated conflicting results. Some studies suggested that early decrease in glomerular filtration rate, CsA dose, time of exposure to CsA therapy, previous renal dysfunction and older age were associated to end stage renal failure. However, the majority of studies did not find any significant correlation among CsA dosages, CsA trough blood levels, patients' age, presence of hypertension or use of antihypertensive drugs and CsA-induced chronic renal failure [244, 449]. The sum of these data suggests that heart transplant recipients present different susceptibility to CsA-induced injury resulting in a large inter-individual variability of renal fibrosis response and progression to end stage renal failure.

The picture observed in lung and lung/heart transplantation is quite similar to that found after heart transplantation. There is a long-term impairment of glomerular filtration rate, structural arteriolar, interstitial and glomerular damage and development of end stage renal failure in up to 6.7% of the patients [448, 460-462].

Information about chronic CsA nephrotoxicity in liver transplantation is scanty, with few papers analyzing renal histology. In a similar way to heart transplantation different authors reported an early and marked glomerular filtration rate decrease with a tendency to subsequent renal function stabilization. Dagöö et al followed 25 liver transplant patients maintained on low CsA dosages for 5 years. There was a sharp decrease of glomerular filtration rate in the first year and after 5 years all recipients presented an average glomerular filtration rate loss of 50% (or a reduction of 3 ml/year). However, the authors did not perform renal biopsies or change immunosuppression, making impossible to differentiate how much of this lesion was functional and reversible or chronic and irreversible [463]. When renal histology was available the usual CsA-induced structural changes were found: preglomerular arteriolopathy, interstitial fibrosis, tubular atrophy and signs of glomerular ischemia [452, 464, 465]. More recently, there have been reports of end stage renal failure in CsA-treated liver transplant recipients. Gonwa et al reported 7% end stage renal failure prevalence in liver recipients who had hepatorenal syndrome compared to 2% in patients who did not present it [466] and Coopersmith performed renal transplantation in 7 liver-transplant recipients that developed CsA-induced end stage renal failure [448]. So far, Fisher et al have published the most complete study on CsA-induced chronic renal failure after liver transplantation. These authors analyzed 883 consecutive liver transplants that were mostly treated with CsA. In patients surviving more than one year there was a severe renal failure prevalence of 4.4 % (25 patients). Twenty-four of these 25 patients were on CsA therapy. Most importantly, 12 of these 25 patients (48%) with severe renal

failure required dialysis despite CsA dose reduction or withdrawal. The median time for severe renal failure was 3 years and for end stage renal failure it was 5 years. Development of severe renal failure and end stage renal failure were associated with higher mortality, 44 and 41%, respectively. Renal biopsies were obtained from 13 of the 25 patients and revealed injuries consistent with CsA chronic nephrotoxicity in 10. The risk-factors analysis found 2 subgroups with diverse characteristics. One consisted of patients with early renal dysfunction preceding later chronic renal failure. These patients presented as risk factors for later development of end stage renal failure older age, perioperative dialysis, cytomegalovirus infection, infection and re-grafting. The second group included patients with late onset renal failure without early renal dysfunction. Trough blood level at month 1 and cumulative CsA dosage at 5 years were predictors of end stage renal failure development in this group [465]. With the increasing survival time of liver transplantation recipients we will probably see higher frequency of CsA-induced chronic renal failure in this setting.

Native kidneys of insulin-dependent diabetes mellitus patients submitted to pancreas transplantation can also suffer the punch of chronic CsA nephrotoxicity. Fioretto et al studied 13 insulin-dependent diabetes mellitus pancreas transplantation recipients, using as control group insulin-dependent diabetes mellitus patients not transplanted or who had early failure of pancreas allograft. Baseline renal function and histology were assessed and repeated up to 5 years of follow-up. The authors found a 34% glomerular filtration rate decrease in the transplant group with subsequent renal function stabilization versus no glomerular filtration rate change in the control group. Renal histology was preserved or slightly impaired in the first 2 years post-transplant, but 5-year biopsies revealed a remarkable increase in interstitial fibrosis, interstitial cortical volume, tubular atrophy and frequency of sclerosed glomeruli. There were no significant histological changes in the control group. Stepwise multiple regression analysis identified intensity of glomerular filtration rate decline in the first year, CsA blood levels and CsA daily dose as good predictors of structural injury at 5 years [467].

Clinical aspects: *Bone marrow transplantation*

CsA therapy is used in bone marrow transplantation for prevention of graft versus host disease for limited periods of time, normally from 6 to 18 months. Dosages are high, around 10 to 12 mg/kg, with target trough blood levels up to 400 ng/ml. Few studies have explored the issue of chronic nephrotoxicity. Nizze et al studied autopsy material from 112 bone marrow transplantation recipients treated either with CsA or CsA-free immunosuppression. They found higher prevalence of interstitial fibrosis, tubular atrophy, arteriolopathy, glomerular and/or arteriolar thrombi and glomerular collapse in the CsA group. When these data were compared with autopsies of heart transplant recipients, the frequency of structural injury was clearly more elevated in bone marrow transplantation (54 versus 19.5%) [468]. Diertele et al found structural renal lesions associated with CsA chronic nephrotoxicity in 67% of 49 bone marrow transplantation recipients. Risk factors associated with worst renal pathology were increasing duration of CsA therapy, more severe renal dysfunction in the first 3 months post-transplant and use of total body irradiation [469]. Indeed, a recent study by Miralbell et al found that total body irradiation and graft versus host disease were associated with increased serum creatinine levels in bone marrow transplantation patients [470].

Clinical aspects: *Autoimmune diseases*

The situation where CsA impact on native kidneys is more evident is probably in the treatment of autoimmune diseases. In this field, rejection is not an issue and the native kidneys are usually healthy and not submitted to other potential aggressions, making easier to demonstrate a direct cause-effect correlation. In fact, Feutren and Mihatsch, on behalf of the International Kidney Biopsy Registry of Cyclosporine in Autoimmune Diseases, found interstitial fibrosis and tubular atrophy and/or arteriolar alterations in 21% of 192 patients with insulin-dependent diabetes mellitus of recent onset, uveitis, psoriasis, Sjögren's syndrome or polychondritis that had been treated with CsA for 4 to 39 months (median 13 m). Use of multivariate logistic-regression analysis found three risk factors for CsA nephropathy: older age, larger initial CsA dose and larger maximal increase in serum creatinine [471]. In a

recent meta-analysis, Vercauteren et al selected 18 papers (from a total of 423) for systematic review of renal function changes. Patients receiving CsA had a risk difference of 20.9% for developing nephrotoxicity as compared to other therapies. More notably, in seven studies CsA withdrawal induced only a partial reversion of renal dysfunction. Renal histology studies were reported in 19 papers and 10 of them were selected for review. These studies comprehended 163 patients with uveitis, psoriasis, or rheumatoid arthritis. Time of biopsy was always over 12 months. All papers reported mild to moderate interstitial fibrosis and/or tubular atrophy and/or arteriolar hyalinosis. Three studies performed baseline and post treatment biopsies and found *de novo* interstitial fibrosis or worsening of pretreatment injury [472].

Autoimmune uveitis was one of the first autoimmune diseases treated with CsA, and in 1986 Palestine et al compared renal biopsies from 17 patients treated for an average time of 2 years (eleven to 39 months) with renal biopsies from patients with idiopathic hematuria not receiving CsA. Interstitial fibrosis and/or tubular atrophy were present in all patients treated with CsA, independently of having impaired or normal renal function at the time of the biopsy, and interstitial infiltrate was found in 14 patients. These 17 patients were young (mean age 35.5 ± 3.1 years) and all had normal baseline serum creatinine (1.0 ± 0.04 mg/dl). At the time of the biopsy mean inulin clearance for CsA-treated group was 69 ± 6 ml/min/1.73 m^2 BSA, serum creatinine was 1.5 ± 0.1 mg/dl and mean CsA dose was 10 ± 0.7 mg/kg [366]. In a subsequent study, the same authors performed sequential renal function and histological evaluation in these patients after two to four years of therapy. Despite CsA dose reductions inulin clearance did not improve in 12 patients and actually, decreased significantly in 3. All patients had arteriolar injury, including hyaline changes. CsA-induced chronic histological damage progressed in three of six follow-up biopsies carried out. It is noteworthy that two of the three patients with progressive interstitial injury had stable renal function [473]. The other study assessing renal histology in CsA-treated patients with autoimmune uveitis found a lymphocytic infiltrate (predominantly T lymphocytes) and arteriolar changes in 80% of them. Patients were treated for three years, with an initial CsA dose of 10 mg/kg that was tapered to 5 mg/kg [474].

Due to its undisputed effectiveness CsA has been largely used in the treatment of psoriasis. The fact that this autoimmune disease does not cause intrinsic renal abnormalities provides a unique opportunity to assess CsA direct impact on the development of chronic structural injury in healthy kidneys. Indeed, most of the papers that evaluated CsA-induced changes in renal histology of autoimmune diseases are about psoriasis. These studies showed that low CsA doses (initial 5 mg/kg, tapered to 1 to 2 mg/kg) consistently induced mild to moderate renal scarring (interstitial fibrosis and tubular atrophy) and frequently caused arteriolopathy associated with variable degrees of renal dysfunction [475-480]. Sequential biopsies found progression of renal scarring and arteriolopathy accompanied by a concomitant glomerular filtration rate decrease, although development and progression of interstitial fibrosis with stable serum creatinine have also been demonstrated [475, 479, 480]. Two studies are particularly relevant because performed pre and post treatment biopsies. Svarstad et al studied 10 psoriatic patients treated with an average CsA dose of 3.23 mg/kg for 12 m. At baseline biopsies just one patient had moderate fibrosis. After one year of therapy renal scarring worsened in this patient and 4 patients had *de novo* interstitial fibrosis [478]. Zachariae reported up to eight years of functional and histological follow-ups in 30 psoriatics treated with low doses of CsA (2.5 to 6 mg/kg). Pretreatment biopsies were done in 25 of them and 17 had totally normal renal tissue. After two years of treatment all patients presented histological changes compatible with chronic CsA nephrotoxicity. Renal injury, consisting of focal tubulointerstitial fibrosis, arteriolopathy and glomerulosclerosis was progressive over time. After four years all biopsied patients had moderate to severe striped fibrosis, with a mean score two times higher than that found after one year. All patients except one developed significant hyalinosis and the percent of sclerotic glomeruli increased from 1% to 8%. These changes were paralleled by glomerular filtration rate decrease and glomerular filtration rate values correlated negatively with the degree of fibrosis [480]. So far there is no clear definition of risk factors related to development of structural renal lesion in CsA-treated psoriatic patients. Correlations with dose or trough levels were negative. One study found that late fibrosis correlated with older age and presence of hypertension [475], but others did not [480].

Powles et al reported failure of renal functional improvement after one month of CsA discontinuation as a good predictor for the finding of chronic changes at biopsy [477].

The use of low doses of CsA in rheumatoid arthritis has also been associated to functional impairment, occasional development of chronic renal failure and chronic structural nephropathy [481-486]. Interpretation of renal biopsies in these patients is complicated by the possibility of AR-induced changes, like tubulointerstitial injury, glomerulosclerosis and amyloidosis or NSAIDS and analgesic-induced tubulointerstitial lesions. In fact, Landewe et al compared renal biopsies from eleven rheumatoid arthritis patients treated with low CsA dose (<5 mg/kg) for a mean of 26 m with renal tissue from autopsy of 22 rheumatoid arthritis patients who did not receive CsA. The two groups were matched for age, disease duration, sex and previous use of gold and/or D-penicillamine. Although glomerular filtration rate decreased 26% from baseline in the CsA group, renal structural injury (glomeruli obsolescence, arteriolopathy, tubular atrophy and interstitial fibrosis) was minimal and similar in both groups [487]. A possible methodological flaw in this study is the lack of report of the cause of death in the control group, and the fact that kidney biopsies were not performed in patients withdrawn from CsA treatment due to decreased renal function [472]. Another study biopsied fourteen AR patients treated with low dosages of CsA (<5 mg/kg) for 6 months. Mean serum creatinine at biopsy was 0.84 mg/dl, thirteen patients showed nonspecific renal injury and only one had moderate striped interstitial fibrosis attributable to CsA nephrotoxicity. However, there were no baseline, control or consecutive biopsies in this study [483]. Reports of the International Kidney Biopsy Registry of Cyclosporine in Autoimmune diseases showed a little more expressive figure for CsA nephrotoxicity in rheumatoid arthritis [481, 484]. The initial paper compared 41 patients treated with CsA at a maximum dose of 4.6 ± 1.2 mg/kg for 16 ± 7 m with 11 rheumatoid arthritis patients not treated with CsA and 41 sex and age matched normal controls (kidney donors). The CsA-treated group showed an interstitial fibrosis and tubular atrophy score higher than values for normal controls and not CsA-treated rheumatoid arthritis, although statistical significance was only seen between normal controls and CsA-treated rheumatoid arthritis. There was no case of typical CsA arteriolopathy. More intense morphological changes (moderate focal interstitial fibrosis with tubular atrophy and/or arteriolopathy) affecting more than 30% of the biopsy area were found in four patients of the CsA-treated group and interpreted as CsA nephropathy. CsA structural injury seemed to correlate with initial serum creatinine level and CsA dose, but not with treatment length [481]. In a subsequent paper the group reported data originated from 60 first and 14 second biopsies in rheumatoid arthritis patients treated with CsA for up to 87 m. At first biopsy they found changes consistent wit CsA nephropathy in five patients, and a further patient presented these changes at second biopsy. It was stressed that none individual developed CsA nephrotoxicity among 22 patients who started treatment with doses lower than 4 mg/kg and subsequently did not receive doses higher than 5 mg/kg [484]. Sund et al conducted the only study that compared pre and post treatment biopsies in rheumatoid arthritis patients given CsA. These authors studied 10 patients, with mean age of 58 years, without known renal disease, who received a low dose of CsA (< 5 mg/kg) for up to 46 months. Following a first (baseline) biopsy, a second one was carried out after a mean of 17.8 months, and a third biopsy after a mean of 38.6 months of treatment. A semiquantitative chronicity index was used for evaluation of renal tissue. Glomerular filtration rate was depressed at the time of both post treatment biopsies. In the baseline biopsy glomeruli were normal or showed slight changes and there was no fibrosis or just a slight increase in interstitial area. In the two subsequent post treatment biopsies the frequency of sclerotic glomeruli increased in two and four patients, respectively, and slight to moderate focal interstitial fibrosis and tubular atrophy developed in about half of the patients. In the seven patients that had three biopsies chronicity index increased from 2.3 to 3.9 in the second biopsy and remained 3.9 in the third. Just one patient presented progressive chronicity index in the three biopsies. Arteriolar changes were nonspecific and similar between the baseline and later biopsies. There was no correlation between structural injury, cumulative CsA dose, and duration of treatment or decrease in glomerular filtration rate. So, this study showed without a doubt that low doses of CsA can cause chronic nephropathy in rheumatoid arthritis patients, but showed too that the injury did not occur equally in all patients and is not obligatory progres-

sive, suggesting an important role for individual susceptibility in the development of CsA-induced structural lesions [485]. Recently, the number of months with elevated serum creatinine was shown to be an independent predictor for development of irreversible CsA-induced renal dysfunction. Van der Borne et al studying 83 CsA-treated rheumatoid arthritis patients found 27% of irreversible increase in serum creatinine among patients that remained with serum creatinine ≥ 30% from baseline more than two months against 6% in patients who serum creatinine remained similarly high less than two months [486].

CsA has also been used in the onset of insulin-dependent diabetes mellitus. Similarly as with the other autoimmune diseases already cited, CsA treatment was related to variable degrees of structural and functional impairment in these patients [488-490]. Mihatsch et al reported that 25% of 40 individuals with recent onset insulin-dependent diabetes mellitus treated with CsA 7 to 9 mg/kg for at least one year had renal interstitial fibrosis and tubular atrophy, and in a less scale, arteriolopathy. Serum creatinine at the time of biopsy was 43% higher than baseline values. There was a significant correlation between tubular atrophy intensity and CsA trough blood level [489]. Another study, by Assan et al, analyzed renal function and histology of 125 insulin-dependent diabetes mellitus patients (74 adults and 51 children) treated with CsA 7.5 to 10 mg/kg for an average of 13 months. All patients were in remission from insulin dependency and without other possible causes of renal dysfunction at the moment of biopsy. Slight interstitial fibrosis and tubular atrophy were found in 26% of the patients. Moderate injury was observed in additional 16% and considered definitely related to CsA. A nonsignificant trend towards glomerular filtration rate decrease was observed in the group with moderate structural lesion, but functional changes reversed with CsA dose reduction. Age, excessive CsA dose and trough blood level were considered risk factors for chronic injury. The magnitude of serum creatinine increase was the best predictor of chronic nephropathy presence at biopsy [488].

Although CsA has been used for treatment of several other autoimmune diseases like atopic dermatitis, myasthenia gravis, systemic sclerosis, primary biliary cirrhosis and other, data about chronic structural injury in these situations are scarce with occasional reports of end stage renal failure or development of interstitial fibrosis in kidney biopsies in demyelinating polyradiculoneuropathy, Sjögren's syndrome, polychondritis and Behçet's syndrome [471, 491, 492].

Therefore, there is no doubt that even low doses of CsA can induce chronic irreversible structural injury and sometimes end stage renal failure in autoimmune patients. The histological lesions seen more consistently are interstitial fibrosis and tubular atrophy. The typical CsA-related arteriolopathy with myocyte necrosis was rarely demonstrated, with a rather more usual finding of nonspecific arteriolar hyalinosis. The available data strongly suggest that the development of chronic injury and its severity depend on different individual susceptibilities to CsA. The best predictors for the presence of fibrosis in renal tissue seem to be the length of time in which renal function remained depressed and the lack of reversibility of functional impairment after CsA discontinuation. Whereas in solid organs or bone marrow transplantation CsA use is necessary and justified by obvious reasons, its use in patients with nonfatal autoimmune diseases should be carefully balanced against the risk of causing progressive renal fibrosis, chronic renal failure or even end stage renal failure.

Clinical aspects: *Primary renal disease*

CsA is a valid option for treatment of steroid-resistant or relapsing nephrotic syndrome and for some forms of glomerulopathies. The clinical and histological differentiation of underlying disease progression from chronic CsA nephrotoxicity in these patients is extremely hard to perform. A baseline biopsy collection is mandatory for reliable renal tissue evaluation in this setting. Some studies approached this problem with the accomplishment of pre and post treatment biopsies originating conflicting results. Clasen et al reported that post treatment renal biopsies in five minimal-change nephrotic syndrome patients treated with CsA for 10 months did not show significant vascular or interstitial changes [493]. The same group published another paper four years later, studying 21 patients with severe steroid-resistant or steroid-dependent nephrotic syndrome treated with CsA for 6 to 71 months. Curiously, at this time possible CsA nephrotoxicity was diagnosed in three and definite CsA-induced nephropathy in two patients. The mean glomerular filtration rate value remained stable over time

[494]. Meyrier et al found different patterns of response for CsA treatment in patients with minimal change disease and focal segmental glomerulosclerosis. In this study, baseline and sequential renal biopsies were done in 36 nephrotic syndrome patients. Those with minimal change disease had stable normal function and less severe tubulointerstitial injury on follow-up biopsy. In contrast, the group with focal segmental glomerulosclerosis increased their serum creatinine as compared to minimal change disease patients and presented a further deterioration of renal histology, i.e. an increased number of sclerotic glomeruli and worsening of interstitial fibrosis. CsA doses higher than 5.5 mg/kg were associated with more severe structural injury [495]. On the other hand, Waldo et al did not find progression of interstitial fibrosis in four follow-up biopsies done after at least two years of CSA therapy in children with steroid-resistant nephrotic syndrome due to focal and segmental glomerulosclerosis [496]. Habib and Niaudet performed baseline and sequential biopsies in 42 CsA-treated children with idiopathic nephrotic syndrome. At pretreatment biopsy just one patient showed moderate tubulointerstitial lesions against nine patients presenting severe interstitial injury in the first follow-up biopsy, done after a mean time of 13 ± 4 months of therapy. A second follow-up biopsy was done after a mean of 29 ± 6 months of CsA therapy and showed progressive tubulointerstitial injury in 13 of 23 children. There was no correlation between the histological changes and length of treatment, CsA blood or trough levels and no impairment of renal function [497]. Other studies found renal structural injury compatible with CsA-induced nephropathy in 7 to 18% of children and adults with nephrotic syndrome and minimal change disease or focal segmental glomerulosclerosis after long term CsA treatment [498-500]. Seikaly et al found mild but progressive tubular atrophy and interstitial fibrosis in 75% of eight children with minimal change disease treated with CsA for more than three years compared to 25% of similar interstitial lesion in eight children with primary nephrotic syndrome. Cortical interstitial fibrosis and tubular atrophy areas were significantly wider in kidneys from children treated with CsA than in kidneys from not treated children that had primary nephrotic syndrome [501].

Clinical management of chronic nephrotoxicity

The first challenge for prevention and treatment of chronic nephrotoxicity is to correctly diagnose it at early stages. Since we are talking about a definition that relies on the presence of irreversible structural injury, a renal biopsy is clearly the gold standard for diagnosis. However, renal biopsies were time consuming, relatively expensive and not free of risks. Moreover, due the focal characteristics of the process and the limited size of a sample biopsy, early or mild lesions may go undiscovered. Changes in renal function or irreversible renal functional impairment are other possible diagnostic tools. Nevertheless, renal function is often followed through serum creatinine levels, a method with notorious little sensitivity. Even more accurate glomerular filtration rate measurements by creatinine, radioisotope or inulin clearance can overestimate real renal function due to the kidney great functional capacity reserve. Urinary enzymes like alanine aminopeptidase or N-acetyl B-D-glucosaminidase own high sensitivity but lack specificity. There is an absolute necessity for an accurate and non-invasive process for early detection of chronic nephrotoxicity, in order to prevent or minimize CsA effects on renal tissue. Recently, some potential diagnostic markers appeared. Urinary collagen III, and to a lesser extent serum collagen III were shown to be good markers for renal fibrosis amount in biopsies from 40 patients with a number of subacute and chronic nephropathies [502]. Haas et al tested the presence of smooth muscle-specific isoform of α-actin in the urine of renal transplant patients submitted to renal biopsy. Patients with renal biopsy showing CsA or tacrolimus nephrotoxicity had increased smooth muscle-specific isoform of a-actin at urine samples when compared to cases without toxicity or healthy controls. Smooth muscle-specific isoform of α-actin correlated well with arteriolopathy severity at biopsy. However, there was an expressive overlapping of values among the three groups, making smooth muscle-specific isoform of α-actin an unreliable marker for clinical diagnosis of chronic CsA nephrotoxicity [503]. Recently, Câmara et al used the rational that CsA-induced interstitial fibrosis would induce subtle proximal tubule dysfunction to test the role of urinary retinol binding protein as an early marker for development of chronic CsA nephropathy. Retinol-binding protein is a small protein filtered by the glomeruli and

almost totally reabsorbed by the proximal tubule, and so increases in urinary concentration point to proximal tubule dysfunction. The authors studied 36 clinically stable CsA-treated heart transplant recipients and identified two subsets of patients, one with high (13 patients) and other with normal (23 patients) urinary concentration of retinol binding protein. During a five years follow-up period 46% of the patients doubled serum creatinine and 38% of then developed end stage renal failure and required dialysis in the high urinary retinol binding protein concentration group against 13% doubling serum creatinine and none developing end stage renal failure in the normal urinary concentration group. These results indicate that urinary retinol binding protein might be a valuable predictor of chronic CsA nephrotoxicity [504].

Currently, preventive measures or treatment for chronic CsA nephrotoxicity are only speculative. A straightforward way to prevent CsA-induced renal fibrosis would be to reduce its dosage or completely withdraw the drug. Recently, some authors have argued about the real need to maintain long-term CsA therapy in transplant recipients, supported by the point that the most noteworthy benefit of CsA use is a remarkable decrease in early acute rejection rate, and so the drug might be decreased or discontinued after the initial months of immunosuppression phase, without compromising long term graft survival [242, 322]. As already discussed in the acute CsA nephrotoxicity section this strategy is still a matter of debate, with some passionate pros and contras. Mourad et al reported that in renal transplant recipients with histologically demonstrated chronic CsA nephropathy after long term therapy, conversion to a sparing or CsA-free immunosuppressive schedule resulted in significant renal function improvement. Unfortunately renal biopsies after CsA regime changes were done only in few patients, who demonstrated improvement of the structural injury [444]. Similarly, Dominguez et al switched 12 renal transplant recipients suffering from chronic CsA or tacrolimus nephrotoxicity to rapamycin therapy, observing a significant decrease in serum creatinine of all patients at 6 months [359].

Recently, a number of authors reported a beneficial effect of CsA dose reduction or discontinuation in conjunction with maintenance or introduction of mycophenolate mofetil in patients with histologically proved or clinically suspected chronic CsA nephropathy. Improvements in renal function were detected in all studies and one reported decrease in serum TGF-β after mofetil mycophenolate introduction. None of the studies performed sequential or follow-up biopsies after immunosuppressive schedule changes [344, 447, 505-508]. The crescent number of papers showing that mofetil mycophenolate arrests renal injury in experimental models of progressive nephropathy [509-511] raises the question if the beneficial effect of mofetil mycophenolate in the renal function of CsA-treated patients is just the result of CsA dose change/withdrawal or a specific consequence of mofetil mycophenolate therapy. In our laboratory, we just finished a study showing the lack of effects of mofetil mycophenolate on the prevention of chronic CsA nephrotoxicity in the salt-depleted rat model, suggesting that the renal function improvement seen in the clinical studies was probably due to CsA reduction or discontinuation [512].

There are few results available about pharmacological management of chronic CsA nephrotoxicity in the clinical field. McCulloch et al studied the effects of nifedipine in CsA-induced interstitial fibrosis in renal transplantation. The authors compared three groups of patients (conventional CsA dose versus conventional CsA dose plus nifedipine versus low CsA dose plus azathioprine) measuring cortical interstitial volume fraction at baseline time and after one, six and 12 months of therapy. After six and 12 months interstitial volume was lower in patients treated with CsA plus nifedipine as compared with the two other groups, but the results only reached statistical significance at 6 months. Glomerular filtration rate was significantly lower in CsA-treated patients as compared to CsA plus nifedipine and CsA plus azathioprine. There was a negative correlation between glomerular filtration rate and interstitial volume fraction [513]. Use of losartan in renal transplant recipients with chronic allograft nephropathy decreased systemic TGF-β levels, indirectly suggesting a possible reduction in the renal fibrogenic process [514].

TACROLIMUS NEPHROTOXICITY

The nephrotoxicity profile of tacrolimus is very similar to that of CsA. Tacrolimus induces acute and reversible functional changes in renal function, chronic renal irreversible structural injury, electrolyte disturbances, renal tubular acidosis and hemolytic-uremic syndrome. There are some few and important differences: tacrolimus induces less hypertension but more glucose metabolism impairment than CsA [242, 515-521]. Also resembling CsA, tacrolimus association with drugs that interfere with the cytochrome P-450 metabolism or with other nephrotoxic drugs, can precipitate acute renal dysfunction [522-526] .

Acute nephrotoxicity

Tacrolimus acute nephrotoxicity can manifest itself as clinically significant acute renal failure, asymptomatic changes in glomerular filtration rate [254, 527-533] or hemolytic-uremic syndrome [534-538]. Even being a cause of hemolytic-uremic syndrome, tacrolimus has been advocated as an alternative treatment for patients with CsA-induced hemolytic-uremic syndrome [539, 540]. However, cases of patients with CsA-induced hemolytic-uremic syndrome that recurred after conversion from CsA to tacrolimus have been reported, indicating that this approach is not completely safe [541].

Tacrolimus causes acute reversible renal dysfunction with the same frequency and intensity as cyclosporine A in renal [527-529, 533], liver [254, 530-532, 542, 543], heart [544-546] and pulmonary [547, 548] transplant recipients. Tacrolimus-induced glomerular filtration rate decrease is associated with an important increase in renal vascular resistance and renal blood flow decrease that occur both in humans and rodents [61, 542, 549-554]. In a consistent way with this pattern of renal hemodynamic changes, the use of calcium channel blockers improved renal function in tacrolimus-treated liver transplant recipient [555] and experimental models of tacrolimus nephrotoxicity [551, 556-558]. Like CsA, tacrolimus acute nephrotoxicity is associated with normal renal histology or with nonspecific changes like isometric cytoplasm vacuolation in tubular epithelial cells, microcalcification, giant mitochondria and giant lysosomes. These changes revert with drug reduction or discontinuation [559].

Tacrolimus dose and exposure did not show correlation, but tacrolimus trough levels have a strong correlation with drug exposure and high trough levels have been associated to episodes of nephrotoxicity [281, 528, 560]. Tacrolimus trough levels < 5 ng/ml are associated with a 5% risk of nephrotoxicity but a 50% risk of rejection. At trough levels of 25 ng/ml there was no rejection but the nephrotoxicity rate raised to 90%. So, a good compromise to obtain efficient immunosuppression with less nephrotoxicity seems to be keeping blood levels between 10 to 15 ng/ml [281].

Experimental studies suggest that tacrolimus may have distinct effects on renal vascular resistance when compared to CsA. Some authors did not find changes in RVR after high single IV doses of tacrolimus *in vivo* or when the drug was used in the isolated auto perfused rat kidney model [561, 562]. Hadad et al performed glomerular hemodynamics studies in rats after a single injection or 10 days of treatment with tacrolimus. The drug caused, both acutely and after 10 days, a significant decrease in single nephron glomerular filtration rate, glomerular plasma flow rate and glomerular ultrafiltration coefficient (Kf) and a significant increase in total arteriolar resistance. It is important to point out that tacrolimus rose in a similar extent afferent and efferent arteriolar resistance. When this drug was added to cultured mesangial cells it significantly reduced cell cross sectional area and increased the intracellular calcium concentration [550]. So, the main determinants for glomerular filtration rate and renal blood flow decrease after tacrolimus administration are likely a generalized increase in renal arterioles resistance and an important decrease in Kf.

Much of the mechanisms associated to CsA-induced acute nephrotoxicity were also evoked and studied in tacrolimus functional nephrotoxicity. Evidence favoring a role for RAS came from studies showing juxtaglomerular apparatus hyperplasia, increase of renin-containing juxtaglomerular apparatus and extent of immunostaining for renin along afferent arterioles, enhancement of tacrolimus nephrotoxicity by salt-depletion and increased plasma renin activity in tacrolimus-treated rats [549, 563-566]. Stillman et al showed that juxtaglomerular apparatus granularity did not correlate with systemic plasma renin activity levels, which suggests a local activation of the renin-angiotensin-aldosterone system [564]. Experimental administration of captopril did not prevent tacrolimus-induced fall in

glomerular filtration rate [567].

A possible role for prostaglandins in tacrolimus acute nephrotoxicity has been explored with conflicting results. Textor et al reported that tacrolimus decreased urinary 6-keto-PGF1α and thromboxane B_2 in liver transplant recipients [542]. In SHR rats, acute tacrolimus nephrotoxicity was associated with increased urinary thromboxane B_2 and decreased 6-keto-PGF1α [557]. Benigni et al did not find changes in the release of thromboxane B_2 and 6-keto-PGF1α by bovine endothelial cells even after 24-hour incubation with increasing concentrations of tacrolimus [561]. In contrast, McCauley et al showed a tacrolimus-induced decrease in thromboxane B_2 and increase in PGE_2 production by mesangial cells [568].

Results about tacrolimus effects on endothelin system and its association with acute nephrotoxicity are also inconsistent. Tacrolimus-induced increase in urinary endothelin was found in denervated isolated perfused rat kidney and in liver transplant recipients [61, 551]. Increased serum levels of endothelin were found in kidney and simultaneous kidney/pancreas transplant recipients suffering from tacrolimus-induced microangiopathy syndrome [534]. In another study, tacrolimus stimulated the secretion of endothelin-1 by cultured tubular cells and increased serum endothelin levels in treated rats [569]. Tacrolimus has been shown to enhance endothelin release by rats' mesangial cells and rabbits' proximal tubule cells [570, 571] but not by LLC-PK1 epithelial cells or cultured bovine endothelial cells [561, 572]. In human endothelial cells, tacrolimus only increased endothelin-1 secretion and endothelin-1 mRNA when extremely high doses were used (0.1 μmol) but not when clinically relevant doses (0.01 μmol) were added to the medium [573]. In a comparative study, tacrolimus showed a weaker effect than CsA on stimulation of prepro endothelin-1 mRNA [574]. Use of endothelin receptor antagonists for prevention of acute tacrolimus nephrotoxicity provided poor results. Although tacrolimus augmented endothelin-1 mRNA levels in SHR, use of an $endothelin_A$/$endothelin_B$ receptor antagonist did not reverse glomerular filtration rate fall and only partially attenuated RVR increase [575]. In the same way, use of an $endothelin_A$ receptor antagonist in a dose that has been previously shown as effective for blocking renal effects of exogenous endothelin infusion did not prevent glomerular filtration rate decrease in tacrolimus-treated rats [576]. Likewise, an $endothelin_A$ receptor antagonist only partially prevented tacrolimus-induced glomerular filtration rate decrease but did not alleviate perfusate flow rate fall and perfusion resistance increase in an isolated perfused rat kidney model [551]. Taken together these data have shown that, as opposed to what has been demonstrated for CsA, the real role of endothelin in acute tacrolimus nephrotoxicity remains to be elucidated.

Tacrolimus capacity to blockade nitric oxide pathway has been well demonstrated. Nitric oxide plays a major role in the pathogenesis of cerebral hypoxia-ischemia injury mediated by glutamate/N-methyl-D-aspartate (NDMA). This injury depends on intracellular calcium influx through NDMA receptor channels, which activate calcineurin with consequent dephosphorylation of constitutive nitric oxide synthase. Tacrolimus addition to cultured neuronal cells reduced NDMA-mediated toxicity, through the inhibition of calcineurin activation, inhibition of constitutive nitric oxide synthase dephosphorylation and consequent decrease in nitric oxide production [577]. In other studies, tacrolimus reduced nitric oxide activity and nitric oxide production in cultured mice macrophage and rat vascular smooth muscle cells. Interestingly, in these studies tacrolimus showed a weaker inhibitory effect than CsA, suggesting that suppression of nitric oxide synthase occurred through distinct mechanisms [94, 578, 579]. More than that, Dusting et al found that only CsA but not tacrolimus suppressed nitric oxide production when clinically relevant doses of these drugs were added to a murine macrophage cell line and rat vascular smooth muscle cells in culture, raising doubts if nitric oxide blockade is really implicated in tacrolimus nephrotoxicity [94]. On the other hand, Strestikova et al have recently published opposing results. These authors, using cultured rat peritoneal macrophages, also showed that tacrolimus and CsA blocked inducible nitric oxide synthase by different mechanisms: at the transcriptional level for tacrolimus and post-transcriptionally for CsA. However, in their studies the inhibitory effect elicited by tacrolimus was clearly more potent than that obtained with CsA [580]. The study of the interplay between tacrolimus and nitric oxide has provided some other disturbing evidences, that did not fit easily in the conception of tacrolimus-induced nitric oxide blockade as a mechanism of tacrolimus nephrotoxicity. For instance, tacrolimus has been shown to up regulate endothelial nitric oxide synthase mRNA

expression in cultured bovine aortic cells [178], tacrolimus-treated rats presented an increase in urinary nitric oxide excretion [581], nitric oxide enhanced tacrolimus-induced proximal tubular epithelial cells apoptosis [582], incubation with L-arginine caused a significant reduction in acetylcholine-induced sensitivity in arteries isolated from rats treated with tacrolimus for eight days [583] and CsA, but not tacrolimus inhibited mRNA expression of inducible nitric oxide synthase in murine macrophage cell [584]. Manipulation of the system has provided contradictory results. L-NAME administration to tacrolimus treated rats resulted in decreased nitric oxide urinary excretion and enhancement of renal dysfunction [581]. Administration of L-arginine simultaneously to renal arteries clamping in a rat model of ischemic acute renal failure in tacrolimus-treated animals induced partial protection on renal function and hemodynamics [585]. In contrast, administration of L-arginine in a rat model of tacrolimus acute nephrotoxicity was unable to prevent functional renal injury, although L-arginine-treated rats presented significantly higher amounts of urinary nitric oxide [586].

Other mediators have been related to tacrolimus acute nephrotoxicity like decreased serum fibrinolytic activity [587], sympathetic overactivity [149, 588], increased renal glutathione levels [589] and increased serotonin production [552].

Tacrolimus may induce tubular dysfunction reflecting as an increased excretion of urinary enzymes, decreased urinary concentrating ability, increased fractional excretion of magnesium in the presence of hypomagnesemia, hyperkalemia, hyperuricemia and tubular acidosis [12, 230, 515, 518, 564, 590, 591]. *In vitro* studies showed that tacrolimus inhibit Na/K – ATPase in rat microdissected cortical collecting duct and medullary thick ascending limb [592], and that high tacrolimus doses added to primary human proximal tubules cultures decreased cell proliferation after 72 hours of incubation [593]. In the same way, only elevated concentrations of tacrolimus had a direct cytotoxic effect on LLC-PK1 tubular cell line [569]. In accordance with these previous results, Cuvello Neto et al recently found that tacrolimus was toxic to oxygenated isolated proximal tubules only in high concentrations. The injury seems to be mediated by a transient increase in intracellular calcium and an increase in oxygen free radicals. On the other hand, FK 506 protected against hypoxia and reoxygenation injury, probably due to the inhibition of nitric oxide synthase activity, reducing nitric oxide and oxygen free radicals generation [594].

If taken together, those data about acute tacrolimus nephrotoxicity suggest that although the net effect of this drug on vascular reactivity, tubular cells and renal function is very similar to that caused by CsA, the mechanisms of tacrolimus-induced functional renal injury are not obligatory the same implicated in CsA-induced acute nephrotoxicity.

Chronic nephrotoxicity

Soon after the beginning of tacrolimus clinical use it became apparent that the new immunosuppressive drug induced chronic renal structural injury identical to that seen in CsA-treated patients. In a blinded analysis of renal biopsy specimens from renal transplant recipients randomized to receive either CsA or tacrolimus, Randhawa et al documented for the first time that chronic renal histological injury caused by the two drugs was indistinguishable qualitatively and quantitatively. In both groups was found a similar prevalence and severity of striped fibrosis, arteriolar hyalinosis and peritubular calcification [595]. Subsequently, several authors confirmed these initial findings, describing tacrolimus-related chronic structural changes absolutely similar to that caused by CsA [559, 596-598]. Moreover, Solez et al reported that CsA and tacrolimus caused a similar prevalence of chronic allograft nephropathy in 144 cadaveric kidney recipients (62% in tacrolimus and 72.3 % in CsA). The authors did not find any apparent histological difference between tacrolimus and CsA biopsies. A multivariate analysis disclosed nephrotoxicity and acute rejection as the most significant predictors for chronic allograft nephropathy [599]. Permanent functional impairment and terminal chronic renal failure have also been related in liver and heart transplant recipients treated with tacrolimus [530, 544].

Andoh et al developed an experimental model of tacrolimus-induced chronic nephrotoxicity through a salt-depletion maneuver in rats [549, 563]. A particular characteristic of this model is that renal functional changes and structural injury occur with tacrolimus blood levels equivalent to those found in tacrolimus treated patients, in a striking contrast with the CsA chronic nephrotoxicity model, where extremely high CsA blood levels are achieved. In this tacrolimus model there is an early and dose-dependent decrease in glom-

erular filtration rate and renal blood flow with a parallel RVR increase followed by a late development of renal interstitial fibrosis involving the inner strip and medullary rays, arteriolar hyalinosis, tubular atrophy and hypertrophy and medullary thick ascending limb size variance. Structural injury showed a statistically significant positive correlation with decreased renal function [549, 563, 564].

Using this salt depletion model Shihab et al explored some of the possible mechanisms of tacrolimus-induced chronic nephrotoxicity. These authors found that tacrolimus induced a progressive increase in renal vessels and tubulointerstitial expression of mRNA for TGF-β, matrix proteins (biglycan, tenascin, fibronectin, type I collagen) and the protease inhibitor plasminogen activator inhibitor 1. So, tacrolimus at the same time that induced a TGF-β-related increase in extracellular matrix proteins blocked its degradation There was an early and sustained increase in systemic plasma renin activity and renal tissue renin mRNA, suggesting a participation of this system in the genesis of interstitial fibrosis [600]. Another evidence pointing for a role of renin-angiotensin-aldosterone system in chronic tacrolimus nephrotoxicity came from the study of Stillman et al, which demonstrated increased juxtaglomerular apparatus granularity in salt-depleted rats given tacrolimus. Juxtaglomerular apparatus granularity did not correlate with systemic renin, suggesting local renin-angiotensin-aldosterone system activation, but strongly correlate with the degree of structural injury [564]. Andoh et al provided an additional support for renin-angiotensin-aldosterone system role showing that cotreatment with losartan partially prevented the development of interstitial fibrosis in salt-depleted tacrolimus-treated rats [601].

Another mechanism that may contribute to tacrolimus-induced renal fibrosis is increased IL-6 production through NF-kappaB activation on non lymphoid cells. Tacrolimus stimulate this inducible transcription factor, which enhances IL-6 production in fibroblasts and mesangial cells. IL-6 in turn triggers mesangial cell proliferation and extracellular matrix production [521, 602].

Recent clinical and experimental studies suggested that tacrolimus might have less renal pro-fibrogenic effects than CsA. Using a rat model of renal ischemia-reperfusion associated with pro fibrotic genes up-regulation, Jain et al found that tacrolimus-treated animals developed less proteinuria and lower serum creatinine than CsA-treated rats. The authors also found that tacrolimus-treated rats had decreased expression of TGF-β and tissue inhibitor of metalloproteinase 1 mRNA as compared to non treated rats submitted to ischemia-reperfusion. CsA did not change TGF-β or tissue inhibitor of metalloproteinase 1 mRNA, but increased collagen III expression and reduced matrix degrading proteins expression (MMP-2 and MMP-9), although those changes did not reach statistical significance [603]. One possible explanation for the discrepancy in TGF-β expression results between this and the previous study that showed enhancement of TGF-β is that Jain et al used 0.2 mg/kg of tacrolimus while Shihab et al used 1 mg/kg of the drug [600, 603]. Differences between the fibrogenic potential of CsA and tacrolimus were also generated by the analysis of fibrosis-associated genes in isolated glomeruli obtained from renal biopsies of tacrolimus and CsA-treated transplant recipients. Expression of collagen III and tissue inhibitor of metalloproteinase 1 were significantly higher in CsA-treated animals. Interestingly, mRNA expression for TGF-β was similar in both groups [604]. The improvement in renal function seen in some (not all) patients with biopsy proved chronic allograft nephropathy after switch from CsA to tacrolimus might be related to this putatively less pro-fibrogenic effect of tacrolimus [605].

In summary, tacrolimus produces clinical and experimental tubulointerstitial fibrosis indistinguishable from that seen with CsA. The pathogenic mechanisms for this structural injury are apparently similar to those described for CsA. If tacrolimus in fact has less fibrogenic renal effect than CsA remains to be conclusively determinate.

SIROLIMUS NEPHROTOXICITY

Rapamycin (sirolimus) is a recently introduced immunosuppressive drug which is isolated from the fungus, *Streptomyces hygroscopicus*. It is a macrocyclic lactone structurally similar to tacrolimus. Unlike tacrolimus and cyclosporine, sirolimus has little nephrotoxicity when administered without a calcineurin inhibitor to renal transplant recipients. Experimental studies have shown that sirolimus when compared in animal models to cyclosporine and tacrolimus produces no decrease in glomerular filtration rate [12]. Despite having little effect on glomerular filtration rate, animal studies have shown that similar to the calcineurin inhibitors, magnesium wasting is observed with sirolimus [12].

In studies prepared for drug approval by the regulatory agencies in the United States, sirolimus plus cyclosporine was compared with regimens containing cyclosporine alone. While rejection rates were markedly decreased with sirolimus added to cyclosporine, renal function at one year was worse with the combination of the two immunosuppressive drugs. These results are thought to be due to a drug interaction such that sirolimus raises cyclosporine blood levels. Somewhat at odds with this interpretation of these data are the studies of Lieberthal showing that rapamycin (sirolimus) impairs recovery from ischemic acute renal failure in the absence of cyclosporine. One hypothesis is that cyclosporine produces some degree of renal ischemia due to vasoconstriction and the rapamycin impairs recovery of those cells due to its antiproliferative action [606].

Sirolimus has been used to spare cyclosporine in the setting of cadaveric renal transplant since unlike the calcineurin inhibitors it is not vasoconstrictive and thus theoretically at least should be of benefit in ischemic reperfusion injury. The studies of Lieberthal mentioned above would lead one to a different strategy. Indeed there are now anecdotes appearing in the literature that similar to experimental animals, sirolimus potentiates ischemic injury following transplantation. There are few data with the combination of sirolimus and tacrolimus. However, limited information suggests that the pattern may be the same. Thus, it is clear that sirolimus used without a calcineurin inhibitor is safe from a nephrotoxic point of view but in combination with a calcineurin inhibitor there are either drug interactions or more fundamental cellular actions of sirolimus that may be adverse to renal tubular cells to impair recovery from ischemic insults such as hypotension and/or acute rejection episodes.

REFERENCES

1. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: a new immunosuppressive agent for organ transplantation. Ann Intern Med 1984; 101: 667-682.
2. Kahan BD. Cyclosporine. N Engl J Med 1989; 321: 1725-1738.
3. de Mattos AM, Olyaei AJ, Bennett WM. Pharmacology of immunosuppressive medications used in renal diseases and transplantation. Am J Kidney Dis 1996; 28: 631-667.
4. Langford CA, Klippel JH, Balow JE, James SP, Sneller MC. Use of cytotoxic agents and cyclosporine in the treatment of autoimmune disease. Part 1: rheumatologic and renal diseases. Ann Intern Med 1998; 128: 1021-1028.
5. Langford CA, Klippel JH, Balow JE, James SP, Sneller MC. Use of cytotoxic agents and cyclosporine in the treatment of autoimmune disease. Part 2: Inflammatory bowel disease, systemic vasculitis, and therapeutic toxicity. Ann Intern Med 1998; 129: 49-58.
6. Ponticelli C. Calcineurin-inhibitors in renal transplantation. Too precious to be abandoned. Nephrol Dial Transplant 2000; 15: 1307-1309.
7. Keown P, Niese D. Cyclosporine microemulsion increases drug exposure and reduces acute rejection without incremental toxicity in de novo renal transplantation. International Sandimmun Neoral Study Group. Kidney Int 1998; 54: 938-944.
8. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK 506 for liver, kidney, and pancreas transplantation. Lancet 1989; 2: 1000-1004.
9. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. The U.S. Multicenter FK 506 Liver Study Group. N Engl J Med 1994; 331: 1110-1115.
10. Spencer CM, Goa KL, Gillis JC. Tacrolimus. An update of its pharmacology and clinical efficacy in the management of organ transplantation. Drugs 1997; 54: 925-975.

11. Opelz G. Immunosuppression with FK 506 does not improve kidney graft survival. Collaborative Transplant Study. Transplant Proc 1999; 31: 1147-1148.
12. Andoh TF, Burdmann EA, Fransechini N, Houghton DC, Bennett WM. Comparison of acute rapamycin nephrotoxicity with cyclosporine and FK 506. Kidney Int 1996; 50: 1110-1117.
13. Campistol JM, Sacks SH. Mechanisms of nephrotoxicity. Transplantation 2000; 69: SS5-SS10.
14. Aramburu J, Yaffe MB, Lopez-Rodriguez C, Cantley LC, Hogan PG, Rao A. Affinity-driven peptide selection of an NFAT inhibitor more selective than cyclosporin A. Science 1999; 285: 2129-2133.
15. English J, Evan A, Houghton DC, Bennett WM. Cyclosporine-induced acute renal dysfunction in the rat. Evidence of arteriolar vasoconstriction with preservation of tubular function. Transplantation 1987; 44: 135-141.
16. Potier M, Wolf A, Cambar J. Comparative study of cyclosporin A, cyclosporin G, and the novel cyclosporin derivative IMM 125 in isolated glomeruli and cultured rat mesangial cells: a morphometric analysis. Nephrol Dial Transplant 1998; 13: 1406-1411.
17. Mihatsch MJ, Thiel G, Ryffel B. Histopathology of cyclosporine nephrotoxicity. Transplant Proc 1988; 20: 759-771.
18. Conger JD, Kim GE, Robinette JB. Effects of ANG II, ETA, and TxA2 receptor antagonists on cyclosporin A renal vasoconstriction. Am J Physiol 1994; 267: F443-F449.
19. L'Azou B, Medina J, Frieauff W, Cordier A, Cambar J, Wolf A. *In vitro* models to study mechanisms involved in cyclosporine A-mediated glomerular contraction. Arch Toxicol 1999; 73: 337-345.
20. Mason J, Muller-Schweinitzer E, Dupont M, Casellas D, Mihatsch M, Moore L, Kaskel F. Cyclosporine and the renin-angiotensin system. Kidney Int (Suppl) 1991; 32: S28-S32.
21. Lee DB. Cyclosporine and the renin-angiotensin axis. Kidney Int 1997; 52: 248-260.
22. Whiting PH, Cunningham C, Thomson AW, Simpson JG. Enhancement of high dose cyclosporin A toxicity by frusemide. Biochem Pharmacol 1984; 33: 1075-1079.
23. Swan SK, Bennett WM. Role of the sodium ion in acute renal failure. Miner Electrolyte Metab 1991; 17: 89-99.
24. Lustig S, Stern N, Eggena P, Tuck ML, Lee DB. Effect of cyclosporin on blood pressure and renin-aldosterone axis in rats. Am J Physiol 1987; 253: H1596-H1600.
25. Perico N, Zoja C, Benigni A, Ghilardi F, Gualandris L, Remuzzi G. Effect of short-term cyclosporine administration in rats on renin-angiotensin and thromboxane A2: possible relevance to the reduction in glomerular filtration rate. J Pharmacol Exp Ther 1986; 239: 229-235.
26. Shehata M, el Nahas M, Barkworth E, Cope GH, Raftery AT. Localization of PDGF-BB in the juxtaglomerular cells of cyclosporin-treated rats. Exp Nephrol 1995; 3: 173-179.
27. Verpooten GA, Wybo I, Pattyn VM, Hendrix PG, Giuliano RA, Nouwen EJ, Roels F, De Broe ME. Cyclosporine nephrotoxicity: comparative cytochemical study of rat kidney and human allograft biopsies. Clin Nephrol 1986; 25 (Suppl 1): S18-S22.
28. Tufro-McReddie A, Gomez RA, Norling LL, Omar AA, Moore LC, Kaskel FJ. Effect of CsA on the expression of renin and angiotensin type 1 receptor genes in the rat kidney. Kidney Int 1993; 43: 615-622.
29. Regitz-Zagrosek V, Auch-Schwelk W, Hess B, Klein U, Duske E, Steffen C, Hildebrandt AG, Fleck E. Tissue- and subtype-specific modulation of angiotensin II receptors by chronic treatment with cyclosporin A, angiotensin-converting enzyme inhibitors and AT1 antagonists. J Cardiovasc Pharmacol 1995; 26: 66-72.
30. Avdonin PV, Cottet-Maire F, Afanasjeva GV, Loktionova SA, Lhote P, Ruegg UT. Cyclosporine A up-regulates angiotensin II receptors and calcium responses in human vascular smooth muscle cells. Kidney Int 1999; 55: 2407-2414.
31. Baxter CR, Duggin GG, Hall BM, Horvath JS, Tiller DJ. Stimulation of renin release from rat renal cortical slices by cyclosporin A. Res Commun Chem Pathol Pharmacol 1984; 43: 417-423.
32. Kurtz A, Della BR, Kuhn K. Cyclosporine A enhances renin secretion and production in isolated juxtaglomerular cells. Kidney Int 1988; 33: 947-953.
33. Hestin D, Mertes PM, Hubert J, Claudon M, Mejat E, Renoult E, Pertek JP, Frimat L, Burlet C, Kessler M. Relationship between blood pressure and renin, angiotensin II and atrial natriuretic factor after renal transplantation. Clin Nephrol 1997; 48: 98-103.
34. Julien J, Farge D, Kreft-Jais C, Guyene TT, Plouin PF, Houssin D, Carpentier A, Corvol P. Cyclosporine-induced stimulation of the renin-angiotensin system after liver and heart transplantation. Transplantation 1993; 56: 885-891.
35. Gardiner DS, Watson MA, Junor BJ, Briggs JD, More IA, Lindop GB. The effect of conversion from cyclosporin to azathioprine on renin- containing cells in renal allograft biopsies. Nephrol Dial Transplant 1991; 6: 363-367.
36. Abraham JS, Bentley FR, Garrison RN. The role of intrarenal prostaglandins and angiotensin II in acute cyclosporine-induced vasoconstriction. Surgery 1991; 110: 343-349.
37. Barros EJ, Boim MA, Ajzen H, Ramos OL, Schor N. Glomerular hemodynamics and hormonal participation on cyclosporine nephrotoxicity. Kidney Int 1987; 32: 19-25.
38. Prevot A, Semama DS, Tendron A, Justrabo E, Guignard JP, Gouyon JB. Endothelin, angiotensin II and adenosine in acute cyclosporine A nephrotoxicity. Pediatr Nephrol 2000; 14: 927-934.

39. Gerkens JF, Smith AJ. Effect of captopril and theophylline treatment on cyclosporine-induced nephrotoxicity in rats. Transplantation 1985; 40: 213-214.
40. Gillum DM, Truong L. Effects of chronic volume expansion and enalapril on chronic cyclosporine nephropathy. Am J Physiol 1990; 258: F934-F939.
41. Grieve EM, Hawksworth GM, Simpson JG, Whiting PH. Effect of thromboxane synthetase inhibition and angiotensin converting enzyme inhibition on acute cyclosporin A nephrotoxicity. Biochem Pharmacol 1990; 40: 2323-2329.
42. Kaskel FJ, Devarajan P, Arbeit LA, Partin JS, Moore LC. Cyclosporine nephrotoxicity: sodium excretion, autoregulation, and angiotensin II. Am J Physiol 1987; 252: F733-F742.
43. Bantle JP, Paller MS, Boudreau RJ, Olivari MT, Ferris TF. Long-term effects of cyclosporine on renal function in organ transplant recipients. J Lab Clin Med 1990; 115: 233-240.
44. Inigo P, Campistol JM, Lario S, Piera C, Campos B, Bescos M, Oppenheimer F, Rivera F. Effects of losartan and amlodipine on intrarenal hemodynamics and TGF- β(1) plasma levels in a crossover trial in renal transplant recipients. J Am Soc Nephrol 2001; 12: 822-827.
45. Rondeau E, Paillard F, Peraldi MN, Violet I, Tasse S, Dussaule JC, Ardaillou R, Sraer JD. Role of the renin-angiotensin system on the renal functional reserve in renal transplant recipients. Kidney Int 1993; 44: 165-172.
46. O'Brien RF, Robbins RJ, McMurtry IF. Endothelial cells in culture produce a vasoconstrictor substance. J Cell Physiol 1987; 132: 263-270.
47. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332: 411-415.
48. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA 1989; 86: 2863-2867.
49. Kohan DE. Endothelins in the normal and diseased kidney. Am J Kidney Dis 1997; 29: 2-26.
50. Watschinger B, Sayegh MH. Endothelin in organ transplantation. Am J Kidney Dis 1996; 27: 151-161.
51. Nakahama H. Stimulatory effect of cyclosporine A on endothelin secretion by a cultured renal epithelial cell line, LLC-PK1 cells. Eur J Pharmacol 1990; 180: 191-192.
52. Nambi P, Pullen M, Contino LC, Brooks DP. Upregulation of renal endothelin receptors in rats with cyclosporine A- induced nephrotoxicity. Eur J Pharmacol 1990; 187: 113-116.
53. Kon V, Sugiura M, Inagami T, Harvie BR, Ichikawa I, Hoover RL. Role of endothelin in cyclosporine-induced glomerular dysfunction. Kidney Int 1990; 37: 1487-1491.
54. Perico N, Dadan J, Remuzzi G. Endothelin mediates the renal vasoconstriction induced by cyclosporine in the rat. J Am Soc Nephrol 1990; 1: 76-83.
55. Takeda M, Breyer MD, Noland TD, Homma T, Hoover RL, Inagami T, Kon V. Endothelin-1 receptor antagonist: effects on endothelin- and cyclosporine-treated mesangial cells. Kidney Int 1992; 41: 1713-1719.
56. Abassi ZA, Pieruzzi F, Nakhoul F, Keiser HR. Effects of cyclosporin A on the synthesis, excretion, and metabolism of endothelin in the rat. Hypertension 1996; 27: 1140-1148.
57. Grekas D, Bamichas G, Karamouzis M, Bacharaki D, Savidis N, Tourkantonis A. Plasma endothelin in cyclosporine A-treated renal transplant patients. Transplant Proc 1999; 31: 1703-1705.
58. Grieff M, Loertscher R, Shohaib SA, Stewart DJ. Cyclosporine-induced elevation in circulating endothelin-1 in patients with solid-organ transplants. Transplantation 1993; 56: 880-884.
59. Kon V, Awazu M. Endothelin and cyclosporine nephrotoxicity. Ren Fail 1992; 14: 345-350.
60. Perico N, Ruggenenti P, Gaspari F, Mosconi L, Benigni A, Amuchastegui CS, Gasparini F, Remuzzi G. Daily renal hypoperfusion induced by cyclosporine in patients with renal transplantation. Transplantation 1992; 54: 56-60.
61. Textor SC, Burnett JC, Jr., Romero JC, Canzanello VJ, Taler SJ, Wiesner R, Porayko M, Krom R, Gores G, Hay E. Urinary endothelin and renal vasoconstriction with cyclosporine or FK 506 after liver transplantation. Kidney Int 1995; 47: 1426-1433.
62. Gonzalez-Santiago L, Lopez-Ongil S, Lamas S, Quereda C, Rodriguez-Puyol M, Rodriguez-Puyol D. Imbalance in endothelial vasoactive factors as a possible cause of cyclosporin toxicity: a role for endothelin-converting enzyme. J Lab Clin Med 2000; 136: 395-401.
63. Takeda Y, Miyamori I, Wu P, Yoneda T, Furukawa K, Takeda R. Effects of an endothelin receptor antagonist in rats with cyclosporine- induced hypertension. Hypertension 1995; 26: 932-936.
64. Nakayama Y, Nonoguchi H, Kiyama S, Ikebe M, Tashima Y, Shimada K, Tanzawa K, Tomita K. Intranephron distribution and regulation of endothelin-converting enzyme-1 in cyclosporin A-induced acute renal failure in rats. J Am Soc Nephrol 1999; 10: 562-571.
65. Marsen TA, Weber F, Egink G, Suckau G, Baldamus CA. Cyclosporin A induces prepro endothelin-1 gene transcription in human endothelial cells. Eur J Pharmacol 1999; 379: 97-106.
66. Fogo A, Hellings SE, Inagami T, Kon V. Endothelin receptor antagonism is protective in *in vivo* acute cyclosporine toxicity. Kidney Int 1992; 42: 770-774.

67. Cavarape A, Endlich K, Feletto F, Parekh N, Bartoli E, Steinhausen M. Contribution of endothelin receptors in renal microvessels in acute cyclosporine-mediated vasoconstriction in rats. Kidney Int 1998; 53: 963-969.
68. Lanese DM, Conger JD. Effects of endothelin receptor antagonist on cyclosporine-induced vasoconstriction in isolated rat renal arterioles. J Clin Invest 1993; 91: 2144-2149.
69. Meyer-Lehnert H, Bokemeyer D, Friedrichs U, Backer A, Kramer HJ. Cellular mechanisms of cyclosporine A-associated side-effects: role of endothelin. Kidney Int (Suppl) 1997; 61: S27-S31.
70. Davis LS, Haleen SJ, Doherty AM, Cody WL, Keiser JA. Effects of selective endothelin antagonists on the hemodynamic response to cyclosporin A. J Am Soc Nephrol 1994; 4: 1448-1454.
71. Binet I, Wallnofer A, Weber C, Jones R, Thiel G. Renal hemodynamics and pharmacokinetics of bosentan with and without cyclosporine A. Kidney Int 2000; 57: 224-231.
72. Storogenko M, Pech-Amsellem MA, Kerdine S, Rousselet F, Pallardy M. Cyclosporin-A inhibits human endothelial cells proliferation through interleukin-6-dependent mechanisms. Life Sci 1997; 60: 1487-1496.
73. Zoja C, Furci L, Ghilardi F, Zilio P, Benigni A, Remuzzi G. Cyclosporin-induced endothelial cell injury. Lab Invest 1986; 55: 455-462.
74. Averna M, Barbagallo CM, Ganci A, Giammarresi C, Cefalu AB, Sparacino V, Caputo F, Basili S, Notarbartolo A, Davi G. Determinants of enhanced thromboxane biosynthesis in renal transplantation. Kidney Int 2001; 59: 1574-1579.
75. Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M. The coagulo-lytic system and endothelial function in cyclosporine- treated kidney allograft recipients. Transplantation 1996; 62: 828-830.
76. Schrama YC, van Dam T, Fijnheer R, Hene RJ, de Groot P, Rabelink TJ. Cyclosporine is associated with endothelial dysfunction but not with platelet activation in renal transplantation. Neth J Med 2001; 59: 6-15.
77. Tiemann C, Prohaska W, Korfer R, Korner M, Brinkmann T, Kleesiek K. Effect of cyclosporine A on the release of tissue factor pathway inhibitor from endothelial cells in heart transplant patients and cell culture. Eur J Clin Chem Clin Biochem 1997; 35: 661-667.
78. Evans SM, Giddings JC, Muraki T, Yamamoto J. Expression of von Willebrand factor, P-selectin (CD62P) and thrombomodulin in human endothelial cells in culture modulated by cyclosporin A. Clin Lab Haematol 1997; 19: 115-122.
79. Bobadilla NA, Gamba G, Tapia E, Garcia-Torres R, Bolio A, Lopez-Zetina P, Herrera-Acosta J. Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression. Am J Physiol 1998; 274: F791-F798.
80. Kone BC, Baylis C. Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. Am J Physiol 1997; 272: F561-F578.
81. Bracht C, Yan XW, LaRocca HP, Sutsch G, Kiowski W. Cyclosporine A and control of vascular tone in the human forearm: influence of post-transplant hypertension. J Hypertens 1999; 17: 357-363.
82. Richards NT, Poston L, Hilton PJ. Cyclosporine A inhibits relaxation but does not induce vasoconstriction in human subcutaneous resistance vessels. J Hypertens 1989; 7: 1-3.
83. Diederich D, Yang Z, Luscher TF. Chronic cyclosporine therapy impairs endothelium-dependent relaxation in the renal artery of the rat. J Am Soc Nephrol 1992; 2: 1291-1297.
84. Gallego MJ, Lopez FA, Riesco A, Monton M, Grandes SM, Barat A, Hernando L, Casado S, Caramelo CA. Blockade of endothelium-dependent responses in conscious rats by cyclosporin A: effect of L-arginine. Am J Physiol 1993; 264: H708-H714.
85. Gallego MJ, Garcia Villalon AL, Lopez Farre AJ, Garcia JL, Garron MP, Casado S, Hernando L, Caramelo CA. Mechanisms of the endothelial toxicity of cyclosporin A. Role of nitric oxide, cGMP, and Ca2+. Circ Res 1994; 74: 477-484.
86. Lee J, Kim SW, Kook H, Kang DG, Kim NH, Choi KC. Effects of L-arginine on cyclosporin-induced alterations of vascular NO/cGMP generation. Nephrol Dial Transplant 1999; 14: 2634-2638.
87. Mikkelsen EO, Poulsen SH, Nyborg NC, Korsgaard N, Sehested J. Difference between aortic and renal vascular reactivity in cyclosporin A treated rats and the effect of cicletanine. Naunyn Schmiedebergs Arch Pharmacol 1992; 345: 356-361.
88. Rego A, Vargas R, Wroblewska B, Foegh ML, Ramwell PW. Attenuation of vascular relaxation and cyclic GMP responses by cyclosporin A. J Pharmacol Exp Ther 1990; 252: 165-170.
89. Roullet JB, Xue H, McCarron DA, Holcomb S, Bennett WM. Vascular mechanisms of cyclosporin-induced hypertension in the rat. J Clin Invest 1994; 93: 2244-2250.
90. Takenaka T, Hashimoto Y, Epstein M. Diminished acetylcholine-induced vasodilation in renal microvessels of cyclosporine-treated rats. J Am Soc Nephrol 1992; 3: 42-50.
91. Assis SM, Monteiro JL, Seguro AC. L-Arginine and allopurinol protect against cyclosporine nephrotoxicity. Transplantation 1997; 63: 1070-1073.
92. Bobadilla NA, Tapia E, Franco M, Lopez P, Mendoza S, Garcia-Torres R, Alvarado JA, Herrera-Acosta J. Role of nitric oxide in renal hemodynamic abnormalities of cyclosporin nephrotoxicity. Kidney Int 1994; 46: 773-779.
93. Vaziri ND, Ni Z, Zhang YP, Ruzics EP, Maleki P, Ding Y. Depressed renal and vascular nitric oxide synthase expression in cyclosporine-induced hypertension. Kidney Int 1998; 54: 482-491.
94. Dusting GJ, Akita K, Hickey H, Smith M, Gurevich V. Cyclosporin A and tacrolimus (FK 506) suppress expression of inducible nitric oxide synthase *in vitro* by different mechanisms. Br J Pharmacol 1999; 128: 337-344.

95. Stroes ES, Luscher TF, de Groot FG, Koomans HA, Rabelink TJ. Cyclosporin A increases nitric oxide activity *in vivo*. Hypertension 1997; 29: 570-575.
96. Morris ST, McMurray JJ, Rodger RS, Farmer R, Jardine AG. Endothelial dysfunction in renal transplant recipients maintained on cyclosporine. Kidney Int 2000; 57: 1100-1106.
97. Gossmann J, Radounikli A, Bernemann A, Schellinski O, Raab HP, Bickeboller R, Scheuermann EH. Pathophysiology of cyclosporine-induced nephrotoxicity in humans: a role for nitric oxide? Kidney Blood Press Res 2001; 24: 111-115.
98. Navarro-Antolin J, Rey-Campos J, Lamas S. Transcriptional induction of endothelial nitric oxide gene by cyclosporine A. J Biol Chem 2000; 275: 3075-3080.
99. Sanchez-Lozada LG, Gamba G, Bolio A, Jimenez F, Herrera-Acosta J, Bobadilla NA. Nifedipine prevents changes in nitric oxide synthase mRNA levels induced by cyclosporine. Hypertension 2000; 36: 642-647.
100. Andoh TF, Gardner MP, Bennett WM. Protective effects of dietary L-arginine supplementation on chronic cyclosporine nephrotoxicity. Transplantation 1997; 64: 1236-1240.
101. Bloom IT, Bentley FR, Spain DA, Garrison RN. An experimental study of altered nitric oxide metabolism as a mechanism of cyclosporin-induced renal vasoconstriction. Br J Surg 1995; 82: 195-198.
102. de Nicola L, Thomsom SC, Wead LM, Brown MR, Gabbai FB. Arginine feeding modifies cyclosporine nephrotoxicity in rats. J Clin Invest 1993; 92: 1859-1865.
103. Gardner MP, Houghton DC, Andoh TF, Lindsley J, Bennett WM. Clinically relevant doses and blood levels produce experimental cyclosporine nephrotoxicity when combined with nitric oxide inhibition. Transplantation 1996; 61: 1506-1512.
104. Lassila M, Finckenberg P, Pere AK, Vapaatalo H, Nurminen ML. Enalapril and valsartan improve cyclosporine A-induced vascular dysfunction in spontaneously hypertensive rats. Eur J Pharmacol 2000; 398: 99-106.
105. Åsberg A, Karsten M, Vassbotn, Hartmann A. Better microvascular function on long-term treatment with lisinopril than nifedipine in renal transplant recipients. Nephrol Dial Transplant 2001; 16: 1465-1470.
106. Gaston RS, Schlessinger SD, Sanders PW, Barker CV, Curtis JJ, Warnock DG. Cyclosporine inhibits the renal response to L-arginine in human kidney transplant recipients. J Am Soc Nephrol 1995; 5: 1426-1433.
107. Koller-Strametz J, Wolzt M, Fuchs C, Putz D, Wisser W, Mensik C, Eichler H, Laufer G, Schmetterer L. Renal hemodynamic effects of L-arginine and sodium nitroprusside in heart transplant recipients. Kidney Int 1999; 55: 1871-1877.
108. Viganò E, Badalamenti G, Paone G, Como G, Finazzi S, Tarantino A, Galmarini D, Ponticelli C. Renal effects of L-arginine infusion in cyclosporine-treated transplant recipients. Transplant Proc 1994; 26: 2622-2623.
109. Andrés A, Morales JM, Praga M, Campo V, Lahera R, García-Robles R, Rodicio JL, Ruilope LM. L-arginine reverses the antinatriuretic effect of cyclosporin in renal transplant patients. Nephrol Dial Transplant 1997; 12: 1437-1440.
110. Bennett WM. Therapeutic implications of arachidonic acid metabolism in transplant-associated acute renal failure. Ren Fail 1992; 14: 261-265.
111. Benigni A, Chiabrando C, Piccinelli A, Perico N, Gavinelli M, Furci L, Patino O, Abbate M, Bertani T, Remuzzi G. Increased urinary excretion of thromboxane B_2 and 2, 3-dinor-TxB_2 in cyclosporin A nephrotoxicity. Kidney Int 1988; 34: 164-174.
112. Coffman TM, Carr DR, Yarger WE, Klotman PE. Evidence that renal prostaglandin and thromboxane production is stimulated in chronic cyclosporine nephrotoxicity. Transplantation 1987; 43: 282-285.
113. Darlametsos IE, Papanikolaou EN, Varonos DD. Effect of nifedipine in cyclosporine-induced nephrotoxicity in rats: roles of the thromboxane and endothelin systems. Prostaglandins Leukot Essent Fatty Acids 2000; 63: 263-269.
114. Grieve EM, Hawksworth GM, Simpson JG, Whiting PH. The reversal of experimental cyclosporin A nephrotoxicity by thromboxane synthetase inhibition. Biochem Pharmacol 1993; 45: 1351-1354.
115. Kawaguchi A, Goldman MH, Shapiro R, Foegh ML, Ramwell PW, Lower R.D. Increase in urinary thromboxane B_2 in rats caused by cyclosporine. Transplantation 1985; 40: 214-216.
116. Kim Y.J., Park Y.H., Moon H.K. Reduction of chronic ciclosporin nephrotoxicity by thromboxane synthase inhibition with OKY-046. Kidney Blood Press Res 1997; 20: 38-43.
117. Perico N, Benigni A, Zoja C, Delaini F, Remuzzi G. Functional significance of exaggerated renal thromboxane A2 synthesis induced by cyclosporin A. Am J Physiol 1986; 251: F581-F587.
118. Spurney RF, Mayros S.D., Collins D, Ruiz P, Klotman P.E., Coffman T. Thromboxane receptor blockade improves cyclosporine nephrotoxicity in rats. Prostaglandins 1990; 39: 135-146.
119. Parra T, de Arriba G, Conejo JR, Cantero M, Arribas I, Rodriguez-Puyol D, Rodriguez-Puyol M, Carballo F. Cyclosporine increases local glomerular synthesis of reactive oxygen species in rats: effect of vitamin E on cyclosporine nephrotoxicity. Transplantation 1998; 66: 1325-1329.
120. Elzinga L, Kelley VE, Houghton DC, Bennett WM. Modification of experimental nephrotoxicity with fish oil as the vehicle for cyclosporine. Transplantation 1987; 43: 271-274.
121. Smeesters C, Chaland P, Giroux L, Moutquin JM, Etienne P, Douglas F, Corman J, St Louis G, Daloze P. Prevention of acute cyclosporine A nephrotoxicity by a thromboxane synthetase inhibitor. Transplant Proc 1988; 20: 658-664.

122. Torras J, Valles J, Sanchez J, Sabate I, Seron D, Carrera M, Castelao AM, Herrero I, Puig-Parellada P, Alsina J, Grino JM. Prevention of experimental cyclosporine nephrotoxicity by dietary supplementation with LSL 90202, a lysine salt of eicosapentaenoic acid. Role of thromboxane and prostacyclin in renal tissue. Nephron 1994; 67: 66-72.
123. Rogers TS, Elzinga L, Bennett WM, Kelley VE. Selective enhancement of thromboxane in macrophages and kidneys in cyclosporine-induced nephrotoxicity. Dietary protection by fish oil. Transplantation 1988; 45: 153-156.
124. Smith SR, Kubacki VB, Rakhit A, Martin LL, Schaffer AV, Jasani MK, Hefty DJ, Johnston T, Cannon C, Bennett WM, Coffman TM. Chronic thromboxane synthase inhibition with CGS 12970 in human cyclosporine nephrotoxicity. Transplantation 1993; 56: 1422-1426.
125. Badalamenti S, Salerno F, Lorenzano E, Paone G, Como G, Finazzi S, Sacchetta AC, Rimola A, Graziani G, Galmarini D, Ponticelli C. Renal effects of dietary supplementation with fish oil in cyclosporine- treated liver transplant recipients. Hepatology 1995; 22: 1695-71.
126. Hansen JM, Johansen NJ, Mollerup HM, Fogh-Andersen N, Strandgaard S. Effects of nitric oxide blockade and cyclosporin A on cardiovascular and renal function in normal man. J Hypertens 1999; 17: 1707-1713.
127. Gladue RP, Newborg MF. The protective effects of the thromboxane synthetase inhibitor Dazmegrel on nephrotoxicity in cyclosporine-treated rats. Transplantation 1991; 52: 837-841.
128. Perico N, Pasini M, Gaspari F, Abbate M, Remuzzi G. Co-participation of thromboxane A2 and leukotriene C4 and D4 in mediating cyclosporine-induced acute renal failure. Transplantation 1991; 52: 873-878.
129. Chouinard ML, Martin LL, Coffman T, Hamilton BH, Linberg LF, Pamidi A, Simke JP, Rakhit A. Pharmacokinetics and biochemical efficacy of pirmagrel, a thromboxane synthase inhibitor, in renal allograft recipients. Clin Pharmacol Ther 1992; 52: 597-604.
130. Weir MR, Klassen DK, Burdick JF. A pilot study to assess the ability of an orally available selective thromboxane synthase inhibitor to improve renal function in cyclosporine-treated renal transplant recipients. J Am Soc Nephrol 1992; 2: 1285-1290.
131. Berthoux FC, Guerin C, Burgard G, Berthoux P, Alamartine E. One-year randomized controlled trial with omega-3 fatty acid-fish oil in clinical renal transplantation. Transplant Proc 1992; 24: 2578-2582.
132. Hansen JM, Hoy CE, Strandgaard S. Fish oil and cyclosporin A-induced renal hypoperfusion in kidney- transplanted patients. Nephrol Dial Transplant 1995; 10: 1745-1750.
133. Homan van der Heide JJ, Bilo HJ, Donker AJ, Wilmink JM, Sluiter WJ, Tegzess AM. The effects of dietary supplementation with fish oil on renal function and the course of early postoperative rejection episodes in cyclosporine-treated renal transplant recipients. Transplantation 1992; 54: 257-263.
134. Butterly DW, Spurney RF, Ruiz P, Griffiths R, Albrightson C, Coffman TM. A role for leukotrienes in cyclosporine nephrotoxicity. Kidney Int 2000; 57: 2586-2593.
135. Bunke M, Wilder L, Martin A. The effect of cyclosporine on agonist-stimulated glomerular and mesangial cell vasodilatory prostaglandin production. Transplantation 1991; 52: 718-722.
136. Copeland KR, Yatscoff RW. Comparison of the effects of cyclosporine and its metabolites on the release of prostacyclin and endothelin from mesangial cells. Transplantation 1992; 53: 640-645.
137. Dumble LJ, Gibbons S, Tejpal N, Chou TC, Redgrave NG, Boyle MJ, Kahan BD. 15 AU81, a prostacyclin analog, potentiates immunosuppression and mitigates renal injury due to cyclosporine. Transplantation 1993; 55: 1124-1128.
138. Scholey JW, Mills DE. Dietary fatty acids and the glomerular hemodynamic response to cyclosporine in borderline hypertensive rats. Kidney Int 1995; 47: 611-617.
139. Moran M, Mozes MF, Maddux MS, Veremis S, Bartkus C, Ketel B, Pollak R, Wallemark C, Jonasson O. Prevention of acute graft rejection by the prostaglandin E1 analogue misoprostol in renal-transplant recipients treated with cyclosporine and prednisone. N Engl J Med 1990; 322: 1183-1188.
140. Le Guen E, Morel D, L'Azou B, Cambar J, Potaux L. Effect of cyclosporin and the prostacyclin analogue iloprost on human glomerular vasoreactivity. Nephrol Dial Transplant 1994; 9: 413-415.
141. Adams MB. Enisoprost in renal transplantation. The Enisoprost Renal Transplant Study Group. Transplantation 1992; 53: 338-345.
142. Manasia AR, Leibowitz AB, Miller CM, Silverstein JH, Schwartz M, Delgiudice R, Vallabhajosula S, Oropello JM, Benjamin E. Postoperative intravenous infusion of alprostadil (PGE1) does not improve renal function in hepatic transplant recipients. J Am Coll Surg 1996; 182: 347-352.
143. Pouteil-Noble C, Chapuis F, Berra N, Hadj-Aissa A, Lacavalerie B, Lefrancois N, Martin X, Touraine JL. Misoprostol in renal transplant recipients: a prospective, randomized, controlled study on the prevention of acute rejection episodes and cyclosporin A nephrotoxicity. Nephrol Dial Transplant 1994; 9: 552-555.
144. Ray JG. Prostaglandin E1 analogs do not improve renal function among either transplant or nontransplant patients: no further trials required. Transplantation 1998; 66: 476-483.

145. Weinblatt ME, Germain BF, Kremer JM, Wall BA, Weisman MH, Maier AL, Coblyn JS. Lack of a renal-protective effect of misoprostol in rheumatoid arthritis patients receiving cyclosporin A. Results of a randomized, placebo-controlled trial. Arthritis Rheum 1994; 37: 1321-1325.
146. Morgan BJ, Lyson T, Scherrer U, Victor RG. Cyclosporine causes sympathetically mediated elevations in arterial pressure in rats. Hypertension 1991; 18: 458-466.
147. Moss NG, Powell SL, Falk RJ. Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. Proc Natl Acad Sci USA 1985; 82: 8222-8226.
148. Scherrer U, Vissing SF, Morgan BJ, Rollins JA, Tindall RS, Ring S, Hanson P, Mohanty PK, Victor RG. Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. N Engl J Med 1990; 323: 693-699.
149. Lyson T, Ermel LD, Belshaw PJ, Alberg DG, Schreiber SL, Victor RG. Cyclosporine- and FK 506-induced sympathetic activation correlates with calcineurin-mediated inhibition of T-cell signaling. Circ Res 1993; 73: 596-602.
150. Lyson T, McMullan DM, Ermel LD, Morgan BJ, Victor RG. Mechanism of cyclosporine-induced sympathetic activation and acute hypertension in rats. Hypertension 1994; 23: 667-675.
151. Reis F, Tavares P, Teixeira F. The distribution of catecholamines between plasma and platelets in cyclosporin A-induced hypertensive rats. Pharmacol Res 2000; 41: 129-135.
152. Rossi NF, Churchill PC, McDonald FD, Ellis VR. Mechanism of cyclosporine A-induced renal vasoconstriction in the rat. J Pharmacol Exp Ther 1989; 250: 896-901.
153. Zhang W, Li JL, Hosaka M, Janz R, Shelton JM, Albright GM, Richardson JA, Sudhof TC, Victor RG. Cyclosporine A-induced hypertension involves synapsin in renal sensory nerve endings. Proc Natl Acad Sci USA 2000; 97: 9765-9770.
154. Grobecker HF, Riebel K, Wellenhofer T. Cyclosporine A-induced hypertension in SHR and WKY: role of the sympatho-adrenal system. Clin Exp Pharmacol Physiol 1995; 22 (Suppl 1): S94-S95.
155. Ishikawa A, Suzuki K, Fujita K. Prevention of cyclosporine-induced nephrotoxicity by prazosin hydrochloride, an α-1-adrenoreceptor blocker. Transplant Proc 1996; 28: 1360-1361.
156. Murray BM, Paller MS, Ferris TF. Effect of cyclosporine administration on renal hemodynamics in conscious rats. Kidney Int 1985; 28: 767-774.
157. Murray BM, Paller MS. Beneficial effects of renal denervation and prazosin on GFR and renal blood flow after cyclosporine in rats. Clin Nephrol 1986; 25 (Suppl 1): S37-S39.
158. Zhong Z, Connor HD, Yin M, Moss N, Mason RP, Bunzendahl H, Forman DT, Thurman RG. Dietary glycine and renal denervation prevents cyclosporin A-induced hydroxyl radical production in rat kidney. Mol Pharmacol 1999; 56: 455-463.
159. Carrier M, Tronc F, Stewart D, Pelletier LC. Dose-dependent effect of cyclosporin on renal arterial resistance in dogs. Am J Physiol 1991; 261: H1791-H1796.
160. Churchill MC, Churchill PC, Bidani AK. The effects of cyclosporine in Lewis rats with native and transplanted kidneys. Transplantation 1993; 55: 1256-1260.
161. Elzinga LW, Rosen S, Burdmann EA, Hatton DC, Lindsley J, Bennett WM. The role of renal sympathetic nerves in experimental chronic cyclosporine nephropathy. Transplantation 2000; 69: 2149-2153.
162. Wyss JM, Mozaffari MS, St John PL, Abrahamson DR. Cyclosporine-induced nephrotoxicity in deoxycorticosterone-NaCl treated rats. Int J Exp Pathol 1993; 74: 615-626.
163. Carvalho MJ, van den Meiracker AH, Boomsma F, Freitas J, Man in 't Veld AJ, Costa O, de Freitas AF. Role of sympathetic nervous system in cyclosporine-induced rise in blood pressure. Hypertension 1999; 34: 102-106.
164. Kaye D, Thompson J, Jennings G, Esler M. Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. Circulation 1993; 88: 1101-1109.
165. Lucini D, Milani RV, Ventura HO, Mehra MR, Messerli FH, Murgo JP, Regenstein F, Copley B, Malliani A, Pagani M. Cyclosporine-induced hypertension: evidence for maintained baroreflex circulatory control. J Heart Lung Transplant 1997; 16: 615-620.
166. Lucini D, Milani RV, Ventura HO, Mehra MR, Messerli F, Pagani M. Study of arterial and autonomic effects of cyclosporine in humans. Hypertension 2000; 35: 1258-1263.
167. Stein CM, He H, Pincus T, Wood AJ. Cyclosporine impairs vasodilation without increased sympathetic activity in humans. Hypertension 1995; 26: 705-710.
168. Lee SH, Yoon YC, Jang YY, Song JH, Han ES, Lee CS. Effect of iron and ascorbate on cyclosporine-induced oxidative damage of kidney mitochondria and microsomes. Pharmacol Res 2001; 43: 161-171.
169. Longoni B, Boschi E, Demontis GC, Ratto GM, Mosca F. Apoptosis and adaptive responses to oxidative stress in human endothelial cells exposed to cyclosporin A correlate with BCL-2 expression levels. FASEB J 2001; 15: 731-740.
170. Parra T, de Arriba G, Arribas I, Perez dL, Rodriguez-Puyol D, Rodriguez-Puyol M. Cyclosporine A nephrotoxicity: role of thromboxane and reactive oxygen species. J Lab Clin Med 1998; 131: 63-70.
171. Tariq M, Morais C, Sobki S, Al Sulaiman M, Al Khader A. N-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. Nephrol Dial Transplant 1999; 14: 923-929.

172. Wang C, Salahudeen AK. Cyclosporine nephrotoxicity: attenuation by an antioxidant-inhibitor of lipid peroxidation *in vitro* and *in vivo*. Transplantation 1994; 58: 940-946.
173. Wang C, Salahudeen AK. Lipid peroxidation accompanies cyclosporine nephrotoxicity: effects of vitamin E. Kidney Int 1995; 47: 927-934.
174. Zhong Z, Arteel GE, Connor HD, Yin M, Frankenberg MV, Stachlewitz RF, Raleigh JA, Mason RP, Thurman RG. Cyclosporin A increases hypoxia and free radical production in rat kidneys: prevention by dietary glycine. Am J Physiol 1998; 275: F595-F604.
175. Zhong Z, Connor HD, Yin M, Wheeler MD, Mason RP, Thurman RG. Viral delivery of superoxide dismutase gene reduces cyclosporine A-induced nephrotoxicity. Kidney Int 2001; 59: 1397-1404.
176. Haberland A, Henke W, Grune T, Siems W, Jung K, Schimke I. Differential response of oxygen radical metabolism in rat heart, liver and kidney to cyclosporine A treatment. Inflamm Res 1997; 46: 452-454.
177. Jenkins JK, Huang H, Ndebele K, Salahudeen AK. Vitamin E inhibits renal mRNA expression of COX II, HO I, TGFβ, and osteopontin in the rat model of cyclosporine nephrotoxicity. Transplantation 2001; 71: 331-334.
178. Navarro-Antolin J, Hernandez-Perera O, Lopez-Ongil S, Rodriguez-Puyol M, Rodriguez-Puyol D, Lamas S. CsA and FK 506 up-regulate eNOS expression: role of reactive oxygen species and AP-1. Kidney Int (Suppl) 1998; 68: S20-S24.
179. Krysztopik RJ, Bentley FR, Spain DA, Wilson MA, Garrison RN. Lazaroids prevent acute cyclosporine-induced renal vasoconstriction. Transplantation 1997; 63: 1215-1220.
180. Kumar KV, Naidu MU, Shifow AA, Prayag A, Ratnakar KS. Melatonin: an antioxidant protects against cyclosporine-induced nephrotoxicity. Transplantation 1999; 67: 1065-1068.
181. Barany P, Stenvinkel P, Ottosson-Seeberger A, Alvestrand A, Morrow J, Roberts JJ, Salahudeen AK. Effect of 6 weeks of vitamin E administration on renal haemodynamic alterations following a single dose of neoral in healthy volunteers. Nephrol Dial Transplant 2001; 16: 580-584.
182. McGrath LT, Treacy R, McClean E, Brown JH. Oxidative stress in cyclosporin and azathioprine treated renal transplant patients. Clin Chim Acta 1997; 264: 1-12.
183. Guieu R, Dussol B, Devaux C, Sampol J, Brunet P, Rochat H, Bechis G, Berland YF. Interactions between cyclosporine A and adenosine in kidney transplant recipients. Kidney Int 1998; 53: 200-204.
184. Halimi G, Sampol J, Clot-Faybesse O, Mercier L, Devaux C, Berland Y, Dussol B, Rochat H, Guieu R. Cyclosporine A and purinergic receptors in rat kidney. Life Sci 1999; 65: 2801-2813.
185. Balakrishnan VS, von Ruhland CJ, Griffiths DF, Coles GA, Williams JD. Effects of a selective adenosine A1 receptor antagonist on the development of cyclosporin nephrotoxicity. Br J Pharmacol 1996; 117: 879-884.
186. Churchill PC, Rossi NF, Churchill MC, Bidani AK, McDonald FD. Acute cyclosporine-induced renal vasoconstriction: lack of effect of theophylline. Am J Physiol 1990; 258: F41-F45.
187. Heyne N, Wolf S, Petersen P, Merten S, Schober W, Erley CM, Risler T, Osswald H. Adenosine receptor antagonism in the prevention of acute cyclosporine A- nephrotoxicity in normal, diabetic and hypertensive rats. Nephrol Dial Transplant 1999; 14 (Suppl 4): 23-24.
188. Stanek B, Kovarik J, Rasoul-Rockenschaub S, Silberbauer K. Renin-angiotensin-aldosterone system and vasopressin in cyclosporine- treated renal allograft recipients. Clin Nephrol 1987; 28: 186-189.
189. Frapier JM, Choby C, Mangoni ME, Nargeot J, Albat B, Richard S. Cyclosporin A increases basal intracellular calcium and calcium responses to endothelin and vasopressin in human coronary myocytes. FEBS Lett 2001; 493: 57-62.
190. Kremer S, Margolis B, Harper P, Skorecki K. Cyclosporine induced alterations in vasopressin signalling in the glomerular mesangial cell. Clin Invest Med 1989; 12: 201-206.
191. Lo RA, Passaquin AC, Ruegg UT. Mechanism of enhanced vasoconstrictor hormone action in vascular smooth muscle cells by cyclosporin A. Br J Pharmacol 1997; 121: 248-252.
192. Cottet-Maire F, Avdonin PV, Roulet E, Buetler TM, Mermod N, Ruegg UT. Upregulation of vasopressin V1A receptor mRNA and protein in vascular smooth muscle cells following cyclosporin A treatment. Br J Pharmacol 2001; 132: 909-917.
193. Bagnis C, Deray G, Dubois M, Pirotzky E, Jacquiaud C, Baghos W, Aupetit B, Braquet P, Jacobs C. Prevention of cyclosporin nephrotoxicity with a platelet-activating factor (PAF) antagonist. Nephrol Dial Transplant 1996; 11: 507-513.
194. dos Santos OF, Boim MA, Bregman R, Draibe SA, Barros EJ, Pirotzky E, Schor N, Braquet P. Effect of platelet-activating factor antagonist on cyclosporine nephrotoxicity. Glomerular hemodynamics evaluation. Transplantation 1989; 47: 592-595.
195. Kawaguchi A, Sugimoto K, Fujimura A. Preventive effect of platelet-activating factor antagonist, Y-24180, against cyclosporine-induced acute nephrotoxicity. Life Sci 2001; 68: 1181-1190.
196. Massicot F, Lamouri A, Martin C, Pham-Huy C, Heymans F, Warnet JM, Godfroid JJ, Claude JR. Preventive effects of two PAF-antagonists, PMS 536 and PMS 549, on cyclosporin-induced LLC-PK1 oxidative injury. J Lipid Mediat Cell Signal 1997; 15: 203-214.
197. Bokemeyer D, Kramer HJ, Meyer-Lehnert H. Atrial natriuretic peptide blunts the cellular effects of cyclosporine in smooth muscle. Hypertension 1993; 21: 166-172.

198. Capasso G, Rosati C, Ciani F, Giordano DR, Russo F, De Santo NG. The beneficial effect of atrial natriuretic peptide on cyclosporine nephrotoxicity. Am J Hypertens 1990; 3: 204-210.
199. Capasso G, Unwin R, Ciani F, Rizzo A, Russo F, Pica A, De Santo NG. Inhibition of neutral endopeptidase potentiates the effects of atrial natriuretic peptide on acute cyclosporin-induced nephrotoxicity. Nephron 2000; 86: 298-305.
200. Lang CC, Henderson IS, Mactier R, Stewart WK, Struthers AD. Atrial natriuretic factor improves renal function and lowers systolic blood pressure in renal allograft recipients treated with cyclosporin A. J Hypertens 1992; 10: 483-488.
201. Lipkin GW, Thuraisingham R, Dawnay AB, Harwood SM, Raine AE. Acute reversal of cyclosporine nephrotoxicity by neutral endopeptidase inhibition in stable renal transplant recipients. Transplantation 1997; 64: 1007-1017.
202. Martinez L, Vio CP, Valdes G, Kychenthal W, Mendoza S. Decreased kallikrein excretion in renal transplant treated with cyclosporine A. Transplant Proc 1990; 22: 294-296.
203. Rodier M, Ribstein J, Parer-Richard C, Mimran A. Renal changes associated with cyclosporine in recent type I diabetes mellitus. Hypertension 1991; 18: 334-340.
204. Spragg J, Coblyn JS, Fraser PA, Austen KF, Weinblatt ME. The effect of cyclosporine on urinary kallikrein excretion in patients with rheumatoid arthritis. Adv Exp Med Biol 1989; 247A: 389-392.
205. Bompart G, Tack I, Castano EM, Pesquero JB, Girolami JP, Bascands JL. Cyclosporine A decreases kallikrein and BK2 mRNA expression in the rat renal cortex. Immunopharmacology 1996; 32: 99-101.
206. Wang C, Ford P, Chao C, Chao L, Chao J. Effect of cyclosporin A on the expression of tissue kallikrein, kininogen, and bradykinin receptor in rat. Am J Physiol 1997; 273: F783-F789.
207. Bohdanecka M, Schuck O, Chadimova M, Sedivy J, Glagolicova A, Skibova J, Kunes J, Dobesova Z, Stuchlik M, Vesela J, Kazdova L. Nephrotoxicity of cyclosporin A in hereditary hypertriglyceridemic rats. Physiol Res 1999; 48: 437-443.
208. Duymelinck C, Dauwe SE, Nouwen EJ, De Broe ME, Verpooten GA. Cholesterol feeding accentuates the cyclosporine-induced elevation of renal plasminogen activator inhibitor type 1. Kidney Int 1997; 51: 1818-1830.
209. Burdmann EA, Andoh TF, Lindsley J, Houghton DC, Bennett WM. Effects of oral magnesium supplementation on acute experimental cyclosporin nephrotoxicity. Nephrol Dial Transplant 1994; 9: 16-21.
210. Pere AK, Lindgren L, Tuomainen P, Krogerus L, Rauhala P, Laakso J, Karppanen H, Vapaatalo H, Ahonen J, Mervaala EM. Dietary potassium and magnesium supplementation in cyclosporine-induced hypertension and nephrotoxicity. Kidney Int 2000; 58: 2462-2472.
211. Bennett WM. Mechanisms of acute and chronic nephrotoxicity from immunosuppressive drugs. Ren Fail 1996; 18: 453-460.
212. Devarajan P, Kaskel FJ, Arbeit LA, Moore LC. Cyclosporine nephrotoxicity: blood volume, sodium conservation, and renal hemodynamics. Am J Physiol 1989; 256: F71-F78.
213. Moore LC, Mason J, Feld L, Van Liew JB, Kaskel FJ. Effect of cyclosporine on endothelial albumin leakage in rats. J Am Soc Nephrol 1992; 3: 51-57.
214. Huang X, Cheng Z, Sunga J, Unemori E, Zsebo K. Systemic administration of recombinant human relaxin (RHRLX) ameliorates the acute cyclosporine nephrotoxicity in rats. J Heart Lung Transplant 2001; 20: 253-
215. Maestri M, Dafoe DC, Adams GA, Gaspari A, Luzzana F, Innocente F, Rademacher J, Dionigi P, Barbieri A, Zonta F, Zonta A, Rabkin R. Insulin-like growth factor-I ameliorates delayed kidney graft function and the acute nephrotoxic effects of cyclosporine. Transplantation 1997; 64: 185-190.
216. Beall A, Epstein A, Woodrum D, Brophy CM. Cyclosporine-induced renal artery smooth muscle contraction is associated with increases in the phosphorylation of specific contractile regulatory proteins. Biochim Biophys Acta 1999; 1449: 41-49.
217. Lo RA, Passaquin AC, Cox C, Ruegg UT. Cyclosporin A potentiates receptor-activated [Ca2+]c increase. J Recept Signal Transduct Res 1997; 17: 149-161.
218. Medina J, Cordier A, Wolf A. Cyclosporine A-induced contraction of isolated rat aortic smooth muscle cells. Life Sci 1997; 61: L-32.
219. Meyer-Lehnert H, Schrier RW. Cyclosporine A enhances vasopressin-induced Ca2+ mobilization and contraction in mesangial cells. Kidney Int 1988; 34: 89-97.
220. Ressureicao FA, Ballejo G, Salgado MC, Ferraz AS. Effect of cyclosporine administration on vascular reactivity of the isolated mesenteric bed. Transplant Proc 1995; 27: 1806-1808.
221. Xue H, Bukoski RD, McCarron DA, Bennett WM. Induction of contraction in isolated rat aorta by cyclosporine. Transplantation 1987; 43: 715-718.
222. Abraham JS, Bentley FR, Garrison RN, Cryer HM. The influence of the cyclosporine vehicle, cremophor EL, on renal microvascular blood flow in the rat. Transplantation 1991; 52: 101-105.
223. Hardy G, Stanke-Labesque F, Deveaux G, Devillier P, Sessa C, Bessard G. Cyclosporine A and cremophor EL induce contractions of human saphenous vein: involvement of thromboxane A2 receptor-dependent pathway. J Cardiovasc Pharmacol 2000; 36: 693-698.
224. Thiel G, Hermle M, Brunner FP. Acutely impaired renal function during intravenous administration of cyclosporine A: a cremophore side-effect. Clin Nephrol 1986; 25 (Suppl 1): S40-S42.

225. Hermida-Rodriguez C, Cantero PJ, Garcia-Valriberas R, Pajares Garcia JM, Mate-Jimenez J. High-dose intravenous cyclosporine in steroid refractory attacks of inflammatory bowel disease. Hepatogastroenterology 1999; 46: 2265-2268.
226. Macris MP, Ford EG, Van Buren CT, Frazier OH. Predictors of severe renal dysfunction after heart transplantation and intravenous cyclosporine therapy. J Heart Transplant 1989; 8: 444-448.
227. Powell-Jackson PR, Young B, Calne RY, Williams R. Nephrotoxicity of parenterally administered cyclosporine after orthotopic liver transplantation. Transplantation 1983; 36: 505-508.
228. Weir MR, Klassen DK, Shen SY, Sullivan D, Buddemeyer EU, Handwerger BS. Acute effects of intravenous cyclosporine on blood pressure, renal hemodynamics, and urine prostaglandin production of healthy humans. Transplantation 1990; 49: 41-47.
229. Burdmann EA, Andoh TF, Prado EAB, Franceschini N, Fujihara C, Zatz R, Bennett WM. Renal, immunosuppressive and pharmacokinetic effects of the substitution of intralipid (LIP) for cremophor (CRE) as vehicle for parenteral cyclosporine (CsA) administration. J Am Soc Nephrol 1995; 6: 995.
230. Burdmann EA, Andoh TF, Lindsley J, Russell J, Bennett WM, Porter G. Urinary enzymes as biomarkers of renal injury in experimental nephrotoxicity of immunosuppressive drugs. Ren Fail 1994; 16: 161-168.
231. Laine J, Holmberg C. Renal and adrenal mechanisms in cyclosporine-induced hyperkalaemia after renal transplantation. Eur J Clin Invest 1995; 25: 670-676.
232. Deppe CE, Heering PJ, Tinel H, Kinne-Saffran E, Grabensee B, Kinne RK. Effect of cyclosporine A on Na+/K(+)-ATPase, Na+/K+/2Cl- cotransporter, and H+/K(+)-ATPase in MDCK cells and two subtypes, C7 and C11. Exp Nephrol 1997; 5: 471-480.
233. Healy E, Dempsey M, Lally C, Ryan MP. Apoptosis and necrosis: mechanisms of cell death induced by cyclosporine A in a renal proximal tubular cell line. Kidney Int 1998; 54: 1955-1966.
234. Lally C, Healy E, Ryan MP. Cyclosporine A-induced cell cycle arrest and cell death in renal epithelial cells. Kidney Int 1999; 56: 1254-1257.
235. Lima R, Serone AP, Schor N, Higa EM. Effect of cyclosporin a on nitric oxide production in cultured LLC-PK1 cells. Ren Fail 2001; 23: 43-52.
236. Yang JJ, Finn WF. Effect of oxypurinol on cyclosporine toxicity in cultured EA, LLC-PK1 and MDCK cells. Ren Fail 1998; 20: 85-101.
237. Gordjani N, Epting T, Fischer-Riepe P, Greger RF, Brandis M, Leipziger J, Nitschke R. Cyclosporin-A-induced effects on the free Ca2+ concentration in LLC-PK1-cells and their mechanisms. Pflugers Arch 2000; 439: 627-633.
238. Paslaru L, Rallu M, Manuel M, Davidson S, Morange M. Cyclosporin A induces an atypical heat shock response. Biochem Biophys Res Commun 2000; 269: 464-469.
239. Stacchiotti A, Rezzani R, Angoscini P, Corsetti G, Bianchi R. Distribution of heat shock proteins in kidneys of rats after immunosuppressive treatment with cyclosporine A. Acta Histochem 2001; 103: 167-177.
240. Ling BN, Eaton DC. Cyclosporin A inhibits apical secretory K^+ channels in rabbit cortical collecting tubule principal cells. Kidney Int 1993; 44: 974-984.
241. da Costa MC, Castro I, Cuvello Neto AL, Burdmann EA, Ferreira AT, Yu L. Role of Ca^{2+} and Mg^{2+} on cyclosporin A acute tubular toxicity. Renal Fail 1999; 21: 571.
242. de Mattos AM, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am J Kidney Dis 2000; 35: 333-346.
243. Fisher NC, Malag M, Gonzlez-Pinto I. The clinical impact of nephrotoxicity in liver transplantation. Transplantation 2000; 69: SS18-SS22.
244. Parry G, Meiser B, Rabago G. The clinical impact of cyclosporine nephrotoxicity in heart transplantation. Transplantation 2000; 69: SS23-SS26.
245. Tsimaratos M, Viard L, Kreitmann B, Remediani C, Picon G, Camboulives J, Sarles J, Metras D. Kidney function in cyclosporine-treated paediatric pulmonary transplant recipients. Transplantation 2000; 69: 2055-2059.
246. Davies DR, Bittmann I, Pardo J. Histopathology of calcineurin inhibitor-induced nephrotoxicity. Transplantation 2000; 69: SS11-SS13.
247. Solez K. International standardization of criteria for histologic diagnosis of chronic rejection in renal allografts. Clin Transplant 1994; 8: 345-350.
248. Curtis JJ, Luke RG, Dubovsky E, Diethelm AG, Whelchel JD, Jones P. Cyclosporin in therapeutic doses increases renal allograft vascular resistance. Lancet 1986; 2: 477-479.
249. Hilbrands LB, Hoitsma AG, Wetzels JF, Koene RA. Detailed study of changes in renal function after conversion from cyclosporine to azathioprine. Clin Nephrol 1996; 45: 230-235.
250. Ruggenenti P, Perico N, Mosconi L, Gaspari F, Benigni A, Amuchastegui CS, Bruzzi I, Remuzzi G. Calcium channel blockers protect transplant patients from cyclosporine- induced daily renal hypoperfusion. Kidney Int 1993; 43: 706-711.
251. Bennett WM, Burdmann EA, Andoh TF, Houghton DC, Lindsley J, Elzinga LW. Nephrotoxicity of immunosuppressive drugs. Nephrol Dial Transplant 1994; 9 (Suppl 4): 141-145.

252. Greenberg A, Egel JW, Thompson ME, Hardesty RL, Griffith BP, Bahnson HT, Bernstein RL, Hastillo A, Hess ML, Puschett JB. Early and late forms of cyclosporine nephrotoxicity: studies in cardiac transplant recipients. Am J Kidney Dis 1987; 9: 12-22.
253. Noel C, Hazzan M, Noel-Walter MP, Jouet JP. Renal failure and bone marrow transplantation. Nephrol Dial Transplant 1998; 13: 2464-2466.
254. Platz KP, Mueller AR, Blumhardt G, Bachmann S, Bechstein WO, Kahl A, Neuhaus P. Nephrotoxicity following orthotopic liver transplantation. A comparison between cyclosporine and FK 506. Transplantation 1994; 58: 170-178.
255. Brann WM, Bennett LE, Keck BM, Hosenpud JD. Morbidity, functional status, and immunosuppressive therapy after heart transplantation: an analysis of the joint International Society for Heart and Lung Transplantation/United Network for Organ Sharing Thoracic Registry. J Heart Lung Transplant 1998; 17: 374-382.
256. Hosenpud JD, Bennett LE, Keck BM, Fiol B, Boucek MM, Novick RJ. The Registry of the International Society for Heart and Lung Transplantation: sixteenth official report—1999. J Heart Lung Transplant 1999; 18: 611-626.
257. Woolfson RG, Neild GH. Cyclosporin nephrotoxicity following cardiac transplantation. Nephrol Dial Transplant 1997; 12: 2054-2056.
258. Zager RA, O'Quigley J, Zager BK, Alpers CE, Shulman HM, Gamelin LM, Stewart P, Thomas ED. Acute renal failure following bone marrow transplantation: a retrospective study of 272 patients. Am J Kidney Dis 1989; 13: 210-216.
259. Zager RA. Acute renal failure in the setting of bone marrow transplantation. Kidney Int 1994; 46: 1443-1458.
260. Zager RA. Acute renal failure syndromes after bone marrow transplantation. Adv Nephrol Necker Hosp 1997; 27: 263-280.
261. Ahuja TS, Niaz N, Agraharkar M. Contrast-induced nephrotoxicity in renal allograft recipients. Clin Nephrol 2000; 54: 11-14.
262. Altman RD, Perez GO, Sfakianakis GN. Interaction of cyclosporine A and nonsteroidal anti-inflammatory drugs on renal function in patients with rheumatoid arthritis. Am J Med 1992; 93: 396-402.
263. Deray G, Le Hoang P, Aupetit B, Achour A, Rottembourg J, Baumelou A. Enhancement of cyclosporine A nephrotoxicity by diclofenac. Clin Nephrol 1987; 27: 213-214.
264. Garcia TM, da Costa JA, Costa RS, Ferraz AS. Acute tubular necrosis in kidney transplant patients treated with enalapril. Ren Fail 1994; 16: 419-423.
265. Luber AD, Maa L, Lam M, Guglielmo BJ. Risk factors for amphotericin B-induced nephrotoxicity. J Antimicrob Chemother 1999; 43: 267-271.
266. Morales JM, Andres A, Prieto C, Diaz Rolon JA, Rodicio JL. Reversible acute renal toxicity by toxic sinergic effect between gentamicin and cyclosporine. Clin Nephrol 1988; 29: 272.
267. Morales JM, Munoz MA, Fernandez ZG, Garcia CC, Garcia Rubiales MA, Andres A, Aguado JM, Gonzalez Pinto I. Reversible acute renal failure caused by the combined use of foscarnet and cyclosporin in organ transplanted patients. Nephrol Dial Transplant 1995; 10: 882-883.
268. Murray BM, Venuto RC, Kohli R, Cunningham EE. Enalapril-associated acute renal failure in renal transplants: possible role of cyclosporine. Am J Kidney Dis 1990; 16: 66-69.
269. Schiff MH, Whelton A. Renal toxicity associated with disease-modifying antirheumatic drugs used for the treatment of rheumatoid arthritis. Semin Arthritis Rheum 2000; 30: 196-208.
270. Termeer A, Hoitsma AJ, Koene RA. Severe nephrotoxicity caused by the combined use of gentamicin and cyclosporine in renal allograft recipients. Transplantation 1986; 42: 220-221.
271. Denton CP, Sweny P, Abdulla A, Black CM. Acute renal failure occurring in scleroderma treated with cyclosporin A: a report of three cases. Br J Rheumatol 1994; 33: 90-92.
272. Dussol B, Reynaud-Gaubert M, Saingra Y, Daniel L, Berland Y. Acute tubular necrosis induced by high level of cyclosporine A in a lung transplant. Transplantation 2000; 70: 1234-1236.
273. Shechter P. Acute tubular necrosis following high-dose cyclosporine A therapy. Eur J Clin Pharmacol 1996; 49: 521-523.
274. Kahn D, Botha JF, Pascoe MD, Pontin AR, Halkett J, Tandon V. Withdrawal of cyclosporine in renal transplant recipients with acute tubular necrosis improves renal function. Transplant Int 2000; 13 (Suppl 1): S82-S83.
275. Young BA, Marsh CL, Alpers CE, Davis CL. Cyclosporine-associated thrombotic microangiopathy/hemolytic uremic syndrome following kidney and kidney-pancreas transplantation. Am J Kidney Dis 1996; 28: 561-571.
276. Mazzali M, Dias EP, Ribeiro-Alves MA, Homsi E, Alves-Filho G. Nonrecurrent hemolytic uremic syndrome (HUS de novo) as cause of acute renal failure after renal transplant. Ren Fail 1997; 19: 271-277.
277. Shulman H, Striker G, Deeg HJ, Kennedy M, Storb R, Thomas ED. Nephrotoxicity of cyclosporin A after allogeneic marrow transplantation: glomerular thromboses and tubular injury. N Engl J Med 1981; 305: 1392-1395.
278. Zarifian A, Meleg-Smith S, O'donovan R, Tesi RJ, Batuman V. Cyclosporine-associated thrombotic microangiopathy in renal allografts. Kidney Int 1999; 55: 2457-2466.
279. Bennett WM, Burdmann E, Andoh T, Elzinga L, Franceschini N. Nephrotoxicity of immunosuppressive drugs. Miner Electrolyte Metab 1994; 20: 214-220.
280. Mahalati K, Belitsky P, West K, Kiberd B, Fraser A, Sketris I, Macdonald AS, McAlister V, Lawen J. Approaching the therapeutic window for cyclosporine in kidney transplantation: a prospective study. J Am Soc Nephrol 2001; 12: 828-833.

281. Macdonald AS. Management strategies for nephrotoxicity. Transplantation 2000; 69: SS31-SS36.
282. Mahalati K, Belitsky P, Sketris I, West K, Panek R. Neoral monitoring by simplified sparse sampling area under the concentration-time curve: its relationship to acute rejection and cyclosporine nephrotoxicity early after kidney transplantation. Transplantation 1999; 68: 55-62.
283. Bunke M, Sloan R, Brier M, Ganzel B. An improved glomerular filtration rate in cardiac transplant recipients with once-a-day cyclosporine dosing. Transplantation 1995; 59: 537-540.
284. Chan C, Maurer J, Cardella C, Cattran D, Pei Y. A randomized controlled trial of verapamil on cyclosporine nephrotoxicity in heart and lung transplant recipients. Transplantation 1997; 63: 1435-1440.
285. Madsen JK, Sorensen SS, Hansen HE, Pedersen EB. The effect of felodipine on renal function and blood pressure in cyclosporin-treated renal transplant recipients during the first three months after transplantation. Nephrol Dial Transplant 1998; 13: 2327-2334.
286. Rahn KH, Barenbrock M, Fritschka E, Heinecke A, Lippert J, Schroeder K, Hauser I, Wagner K, Neumayer HH. Effect of nitrendipine on renal function in renal-transplant patients treated with cyclosporin: a randomised trial. Lancet 1999; 354: 1415-1420.
287. Rodicio JL. Calcium antagonists and renal protection from cyclosporine nephrotoxicity: long-term trial in renal transplantation patients. J Cardiovasc Pharmacol 2000; 35: S7-S11.
288. Shin GT, Cheigh JS, Riggio RR, Suthanthiran M, Stubenbord WT, Serur D, Wang JC, Rubin AL, Stenzel KH. Effect of nifedipine on renal allograft function and survival beyond one year. Clin Nephrol 1997; 47: 33-36.
289. van Riemsdijk IC, Mulder PG, de Fijter JW, Bruijn JA, van Hooff JP, Hoitsma AJ, Tegzess AM, Weimar W. Addition of isradipine (Lomir) results in a better renal function after kidney transplantation: a double-blind, randomized, placebo-controlled, multicenter study. Transplantation 2000; 70: 122-126.
290. Wilkie ME, Beer JC, Evans SJ, Raftery MJ, Lord RH, Moore R, Marsh FP. A double-blind, randomized, placebo-controlled study of nifedipine on early renal allograft function. Nephrol Dial Transplant 1994; 9: 800-804.
291. Neumayer HH, Kunzendorf U, Schreiber M. Protective effects of calcium antagonists in human renal transplantation. Kidney Int (Suppl) 1992; 36: S87-S93.
292. Harper SJ, Moorhouse J, Abrams K, Jurewicz A, Nicholson M, Horsburgh T, Harris K, Combe C, Bell PR, Walls J, Donnelly PK, Veitch PS, Feehally J. The beneficial effects of oral nifedipine on cyclosporin-treated renal transplant recipients—a randomised prospective study. Transplant Int 1996; 9: 115-125.
293. Lapointe N, Chen H, Qi S, Xu D, Daloze P, Dumont L. Immunomodulating effects of second-generation calcium channel blockers on experimental heart transplantation. Eur Surg Res 1999; 31: 259-266.
294. Blaheta RA, Hailer NP, Brude N, Wittig B, Leckel K, Oppermann E, Bachmann M, Harder S, Cinatl J, Scholz M, Bereiter-Hahn J, Weber S, Encke A, Markus BH. *In vitro* analysis of verapamil-induced immunosuppression: potent inhibition of T cell motility and lymphocytic transmigration through allogeneic endothelial cells. Transplantation 2000; 69: 588-597.
295. Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999; 70: 560S-569S.
296. Stoof TJ, Korstanje MJ, Bilo HJ, Starink TM, Hulsmans RF, Donker AJ. Does fish oil protect renal function in cyclosporin-treated psoriasis patients? J Intern Med 1989; 226: 437-441.
297. Homan van der Heide JJ, Bilo HJ, Tegzess AM, Donker AJ. The effects of dietary supplementation with fish oil on renal function in cyclosporine-treated renal transplant recipients. Transplantation 1990; 49: 523-527.
298. van der Heide JJ, Bilo HJ, Donker JM, Wilmink JM, Tegzess AM. Effect of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. N Engl J Med 1993; 329: 769-773.
299. Bennett WM, Carpenter CB, Shapiro ME, Strom TB, Hefty D, Tillman M, Abrams J, Ryan D, Kelley VR. Delayed omega-3 fatty acid supplements in renal transplantation. A double-blind, placebo-controlled study. Transplantation 1995; 59: 352-356.
300. Kooijmans-Coutinho MF, Rischen-Vos J, Hermans J, Arndt JW, van der Woude FJ. Dietary fish oil in renal transplant recipients treated with cyclosporin-A: no beneficial effects shown. J Am Soc Nephrol 1996; 7: 513-518.
301. Schut NH, Bilo HJ, Popp-Snijders C, Goedhart PT, Wilmink JM. Erythrocyte deformability, endothelin levels, and renal function in cyclosporin-treated renal transplant recipients: effects of intervention with fish oil and corn oil. Scand J Clin Lab Invest 1993; 53: 499-506.
302. Holm T, Andreassen AK, Aukrust P, Andersen K, Geiran OR, Kjekshus J, Simonsen S, Gullestad L. Omega-3 fatty acids improve blood pressure control and preserve renal function in hypertensive heart transplant recipients. Eur Heart J 2001; 22: 428-436.
303. Carmellini M, Frosini F, Filipponi F, Boggi U, Mosca F. Effect of cilastatin on cyclosporine-induced acute nephrotoxicity in kidney transplant recipients. Transplantation 1997; 64: 164-166.
304. Carmellini M, Matteucci E, Boggi U, Cecconi S, Giampietro O, Mosca F. Imipenem/cilastatin reduces cyclosporin-induced tubular damage in kidney transplant recipients. Transplant Proc 1998; 30: 2034-2035.
305. Gruss E, Tomas JF, Bernis C, Rodriguez F, Traver JA, Fernandez-Ranada JM. Nephroprotective effect of cilastatin in allogeneic bone marrow transplantation. Results from a retrospective analysis. Bone Marrow Transplant 1996; 18: 761-765.
306. Markewitz A, Hammer C, Pfeiffer M, Zahn S, Drechsel J, Reichenspurner H, Reichart B. Reduction of cyclosporine-induced nephrotoxicity by cilastatin following clinical heart transplantation. Transplantation 1994; 57: 865-870.

307. Burdmann EA, Andoh TF, Rosen S, Lindsley J, Munar MY, Elzinga LW, Bennett WM. Experimental nephrotoxicity, hepatotoxicity and pharmacokinetics of cyclosporin G versus cyclosporin A. Kidney Int 1994; 45: 684-691.
308. Gruber SA, Gallichio M, Rosano TG, Kaplan SS, Hughes SE, Urbauer DL, Singh TP, Lempert N, Conti DJ, Stein DS, Drusano G. Comparative pharmacokinetics and renal effects of cyclosporin A and cyclosporin G in renal allograft recipients. J Clin Pharmacol 1997; 37: 575-586.
309. Nussenblatt RB, de Smet MD, Rubin B, Freidlin V, Whitcup SM, Davis J, Herman D, Bloom JN, Sran PK, Whitcher S, et al. A masked, randomized, dose-response study between cyclosporine A and G in the treatment of sight-threatening uveitis of noninfectious origin. Am J Ophthalmol 1993; 115: 583-591.
310. Hiestand PC, Graber M, Hurtenbach U, Herrmann P, Cammisuli S, Richardson BP, Eberle MK, Donatsch P, Ryffel B, Borel JF. New cyclosporine derivative SDZ IMM 125: *in vitro* and *in vivo* pharmacologic effects and toxicologic evaluation. Transplant Proc 1993; 25: 691-692.
311. Witkamp L, Zonneveld IM, Jung EG, Schopf RE, Christophers E, Grossman R, Meffert H, Belaich S, Mahrle G, van Joost T, et al. Efficacy and tolerability of multiple-dose SDZ IMM 125 in patients with severe psoriasis. Br J Dermatol 1995; 133: 95-103.
312. Bennett WM. The nephrotoxicity of new and old immunosuppressive drugs. Ren Fail 1998; 20: 687-690.
313. Noble S, Markham A. Cyclosporin. A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral). Drugs 1995; 50: 924-941.
314. Bartucci M, Koshla B, Fleming-Brooks S, Weigel K, Donley V, Schulak JA, Knauss TC, Hricik DE. Renal function following conversion from Sandimmune to Neoral in stable renal transplant recipients. J Transpl Coord 1997; 7: 78-81.
315. Gennery AR, O'Sullivan JJ, Hasan A, Hamilton JR, Dark JH. Changing cyclosporin A formulation: an analysis in paediatric cardiac transplant recipients. Pediatr Transplant 1999; 3: 215-218.
316. Neumayer HH, Budde K, Farber L, Haller P, Kohnen R, Maibucher A, Schuster A, Vollmar J, Waiser J, Luft FC. Conversion to microemulsion cyclosporine in stable renal transplant patients: results after one year. Clin Nephrol 1996; 45: 326-331.
317. Hemming AW, Greig PD, Cattral MS, Chung SW, Lilly LB, Aljumah AA, Levy GA. A microemulsion of cyclosporine without intravenous cyclosporine in liver transplantation. Transplantation 1996; 62: 1798-1802.
318. Cole E, Keown P, Landsberg D, Halloran P, Shoker A, Rush D, Jeffrey J, Russell D, Stiller C, Muirhead N, Paul L, Zaltzman J, Loertscher R, Daloze P, Dandavino R, Boucher A, Handa P, Lawen J, Belitsky P, Parfrey P, Tan A, Hendricks L. Safety and tolerability of cyclosporine and cyclosporine microemulsion during 18 months of follow-up in stable renal transplant recipients: a report of the Canadian Neoral Renal Study Group. Transplantation 1998; 65: 505-510.
319. Pollard SG, Lear PA, Ready AR, Moore RH, Johnson RW. Comparison of microemulsion and conventional formulations of cyclosporine A in preventing acute rejection in de novo kidney transplant patients. The U.K. Neoral Renal Study Group. Transplantation 1999; 68: 1325-1331.
320. Olyaei AJ, deMattos AM, Bennett WM. Switching between cyclosporin formulations. What are the risks ? Drug Saf 1997; 16: 366-373.
321. Hricik DE, Dixit A, Knauss TC, Donley V, Bartucci MR, Schulak JA. Benefits of pre-emptive dose reduction for Sandimmune to Neoral conversion in stable renal transplant recipients. Clin Transplant 1998; 12: 575-578.
322. Kasiske BL, Chakkera HA, Louis TA, Ma JZ. A meta-analysis of immunosuppression withdrawal trials in renal transplantation. J Am Soc Nephrol 2000; 11: 1910-1917.
323. Helderman JH, Goral S. Cyclosporin withdrawal: to be or not to be! Nephrol Dial Transplant 1998; 13: 31-33.
324. Newstead CG, Johnston PA, Will EJ, Davison AM. The case for withdrawal of cyclosporin after renal transplantation. Nephrol Dial Transplant 1998; 13: 28-31.
325. Almond PS, Matas A, Gillingham K, Dunn DL, Payne WD, Gores P, Gruessner R, Najarian JS. Risk factors for chronic rejection in renal allograft recipients. Transplantation 1993; 55: 752-756.
326. Opelz G. Effect of the maintenance immunosuppressive drug regimen on kidney transplant outcome. Transplantation 1994; 58: 443-446.
327. Jha V, Muthukumar T, Kohli HS, Sud K, Gupta KL, Sakhuja V. Impact of cyclosporine withdrawal on living related renal transplants: a single-center experience. Am J Kidney Dis 2001; 37: 119-124.
328. Kasiske BL, Heim-Duthoy K, Ma JZ. Elective cyclosporine withdrawal after renal transplantation. A meta- analysis. JAMA 1993; 269: 395-400.
329. Rocher LL, Milford EL, Kirkman RL, Carpenter CB, Strom TB, Tilney NL. Conversion from cyclosporine to azathioprine in renal allograft recipients. Transplantation 1984; 38: 669-674.
330. Hunt SA, Stinson EB, Oyer PE, Billingham ME, Gamberg P, Miller J, Schroeder JS, Baldwin JC, Shumway NE. Results of "immunoconversion" from cyclosporine to azathioprine in heart transplant recipients with progressive nephrotoxicity. Transplant Proc 1987; 19: 2522-2524.
331. Sandborn WJ, Hay JE, Porayko MK, Gores GJ, Steers JL, Krom RA, Wiesner RH. Cyclosporine withdrawal for nephrotoxicity in liver transplant recipients does not result in sustained improvement in kidney function and causes cellular and ductopenic rejection. Hepatology 1994; 19: 925-932.

332. Dubey D, Kumar A, Srivastava A, Mandhani A, Sharma AP, Gupta A, Sharma RK. Cyclosporin A withdrawal in live related renal transplantation: long-term results. Clin Transplant 2001; 15: 136-141.
333. Heim-Duthoy KL, Chitwood KK, Tortorice KL, Massy ZA, Kasiske BL. Elective cyclosporine withdrawal 1 year after renal transplantation. Am J Kidney Dis 1994; 24: 846-853.
334. Hollander AA, van Saase JL, Kootte AM, van Dorp WT, van Bockel HJ, van Es LA, van der Woude FJ. Beneficial effects of conversion from cyclosporin to azathioprine after kidney transplantation. Lancet 1995; 345: 610-614.
335. MacPhee IA, Bradley JA, Briggs JD, Junor BJ, MacPherson SG, McMillan MA, Rodger RS, Watson MA. Long-term outcome of a prospective randomized trial of conversion from cyclosporine to azathioprine treatment one year after renal transplantation. Transplantation 1998; 66: 1186-1192.
336. Pescovitz MD, Govani M. Sirolimus and mycophenolate mofetil for calcineurin-free immunosuppression in renal transplant recipients. Am J Kidney Dis 2001; 38: S16-S21.
337. de Sevaux RG, Gregoor PJ, Hene RJ, Hoitsma AJ, Vos P, Weimar W, Van Gelder T, Hilbrands LB. A controlled trial comparing two doses of cyclosporine in conjunction with mycophenolate mofetil and corticosteroids. J Am Soc Nephrol 2001; 12: 1750-1757.
338. Schrama YC, Joles JA, van Tol A, Boer P, Koomans HA, Hene RJ. Conversion to mycophenolate mofetil in conjunction with stepwise withdrawal of cyclosporine in stable renal transplant recipients. Transplantation 2000; 69: 376-383.
339. Weir MR, Anderson L, Fink JC, Gabregiorgish K, Schweitzer EJ, Hoehn-Saric E, Klassen DK, Cangro CB, Johnson LB, Kuo PC, Lim JY, Bartlett ST. A novel approach to the treatment of chronic allograft nephropathy. Transplantation 1997; 64: 1706-1710.
340. Zanker B, Schneeberger H, Rothenpieler U, Hillebrand G, Illner WD, Theodorakis I, Stangl M, Land W. Mycophenolate mofetil-based, cyclosporine-free induction and maintenance immunosuppression: first-3-months analysis of efficacy and safety in two cohorts of renal allograft recipients. Transplantation 1998; 66: 44-49.
341. Aleksic I, Baryalei M, Busch T, Pieske B, Schorn B, Strauch J, Sirbu H, Dalichau H. Improvement of impaired renal function in heart transplant recipients treated with mycophenolate mofetil and low-dose cyclosporine. Transplantation 2000; 69: 1586-1590.
342. Herrero JI, Quiroga J, Sangro B, Girala M, Gomez-Manero N, Pardo F, Alvarez-Cienfuegos J, Prieto J. Conversion of liver transplant recipients on cyclosporine with renal impairment to mycophenolate mofetil. Liver Transpl Surg 1999; 5: 414-420.
343. Schlitt HJ, Barkmann A, Boker KH, Schmidt HH, Emmanouilidis N, Rosenau J, Bahr MJ, Tusch G, Manns MP, Nashan B, Klempnauer J. Replacement of calcineurin inhibitors with mycophenolate mofetil in liver-transplant patients with renal dysfunction: a randomised controlled study. Lancet 2001; 357: 587-591.
344. David-Neto E, Araujo LM, Lemos FC, David DS, Mazzucchi E, Nahas WC, Arap S, Ianhez LE. Introduction of mycophenolate mofetil and cyclosporin reduction in children with chronic transplant nephropathy. Pediatr Transplant 2001; 5: 302-309.
345. Smak Gregoor PJ, Van Gelder T, van Besouw NM, van der Mast BJ, Ijzermans JN, Weimar W. Randomized study on the conversion of treatment with cyclosporine to azathioprine or mycophenolate mofetil followed by dose reduction. Transplantation 2000; 70: 143-148.
346. Theodorakis J, Schneeberger H, Illner WD, Stangl M, Zanker B, Land W. Nephrotoxicity-free, mycophenolate mofetil based induction/maintenance immunosuppression in elderly recipients of renal allografts from elderly cadaveric donors. Transplant Proc 2000; 32(1A Suppl): 9S-11S.
347. Lecornu-Heuze L, Ducloux D, Rebibou JM, Martin L, Billerey C, Chalopin JM. Mycophenolate mofetil in cyclosporin-associated thrombotic microangiopathy. Nephrol Dial Transplant 1998; 13: 3212-3213.
348. Davison SC, Morris-Jones R, Powles AV, Fry L. Change of treatment from cyclosporin to mycophenolate mofetil in severe psoriasis. Br J Dermatol 2000; 143: 405-407.
349. Thervet E, Morelon E, Ducloux D, Bererhi L, Noel LH, Janin A, Bedrossian J, Puget S, Chalopin JM, Mihatsch M, Legendre C, Kreis H. Cyclosporine withdrawal in stable renal transplant recipients after azathioprine-mycophenolate mofetil conversion. Clin Transplant 2000; 14: 561-566.
350. Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. Clin Biochem 1998; 31: 335-340.
351. Groth CG, Backman L, Morales JM, Calne R, Kreis H, Lang P, Touraine JL, Claesson K, Campistol JM, Durand D, Wramner L, Brattstrom C, Charpentier B. Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. Transplantation 1999; 67: 1036-1042.
352. Kreis H, Cisterne JM, Land W, Wramner L, Squifflet JP, Abramowicz D, Campistol JM, Morales JM, Grinyo JM, Mourad G, Berthoux FC, Brattstrom C, Lebranchu Y, Vialtel P. Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. Transplantation 2000; 69: 1252-1260.
353. Morelon E, Mamzer-Bruneel MF, Peraldi MN, Kreis H. Sirolimus: a new promising immunosuppressive drug. Towards a rationale for its use in renal transplantation. Nephrol Dial Transplant 2001; 16: 18-20.

354. Andoh TF, Lindsley J, Franceschini N, Bennett WM. Synergistic effects of cyclosporine and rapamycin in a chronic nephrotoxicity model. Transplantation 1996; 62: 311-316.
355. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. Lancet 2000; 356: 194-202.
356. Macdonald AS. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. Transplantation 2001; 71: 271-280.
357. Podder H, Stepkowski SM, Napoli KL, Clark J, Verani RR, Chou TC, Kahan BD. Pharmacokinetic interactions augment toxicities of sirolimus/cyclosporine combinations. J Am Soc Nephrol 2001; 12: 1059-1071.
358. Kahan BD, Kramer WG. Median effect analysis of efficacy versus adverse effects of immunosuppressants. Clin Pharmacol Ther 2001; 70: 74-81.
359. Dominguez J, Mahalati K, Kiberd B, McAlister VC, Macdonald AS. Conversion to rapamycin immunosuppression in renal transplant recipients: report of an initial experience. Transplantation 2000; 70: 1244-1247.
360. Bennett WM, DeMattos A, Meyer MM, Andoh T, Barry JM. Chronic cyclosporine nephropathy: the Achilles' heel of immunosuppressive therapy. Kidney Int 1996; 50: 1089-1100.
361. Mihatsch MJ, Ryffel B, Gudat F. The differential diagnosis between rejection and cyclosporine toxicity. Kidney Int (Suppl) 1995; 52: S63-S69.
362. Franceschini N, Alpers CE, Bennett WM, Andoh TF. Cyclosporine arteriolopathy: effects of drug withdrawal. Am J Kidney Dis 1998; 32: 247-253.
363. Hamahira K, Iijima K, Tanaka R, Nakamura H, Yoshikawa N. Recovery from cyclosporine-associated arteriolopathy in childhood nephrotic syndrome. Pediatr Nephrol 2001; 16: 723-727.
364. Iijima K, Hamahira K, Kobayashi A, Nakamura H, Yoshikawa N. Immunohistochemical analysis of renin activity in chronic cyclosporine nephropathy in childhood nephrotic syndrome. J Am Soc Nephrol 2000; 11: 2265-2271.
365. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. N Engl J Med 1984; 311: 699-705.
366. Palestine AG, Austin HA, III, Balow JE, Antonovych TT, Sabnis SG, Preuss HG, Nussenblatt RB. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. N Engl J Med 1986; 314: 1293-1298.
367. Gerkens JF, Bhagwandeen SB, Dosen PJ, Smith AJ. The effect of salt intake on cyclosporine-induced impairment of renal function in rats. Transplantation 1984; 38: 412-417.
368. Jackson NM, Hsu CH, Visscher GE, Venkatachalam MA, Humes HD. Alterations in renal structure and function in a rat model of cyclosporine nephrotoxicity. J Pharmacol Exp Ther 1987; 242: 749-756.
369. Elzinga LW, Rosen S, Bennett WM. Dissociation of glomerular filtration rate from tubulointerstitial fibrosis in experimental chronic cyclosporine nephropathy: role of sodium intake. J Am Soc Nephrol 1993; 4: 214-221.
370. Rosen S, Greenfeld Z, Brezis M. Chronic cyclosporine-induced nephropathy in the rat. A medullary ray and inner stripe injury. Transplantation 1990; 49: 445-452.
371. Young BA, Burdmann EA, Johnson RJ, Andoh T, Bennett WM, Couser WG, Alpers CE. Cyclosporine A induced arteriolopathy in a rat model of chronic cyclosporine nephropathy. Kidney Int 1995; 48: 431-438.
372. Myers BD, Newton L, Boshkos C, Macoviak JA, Frist WH, Derby GC, Perlroth MG, Sibley RK. Chronic injury of human renal microvessels with low-dose cyclosporine therapy. Transplantation 1988; 46: 694-703.
373. Myers BD, Sibley R, Newton L, Tomlanovich SJ, Boshkos C, Stinson E, Luetscher JA, Whitney DJ, Krasny D, Coplon NS, Perlroth MG. The long-term course of cyclosporine-associated chronic nephropathy. Kidney Int 1988; 33: 590-600.
374. Truong LD, Farhood A, Tasby J, Gillum D. Experimental chronic renal ischemia: morphologic and immunologic studies. Kidney Int 1992; 41: 1676-1689.
375. Burdmann EA, Young B, Andoh TF, Evans A, Alpers CE, Lindsley J, Johnson RJ, Couser W, Bennett WM. Mechanisms of cyclosporine-induced interstitial fibrosis. Transplant Proc 1994; 26: 2588-2589.
376. Dieperink H, Hansen HV, Kemp M, Leyssac PP, Starklint H, Kemp E. Antagonist capacity of felodipine on cyclosporin A nephrotoxicity in the rat. Nephrol Dial Transplant 1992; 7: 1124-1129.
377. Wolf G, Neilson EG. Increases in levels of collagen types I and IV messenger ribonucleic acid in murine kidneys after treatment with ciclosporin. Nephron 1992; 60: 87-91.
378. Hunley TE, Fogo A, Iwasaki S, Kon V. Endothelin A receptor mediates functional but not structural damage in chronic cyclosporine nephrotoxicity. J Am Soc Nephrol 1995; 5: 1718-1723.
379. Kon V, Hunley TE, Fogo A. Combined antagonism of endothelin A/B receptors links endothelin to vasoconstriction whereas angiotensin II effects fibrosis. Studies in chronic cyclosporine nephrotoxicity in rats. Transplantation 1995; 60: 89-95.
380. Burdmann EA, Andoh TF, Nast CC, Evan A, Connors BA, Coffman TM, Lindsley J, Bennett WM. Prevention of experimental cyclosporin-induced interstitial fibrosis by losartan and enalapril. Am J Physiol 1995; 269: F491-F499.
381. Vieira JM, Jr., Noronha IL, Malheiros DM, Burdmann EA. Cyclosporine-induced interstitial fibrosis and arteriolar TGF-β expression with preserved renal blood flow. Transplantation 1999; 68: 1746-1753.

382. Ramirez C, Olmo A, O'Valle F, Masseroli M, Aguilar M, Gomez-Morales M, Revelles F, Garcia-Chicano MJ, Arrebola F, Reguero ME, del Moral RG. Role of intrarenal endothelin 1, endothelin 3, and angiotensin II expression in chronic cyclosporin A nephrotoxicity in rats. Exp Nephrol 2000; 8: 161-172.
383. Fox J, Guan S, Hymel AA, Navar LG. Dietary Na and ACE inhibition effects on renal tissue angiotensin I and II and ACE activity in rats. Am J Physiol 1992; 262: F902-F909.
384. Holmer S, Eckardt KU, LeHir M, Schricker K, Riegger G, Kurtz A. Influence of dietary NaCl intake on renin gene expression in the kidneys and adrenal glands of rats. Pflugers Arch 1993; 425: 62-67.
385. Ruiz-Ortega M, Egido J. Angiotensin II modulates cell growth-related events and synthesis of matrix proteins in renal interstitial fibroblasts. Kidney Int 1997; 52: 1497-1510.
386. Wolf G, Neilson EG. Angiotensin II as a renal growth factor. J Am Soc Nephrol 1993; 3: 1531-1540.
387. Wolf G, Kalluri R, Ziyadeh FN, Neilson EG, Stahl RA. Angiotensin II induces α3(IV) collagen expression in cultured murine proximal tubular cells. Proc Assoc Am Physicians 1999; 111: 357-364.
388. Johnson RJ, Alpers CE, Yoshimura A, Lombardi D, Pritzl P, Floege J, Schwartz SM. Renal injury from angiotensin II-mediated hypertension. Hypertension 1992; 19: 464-474.
389. Meister B, Lippoldt A, Bunnemann B, Inagami T, Ganten D, Fuxe K. Cellular expression of angiotensin type-1 receptor mRNA in the kidney. Kidney Int 1993; 44: 331-336.
390. Sechi LA, Grady EF, Griffin CA, Kalinyak JE, Schambelan M. Distribution of angiotensin II receptor subtypes in rat and human kidney. Am J Physiol 1992; 262: F236-F240.
391. Zhuo J, Alcorn D, Allen AM, Mendelsohn FA. High resolution localization of angiotensin II receptors in rat renal medulla. Kidney Int 1992; 42: 1372-1380.
392. Lafayette RA, Mayer G, Meyer TW. The effects of blood pressure reduction on cyclosporine nephrotoxicity in the rat. J Am Soc Nephrol 1993; 3: 1892-1899.
393. Lassila M, Finckenberg P, Pere AK, Krogerus L, Ahonen J, Vapaatalo H, Nurminen ML. Comparison of enalapril and valsartan in cyclosporine A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats on high-sodium diet. Br J Pharmacol 2000; 130: 1339-1347.
394. Johnson DW, Saunders HJ, Vesey DA, Qi W, Field MJ, Pollock CA. Enalaprilat directly ameliorates *in vitro* cyclosporin nephrotoxicity in human tubulo-interstitial cells. Nephron 2000; 86: 473-481.
395. Yang CW, Ahn HJ, Kim WY, Shin MJ, Kim SK, Park JH, Kim YO, Kim YS, Kim J, Bang BK. Influence of the renin-angiotensin system on epidermal growth factor expression in normal and cyclosporine-treated rat kidney. Kidney Int 2001; 60: 847-857.
396. Islam M, Burke JF, Jr., McGowan TA, Zhu Y, Dunn SR, McCue P, Kanalas J, Sharma K. Effect of anti-transforming growth factor-β antibodies in cyclosporine-induced renal dysfunction. Kidney Int 2001; 59: 498-506.
397. Khanna A, Kapur S, Sharma V, Li B, Suthanthiran M. *In vivo* hyperexpression of transforming growth factor-β1 in mice: stimulation by cyclosporine. Transplantation 1997; 63: 1037-1039.
398. Pichler RH, Franceschini N, Young BA, Hugo C, Andoh TF, Burdmann EA, Shankland SJ, Alpers CE, Bennett WM, Couser WG. Pathogenesis of cyclosporine nephropathy: roles of angiotensin II and osteopontin. J Am Soc Nephrol 1995; 6: 1186-1196.
399. Shin GT, Khanna A, Ding R, Sharma VK, Lagman M, Li B, Suthanthiran M. *In vivo* expression of transforming growth factor-β1 in humans: stimulation by cyclosporine. Transplantation 1998; 65: 313-318.
400. Wolf G, Thaiss F, Stahl RA. Cyclosporine stimulates expression of transforming growth factor-β in renal cells. Possible mechanism of cyclosporines antiproliferative effects. Transplantation 1995; 60: 237-241.
401. Campistol JM, Inigo P, Larios S, Bescos M, Oppenheimer F. Role of transforming growth factor-β1 in the progression of chronic allograft nephropathy. Nephrol Dial Transplant 2001; 16 (Suppl 1): 114-116.
402. Shihab FS, Andoh TF, Tanner AM, Noble NA, Border WA, Franceschini N, Bennett WM. Role of transforming growth factor-β 1 in experimental chronic cyclosporine nephropathy. Kidney Int 1996; 49: 1141-1151.
403. Cuhaci B, Kumar MS, Bloom RD, Pratt B, Haussman G, Laskow DA, Alidoost M, Grotkowski C, Cahill K, Butani L, Sturgill BC, Pankewycz OG. Transforming growth factor-β levels in human allograft chronic fibrosis correlate with rate of decline in renal function. Transplantation 1999; 68: 785-790.
404. Baan CC, Balk AH, Holweg CT, van Riemsdijk IC, Maat LP, Vantrimpont PJ, Niesters HG, Weimar W. Renal failure after clinical heart transplantation is associated with the TGF-β 1 codon 10 gene polymorphism. J Heart Lung Transplant 2000; 19: 866-872.
405. Wolf G. Link between angiotensin II and TGF-β in the kidney. Miner Electrolyte Metab 1998; 24: 174-180.
406. Shihab FS, Andoh TF, Tanner AM, Bennett WM. Sodium depletion enhances fibrosis and the expression of TGF-β1 and matrix proteins in experimental chronic cyclosporine nephropathy. Am J Kidney Dis 1997; 30: 71-81.
407. Shihab FS, Bennett WM, Tanner AM, Andoh TF. Angiotensin II blockade decreases TGF-β1 and matrix proteins in cyclosporine nephropathy. Kidney Int 1997; 52: 660-673.
408. Gillum DM, Truong L, Tasby J. Characterization of the interstitial cellular infiltrate in experimental chronic cyclosporine nephropathy. Transplantation 1990; 49: 793-797.

409. Young BA, Burdmann EA, Johnson RJ, Alpers CE, Giachelli CM, Eng E, Andoh T, Bennett WM, Couser WG. Cellular proliferation and macrophage influx precede interstitial fibrosis in cyclosporine nephrotoxicity. Kidney Int 1995; 48: 439-448.
410. Benigni A, Bruzzi I, Mister M, Azzollini N, Gaspari F, Perico N, Gotti E, Bertani T, Remuzzi G. Nature and mediators of renal lesions in kidney transplant patients given cyclosporine for more than one year. Kidney Int 1999; 55: 674-685.
411. Hudkins KL, Le QC, Segerer S, Johnson RJ, Davis CL, Giachelli CM, Alpers CE. Osteopontin expression in human cyclosporine toxicity. Kidney Int 2001; 60: 635-640.
412. Ghielli M, Verstrepen WA, Nouwen EJ, De Broe ME. Inflammatory cells in renal regeneration. Ren Fail 1996; 18: 355-375.
413. Rodriguez-Iturbe B, Pons H, Herrera-Acosta J, Johnson RJ. Role of immunocompetent cells in nonimmune renal diseases. Kidney Int 2001; 59: 1626-1640.
414. Becker GJ, Hewitson TD. The role of tubulointerstitial injury in chronic renal failure. Curr Opin Nephrol Hypertens 2000; 9: 133-138.
415. Iwai N, Inagami T, Ohmichi N, Kinoshita M. Renin is expressed in rat macrophage/monocyte cells. Hypertension 1996; 27: 399-403.
416. Okamura A, Rakugi H, Ohishi M, Yanagitani Y, Takiuchi S, Moriguchi K, Fennessy PA, Higaki J, Ogihara T. Upregulation of renin-angiotensin system during differentiation of monocytes to macrophages. J Hypertens 1999; 17: 537-545.
417. Wolf G, Killen PD, Neilson EG. Cyclosporin A stimulates transcription and procollagen secretion in tubulointerstitial fibroblasts and proximal tubular cells. J Am Soc Nephrol 1990; 1: 918-922.
418. Ghiggeri GM, Altieri P, Oleggini R, Valenti F, Ginevri F, Perfumo F, Gusmano R. Cyclosporine enhances the synthesis of selected extracellular matrix proteins by renal cells "in culture". Different cell responses and phenotype characterization. Transplantation 1994; 57: 1382-1388.
419. Johnson DW, Saunders HJ, Johnson FJ, Huq SO, Field MJ, Pollock CA. Cyclosporin exerts a direct fibrogenic effect on human tubulointerstitial cells: roles of insulin-like growth factor I, transforming growth factor β1, and platelet-derived growth factor. J Pharmacol Exp Ther 1999; 289: 535-542.
420. Esposito C, Fornoni A, Cornacchia F, Bellotti N, Fasoli G, Foschi A, Mazzucchelli I, Mazzullo T, Semeraro L, Dal Canton A. Cyclosporine induces different responses in human epithelial, endothelial and fibroblast cell cultures. Kidney Int 2000; 58: 123-130.
421. Duymelinck C, Deng JT, Dauwe SE, De Broe ME, Verpooten GA. Inhibition of the matrix metalloproteinase system in a rat model of chronic cyclosporine nephropathy. Kidney Int 1998; 54: 804-818.
422. Fornoni A, Lenz O, Tack I, Potier M, Elliot SJ, Striker LJ, Striker GE. Matrix accumulation in mesangial cells exposed to cyclosporine A requires a permissive genetic background. Transplantation 2000; 70: 587-593.
423. Shihab FS, Andoh TF, Tanner AM, Yi H, Bennett WM. Expression of apoptosis regulatory genes in chronic cyclosporine nephrotoxicity favors apoptosis. Kidney Int 1999; 56: 2147-2159.
424. Thomas SE, Andoh TF, Pichler RH, Shankland SJ, Couser WG, Bennett WM, Johnson RJ. Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. Kidney Int 1998; 53: 897-908.
425. Amore A, Emancipator SN, Cirina P, Conti G, Ricotti E, Bagheri N, Coppo R. Nitric oxide mediates cyclosporine-induced apoptosis in cultured renal cells. Kidney Int 2000; 57: 1549-1559.
426. del Moral RG, Andujar M, Ramirez C, Gomez-Morales M, Masseroli M, Aguilar M, Olmo A, Arrebola F, Guillen M, Garcia-Chicano MJ, Nogales FF, O'Valle F. Chronic cyclosporin A nephrotoxicity, P-glycoprotein overexpression, and relationships with intrarenal angiotensin II deposits. Am J Pathol 1997; 151: 1705-1714.
427. Koziolek MJ, Riess R, Geiger H, Thevenod F, Hauser IA. Expression of multidrug resistance P-glycoprotein in kidney allografts from cyclosporine A-treated patients. Kidney Int 2001; 60: 156-166.
428. Shihab FS, Yi H, Bennett WM, Andoh TF. Effect of nitric oxide modulation on TGF-β1 and matrix proteins in chronic cyclosporine nephrotoxicity. Kidney Int 2000; 58: 1174-1185.
429. Hutchinson IV. An endothelin-transforming growth factor β pathway in the nephrotoxicity of immunosuppressive drugs. Curr Opin Nephrol Hypertens 1998; 7: 665-671.
430. Shihab FS, Bennett WM, Yi H, Andoh TF. Expression of vascular endothelial growth factor and its receptors Flt-1 and KDR/Flk-1 in chronic cyclosporine nephrotoxicity. Transplantation 2001; 72: 164-168.
431. Kang DH, Kim YG, Andoh TF, Gordon KL, Suga S, Mazzali M, Jefferson JA, Hughes J, Bennett W, Schreiner GF, Johnson RJ. Post-cyclosporine-mediated hypertension and nephropathy: amelioration by vascular endothelial growth factor. Am J Physiol Renal Physiol 2001; 280: F727-F736.
432. Mazzali M, Kim YG, Suga S, Gordon KL, Kang DH, Jefferson JA, Hughes J, Kivlighn SD, Lan HY, Johnson RJ. Hyperuricemia exacerbates chronic cyclosporine nephropathy. Transplantation 2001; 71: 900-905.
433. Perico N, Rossini M, Imberti O, Malanchini B, Cornejo RP, Gaspari F, Bertani T, Remuzzi G. Thromboxane receptor blockade attenuates chronic cyclosporine nephrotoxicity and improves survival in rats with renal isograft. J Am Soc Nephrol 1992; 2: 1398-1404.

434. Stillman IE, English J, Burdmann EA, Andoh TF, Franschini N, Bennett WM, Rosen S. Prednisone alters the histopathology of chronic cyclosporine nephropathy. Exp Nephrol 1997; 5: 61-68.
435. Sobh M, Sabry A, Moustafa F, Foda MA, Sally S, Ghoneim M. Effect of colchicine on chronic ciclosporin nephrotoxicity in Sprague- Dawley rats. Nephron 1998; 79: 452-457.
436. Johnson DW, Saunders HJ, Field MJ, Pollock CA. *In vitro* effects of simvastatin on tubulointerstitial cells in a human model of cyclosporin nephrotoxicity. Am J Physiol 1999; 276: F467-F475.
437. Abrass CK, Berfield AK, Stehman-Breen C, Alpers CE, Davis CL. Unique changes in interstitial extracellular matrix composition are associated with rejection and cyclosporine toxicity in human renal allograft biopsies. Am J Kidney Dis 1999; 33: 11-20.
438. Burke JF, Jr., Pirsch JD, Ramos EL, Salomon DR, Stablein DM, Van Buren DH, West JC. Long-term efficacy and safety of cyclosporine in renal-transplant recipients. N Engl J Med 1994; 331: 358-363.
439. Lewis RM. Long-term use of cyclosporine A does not adversely impact on clinical outcomes following renal transplantation. Kidney Int (Suppl) 1995; 52: S75-S78.
440. Jacobson SH, Jaremko G, Duraj FF, Wilczek HE. Renal fibrosis in cyclosporin A-treated renal allograft recipients: morphological findings in relation to renal hemodynamics. Transplant Int 1996; 9: 492-498.
441. Kon SP, Templar J, Dodd SM, Rudge CJ, Raftery MJ. Diagnostic contribution of renal allograft biopsies at various intervals after transplantation. Transplantation 1997; 63: 547-550.
442. Klintmalm G, Bohman SO, Sundelin B, Wilczek H. Interstitial fibrosis in renal allografts after 12 to 46 months of cyclosporin treatment: beneficial effect of low doses in early post- transplantation period. Lancet 1984; 2: 950-954.
443. Ruiz P, Kolbeck PC, Scroggs MW, Sanfilippo F. Associations between cyclosporine therapy and interstitial fibrosis in renal allograft biopsies. Transplantation 1988; 45: 91-95.
444. Mourad G, Vela C, Ribstein J, Mimran A. Long-term improvement in renal function after cyclosporine reduction in renal transplant recipients with histologically proven chronic cyclosporine nephropathy. Transplantation 1998; 65: 661-667.
445. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000; 342: 605-612.
446. Marcen R, Pascual J, Teruel JL, Villafruela JJ, Rivera ME, Mampaso F, Burgos FJ, Ortuno J. Outcome of cadaveric renal transplant patients treated for 10 years with cyclosporine: is chronic allograft nephropathy the major cause of late graft loss? Transplantation 2001; 72: 57-62.
447. Weir MR, Ward MT, Blahut SA, Klassen DK, Cangro CB, Bartlett ST, Fink JC. Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy. Kidney Int 2001; 59: 1567-1573.
448. Coopersmith CM, Brennan DC, Miller B, Wang C, Hmiel P, Shenoy S, Ramachandran V, Jendrisak MD, Ceriotti CS, Mohanakumar T, Lowell JA. Renal transplantation following previous heart, liver, and lung transplantation: an 8-year single-center experience. Surgery 2001; 130: 457-462.
449. Herlitz H, Lindelow B. Renal failure following cardiac transplantation. Nephrol Dial Transplant 2000; 15: 311-314.
450. Myers BD, Newton L. Cyclosporine-induced chronic nephropathy: an obliterative microvascular renal injury. J Am Soc Nephrol 1991; 2: S45-S52.
451. Bertani T, Ferrazzi P, Schieppati A, Ruggenenti P, Gamba A, Parenzan L, Mecca G, Perico N, Imberti O, Remuzzi A, Remuzzi G. Nature and extent of glomerular injury induced by cyclosporine in heart transplant patients. Kidney Int 1991; 40: 243-250.
452. Falkenhain ME, Cosio FG, Sedmak DD. Progressive histologic injury in kidneys from heart and liver transplant recipients receiving cyclosporine. Transplantation 1996; 62: 364-370.
453. Goldstein DJ, Zuech N, Sehgal V, Weinberg AD, Drusin R, Cohen D. Cyclosporine-associated end-stage nephropathy after cardiac transplantation: incidence and progression. Transplantation 1997; 63: 664-668.
454. Gonwa TA, Mai ML, Pilcher J, Johnson C, Pierson S, Capehart JE, Alivizatos PA. Stability of long-term renal function in heart transplant patients treated with induction therapy and low-dose cyclosporine. J Heart Lung Transplant 1992; 11: 926-928.
455. Goral S, Ynares C, Shyr Y, Yeoh TK, Johnson HK. Long-term renal function in heart transplant recipients receiving cyclosporine therapy. J Heart Lung Transplant 1997; 16: 1106-1112.
456. Kuo PC, Luikart H, Busse-Henry S, Hunt SA, Valantine HA, Stinson EB, Oyer PE, Scandling JD, Alfrey EJ, Dafoe DC. Clinical outcome of interval cadaveric renal transplantation in cardiac allograft recipients. Clin Transplant 1995; 9: 92-97.
457. Lewis RM, Van Buren CT, Radovancevic B, Frazier OH, Janney RP, Powers PL, Golden DL, Giannakis JG, Macris MP, Kerman RH, et al. Impact of long-term cyclosporine immunosuppressive therapy on native kidneys versus renal allografts: serial renal function in heart and kidney transplant recipients. J Heart Lung Transplant 1991; 10: 63-70.
458. Van Gelder T, Balk AH, Zietse R, Hesse C, Mochtar B, Weimar W. Renal insufficiency after heart transplantation: a case-control study. Nephrol Dial Transplant 1998; 13: 2322-2326.
459. Zietse R, Balk AH, vd Dorpel MA, Meeter K, Bos E, Weimar W. Time course of the decline in renal function in cyclosporine-treated heart transplant recipients. Am J Nephrol 1994; 14: 1-5.
460. Griffiths MH, Crowe AV, Papadaki L, Banner NR, Yacoub MH, Thompson FD, Neild GH. Cyclosporin nephrotoxicity in heart and lung transplant patients. QJM 1996; 89: 751-763.

461. Pattison JM, Petersen J, Kuo P, Valantine V, Robbins RC, Theodore J. The incidence of renal failure in one hundred consecutive heart-lung transplant recipients. Am J Kidney Dis 1995; 26: 643-648.
462. Zaltzman JS, Pei Y, Maurer J, Patterson A, Cattran DC. Cyclosporine nephrotoxicity in lung transplant recipients. Transplantation 1992; 54: 875-878.
463. Dagoo T, Backman L, Mjornstedt L, Olausson M, Friman S. Progressive renal impairment in liver transplant recipients on a cyclosporine-based protocol: 5 year follow-up with glomerular filtration rate measurements. Transplant Proc 1997; 29: 3116-3118.
464. Dische FE, Neuberger J, Keating J, Parsons V, Calne RY, Williams R. Kidney pathology in liver allograft recipients after long-term treatment with cyclosporin A. Lab Invest 1988; 58: 395-402.
465. Fisher NC, Nightingale PG, Gunson BK, Lipkin GW, Neuberger JM. Chronic renal failure following liver transplantation: a retrospective analysis. Transplantation 1998; 66: 59-66.
466. Gonwa TA, Klintmalm GB, Levy M, Jennings LS, Goldstein RM, Husberg BS. Impact of pretransplant renal function on survival after liver transplantation. Transplantation 1995; 59: 361-365.
467. Fioretto P, Steffes MW, Mihatsch MJ, Strom EH, Sutherland DE, Mauer M. Cyclosporine associated lesions in native kidneys of diabetic pancreas transplant recipients. Kidney Int 1995; 48: 489-495.
468. Nizze H, Mihatsch MJ, Zollinger HU, Brocheriou C, Gokel JM, Henry K, Sloane JP, Stovin PG. Cyclosporine-associated nephropathy in patients with heart and bone marrow transplants. Clin Nephrol 1988; 30: 248-260.
469. Dieterle A, Gratwohl A, Nizze H, Huser B, Mihatsch MJ, Thiel G, Tichelli A, Signer E, Nissen C, Speck B. Chronic cyclosporine-associated nephrotoxicity in bone marrow transplant patients. Transplantation 1990; 49: 1093-1100.
470. Miralbell R, Bieri S, Mermillod B, Helg C, Sancho G, Pastoors B, Keller A, Kurtz JM, Chapuis B. Renal toxicity after allogeneic bone marrow transplantation: the combined effects of total-body irradiation and graft-versus-host disease. J Clin Oncol 1996; 14: 579-585.
471. Feutren G, Mihatsch MJ. Risk factors for cyclosporine-induced nephropathy in patients with autoimmune diseases. International Kidney Biopsy Registry of Cyclosporine in Autoimmune Diseases. N Engl J Med 1992; 326: 1654-1660.
472. Vercauteren SB, Bosmans JL, Elseviers MM, Verpooten GA, De Broe ME. A meta-analysis and morphological review of cyclosporine-induced nephrotoxicity in auto-immune diseases. Kidney Int 1998; 54: 536-545.
473. Austin HA, III, Palestine AG, Sabnis SG, Balow JE, Preuss HG, Nussenblatt RB, Antonovych TT. Evolution of ciclosporin nephrotoxicity in patients treated for autoimmune uveitis. Am J Nephrol 1989; 9: 392-402.
474. Andersen CB, Larsen S, Elmgreen J, Tvede N, Andersen V. Cyclosporine-induced renal morphologic and immunohistologic changes in patients with chronic uveitis. APMIS 1991; 99: 576-582.
475. Lowe NJ, Wieder JM, Rosenbach A, Johnson K, Kunkel R, Bainbridge C, Bourget T, Dimov I, Simpson K, Glass E, Grabie MT. Long-term low-dose cyclosporine therapy for severe psoriasis: effects on renal function and structure. J Am Acad Dermatol 1996; 35: 710-719.
476. Messana JM, Johnson KJ, Mihatsch MJ. Renal structure and function effects after low dose cyclosporine in psoriasis patients: a preliminary report. Clin Nephrol 1995; 43: 150-153.
477. Powles AV, Cook T, Hulme B, Baker BS, Lewis HM, Thomas E, Valdimarsson H, Fry L. Renal function and biopsy findings after 5 years' treatment with low- dose cyclosporin for psoriasis. Br J Dermatol 1993; 128: 159-165.
478. Svarstad E, Helland S, Morken T, Bostad L, Myking A, Iversen BM, Ofstad J. Renal effects of maintenance low-dose cyclosporin A treatment in psoriasis. Nephrol Dial Transplant 1994; 9: 1462-1467.
479. Young EW, Ellis CN, Messana JM, Johnson KJ, Leichtman AB, Mihatsch MJ, Hamilton TA, Groisser DS, Fradin MS, Voorhees JJ. A prospective study of renal structure and function in psoriasis patients treated with cyclosporin. Kidney Int 1994; 46: 1216-1222.
480. Zachariae H, Kragballe K, Hansen HE, Marcussen N, Olsen S. Renal biopsy findings in long-term cyclosporin treatment of psoriasis. Br J Dermatol 1997; 136: 531-535.
481. Renal morphology after cyclosporin A therapy in rheumatoid arthritis patients. International Kidney Biopsy Registry of Cyclosporin (Sandimmun) in Autoimmune Diseases. Br J Rheumatol 1993; 32 (Suppl 1): 65-71.
482. Berg KJ, Forre O, Bjerkhoel F, Amundsen E, Djoseland O, Rugstad HE, Westre B. Side effects of cyclosporin A treatment in patients with rheumatoid arthritis. Kidney Int 1986; 29: 1180-1187.
483. Ludwin D, Alexopoulou I, Esdaile JM, Tugwell P. Renal biopsy specimens from patients with rheumatoid arthritis and apparently normal renal function after therapy with cyclosporine. Canadian Multicentre Rheumatology Group. Am J Kidney Dis 1994; 23: 260-265.
484. Rodriguez F, Krayenbuhl JC, Harrison WB, Forre O, Dijkmans BA, Tugwell P, Miescher PA, Mihatsch MJ. Renal biopsy findings and followup of renal function in rheumatoid arthritis patients treated with cyclosporin A. An update from the International Kidney Biopsy Registry. Arthritis Rheum 1996; 39: 1491-1498.
485. Sund S, Forre O, Berg KJ, Kvien TK, Hovig T. Morphological and functional renal effects of long-term low-dose cyclosporin A treatment in patients with rheumatoid arthritis. Clin Nephrol 1994; 41: 33-40.

486. van den Borne BE, Landewe RB, Goei The HS, Breedveld FC, Dijkmans BA. Cyclosporin A therapy in rheumatoid arthritis: only strict application of the guidelines for safe use can prevent irreversible renal function loss. Rheumatology (Oxford) 1999; 38: 254-259.
487. Landewe RB, Dijkmans BA, van der Woude FJ, Breedveld FC, Mihatsch MJ, Bruijn JA. Longterm low dose cyclosporine in patients with rheumatoid arthritis: renal function loss without structural nephropathy. J Rheumatol 1996; 23: 61-64.
488. Assan R, Timsit J, Feutren G, Bougneres P, Czernichow P, Hannedouche T, Boitard C, Noel LH, Mihatsch MJ, Bach JF. The kidney in cyclosporin A-treated diabetic patients: a long-term clinicopathological study. Clin Nephrol 1994; 41: 41-49.
489. Mihatsch MJ, Helmchen U, Casanova P, Habib R, Larsen S, Magil A, Noel LH, Rapola J, Ulrich J, Wallace AC. Kidney biopsy findings in cyclosporine-treated patients with insulin- dependent diabetes mellitus. Klin Wochenschr 1991; 69: 354-359.
490. Parving HH, Tarnow L, Nielsen FS, Rossing P, Mandrup-Poulsen T, Osterby R, Nerup J. Cyclosporine nephrotoxicity in type 1 diabetic patients. A 7-year follow-up study. Diabetes Care 1999; 22: 478-483.
491. Kolkin S, Nahman NS, Jr., Mendell JR. Chronic nephrotoxicity complicating cyclosporine treatment of chronic inflammatory demyelinating polyradiculoneuropathy. Neurology 1987; 37: 147-149.
492. Svenson K, Bohman SO, Hallgren R. Renal interstitial fibrosis and vascular changes. Occurrence in patients with autoimmune diseases treated with cyclosporine. Arch Intern Med 1986; 146: 2007-2010.
493. Clasen W, Kindler J, Mihatsch MJ, Sieberth HG. Long-term treatment of minimal-change nephrotic syndrome with cyclosporin: a control biopsy study. Nephrol Dial Transplant 1988; 3: 733-737.
494. Sieberth HG, Clasen W, Fuhs M, Ittel T, Kindler J, Mihatsch MJ. Serial kidney biopsies in patients with nephrotic syndrome treated with cyclosporin. J Autoimmun 1992; 5 (Suppl A): 355-361.
495. Meyrier A, Noel LH, Auriche P, Callard P. Long-term renal tolerance of cyclosporin A treatment in adult idiopathic nephrotic syndrome. Collaborative Group of the Societe de Nephrologie. Kidney Int 1994; 45: 1446-1456.
496. Waldo FB, Benfield MR, Kohaut EC. Therapy of focal and segmental glomerulosclerosis with methylprednisolone, cyclosporine A, and prednisone. Pediatr Nephrol 1998; 12: 397-400.
497. Habib R, Niaudet P. Comparison between pre- and posttreatment renal biopsies in children receiving ciclosporine for idiopathic nephrosis. Clin Nephrol 1994; 42: 141-146.
498. Hino S, Takemura T, Okada M, Murakami K, Yagi K, Fukushima K, Yoshioka K. Follow-up study of children with nephrotic syndrome treated with a long- term moderate dose of cyclosporine. Am J Kidney Dis 1998; 31: 932-939.
499. Ittel TH, Clasen W, Fuhs M, Kindler J, Mihatsch MJ, Sieberth HG. Long-term ciclosporine A treatment in adults with minimal change nephrotic syndrome or focal segmental glomerulosclerosis. Clin Nephrol 1995; 44: 156-162.
500. Kano K, Kyo K, Yamada Y, Ito S, Ando T, Arisaka O. Comparison between pre- and posttreatment clinical and renal biopsies in children receiving low dose ciclosporine-A for 2 years for steroid- dependent nephrotic syndrome. Clin Nephrol 1999; 52: 19-24.
501. Seikaly MG, Prashner H, Nolde-Hurlbert B, Browne R. Long-term clinical and pathological effects of cyclosporin in children with nephrosis. Pediatr Nephrol 2000; 14: 214-217.
502. Soylemezoglu O, Wild G, Dalley AJ, MacNeil S, Milford-Ward A, Brown CB, el Nahas AM. Urinary and serum type III collagen: markers of renal fibrosis. Nephrol Dial Transplant 1997; 12: 1883-1889.
503. Haas M, Meehan SM, Josephson MA, Wit EJ, Woodle ES, Thistlethwaite JR. Smooth muscle-specific actin levels in the urine of renal transplant recipients: correlation with cyclosporine or tacrolimus nephrotoxicity. Am J Kidney Dis 1999; 34: 69-84.
504. Camara NO, Matos AC, Rodrigues DA, Pereira AB, Pacheco-Silva A. Early detection of heart transplant patients with increased risk of ciclosporin nephrotoxicity. Lancet 2001; 357: 856-857.
505. Ducloux D, Fournier V, Bresson-Vautrin C, Rebibou JM, Billerey C, Saint-Hillier Y, Chalopin JM. Mycophenolate mofetil in renal transplant recipients with cyclosporine- associated nephrotoxicity: a preliminary report. Transplantation 1998; 65: 1504-1506.
506. Filler G, Gellermann J, Zimmering M, Mai I. Effect of adding Mycophenolate mofetil in paediatric renal transplant recipients with chronical cyclosporine nephrotoxicity. Transplant Int 2000; 13: 201-206.
507. Houde I, Isenring P, Boucher D, Noel R, Lachanche JG. Mycophenolate mofetil, an alternative to cyclosporine A for long-term immunosuppression in kidney transplantation ? Transplantation 2000; 70: 1251-1253.
508. Hueso M, Bover J, Seron D, Gil-Vernet S, Sabate I, Fulladosa X, Ramos R, Coll O, Alsina J, Grinyo JM. Low-dose cyclosporine and mycophenolate mofetil in renal allograft recipients with suboptimal renal function. Transplantation 1998; 66: 1727-1731.
509. Badid C, Vincent M, McGregor B, Melin M, Hadj-Aissa A, Veysseyre C, Hartmann DJ, Desmouliere A, Laville M. Mycophenolate mofetil reduces myofibroblast infiltration and collagen III deposition in rat remnant kidney. Kidney Int 2000; 58: 51-61.
510. Fujihara CK, De LN, I, Malheiros, Antunes GR, de Oliveira IB, Zatz R. Combined mycophenolate mofetil and losartan therapy arrests established injury in the remnant kidney. J Am Soc Nephrol 2000; 11: 283-290.
511. Remuzzi G, Zoja C, Gagliardini E, Corna D, Abbate M, Benigni A. Combining an antiproteinuric approach with mycophenolate mofetil fully suppresses progressive nephropathy of experimental animals. J Am Soc Nephrol 1999; 10: 1542-1549.

512. Araujo IM, Mendes GEF, Baptista MAF, Burdmann EA. Effects of mycophenolate mofetil (MMF) on chronic cyclosporine (CsA) nephrotoxicity. J Am Soc Nephrol 2001; 12: 797A.
513. McCulloch TA, Harper SJ, Donnelly PK, Moorhouse J, Bell PR, Walls J, Feehally J, Furness PN. Influence of nifedipine on interstitial fibrosis in renal transplant allografts treated with cyclosporin A. J Clin Pathol 1994; 47: 839-842.
514. Campistol JM, Inigo P, Jimenez W, Lario S, Clesca PH, Oppenheimer F, Rivera F. Losartan decreases plasma levels of TGF-β1 in transplant patients with chronic allograft nephropathy. Kidney Int 1999; 56: 714-719.
515. Heering P, Ivens K, Aker S, Grabensee B. Distal tubular acidosis induced by FK 506. Clin Transplant 1998; 12: 465-471.
516. McCauley J. The nephrotoxicity of FK 506 as compared with cyclosporine. Curr Opin Nephrol Hypertens 1993; 2: 662-669.
517. Morales JM, Andres A, Rengel M, Rodicio JL. Influence of cyclosporin, tacrolimus and rapamycin on renal function and arterial hypertension after renal transplantation. Nephrol Dial Transplant 2001; 16 (Suppl 1): 121-124.
518. O'Gorman MA, Fivush B, Wise B, Colombani P, Burdick J, Schwarz KB. Proximal renal tubular acidosis secondary to FK 506 in pediatric liver transplant patients. Clin Transplant 1995; 9: 312-316.
519. Plosker GL, Foster RH. Tacrolimus: a further update of its pharmacology and therapeutic use in the management of organ transplantation. Drugs 2000; 59: 323-389.
520. Sakamoto K, Yamada K, Arita S, Hamaguchi K, Kashiwabara H, Yokoyama T. Sodium-losing nephropathy and distal tubular damage of transplant kidneys with FK 506 administration. Transplant Proc 1995; 27: 826-828.
521. Finn WF. FK 506 nephrotoxicity. Renal Failure 1999; 21: 319-329.
522. Chenhsu RY, Loong CC, Chou MH, Lin MF, Yang WC. Renal allograft dysfunction associated with rifampin-tacrolimus interaction. Ann Pharmacother 2000; 34: 27-31.
523. Gomez G, Alvarez ML, Errasti P, Lavilla FJ, Garcia N, Ballester B, Garcia I, Purroy A. Acute tacrolimus nephrotoxicity in renal transplant patients treated with clarithromycin. Transplant Proc 1999; 31: 2250-2251.
524. Ocran KW, Plauth M, Mai I, Lochs H. Tacrolimus toxicity due to drug interaction with mibefradil in a patient after liver transplantation. Z Gastroenterol 1999; 37: 1025-1028.
525. Olyaei AJ, deMattos AM, Norman DJ, Bennett WM. Interaction between tacrolimus and nefazodone in a stable renal transplant recipient. Pharmacotherapy 1998; 18: 1356-1359.
526. Sheiner PA, Mor E, Chodoff L, Glabman S, Emre S, Schwartz ME, Miller CM. Acute renal failure associated with the use of ibuprofen in two liver transplant recipients on FK 506. Transplantation 1994; 57: 1132-1133.
527. Ahsan N, Johnson C, Gonwa T, Halloran P, Stegall M, Hardy M, Metzger R, Shield C, III, Rocher L, Scandling J, Sorensen J, Mulloy L, Light J, Corwin C, Danovitch G, Wachs M, VanVeldhuisen P, Salm K, Tolzman D, Fitzsimmons WE. Randomized trial of tacrolimus plus mycophenolate mofetil or azathioprine versus cyclosporine oral solution (modified) plus mycophenolate mofetil after cadaveric kidney transplantation: results at 2 years. Transplantation 2001; 72: 245-250.
528. Katari SR, Magnone M, Shapiro R, Jordan M, Scantlebury V, Vivas C, Gritsch A, McCauley J, Starzl T, Demetris AJ, Randhawa PS. Clinical features of acute reversible tacrolimus (FK 506) nephrotoxicity in kidney transplant recipients. Clin Transplant 1997; 11: 237-242.
529. Mayer AD, Dmitrewski J, Squifflet JP, Besse T, Grabensee B, Klein B, Eigler FW, Heemann U, Pichlmayr R, Behrend M, Vanrenterghem Y, Donck J, van Hooff J, Christiaans M, Morales JM, Andres A, Johnson RW, Short C, Buchholz B, Rehmert N, Land W, Schleibner S, Forsythe JL, Talbot D, Neumayer H-H, Hauser I, Ericzon B-G, Brattstrom C, Claesson K, Muhlbacher F, Pohanka E. Multicenter randomized trial comparing tacrolimus (FK 506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. Transplantation 1997; 64: 436-443.
530. Mor E, Patel T, Glabman S, Sheiner P, Emre S, Guy S, Schwartz M, Miller C. Comparison of short and long-term renal function in liver transplant patients receiving cyclosporin or FK 506. Transplant Int 1994; 7 (Suppl 1): S77-S80.
531. Porayko MK, Textor SC, Krom RA, Hay JE, Gores GJ, Richards TM, Crotty PH, Beaver SJ, Steers JL, Wiesner RH. Nephrotoxic effects of primary immunosuppression with FK-506 and cyclosporine regimens after liver transplantation. Mayo Clin Proc 1994; 69: 105-111.
532. Porayko MK, Gonwa TA, Klintmalm GB, Wiesner RH. Comparing nephrotoxicity of FK 506 and cyclosporine regimens after liver transplantation: preliminary results from US Multicenter trial. U.S. Multicenter Liver Study Group. Transplant Proc 1995; 27: 1114-1116.
533. Vincenti F, Laskow DA, Neylan JF, Mendez R, Matas AJ. One-year follow-up of an open-label trial of FK 506 for primary kidney transplantation. A report of the U.S. Multicenter FK 506 Kidney Transplant Group. Transplantation 1996; 61: 1576-1581.
534. Burke GW, Ciancio G, Cirocco R, Markou M, Olson L, Contreras N, Roth D, Esquenazi V, Tzakis A, Miller J. Microangiopathy in kidney and simultaneous pancreas/kidney recipients treated with tacrolimus: evidence of endothelin and cytokine involvement. Transplantation 1999; 68: 1336-1342.
535. Gharpure VS, Devine SM, Holland HK, Geller RB, O'Toole K, Wingard JR. Thrombotic thrombocytopenic purpura associated with FK 506 following bone marrow transplantation. Bone Marrow Transplant 1995; 16: 715-716.
536. Schmidt RH, Lenz T, Grone HJ, Geiger H, Scheuermann EH. Haemolytic-uraemic syndrome after tacrolimus rescue therapy for cortisone-resistant rejection. Nephrol Dial Transplant 1999; 14: 979-983.

537. Schmidt RJ, Venkat KK, Dumler F. Hemolytic-uremic syndrome in a renal transplant recipient on FK 506 immunosuppression. Transplant Proc 1991; 23: 3156-3157.
538. Pham PT, Peng A, Wilkinson AH, Gritsch HA, Lassman C, Pham PC, Danovitch GM. Cyclosporine and tacrolimus-associated thrombotic microangiopathy. Am J Kidney Dis 2000; 36: 844-850.
539. Abdalla AH, al Sulaiman MH, al Khader AA. FK 506 as an alternative in cyclosporin-induced hemolytic uremic syndrome in a kidney transplant recipient. Transplant Int 1994; 7: 382-384.
540. McCauley J, Bronsther O, Fung J, Todo S, Starzl TE. Treatment of cyclosporin-induced haemolytic uraemic syndrome with FK 506. Lancet 1989; 2: 1516.
541. Abraham KA, Little MA, Dorman AM, Walshe JJ. Hemolytic-uremic syndrome in association with both cyclosporine and tacrolimus. Transplant Int 2000; 13: 443-447.
542. Textor SC, Wiesner R, Wilson DJ, Porayko M, Romero JC, Burnett JC, Jr., Gores G, Hay E, Dickson ER, Krom RA. Systemic and renal hemodynamic differences between FK 506 and cyclosporine in liver transplant recipients. Transplantation 1993; 55: 1332-1339.
543. McDiarmid SV, Colonna JO, Shaked A, Ament ME, Busuttil RW. A comparison of renal function in cyclosporine- and FK-506-treated patients after primary orthotopic liver transplantation. Transplantation 1993; 56: 847-853.
544. Asante-Korang A, Boyle GJ, Webber SA, Miller SA, Fricker FJ. Experience of FK 506 immune suppression in pediatric heart transplantation: a study of long-term adverse effects. J Heart Lung Transplant 1996; 15: 415-422.
545. Reichart B, Meiser B, Vigano M, Rinaldi M, Martinelli L, Yacoub M, Banner NR, Gandjbakhch I, Dorent R, Hetzer R, Hummel M. European Multicenter Tacrolimus (FK 506) Heart Pilot Study: one-year results—European Tacrolimus Multicenter Heart Study Group. J Heart Lung Transplant 1998; 17: 775-781.
546. Taylor DO, Barr ML, Radovancevic B, Renlund DG, Mentzer RM, Jr., Smart FW, Tolman DE, Frazier OH, Young JB, VanVeldhuisen P. A randomized, multicenter comparison of tacrolimus and cyclosporine immunosuppressive regimens in cardiac transplantation: decreased hyperlipidemia and hypertension with tacrolimus. J Heart Lung Transplant 1999; 18: 336-345.
547. Griffith BP, Bando K, Hardesty RL, Armitage JM, Keenan RJ, Pham SM, Paradis IL, Yousem SA, Komatsu K, Konishi H, Fung JJ, Starzl TE. A prospective randomized trial of FK 506 versus cyclosporine after human pulmonary transplantation. Transplantation 1994; 57: 848-851.
548. Kur F, Reichenspurner H, Meiser BM, Welz A, Furst H, Muller C, Vogelmeier C, Schwaiblmaier M, Briegel J, Reichart B. Tacrolimus (FK 506) as primary immunosuppressant after lung transplantation. Thorac Cardiovasc Surg 1999; 47: 174-178.
549. Andoh TF, Burdmann EA, Lindsley J, Houghton DC, Bennett WM. Functional and structural characteristics of experimental FK 506 nephrotoxicity. Clin Exp Pharmacol Physiol 1995; 22: 646-654.
550. Hadad SJ, Souza ER, Ferreira AT, Oshiro ME, Boim MA, Razvickas CV, Moura LA, Schor N. FK 506: effects on glomerular hemodynamics and on mesangial cells in culture. Kidney Int 1995; 48: 56-64.
551. Kumano K, Chen J, He M, Endo T, Masaki Y. Role of endothelin in FK 506-induced renal hypoperfusion in rats. Transplant Proc 1995; 27: 550-553.
552. Mugiya S, Fujita K, Malyszko J, Takada Y, Takada A. Renal blood flow and serotonin metabolism in tacrolimus treated rats. Int J Urol 1996; 3: 187-190.
553. Nielsen FT, Leyssac PP, Kemp E, Starklint H, Dieperink H. Nephrotoxicity of FK-506 in the rat. Studies on glomerular and tubular function, and on the relationship between efficacy and toxicity. Nephrol Dial Transplant 1995; 10: 334-340.
554. Ueda D, Suzuki K, Kawabe K. Influence of FK 506 on renal blood flow. Transplant Proc 1993; 25: 2722-2723.
555. Seifeldin R, Marcos-Alvarez A, Lewis WD, Gordon FD, Jenkins RL. Effect of nifedipine on renal function in liver transplant recipients receiving tacrolimus. Clin Ther 1996; 18: 491-496.
556. Andoh TF, Burdmann EA, Houghton DC, Lindsley J, Bennett WM. Protective effects of nilvadipine on experimental acute FK 506 nephrotoxicity. J Am Soc Nephrol 1993; 4: 760.
557. Mitamura T, Yamada A, Ishida H, Fujihira S, Ohara K, Noguchi H, Mine Y. Tacrolimus (FK 506)-induced nephrotoxicity in spontaneous hypertensive rats. J Toxicol Sci 1994; 19: 219-226.
558. Tada H, Yanagiwara S, Ito K, Suzuki T. Role of diltiazem on tacrolimus pharmacokinetics in tacrolimus-induced nephrotoxic rats. Pharmacol Toxicol 1999; 84: 241-246.
559. Mihatsch MJ, Kyo M, Morozumi K, Yamaguchi Y, Nickeleit V, Ryffel B. The side-effects of ciclosporine-A and tacrolimus. Clin Nephrol 1998; 49: 356-363.
560. Shimizu T, Tanabe K, Tokumoto T, Ishikawa N, Shinmura H, Oshima T, Toma H, Yamaguchi Y. Clinical and histological analysis of acute tacrolimus (TAC) nephrotoxicity in renal allografts. Clin Transplant 1999; 13 (Suppl 1): 48-53.
561. Benigni A, Morigi M, Perico N, Zoja C, Amuchastegui CS, Piccinelli A, Donadelli R, Remuzzi G. The acute effect of FK 506 and cyclosporine on endothelial cell function and renal vascular resistance. Transplantation 1992; 54: 775-780.
562. Bagnis C, Deray G, Dubois M, Adabra Y, Jacquiaud C, Jaudon MC, Jacobs C. Comparative acute nephrotoxicity of FK-506 and ciclosporin in an isolated in situ autoperfused rat kidney model. Am J Nephrol 1997; 17: 17-24.

563. Andoh TF, Burdmann EA, Lindsley J, Houghton DC, Bennett WM. Enhancement of FK 506 nephrotoxicity by sodium depletion in an experimental rat model. Transplantation 1994; 57: 483-489.
564. Stillman IE, Andoh TF, Burdmann EA, Bennett WM, Rosen S. FK 506 nephrotoxicity: morphologic and physiologic characterization of a rat model. Lab Invest 1995; 73: 794-803.
565. Su Q, Weber L, Le Hir M, Zenke G, Ryffel B. Nephrotoxicity of cyclosporin A and FK 506: inhibition of calcineurin phosphatase. Ren Physiol Biochem 1995; 18: 128-139.
566. Yamada K, Sugisaki Y, Akimoto M, Yamanaka N. FK 506-induced juxtaglomerular apparatus hyperplasia and tubular damage in rat kidney - morphologic and biologic analysis. Transplant Proc 1992; 24: 1396-1398.
567. Kumano K, Wang G, Endo T, Kuwao S. FK 506-induced nephrotoxicity in rats. Transplant Proc 1991; 23: 512-515.
568. McCauley J, Studer R, Craven P, Murray S. The effects of cyclosporine A, cyclosporine G, and FK 506 upon prostaglandin production in renal mesangial cells in culture. Transplant Proc 1991; 23: 3141-3142.
569. Moutabarrik A, Ishibashi M, Fukunaga M, Kameoka H, Kawaguchi N, Takano Y, Kokado Y, Sonoda T, Onishi S, Takahara S, Okuyama A. FK 506-induced kidney tubular cell injury. Transplantation 1992; 54: 1041-1047.
570. Goodall T, Kind CN, Hammond TG. FK 506-induced endothelin release by cultured rat mesangial cells. J Cardiovasc Pharmacol 1995; 26 (Suppl 3): S482-S485.
571. Haug C, Grill C, Schmid-Kotsas A, Gruenert A, Jehle PM. Endothelin release by rabbit proximal tubule cells: modulatory effects of cyclosporine A, tacrolimus, HGF and EGF. Kidney Int 1998; 54: 1626-1636.
572. Nakahama H, Fukunaga M, Kakihara M, Horio M, Fujiwara Y, Fukuhara Y, Ueda N, Orita Y, Kamada T. Comparative effects of cyclosporine A and FK-506 on endothelin secretion by a cultured renal cell line, LLC-PK1. J Cardiovasc Pharmacol 1991; 17 (Suppl 7): S172-S173.
573. Takeda Y, Yoneda T, Ito Y, Miyamori I, Takeda R. Stimulation of endothelin mRNA and secretion in human endothelial cells by FK 506. J Cardiovasc Pharmacol 1993; 22 (Suppl 8): S310-S312.
574. Marsen TA, Weber F, Egink G, Suckau G, Baldamus CA. Differential transcriptional regulation of endothelin-1 by immunosuppressants FK 506 and cyclosporin A. Fundam Clin Pharmacol 2000; 14: 401-408.
575. Uchida J, Miura K, Yamanaka S, Kim S, Iwao H, Nakatani T, Kishimoto T. Renal endothelin in FK 506-induced nephrotoxicity in spontaneously hypertensive rats. Jpn J Pharmacol 1998; 76: 39-49.
576. Burdmann EA, Mattar AL, Fujihara C, Andoh TF, Zatz R, Bennett WM. Effects of an endothelin Et_A receptor antagonist on experimental FK 506-induced acute renal dysfunction. J Am Soc Nephrol 1994; 5: 918.
577. Dawson TM, Steiner JP, Dawson VL, Dinerman JL, Uhl GR, Snyder SH. Immunosuppressant FK 506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. Proc Natl Acad Sci USA 1993; 90: 9808-9812.
578. Akita K, Dusting GJ, Hickey H. Suppression of nitric oxide production by cyclosporin A and FK 506A in rat vascular smooth muscle cells. Clin Exp Pharmacol Physiol 1994; 21: 231-233.
579. Conde M, Andrade J, Bedoya FJ, Santa MC, Sobrino F. Inhibitory effect of cyclosporin A and FK 506 on nitric oxide production by cultured macrophages. Evidence of a direct effect on nitric oxide synthase activity. Immunology 1995; 84: 476-481.
580. Strestikova P, Otova B, Filipec M, Masek K, Farghali H. Different mechanisms in inhibition of rat macrophage nitric oxide synthase expression by FK 506 and cyclosporin A. Immunopharmacol Immunotoxicol 2001; 23: 67-74.
581. Wang L, Kubodera S, Ueno A, Takeda M. Effects of nitric oxide synthesis inhibition on FK 506-induced nephrotoxicity in rats. Renal Failure 2001; 23: 11-19.
582. Hortelano S, Castilla M, Torres AM, Tejedor A, Bosca L. Potentiation by nitric oxide of cyclosporin A and FK 506-induced apoptosis in renal proximal tubule cells. J Am Soc Nephrol 2000; 11: 2315-2323.
583. De Lima JJ, Xue H, Coburn L, Andoh TF, McCarron DA, Bennett WM, Roullet JB. Effects of FK 506 in rat and human resistance arteries. Kidney Int 1999; 55: 1518-1527.
584. Attur MG, Patel R, Thakker G, Vyas P, Levartovsky D, Patel P, Naqvi S, Raza R, Patel K, Abramson D, Bruno G, Abramson SB, Amin AR. Differential anti-inflammatory effects of immunosuppressive drugs: cyclosporin, rapamycin and FK-506 on inducible nitric oxide synthase, nitric oxide, cyclooxygenase-2 and PGE2 production. Inflamm Res 2000; 49: 20-26.
585. Lopau K, Kleinert D, Erler J, Schramm L, Heidbreder E, Wanner C. Tacrolimus in acute renal failure: does L-arginine-infusion prevent changes in renal hemodynamics ? Transplant Int 2000; 13: 436-442.
586. Tome LA, Castro I, Yu L, Andoh TF, Seguro AC, Burdmann EA. Role of nitric oxide (NO) on FK 506-induced acute nephrotoxicity - *in vivo* and *in vitro* studies. J Am Soc Nephrol 1996; 7: 1897.
587. Mugiya S, Suzuki K, Ueda D, Ohtawara Y, Fujita K, Malyszko J, Urano T, Knofler R, Takada Y, Takada A. Renal blood flow, fibrinolysis, and platelet aggregation following tacrolimus (FK 506) treatment in rats. Thromb Res 1995; 79: 337-341.
588. Zhang W, Victor RG. Calcineurin inhibitors cause renal afferent activation in rats: a novel mechanism of cyclosporine-induced hypertension. Am J Hypertens 2000; 13: 999-1004.
589. Edkins RD, Rybak LP, Hoffman DW. Comparison of cyclosporine and FK 506 effects on glutathione levels in rat cochlea, brain, liver and kidney. Biochem Pharmacol 1992; 43: 911-913.

590. Jain A, Reyes J, Kashyap R, Rohal S, Abu-Elmagd K, Starzl T, Fung J. What have we learned about primary liver transplantation under tacrolimus immunosuppression ? Long-term follow-up of the first 1000 patients. Ann Surg 1999; 230: 441-448.
591. Ryffel B, Weber E, Mihatsch MJ. Nephrotoxicity of immunosuppressants in rats: comparison of macrolides with cyclosporin. Exp Nephrol 1994; 2: 324-333.
592. Lea JP, Sands JM, McMahon SJ, Tumlin JA. Evidence that the inhibition of Na+/K(+)-ATPase activity by FK 506 involves calcineurin. Kidney Int 1994; 46: 647-652.
593. Blaehr H, Andersen CB, Ladefoged J. Acute effects of FK 506 and cyclosporine A on cultured human proximal tubular cells. Eur J Pharmacol 1993; 228: 283-288.
594. Cuvello Neto AL, Castro I, Costa MC, Ferreira AT, Burdmann EA, Yu L. Tacrolimus (FK-506)-induced tubular toxicity: role of intracellular calcium, nitric oxide (NO) and hydroperoxides. J Am Soc Nephrol 2001; 12: 799A.
595. Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ. The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK 506. Clinical significance and comparison with cyclosporine. Am J Surg Pathol 1993; 17: 60-68.
596. Hatori M, Takahara S, Kyo M, Kokado Y, Nagano S, Okuyama A. Clinical and histopathologic examination of renal allografts treated with tacrolimus (FK 506) for at least one year. Int J Urol 1998; 5: 526-533.
597. Kyo M, Hatori M, Takahara S, Kyakuno M, Nakamura T, Okada M, Kokado Y, Toki K, Ding XQ, Miki T, Miyamoto M, Okuyama A. Morphological findings in non-episode biopsies of kidney transplant allografts treated with FK 506 or cyclosporine. Transplant Int 1998; 11 (Suppl 1): S100-S103.
598. Sugito K, Morozumi K, Oikawa T. A comparative study of clinicopathologic characteristics of renal allografts under cyclosporine and FK 506 immunosuppression. Transplant Proc 1996; 28: 1335-1337.
599. Solez K, Vincenti F, Filo RS. Histopathologic findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tarolimus versus cyclosporine: a report of the FK 506 Kidney Transplant Study Group. Transplantation 1998; 66: 1736-1740.
600. Shihab FS, Bennett WM, Tanner AM, Andoh TF. Mechanism of fibrosis in experimental tacrolimus nephrotoxicity. Transplantation 1997; 64: 1829-1837.
601. Andoh TF, Burdmann EA, Houghton DC, Lindsley J, Franceschini N, Bennett WM. Calcium antagonist (CA) versus angiotensin II antagonist (ATA) in a rat model of chronic FK 506 nephrotoxicity. J Am Soc Nephrol 1994; 5: 915.
602. Muraoka K, Fujimoto K, Sun X, Yoshioka K, Shimizu K, Yagi M, Bose H, Jr., Miyazaki I, Yamamoto K. Immunosuppressant FK 506 induces interleukin-6 production through the activation of transcription factor nuclear factor (NF)-kappa(B). Implications for FK 506 nephropathy. J Clin Invest 1996; 97: 2433-2439.
603. Jain S, Bicknell GR, Nicholson ML. Tacrolimus has less fibrogenic potential than cyclosporin A in a model of renal ischaemia-reperfusion injury. Br J Surg 2000; 87: 1563-1568.
604. Bicknell GR, Williams ST, Shaw JA, Pringle JH, Furness PN, Nicholson ML. Differential effects of cyclosporin and tacrolimus on the expression of fibrosis-associated genes in isolated glomeruli from renal transplants. Br J Surg 2000; 87: 1569-1575.
605. Morris-Stiff G, Baboolal K, Singh J, Balaji V, Ostrowski K, Moore R, Lord R, Jurewicz A. Conversion from cyclosporine to tacrolimus in renal allograft recipients with chronic graft nephropathy: preliminary report. Transplant Proc 1998; 30: 1245-1246.
606. Lieberthal W, Fuhro R, Andry CC, Rennke H, Abernathy VE, Koh JS, Valeri R, Levine JS. Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. Am J Physiol Renal Physiol 281:F693-F706, 2001.

22

Immunomodulators: interleukins, interferons, and the OKT3 monoclonal antibody

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Introduction

The term cytokines includes interleukins, chemokines, growth factors, colony stimulating factors, and tumor necrosis factors (TNF). These molecules are involved in cell injury and repair, inflammation and its regulation, and apoptosis.

In the first part of this chapter, we review the renal toxicity of the cytokines currently in clinical use. In the second part, we describe the cytokine-mediated nephrotoxicity associated with the use of the OKT3 monoclonal antibody in transplant recipients.

Cytokine associated renal dysfunction is regularly observed in the setting of sepsis syndrome or systemic inflammatory response syndrome. Systemic inflammatory response syndrome is often used as a model for evaluating the renal effects of various cytokines. During systemic inflammatory response syndrome, it has been observed that even in the absence of systemic hypotension, acute tubular necrosis can occur. Certain cytokines are released during systemic inflammatory response syndrome that mediate peripheral vasodila-

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tion in the absence of systemic hypotension. The renal response to peripheral vasodilation is renal vasoconstriction and reduced renal blood flow. One can appreciate that in the setting of renal ischemia, it is difficult to conclusively attribute the etiology of acute tubular necrosis to the direct nephrotoxic effects of cytokines much less any individual cytokine among the cascade of mediators which produce the shock syndrome. The cytokine release syndrome associated with OKT3 administration, as discussed later in this chapter, is similar to systemic inflammatory response syndrome. In both syndromes, TNF-α is the initiator or central mediator of the cytokine cascade.

The crucial role of TNF-alpha in cytokine-associated renal dysfunction

Tumor necrosis factor α (TNF-α) is a pro-inflammatory cytokine which augments its own production and the synthesis of other inflammatory mediators. It stimulates the pyrogen, IL-1β and regulates genes coding for other inflammatory mediators such as IL-1, IL-6, IL-8, and macrophage inflammatory protein. Endotoxin is the most studied stimulator of TNF-α but other cytokines, various phospholipases and protein kinases, and some toxic agents also participate in its activation. There are two TNF-α receptors and two distinct families of adaptor proteins or TNF-α receptor-associated factors that can activate different cytosolic signaling cascades (death domain homologs and signaling pathways such as mitogen-activated protein kinase and extracellular signal-regulated kinase) leading to activation of a variety of nuclear transcription factors, especially NF-kappaB [1]. The plasticity of this system helps to explain the number and complexity of TNF-α activities (Table 1).

TNF-α and interleukin-1 (IL-1) are the two major cytokines implicated in the pathogenesis of systemic inflammatory response syndrome. Inhibition of these cytokines with anti-cytokine antibodies or receptor deficient knockout mice is associated with prevention of renal injury, but the mechanism may be simply preventing hypotension. The data do not support a direct role of IL-1 in the pathogenesis of acute renal failure

Table 1. TNF-α actions [2].

1. Induces synthesis of active proteins by causing changes in gene expression.
2. Induces both cell necrosis and cell apoptosis
3. Mitogenic for fibroblasts, hepatocytes, smooth muscle cells, and lymphocytes.
4. Increases natural killer, endothelial, macrophage/monocyte, neutrophil, and lymphocyte cell activity.
5. Stimulates production of adrenocorticotrophic hormone and thyroid stimulating hormone.
6. Stimulates muscle cell glycolysis and glycogenesis
7. Stimulates synovial fluid synthesis of prostaglandine-D2, plasminogen activator, collagenase, hyaluronic acid
8. Major role in inflammation by providing cell signals and regulating genes that code for IL-1, IL-6, IL-8, macrophage inflammatory protein, granulocyte macrophage-colony stimulating factor, intercellular adhesion molecule 1 (ICAM-1), and endothelial leukocyte adhesion molecule 1 (ECAM-1)

Table 2. Tumor necrosis factor-mediated renal diseases [3].

1. Ischemia and reperfusion-induced acute tubular necrosis
2. Endotoxin induced acute renal failure
3. Renal injury models such as aminonucleoside-induced nephrosis and adriamycin-induced nephrosis
4. Anti-glomerular basement membrane glomerulonephritis
5. Immune complex glomerulonephritis
6. Focal proliferative and exudative glomerulonephritis
7. Lupus nephritis
8. Diabetic nephropathy

during sepsis. IL-1 infusions do not alter GFR or renal blood flow or blood pressure [4]. Very high doses of systemic TNF-α can cause renal injury as observed by Bertani in an experimental rabbit model. He showed that an intravenous TNF-α infusion did induce glomerular endothelial damage, neutrophil accumulation, and fibrin deposition within capillary lumens [5].

While systemically administer TNF-α and IL-1 does not appear to have a direct effect on the kidney, they may stimulate local synthesis of cytokines. Giroir et al. showed that endotoxin infusions in mice increased TNF-α synthesis in the kidney [6]. Other investigators have shown that TNF-α and IL-1 induce glomerular endothelial and mesangial cell production of vasoconstrictive mediators, platelet activating factor, endothelin-1 and adenosine plus release of the vasodilators nitric oxide and prostaglandin E2. Local release of TNF-α reduces glomerular blood flow and glomerular filtration rate, induces the synthesis of other proinflammatory mediators, and, along with reactive oxygen species, increases glomerular albumin permeability. TNF-α recruits neutrophils and monocytes to the kidney and enhances their adhesion to glomerular cells and induces expression of intercellular adhesion molecules and ligands for L-selectin. Thus TNF-α promotes aggregation and adhesion of neutrophils and monocytes to glomerular cells and release of other toxic products. TNF-α induces production of reactive oxygen species by mesangial cells and tissue factor production by mesangial and endothelial cells, leading to fibrin deposition. Reactive oxygen species and IL-1 activate transcription factors involved in inflammatory gene expression [7, 8].

Local over-expression of cytokines, especially TNF-α and IL-1, are involved in many of the structural and functional changes in inflammatory kidney diseases [9, 10, 11]. Ischemia-reperfusion induces TNF-α production not only in the kidney, but also in liver, heart, lung and brain. Ischemia also results in monocyte infiltration into the kidney and may be the initial source of TNF-α. TNF-α has been shown to decrease glomerular blood flow, increase basement membrane permeability, activate glomerular endothelial and mesangial cells, and induce proteinuria and tubulointerstitial nephritis [12]. At one time it was thought that infiltrating monocytes were the only source of TNF-α. It is now known that glomerular mesangial cells and possibly proximal convoluted tubule cells are a local source of TNF-α [3].

How does one unscramble the direct effects or indirect effects of various cytokines when the pathways are numerous, complex and inter-related and individual cytokines may have opposite roles depending on the biologic setting? Many of these cytokines have been produced by recombinant technology and are used clinically in the treatment of cancer, autoimmune disease, viral disease (e.g. hepatitis B and C), and even some types of glomerulonephritis [13].

This chapter will examine the nephrotoxic potential of the cytokines or monoclonal antibodies at the doses being used in the treatment of cancer, autoimmune diseases, and transplantation. Non-specific immunomodulators and highly specific monoclonal antibodies are being used singly or in various combinations to treat cancer, autoimmune disease, and solid organ transplants. Many of these therapies achieve their effect by stimulating the release of cytokines or release cytokines as a by-product of therapy.

Adjuvants and immunomodulators

Melatonin: T-helper cells bear G-protein coupled melatonin cell membrane receptors and activation of melatonin receptors, induce the release of T-helper cell type 1 (Th1) cytokines such as interferon-γ (IFN-γ) and IL-2. Melatonin also stimulates the release of novel opioid cytokines, which cross-react immunologically with both IL-4 and dynorphin B. Melatonin may enhance the production of IL-6 from human monocytes. Conversely, IFN-γ and various colony-stimulating factors may feedback to modulate melatonin production by the pineal gland [14].

European Mistletoe (Viscum album) extract: {Iscador Qu Spezial or Helixor (aqueous VAL extract) or Lectin-1 (galactoside-specific lectin)}. The various extracts of Viscum album (VAL) differ in composition and immunomodulatory effect. Some of these extracts stimulate release of γ-IFN, IL-4, TNF-α, IL-12 and IL-6 and are used as unconventional adjuvant therapy in the treatment of HIV and cancer [15-18]. Controlled studies of the galactoside-specific lectin component showed no evidence of cellular reaction in bladders with urothelial carcinoma [19]. The specific agents contained in the extract that are purported to have an antitumor effect have not yet been characterized.

Non-specific adjuvant: boosts the cellular and humoral

immune response at the local or systemic level by unknown mechanisms, which result in cytokine release [20]. These non-specific adjuvants include:

- Bacillus Calmette-Guerin (BCG): BCG is used in the treatment of multiple sclerosis and superficial transitional cell carcinoma.
- Corynebacterium parvum
- Incomplete Freunds adjuvant
- Keyhole Limpet Hemocyanin

Peptide Vaccines: tumor-specific antigens derived from the tumor tissue itself are used as vaccines against the tumor. This therapy has limited success because of the development of antigen-loss tumor variants and impaired HLA class I expression and antigen presentation [20].

Bcr-abl fusion peptide: vaccine against chronic myelogenous leukemia which generates prolonged peptide-specific T-cell proliferative responses and delayed type hypersensitivity responses that initiate cytokine release [21].

Immunoglobulins and monoclonal antibodies

Intravenous immunoglobulin: modulates cytokine production and downregulates IL-1, IL-2, IL-3, IL-4, IL-5, IL-10, TNF-α, and granulocyte-macrophage colony-stimulating factor. However, flushing, myalgia, headache, fever, chills, wheezing, hypotension and tachycardia have been noted after the start of the infusion and have been attributed to activation of complement and the complement cascade. Reversible increases in serum creatinine occurred in 4/17 patients treated for ANCA-associated vasculitis [22]. Acute renal failure has been reported with IV immunoglobulin and is thought to be related to a high solute load-induced injury to the proximal tubule. Generally, the injury is reversible [23, 24]. More severe acute renal failure was noted in a patient who had underlying mixed cryoglobulinemia [25]. Other studies have confirmed the notion that I.V. immunoglobulin infusions are more likely to result in acute renal failure in the presence of underlying renal disease or in combination with such drugs as non-steroidals and angiotensin converting enzyme inhibitors [26].

Monoclonal Antibody Therapy

Monoclonal antibodies can vary tremendously in terms of isotype, construction (animal derived, chimeric, humanized, bound to toxin), ability to activate complement, binding avidity, target specificity, and whether it binds and blocks or binds and activates the receptor. Monoclonal antibodies may be directed toward soluble or membrane bound receptors or receptor ligands, tumor antigens, growth factor or their receptors. Therefore the toxicity and side effects are equally variable [27].

In general, complement-binding monoclonal antibodies are more likely to cause a first dose response with cytokine release and potentially renal failure.

Monoclonal antibodies that are associated with systemic response consistent with cytokine release include:

- *Herceptin (trastuazmab):* recombinant, humanized, monoclonal antibody against HER2/neu (c-erb-B2), which is a glycoprotein receptor with intrinsic tyrosine kinase activity that is over-expressed in a percentage of patients with breast cancer. Side effects worsen at higher doses: fever, nausea, vomiting, diarrhea, rash and headaches [28].
- *Anti-CD-20 antibodies:* (1F5, anti-B1 (tositumomab) murine IgG2a antibody conjugated to iodine 131, IDEC-C2B8 (rituximab) murine antigen-combining variable regions grafted onto a human constant region with human IgG1-Fc domains.). First dose effect was noted with rituximab (fever, rigors, hypotension) suggesting cytokine release. Some patients have experienced severe hypotension with the first 2 infusions. Eight deaths have been recorded which seemed to be related to tumor burden, tumor lysis, and ARDS [29, 30].
- *Anti-TNF antibodies:* human-mouse chimeric antibody (infliximab or Remicade). A trial in the treatment of Crohn's disease noted infusion reactions, transient increased of anti-dsDNA antibodies, and serum sickness-like delayed hypersensitivity with retreatment. Induction of human-antichimeric-antibodies was suggested as the cause of some of the infusion reactions [31].
- *Anti-CD4:* (humanized hIgG1-CD4 modulating, non-depleting monoclonal antibody). Anti-CD4 monoclonal antibodies have been used in the treatment of rheumatoid arthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis. First dose reactions were observed (dyspnea, chills, hypotension [32]. Of note, the *anti-IL-2 Receptor (α chain) antibodies* daclizumab (Zenapax) and basiliximab (Simulect), widely studied in renal transplant recipients, do not induce cytokine release or first dose reactions [33].

Interleukin-2

IL-2 is a 15 kilo Dalton glycoprotein that is normally produced by antigen or mitogen activated circulating T-lymphocytes. It induces natural killer cell activity, enhances the allogeneic response, and activates cytolytic T-lymphocytes [34].

IL-2, with or without leukapheresis and reinfusion of lymphokine-activated killer cells, has been used in the treatment of solid tumors such as metastatic melanoma, metastatic renal cell carcinoma, and colorectal carcinoma. Unless bacterial or viral contamination is inadvertently introduced at the time of cell culture, lymphokine activated killer cell infusion is associated with only minor side effects of mild chills and fever and occasional dyspnea or bronchospasm similar to that seen with granulocyte transfusion reactions [35]. IL-2 infusions are associated with significant dose-dependent toxicity characterized by fevers, malaise, nausea, vomiting, diarrhea, hepatic dysfunction, pulmonary edema, somnolence, confusion, dysrhythmias, myocardial infarction, hematopoietic suppression, and renal insufficiency [35]. IL-2 appears to cause a generalized increase in capillary permeability, reduced systemic vascular resistance, fluid shifts and low effective circulating blood volume. It is not known if the vascular effects are a direct effect of IL-2 or due to IL-2 induced release of other mediators such as interferon (IFN), IL-1, TNF-α, and lymphotoxin [36, 37].

IL-2 has a short serum half-life of 6-10 min and a plasma elimination of 30-60 min after bolus intravenously infusion [36]. Resultant toxicity is generally transient and reversible.

Rosentein et al. [38] injected high dose IL-2 into mice followed by intravenous I^{125} bovine serum albumin as a marker of capillary leak. The severity of the vascular leak syndrome was dependent upon the number of days of treatment and the dose given. Severity could be reduced by immune suppression with cyclophosphamide, corticosteroids, or whole body irradiation implying that lymphokines released by lymphocytes played a role in the induction of the vascular leak phenomenon.

Schomburg [39] demonstrated that low to intermediate palliative doses of IL-2 in combination with IFN-α therapy was less nephrotoxic and less vasculo-toxic, especially if given subcutaneously rather than intravenously. Although there was a significant increase in serum creatinine and blood urea nitrogen (mean peak of 115.1 ± 21.4 mmol/L, 6.5 ± 2.5 mmol/L), there was no clinical evidence of uremia.

Renal toxicity has been attributed to sequelae from the development of the capillary leak syndrome. Vascular leak resulted in significant extravascular fluid accumulation (ascites, pleural effusions, peripheral edema) and weight gains of as much as 17 kg in 3 weeks [36]. As in sepsis syndrome, hypotension, oliguria and reduced fractional excretion of sodium accompanied the capillary leak.

Ponce treated 5 patients who had metastatic colorectal carcinoma with continuous intravenous infusions of IL-2 for 5 days and 9 cycles. They attempted to maintain a stable blood pressure with aggressive fluid replacement. However systemic vascular resistance declined from 1304 to 871 dyn/s/cm^{-5} and mean arterial blood pressure still dropped from 105 to 86 mmHg. Urine output dropped significantly and serum creatinine rose significantly. Urine sediment was normal on day 1 but contained multiple epithelial cells and brown casts by day 5 [40].

Others have shown that oliguria accompanying IL-2 infusions, responds to low-dose dopamine infusions, fluid resuscitation, and α agonists such as phenylephrine [37, 41, 42].

Rafi-Janajreh et al. [43] examined the mechanism of IL-2 induced vascular leak syndrome in a mouse model. The vascular leak was especially significant in the lung and liver of wild-type mice but was markedly reduced in the lungs and liver of CD44 knockout mice. Both groups had similar levels of perivascular infiltration with lymphocytes but the CD44 knockout mice did not have endothelial cell damage and also exhibited a marked decrease in IL-2-induced lymphokine-activated killer cell activity. These investigators also showed that the vascular leak syndrome was dependent on the expression of CD44 on immune cells and not the endothelial cells.

It is possible that IL-2 induced renal failure only occurs in the setting of profound hypotension, prior volume depletion, concurrent administration of potentially nephrotoxic drugs, or the presence of underlying renal disease.

Nausea, vomiting, and profuse diarrhea are relatively common side effects that may contribute to renal dysfunction because of intravascular volume depletion and activation of angiotensin II and renal sympa-

thetic nervous system.

In the past indomethacin was commonly given as prophylaxis against the chills, fever, arthralgias, myalgia's, and malaise associated with IL-2 administration. Non-steroidal anti-inflammatory drugs block prostaglandin-mediated glomerular afferent arteriolar vasodilation that is part of the auto-regulatory response to hypotension and renal hypoperfusion. Co-administration of a non-steroidal anti-inflammatory drug with IL-2 sometimes precipitated acute renal failure.

Memoli studied 9 patients being treated for metastatic renal cancer with a continuous intravenous infusion of rIL-2. All nine of the patients had undergone a unilateral nephrectomy and may have had baseline renal impairment. All 9 experienced a progressive decline in creatinine clearance. Low dose dopamine seemed to improve creatinine clearance after renal impairment had occurred [41].

Morroquin et al. [44] studied the effect of high-dose IL-2 therapy in the treatment of patients with metastatic melanoma and renal cell cancer. Animal models have shown that successful treatment with IL-2 is dose and schedule dependent. They found that there was a subset of patients who could not tolerate high doses or retreatment due to renal toxicity. Pretreatment factors that were significantly associated with renal toxicity were male sex, diagnosis of renal cancer, previous nephrectomy, and older age. These patients also had higher baseline creatinine.

Beldegrun [34] studied 99 patients with various types of metastatic cancer who had no identified renal disease, had a serum creatinine < 1.9 mg/dl (despite unilateral nephrectomy in some) and had no autoimmune disorders and no exposure to immunosuppressive drugs. A confounding factor in the study was the prophylactic administration of indomethacin. Mean baseline creatinine was 1.06 ± 0.03 mg/dl. Mean peak creatinine was 3.44 ± 0.19 mg/ml. The mean percentage increased in creatinine was 219 ± 15%. Mean peak serum creatinine level correlated with dose of IL-2 administered. In 62%, 84.3% and 95.2% of patients the serum creatinine level returned to baseline level within 7 days, 14 days and 30 days respectively. Patients with baseline elevation of serum creatinine greater than 1.4 mg/dl, renal cell carcinoma and radical nephrectomy represented a high-risk group who were more sensitive to the IL-2 regimen and had a prolonged recovery of renal function. Weight gain and edema were observed in conjugation with the renal dysfunction. The mean acute weight gain was 11.0 ± 0.66%, and the mean decrease in urine volume was 77.5%.

The following study suggests that IL-2 causes direct injury to the kidney. Textor et al. [45] noted universal progressive hypotension, sodium avidity, weight gain and edema, diminished glomerular filtration rate and evidence of ongoing tubular injury after administration of recombinant IL-2 to 12 patients. Serum creatinine and blood urea nitrogen rose in all patients and fractional excretion of sodium (FE_{Na}) fell to extremely low values. Urinary excretion of N-acetylglucosaminidase corrected for creatinine excretion was used as a marker of tubular lysosomal injury. N-acetylglucosaminidase was noted to rise with the peak in serum creatinine and to remain elevated after discontinuation of therapy. Plasma renin activity nearly doubled during therapy. Serum creatinine returned to normal one week following discontinuation of therapy. All patients received indomethacin, which may have contributed to the development of acute renal failure. Several patients required low dose dopamine for oliguria and phenylephrine for hypotension.

The above two studies are difficult to interpret because of the co-administration of non-steroidal anti-inflammatory drugs. Christiansen et al. [46] reported adverse effects of IL-2 in a patient who did not receive non-steroidal anti-inflammatory drugs. He developed hypotension, oliguria, creatinine elevation, and sodium retention. Serum phosphate was low but the fractional excretion of both phosphate and sodium was also very low. Morning venous aldosterone and plasma renin activity were significantly elevated and urinary prostaglandin excretion was depressed. The authors postulated that IL-2 had a direct effect on renal prostaglandin synthesis. Reduced production of vasodilatory prostaglandins could contribute to intense renal vasoconstriction and sodium retention by the proximal tubule. The presence of high aldosterone levels would enhance the sodium retention via the distal tubule.

Kozeny [47] evaluated IL-2 associated fluid and electrolyte disorders in 8 patients with metastatic cancer. All patients developed capillary leak syndrome, prerenal azotemia, hypophosphatemia, hypocalcemia, hypomagnesemia, and respiratory alkalosis. As noted in other studies, albumin fell precipitously with an associated fall in serum calcium. However, measurement

of ionized calcium and urinary calcium demonstrated true hypocalcemia and hypocalciuria. There was an associated hypomagnesemia and hypomagnesuria, hypophosphatemia, hypophosphaturea. Primary hyperventilation and respiratory alkalosis were thought to have caused an increased binding to albumin and intracellular shifts of these ions. Likewise, severe hypophosphatemia can be seen in gram-negative sepsis in association with respiratory alkalosis.

All patients developed a compensatory metabolic acidosis due to chronic hyperventilation. Respiratory alkalosis was thought to have developed because of capillary leak into the lungs producing borderline or frank pulmonary edema. After several days a superimposed normal anion gap acidosis developed from dilution by large volumes of saline fluid resuscitation. The authors found no defects in renal handling of calcium, phosphorous, or magnesium. There was no evidence of a renal acidification defect or renal tubular acidosis.

Shalmi et al. [48] suggested that an intrinsic renal defect may contribute to the renal dysfunction since the creatinine appeared to increase out of proportion to the blood urea nitrogen. Radionuclide studies utilizing Tc-99 DTPA and ^{131}I-Hippuran demonstrated impaired glomerular filtration rate out of proportion to the slight reduction in renal plasma flow. Glomerular filtration rate as measured by serial 2 hour creatinine clearances, showed an average reduction of 43% compared to an average decline in renal plasma flow of only 5%. If the predominant lesions were prerenal azotemia, one would expect relative preservation of glomerular filtration rate in the face of renal hypoperfusion, thereby increasing the filtration fraction. The urinalysis did not show an active sediment as one might see in acute tubular necrosis. The authors suggested that the generalized capillary leak syndrome associated with the administration of IL-2 might have contributed to intrarenal edema and congestion leading to increased backpressure and a decrease in ultrafiltration pressure and glomerular filtration rate.

The above-mentioned studies have suggested or proposed a direct renal injury by IL-2, but none of them have been able to conclusively distinguish a direct IL-2 renal effect from simple renal under-perfusion severe enough to cause ischemia and acute tubular necrosis. The toxicity of IL-2 has been clearly associated with widespread endothelial cell damage and capillary leak [43]. This is consistent with a generalized, systemic effect of IL-2 rather than proof of a specific direct effect on the kidney.

Hall et al. [49] examined the nephrotoxic effects of IL-2 and its putative mediator, TNF-α in the LLC-PK1 pig kidney cell line. Levels of IL-2 comparable to those used in human studies, caused vacuolization, cell shrinkage and growth inhibition. Dexamethasone, which is used clinically to inhibit TNF-α, failed to protect the cultured cells from the effects of IL-2. TNF-α when given alone had no apparent effect on morphology or cell growth, suggesting that the nephrotoxic effect of IL-2 was direct.

Vlasveld [50] obtained biopsy material from a patient with renal cell cancer who developed acute renal failure in the sixth week of a continuous rIL-2 infusion. The pathology was that of acute tubulo-interstitial nephritis. Further studies on cryopreserved peripheral blood lymphocytes revealed specific cytolytic activity against an autologous renal cell line cultured from the biopsy specimen. These findings are consistent with an allergic reaction to IL-2.

Administration of cytokine combinations may be synergistic in their toxicity. Dutcher et al. reported a phase II outpatient trial of subcutaneous IL-2 plus IFN-α [51]. They noted higher-grade toxicity of fatigue, nausea, vomiting, diarrhea, anorexia, fluid overload, rash, aseptic meningitis, chest pain, atrial fibrillation, and hypotension. One patient developed irreversible, dialysis dependent renal failure with crescentic glomerulonephritis.

Carson et al. administered IL-12 in combination with IL-2 or IL-15 to several mouse strains. The combination induced systemic inflammatory response syndrome that rapidly progressed to a fatal shock-like state. They showed that the natural killer cell was the only cell out of the total lymphocyte found responsible for mediating the syndrome [52].

Several interleukins are being tested as therapeutic agents (IL-3, IL-4, IL-7, IL-12). At this time there is insufficient information to comment on nephrotoxicity.

Interferons

Based on differences in receptor occupancy, 2 classes of interferons have been identified: Type I includes IFN-α synthesized by leukocytes and IFN-β synthesized by fibroblasts and epithelial cells. Type II

includes IFN-γ synthesized by lymphocytes and monocyte/macrophage. The three interferons have different biologic activity. All have antiviral activity and all have active roles in resistance to tumors, control of cell growth and differentiation, expression of cell surface molecules, and immune-modulating effects.

Interferon-alpha

IFN-α has antiviral and antiproliferative effects, which have proven useful in the treatment of hepatitis B and C, cryoglobulinemia, and various tumors including rectal cancer, lymphoma, breast cancer, ovarian malignancies, cutaneous T-cell leukemia (mycosis fungoides), bladder cancer, cervical dysplasia, melanoma, and chronic lymphocytic lymphoma. Side effects include fever, chills, malaise, headache, myalgia's, neuropathy, somnolence, confusion, and fatigue. Leukopenia and elevation of serum transaminases are the most common dose limiting side effects. Nephrotoxicity is uncommon and usually noted in individual case reports as an association with administration of IFN-α. Often there are other factors contributing to acute renal failure such as concomitant renal disease (nephrectomy, Hepatitis C, or nephrotoxic drugs). Phillips reviewed this topic in 1996 [53]. Gutterman [54] reported no effects of treatment on serum creatinine and blood urea nitrogen, although transient pyuria was noted in 5 of 16 patients. Abdullhay [55] noted mild elevations of blood urea nitrogen and creatinine in 10 patients and more severe dysfunction in 2 of 36 patients with ovarian malignancies. The latter 2 patients had prior renal impairment.

Reports of isolated proteinuria associated with IFN-α therapy have appeared in the literature. Sherwin [56] observed 2 patients with transient proteinuria of less than 2 g/day, which recurred with rechallenge with IFN-α. Quesada [57] initially reported proteinuria of less than 2 g/day, which recurred with rechallenge with IFN-α. Quesada [57] initially reported proteinuria in 13 of 38 (34%) of patients, excluding those with multiple myeloma. In 2 of 13 patients, the proteinuria persisted after discontinuation of the drug. In a later publication, Quesada [58] cited a 15-20% incidence of dose independent proteinuria. Quantitation rarely exceeded 1 g/24hr and was not associated with a decline in glomerular filtration rate. Ferri [59] also noted proteinuria in patients being treated for mixed cryoglobulinemia but admitted that subclinical glomerular involvement with cryoglobulins could have been present. There are also reports of acute renal failure and nephrotic syndrome associated with IFN-α therapy [60, 61].

As far back as 1976 IFN-α was shown to be able to induce glomerulonephritis in animal models. Gresser [62] was able to develop an animal model of acute glomerulonephritis by injecting high dose IFN-α into mice. Experimental evidence supports an immunologic effect of IFN-α on the kidney. Morel-Maroger [63] injected partially purified mouse IFN into newborn mice and found marked thickening of the glomerular basement membrane preceding the deposition of immunoglobulin and complement. Since then there have been a number of case reports of IFN-α associated glomerulonephritis (GN) in humans. A variety of lesions have been reported including minimal change disease, pauci-immune GN, rapidly progressive GN, and focal segmental glomerulosclerosis [64, 65].

Selby [66] reported a patient with myeloma who developed nephritic syndrome during treatment with IFN-α. Creatinine rose 2 mg/dl associated with protein excretion of 6 g in 24 hours. The nephritic syndrome reversed after treatment was withdrawn. Similar findings were reported by Quesada [58] in 2 patients with multiple myeloma and renal cell carcinoma.

Herman [67] published a case report of a patient with hairy cell leukemia who developed membranoproliferative glomerulonephritis during treatment with IFN-α. He developed hematuria, pyuria, and depressed complement levels. Renal biopsy revealed foot process effacement and subendothelial deposits.

Averbuch [68] reported a patient with Mycosis Fungoides who developed 6 kg weight gain, edema and a rise in serum creatinine and BUN to 4.1 mg/dl and 55 mg/dl respectively after 6 doses of IFN-α. The urinary sediment showed 15-20 white cells/high-power field with many eosinophils, tubular epithelial cells, and granular casts. The patient excreted 28 g of nonselective proteinuria in 24 hours. A kidney biopsy revealed extensive interstitial edema and moderately severe, patchy interstitial infiltration with lymphocytes, plasma cells and eosinophils. Electron microscopy showed foot process effacement consistent with minimal change disease. After IFN-α was discontinued, renal function returned to normal, but low-grade proteinuria continued for 2 months. Rechallenge with IFN-α again produced azotemia and nephritic range pro-

teinuria. The authors commented that the lesion of minimal change nephropathy and acute interstitial nephritis is similar to the histologic pattern noted with toxicity from nonsteroidal anti-inflammatory agents. They suggested that activated cytotoxic T cells were responsible for a cell mediated delayed hypersensitivity mechanism of injury. Similar cases of minimal change disease associated with IFN-α were reported by Traynor et al. [69] and Rettmar et al. [70]. Shah et al. reported 2 cases of renal failure associated with IFN-α treatment of chronic myeloid leukemia. Both patients had proteinuria and focal segmental glomerulosclerosis on biopsy. The authors reported 15 other cases of renal failure and proteinuria associated with IFN-α in which the pathology was less well defined [71].

Unusual immune side effects have been reported in association with rIFN-α therapy. Chronic hemolytic-uremic syndrome was observed in a patient with multiple myeloma treated with IFN-α (De Broe ME, personal communication). The post bone marrow transplantation course was complicated and he received several nephrotoxic antibiotics. Three months later treatment with IFN-α was started. Towards the end of the treatment renal function deteriorated. There was partial renal recovery after cessation of therapy. Renal biopsy showed focal membranoproliferative lesions, mesangiolysis and intracapillary thrombosis consistent with a chronic form of hemolytic uremic syndrome. A similar observation has been reported by Ravandi-Kashani et al. [72]. Harvey et al. [73] reported 3 cases of HUS/TTP. Two patients developed renal failure requiring dialysis. E. coli OH157.H7 was grown from the stool of one patient.

Acute renal failure or deterioration has frequently been cited in association with IFN-α treatment of Hepatitis C and even Hepatitis B. It is well known that Hepatitis C virus infection causes glomerulonephritis (GN). Membranoproliferative GN is the most common manifestation and biopsy specimens have shown deposition of immune complexes composed of HCV related antigen and cryoglobulin. The difficulty lies in distinguishing glomerulonephritis caused by Hepatitis C from glomerulonephritis seen in association with IFN-α therapy or from occult underlying renal disease that is exacerbated by IFN-α.

There have been reports of nephrotic range proteinuria and focal segmental glomerulosclerosis on biopsy, in patients who are being treated with IFN-α for Hepatitis C [74, 75]. Gordon et al. [76] reported a case of IFN-α induced exacerbation of vasculitis (rash and renal impairment) in a patient with hepatitis C-associated cryoglobulinemia.

Ohta et al. [77] examined 24 patients who manifested the appearance of/or exacerbation of proteinuria after IFN therapy for chronic HCV infection. One patient had known HCV related glomerulopathy and cryoglobulinemia and showed a good response to therapy including improved renal function and remission of proteinuria. Yamabe et al. and also Sarac et al. confirmed good responses to IFN therapy without renal deterioration in patients with HCV-related glomerulonephritis [78, 79]. In Ohta's study only 3 subjects were treated with IFN-α, the remainder were treated with IFN-β. As was shown by Johnson [80] improvement in MPGN with IFN-α correlated with clearance of viremia but did not correlate with remission of proteinuria. Other results were quite variable. The authors looked at permselectivity, presence or absence of glomerular cryoglobulin or HCV antigen, deterioration of renal function, and reversibility of proteinuria. 11 of 24 patients underwent biopsy of the kidney. The authors felt that absence (or minimal presence) of HCV core antigens or cryoglobulin deposits in the glomeruli indicated non-HCV related or primary glomerulonephritis and that these patients might be at higher risk for exacerbation or direct injury from IFN. There was no clear explanation of which patients would benefit from or fail to respond to IFN and which patients might develop irreversible renal injury.

To complicate the issue, Jamal Al-Wakeel treated 4 patients with IFN-α who had primary GN and had refused conventional therapy with cyclophosphamide and/or prednisone. One patient with membranous GN and 2 with mesangial GN had remission of nephrotic syndrome [13]. The authors suggested that IFN-α might prove to be a useful alternative therapy in the treatment of inflammatory glomerular diseases.

Renal transplant patients with Hepatitis C seem to be especially susceptible to injury from IFN-α. IFN-α triggers renal graft rejection in a substantial number of patients, and is now considered contra-indicated in this setting [81-83].

In summary, other symptoms of IFN-α toxicity are far more common than nephrotoxicity (fevers, chills, malaise, arthralgias, fatigue anorexia, weight loss, depression, impaired cognitive function, diminished li-

bido, abnormal thyroid function). Nevertheless, IFN-α has a complicated and important relationship to the kidney but there are many confounding factors that tend to obscure the molecular dynamics of that relationship.

Interferon-beta

IFN-β shares 29% amino acid homology with IFN-α and has been used in the treatment of multiple sclerosis. Type I interferons (α and β) differ from Type II interferons (γ) in biochemical properties, biological function, and receptor specificity. Side effects common to both classes of interferons include chills, fever, rigors, headache, myalgia's, hypotension, nausea, vomiting, anorexia, constipation, fatigue, neutropenia, and elevated transaminases. This constellation of symptoms frequently results in mild to moderate hypotension and volume depletion and could potentially contribute to prerenal azotemia or acute tubular necrosis.

IFN-β has been used in combination with IFN-γ because of synergistic anti-tumor effects. The combinations of interferons appear to have potentiate systemic effects and cumulative toxicity compared to administration of either interferon alone. Synergistic toxicity limits both the tolerated dose maximum and therapeutic efficacy. Low doses of β and γ interferons given in combination, either by intravenous bolus or continuous infusion, do not appear to cause renal damage or dysfunction [63, 84].

The specific renal effects of IFN-β have not been evaluated, although the toxicity profile of all interferons appears to be quite similar. Increased insensible fluid losses via skin or the gastrointestinal tract or fluid sequestration from capillary leak and hypoalbuminemia all contribute to the development of prerenal azotemia. Volume depletion and hypotension activate angiotensin II and renal sympathetic nerves to try to maintain the glomerular filtration fraction. Angiotensin II is a potent vasoconstrictor and also up-regulates the expression of growth factors and cytokines such as TGF-β, TNF-α, vascular cell adhesion molecule-1 (VCAM-1), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and insulin-like growth factor that are involved in renal injury and repair.

Interferon-gamma

There are several subspecies of IFN-γ determined by differential glycosylation and IFN-γ is a more potent immunomodulator than other interferons.

IFN-γ is cidal to human tumor cell lines, activates monocyte/macrophages, upregulates Class II MHC expression and increases natural killer cell activity [85]. In the kidney, IFN-γ regulates Class I and II MHC expression in the basal state, in response to inflammatory stimuli, and after ischemia or ischemia-reperfusion renal injury [86].

Systemic side effects are similar to those of other interferons, namely fever, chills, rigors, hypotension, confusion, disorientation, anorexia, lethargy, nausea, vomiting, diarrhea, myalgia, leukopenia, hepatotoxicity. Side effects are reversible and limited to the time of administration of the drug. Mild changes in liver function have been observed at higher dose levels and include hypoalbuminemia. There have been no significant changes noted in blood urea nitrogen and creatinine, although a small degree of proteinuria has occasionally been observed [87, 88].

Ault [89] reported a case of acute renal failure in a 12-year old child, which required temporary hemodialysis after 19 days of therapy with IFN-γ. The urinary sediment contained numerous white cells, red cells, and waxy and granular casts. Open renal biopsy revealed focal segmental glomerulosclerosis in 3 of 43 glomeruli and irregular wrinkling of the peripheral basement membrane in others. A tuft adhesion to Bowman's capsule was seen in one glomerulus. There was also diffuse tubular damage and interstitial edema consistent with acute tubular necrosis. Electron microscopy demonstrated foot process effacement. Direct immunofluorescence was negative for IgG, IgA, IgM, kappa and lambda light chains, C3, Clq, properdin, and fibrin reactive products. Renal function returned to normal after withdrawal of the drug. The authors suggested that structural distortion of the basement membrane and absence of immune complexes was evidence for direct glomerular injury by the cytokine. However the authors could not exclude prior subclinical FSGS in the child.

To support their hypothesis, the authors cited studies in newborn Swiss mice exposed to mouse IFN in which there was diffuse glomerular basement membrane thickening and capillary IgG and C3 deposition,

which progressed to focal segmental glomerulosclerosis.

OKT3 nephrotoxicity: *From acute tubular necrosis to hemolytic-uremic syndrome*

Introduction

OKT3 is a murine monoclonal antibody (mAb) recognizing the CD3 complex closely associated with the antigen receptor of mature T cells (TCR) [90]. T-lymphocytes are of paramount importance in allograft rejection, and OKT3 has proved very efficient in both the treatment and the prevention of allograft rejection [91, 92]. The immunosuppressive properties of OKT3 are related to its ability to deplete CD3+ cells from the circulation, to induce the internalization of CD3-TCR complexes (modulation), and to sterically inhibit residual CD3-TCR complexes [93]. Before exerting its immunosuppressive effects, OKT3 induces a transient activation of leukocytes. The ability of OKT3 to induce multivalent cross-linking of both the T cell-receptor/CD3 complex and the monocyte Fc receptor results in T cell and monocyte activation [94]. This is accompanied by the release of several proinflammatory cytokines including TNF-α, IFN-γ, IL-2 and IL-6 into the circulation within hours after the initial OKT3 injection [95-100]. The investigation of cytokine gene expression in purified cell populations obtained from spleens of mice injected with an activating anti-CD3 antibody, indicate that T cells are the main source of TNF-α in this setting. Monocytes are also activated, as shown by their production of IL-1 and IL-6 [101]. The toxicity of OKT3 is due to the synergy between TNF-α and IFN-γ, as can also be observed after injection of both endotoxin and Staphylococcal enterotoxin B in mice. Indeed, mAbs directed against either TNF-α [102, 103] or IFN-γ [104] can prevent hypothermia, hypomotility, diarrhea, piloerection, and even death induced by the activating 145-2C11 anti-CD3 mAb in mice.

In addition to cytokines, OKT3 activates the complement system via the classical pathway as shown by increased levels of C3a and C4 metabolites within minutes of OKT3 injection [105, 106]. Complement activation could synergize with cytokine and in particular could trigger early respiratory manifestations [107]. However, the occurrence of full-blown anti-CD3-induced toxicity in complement-deficient mice [108] as well as the lack of toxicity of a complement-binding non-mitogenic anti-T-cell receptor IgM mAb [109] argues against a major role for complement activation in the pathogenesis of OKT3-associated toxicity.

Peak serum levels of TNF-α and IFN-γ occur 1 to 2 hours after the first injection of the mAb, and are followed by a complex of symptoms referred to as "cytokine release syndrome". Thus, patients may develop fever, chills, headaches, myalgias, nausea, vomiting, diarrhea, and respiratory symptoms after the first OKT3 injections [91, 110, 111]. Occasionally, more serious complications such as pulmonary edema, aseptic meningitis or convulsions can also occur. In addition, attention was recently drawn to the nephrotoxic properties of OKT3 when used in the prevention or in the treatment of renal allograft rejection. We here describe the clinical characteristics, the pathology, the precipitating factors and the possible prevention of the renal lesions induced by OKT3 in kidney transplant recipients.

Clinico-pathological observations

Transient renal dysfunction after therapy of acute rejection with OKT3

OKT3 mAb has showed greater potency than corticosteroids in the treatment of kidney graft rejection, allowing for improved long-term graft survival after the acute rejection episode [91]. However, transient renal dysfunction has been observed at the initiation of OKT3 therapy. Simpson et al. observed signs of tubular toxicity in the urine sediment and a sharp increase in serum creatinine during the first 3 days of OKT3 therapy [112]. We extended these observations by comparing, in a retrospective study, the evolution of renal function during kidney graft rejection treated with either OKT3 or mPDS. The clinical observation that high-dose corticosteroids precipitate the development of intragraft thromboses led us to study the effects of methylprednisolone (mPDS) on the procoagulant activity induced by OKT3 on peripheral blood mononuclear cells (PBMC) *in vitro*. The procoagulant activity of unstimulated PBMC (mean ± sem: 0.6 ± 0.1 mU/ml) reached 3.0 ± 0.7 mU/ml after OKT3 stimulation ($p=0.0062$) and further increased to 7.4 ± 2.0 mU/ml when PBMC were first preincubated overnight with mPDS before OKT3 stimulation ($p=0.018$ as compared

to OKT3 alone). This process involved the tissue factor/factor VII pathway, as shown by increased membrane expression of tissue factor on monocytes as well as by a marked reduction of the induced procoagulant activity when the clotting assay was performed with factor VII-deficient plasma [124]. Thus, high-dose mPDS represents a major risk factor for thrombosis after OKT3 prophylaxis, probably because of the ability of mPDS to potentiate OKT3-induced tissue factor expression and activity on monocytes. Furthermore, corticosteroids increase plasminogen activator inhibitor-1 secretion by hepatocytes [184], while at the same time they decrease tissue-type plasminogen activator [185] as well as prostacyclin [186, 187] and nitric oxide production [187, 188]. That the procoagulant effects of steroids can affect the kidneys has been clearly demonstrated by the extensive glomerular fibrin deposits occurring in rabbits pretreated with large doses of corticosteroids before endotoxin challenge [189, 190]. The early increase in serum creatinine was significantly higher in OKT3 *vs* mPDS-treated patients [113]. As with prophylactic treatment (see below), OKT3 nephrotoxicity appeared reversible and did not jeopardize the long-term graft outcome.

Alteration of renal function during therapy of allograft rejection by OKT3 is not limited to the transplanted kidney. Indeed, four of sixteen cardiac transplant patients developed a rise in creatinine following therapy of rejection with OKT3. Renal dysfunction resolved spontaneously in all cases [114].

Interestingly, the nephrotoxicity of anti-CD3/TCR mAbs appears related to the magnitude of cytokine release they induce. Thus, T10B9, a murine IgM anti-TCR mAb that does not bind to Fcg receptors on monocytes and therefore leads to only minor increases in IFN-γ and TNF-α serum levels, does not induce nephrotoxicity [115]. Along the same line, therapy of rejection with a humanized, Fc receptor non-binding, non-activating OKT3 (huOKT3g1 Ala-Ala) antibody did not induce the early rise of serum creatinine seen after OKT3 therapy [116].

Increased incidence of acute tubular necrosis and renal dysfunction during OKT3 prophylaxis

Part of the rationale for the prophylactic use of OKT3 in cadaver kidney transplantation was to delay cyclosporine therapy in the hope to reduce the incidence of postoperative acute tubular necrosis. While OKT3 prophylaxis improves long-term kidney graft survival especially in high-risk patients [92, 117-120], the incidence of postoperative acute tubular necrosis has not been reduced by OKT3 use [117, 121]. Furthermore, we made the unexpected observation in a prospective, randomized study that patients receiving OKT3 display an increased rate of postoperative dialysis requirement (14 out of 21 patients) as compared to those treated with cyclosporine (6 out of 21, p=0.03) [122]. There were no long-term sequelae, as both groups showed similar graft function up to 3 years after transplantation [121].

In addition, patients who achieved immediate postoperative graft function may also develop renal dysfunction early during the course of OKT3 prophylaxis. Indeed, 10 out of 133 patients (7.5%) treated with OKT3, azathioprine and steroids experienced an abrupt rise in serum creatinine between postoperative days 2 to 5. None of these patients had received cyclosporine. Six patients underwent allograft biopsies at the time of dysfunction; 4 were normal, and 2 showed only mild interstitial edema. All patients recovered without sequelae. This side effect was considered related to the cytokines released after OKT3 therapy [114].

Induction of intragraft thrombosis by prophylactic OKT3 therapy

Besides the transient kidney dysfunction described above, OKT3 exerts procoagulant effects, which can precipitate intragraft thromboses and result in transplant loss. This can occur when OKT3 is used as either prophylaxis [123, 124] or treatment of rejection [125-127]. All the thromboses observed in our center took place during prophylactic administration of OKT3 [123, 124]. They occurred between postoperative day 1 and 11, always before the introduction of cyclosporine A. Thromboses involved graft arteries (n=2), veins (n=5), or glomerular capillaries (n=6). In the latter cases, the most striking finding in the renal biopsies was the formation of thrombi in some glomerular capillary loops and in afferent arterioles. Moreover, glomerular tufts showed thickening and wrinkling of their capillary walls with formation of "double contours". Endothelial cells were swollen and took a foamy appearance, this process causing diminished patency of capillary lumens (Figure 1). A few glomerular tufts were totally ischemic and had collapsed. Except for one case in which signs of discrete cellular rejection were present,

the interstitium was only slightly enlarged by edema without significant influx of inflammatory cells or tubular damages. However, increased numbers of polymorphonuclear cells, especially eosinophils, were present in peritubular and glomerular capillaries. Small and medium size arteries never displayed intimal infiltration of inflammatory cells or increase in intimal stromal matrix. Immunofluorescence staining was consistently negative for complement and immunoglobulin deposits in both glomeruli as well as in vessels. The formation of thrombi was confirmed by a strong reactivity for fibrinogen. The diagnosis of thrombotic microangiopathy was corroborated by electron microscopy, which showed detachment of endothelial cells from the glomerular basement membrane, accumulation of fluffy material in subendothelial position (Figure 2) and fibrin deposition in glomerular capillaries. These lesions of thrombotic microangiopathy are similar to those described in hemolytic-uremic syndrome [128].

Along this line, 4 of the 6 patients with glomerular thromboses had decreased hematocrit, platelets counts, and haptoglobin levels together with schistocytes on blood smears indicating microangiopathic hemolytic anemia. Treatment of thrombotic microangiopathies with anti-aggregants, steroids, and plasmapheresis was unsuccessful except in one case. Furthermore, all grafts with arterial or venous thromboses were lost. The occurrence of hemolytic uremic syndrome associated with OKT3 therapy, given either as prophylaxis or as treatment of rejection has since been observed by others [129, 130].

Although the first OKT3 dose induces a transient activation of the coagulation system in all patients (see below), only a small number develop intragraft thrombosis [124]. Possible risk factors for thrombosis were found in 6 out of the 13 patients who developed this complication: 2 received pediatric kidneys, 1 received a kidney large for his size, 1 had hemolytic-uremic syndrome as initial nephropathy, 1 had a lupus anticoagulant and 1 had a sequela of venous thrombosis on the iliac vessels used for graft anastomosis. We then searched for additional risk factors by comparing the clinical parameters of the 13 recipients with thromboses to those of 218 patients free of this complication. Cold or warm ischemia times, numbers of HLA-A, -B, or -DR mismatches, dose of first OKT3 injection (5 or 10 mg), numbers of patients with preformed anti-HLA

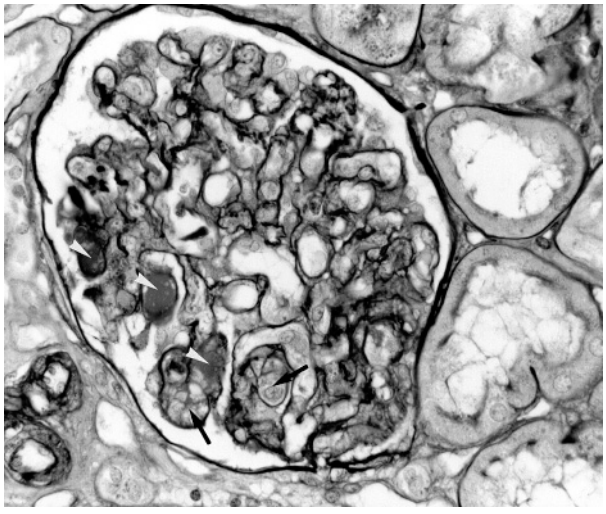

***Figure 1.**: Glomerulus from a patient with OKT3-induced thrombotic microangiopathy. The capillaries are occluded by swollen endothelial cells (black arrows) and by fibrin-like material (white arrows). (Periodic acid-Schiff; magnification x240)*

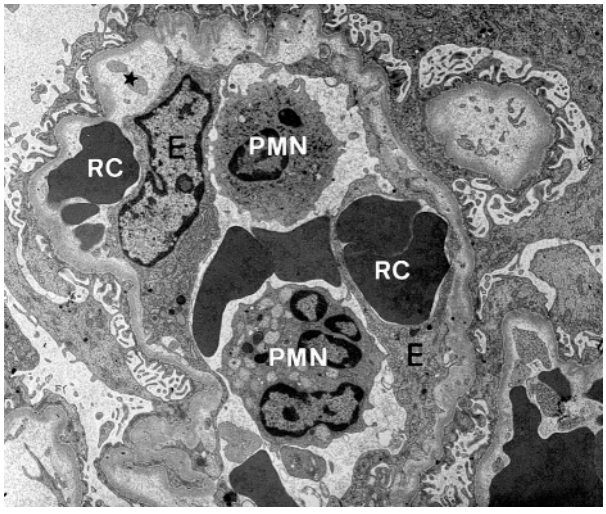

***Figure 2.**: Electron micrograph of glomerular capillary loop from a patient with OKT3-induced thrombotic microangiopathy to show detachment of endothelial cells (E), lucent expanded subendothelial zone (*) and red cells (RC) which appear to be trapped between endothelium and basement membrane and accumulation of polymorphonuclear cells (PMN) in the narrowed lumen. (magnification x3360)*

antibodies and numbers of retransplant patients were not significantly different in either group. The only relevant parameter appeared to be the mPDS dose given as pretreatment before the first OKT3 injection. Indeed, 6 out of the 42 patients (14%) who received 30 mg/kg mPDS experienced a thrombotic event, as compared to 7 out of the 189 patients (3.7%) who received the regular 8 mg/kg m-PDS (p=0.016). This latter incidence is similar to the 2 to 3% of graft vessels thromboses reported in the absence of OKT3 prophylaxis [131, 132] suggesting that the intrinsic risk of thrombotic event related to OKT3 is low. The precipitating role of high dose steroids is supported by several experimental data (see below).

Prevention of OKT3 nephrotoxicity after kidney transplantation

An increased incidence of delayed graft function may be traced to the policy of restriction of peri-operative fluid infusion, implemented initially because of the known risk of life-threatening pulmonary edema in hypervolemic patients receiving OKT3 [91]. Postoperative hypovolemia together with the use of high dose of mPDS (30 mg/kg) before the first OKT3 injection probably combined to trigger graft vessels thromboses. In January of 1992, this led us to modify our perioperative management of kidney transplant recipients receiving OKT3 prophylaxis in three ways: 1) preoperative hypovolemia was corrected; 2) the calcium-channel blocker diltiazem, considered beneficial for recovery of graft function, was administered on the day of transplantation; and 3) the high dose of mPDS previously shown to precipitate OKT3 coagulopathy was avoided [133]. Comparison of two consecutive series of patients (group 1, control patients, n=172; group 2, managed as described above, n=173) showed that: 1) the incidence of delayed graft function fell from 52% in group 1 to 22% in group 2 ($p<0.0001$); 2) pulmonary edema was not more frequent in group 2 (3.5% vs 1.7% in group 1, p=0.5); 3) the frequency of intragraft thrombosis fell from 7.6% in group 1 to 1.2% in group 2 (p=0.0034). Multivariate analysis showed that the volemia/diltiazem program and avoidance of high mPDS dose were the most important factors responsible for the reduced occurrence of delayed graft function and graft vessels thrombosis, respectively. Thus, a combined strategy of avoidance of high steroid doses, administration of a calcium-channel blocker and optimization of volume status is safe and efficiently prevents OKT3 nephrotoxicity (Table 3) [133].

Pathophysiological considerations

Transient kidney graft dysfunction and acute tubular necrosis

The cytokines released after the first OKT3 injection probably play a major role in the pathogenesis of this type of OKT3 nephrotoxicity. Indeed, injections in rodent and man of recombinant IL-2 [134, 135], IFN-γ [136, 137] or TNF-α [138] have been consistently reported to induce renal dysfunction, sometimes in association with acute tubular necrosis. The following hypotheses can be put forward to account for OKT3-induced renal dysfunction in kidney transplant recipients:

1. ***Renal ischemia***. This is due to several factors. First, OKT3 induces a transient decrease in myocardial contractility [139], which also occurs after infusion of IL-2 [140] and TNF-α [141, 142]. Second, the same mediators cause extravasation of intravascular fluid, the so-called "vascular leak syndrome" [138, 143, 144]. This reduces circulating blood volume and further compromises renal perfusion. Finally, OKT3 leads to systemic release of the vasoconstrictor molecule endothelin [145], to which the renal vasculature is particularly sensitive [146].
2. ***Induction of cells and molecules able to mediate kidney injury***.

First, cytokines such as IFN-γ and IL-1 exert direct toxic effects on the kidney, as indicated by their ability to induce death of renal tubular cells in culture [147]. Second, activation of leukocytes by pro-inflammatory cytokines renders them capable of mediating tissue lesions [148-150]. For instance, neutrophils, which are activated after OKT3 injection [151], can cause renal failure through the release of oxygen radicals and proteases [152].

To get further insights into the anti-CD3-induced nephrotoxicity, we investigated in a murine model the ability of anti-CD3 mAb to directly damage native kidneys. For this purpose, we injected mice with the hamster anti-mouse CD3 mAb 145-2C11 [153]. Like OKT3, this mAb is immunosuppressive [154] and first induces a transient release of several cytokines in the circulation, such as IL-2, IL-6, TNF-α, and IFN-γ [102, 155, 156]. Histologic analysis revealed signs of tubular ne-

crosis 48 hours after the injection of the 145-2C11 mAb [156]. Lesions were prominent at the corticomedullary junction where abrasion of the brush border of the tubular epithelium and desquamation of tubular epithelial cells were commonly seen. This tubular injury was associated with increased blood urea nitrogen levels and enhanced urinary excretion of endopeptidase 24.11 [156], an enzyme of the brush border of the proximal tubular epithelium.

The role of cytokines in anti-CD3-mAb-mediated nephropathy was analyzed by pretreating mice with either neutralizing anti-TNF antibodies or mPDS [156]. The histologic lesions and the renal excretion of endopeptidase 24.11 were moderately prevented by anti-TNF antibodies, suggesting the involvement of other mediators, which could act in synergy with TNF-α. In support of this view, steroid pretreatment before anti-CD3 challenge almost completely abolished IL-2, TNF-α and IL6 release and resulted in preservation of renal histology.

In addition to the ability of activating anti-CD3 mAbs to mediate renal injury of normal, native kidneys, the renal transplant might be more susceptible to injury at the time of OKT3 administration for the following reasons:

1. Increased expression of adhesion molecules on the kidney graft.

Cold and warm ischemia associated with organ procurement and the inflammatory lesions present during acute rejection are likely to increase the risk of acute failure after OKT3 exposure. Indeed, both ischemia and rejection increase the expression of adhesion molecules on endothelial and parenchymal cells [157]. In addition, administration of OKT3 in man leads to increased expression of the adhesion molecules ICAM-1 and VCAM-1 on dermal vessels [158]. This was associated with an increased recruitment and migration of T cells within the skin. Along the same line, injection of activating anti-CD3 antibodies in mice also induced patchy endothelial expression of VCAM-1 on large arteries in all tissues examined [159]. Of particular relevance was the observation that VCAM-1 expression was much more abundant, and not restricted to large arteries but now also involving capillary endothelial cells, in recently implanted cardiac isograft. Thus, OKT3 may synergize with ischemia to induce massive expression of adhesion molecules on graft endothelial cells.

2. Preferential recruitment of effector cells in the kidney graft.

Cross-linking of CD3 and Fcg receptors, together with the release of pro-inflammatory cytokines, triggers enhanced expression of adhesion molecules on leukocytes. Thus, *in vivo* and *in vitro* experiments have shown that OKT3 upregulates the expression of activation epitopes of CD11a/CD18, as well as increased expression of CD11b/CD18 on T cells [158]. This resulted in increased adhesion of these cells to vascular endothelium. Likewise, OKT3-treated patients display increased expression of CD11b on granulocytes [160], and of CD11b, CD11c, and CD29 on monocytes [160, 161]. This could obviously lead to sustained recruitment and activation of potentially damaging leukocytes in the renal allograft.

Intragraft thromboses

This adverse effect is related to the ability of OKT3 to activate the coagulation system as indicated by sequential determinations of plasma levels of prothrombin fragments 1+2 (F 1+2). These molecules are released during conversion of prothrombin to thrombin and witness activation of the common pathway of the coagulation system [162]. All kidney transplant recipients tested in our center displayed increased plasma levels of prothrombin fragments, which peaked 4 hours after the first injection of OKT3 (mean ± SEM: 4.82 ± 0.73 vs. 1.75 ± 0.37 nmol/L in controls, $p < 0.01$) [163]. The magnitude and the time course of the changes in F 1+2 plasma levels were similar whether the patients received 5 or 10 mg OKT3, and whether OKT3 was given as prophylaxis or for treatment of rejection. Fibrin degradation products, indicative of a fibrinolytic process, were already above baseline values at 4 hours, and continued to increase until 24 hours [163]. Finally, the levels of von Willebrand factor antigen, a molecule released by activated or damaged endothelial cells, were also significantly increased after OKT3 injection. This systemic activation of coagulation is transient, as it occurs only after the first injection of the mAb [163]. The hemostatic changes induced by OKT3 have been confirmed in two independent studies [164, 165]. These investigators found increased levels of thrombin-antithrombin-III complexes, indicative of activation of the coagulation, as well as of tissue-type plasminogen-activator [164, 165] and plasmin-α2-antiplasmin complexes [165] indicative of fibrinolysis, after the first injection of OKT3. In addition, Raasveld et al. found no

changes in plasma kallikrein-C1-inhibitor complex, suggesting that activation of the coagulation occurs via the extrinsic rather than the intrinsic pathway [164].

In vitro studies showed that the mechanisms underlying activation of the coagulation by OKT3 are related to the induction of procoagulant activity of the tissue-factor type on both endothelial cells and monocytes.

1. Endothelial cells

We investigated the ability of OKT3 to induce tissue factor activity at the surface of human umbilical vein endothelial cells (HUVEC). While both OKT3 and supernatants of unstimulated peripheral blood mononuclear cells (PBMC) were inactive, supernatants of OKT3-stimulated PBMC induced a massive increase of tissue factor activity at the HUVEC surface as measured by thrombin generation [163]. The marked inhibition of this procoagulant activity by anti-TNF mAb showed that TNF-α is an important mediator of the procoagulant effect of OKT3 at the endothelial cell level [163]. This is in line with the well-known ability of TNF-α to induce expression of tissue factor on the endothelium, thereby promoting microvascular thromboses [166, 167].

2. Monocytes

We and others observed that monocytes display increased procoagulant activity after culture of PBMC with OKT3 [124, 168, 169]. As for endothelial cells, this activity is due to increased tissue factor expression [124], confirming that the extrinsic pathway of the coagulation plays a major role in the hemostatic changes induced by OKT3. The mechanisms by which OKT3 induces tissue factor on monocytes are still unclear. While products of activated T cells such as monocyte procoagulant inducing factor [170] or IFN-γ [171] could play a role, TNF-α does not seem to be involved [172]. Beside soluble factors, cognate interactions between activated T cells and monocytes appear to be necessary to elicit monocyte procoagulant activity [173]. Whatever the mechanism, recent observations indicate that the first injection of OKT3 triggers circulating monocytes to display increased tissue factor expression and activity *in vivo* like it does *in vitro* [174]. Thus, the procoagulant activity of circulating monocytes was increased 3- to 5- fold at 3 and 5 hours after the initial OKT3 injection. These monocytes displayed increased tissue factor expression at the same moments. Tissue factor mRNA was detected in blood by PCR as early as 2 hours after OKT3 administration. *In vitro* experiments showed that OKT3 as well as 2 mitogenic, humanized anti-CD3 antibodies potently induced monocytic procoagulant activity whereas the 4 non-mitogenic anti-CD3 antibodies tested were over 1000-fold less potent than OKT3 [174].

In addition to its ability to induce tissue factor expression and activity on endothelial cells and monocytes, OKT3 probably also promotes intravascular thromboses by several other mechanisms. First, proinflammatory cytokines such as TNF-α, IL-1 and IFN-γ synergize to decrease endothelial cell expression of thrombomodulin [166, 175], thereby markedly inhibiting the anticoagulant effects of protein C and S [176]. Second, these mediators also increase the release of plasminogen activator inhibitor-1 [177, 178], one of the key anti-fibrinolytic molecule. However, at the same time proinflammatory cytokines induce the production of molecules such as tissue-type plasminogen activator [178], prostacyclin [179] and endothelium-derived relaxing factor (nitric oxide) [180, 181] that will help to maintain homeostasis. Indeed, tissue-type plasminogen activator will initiate fibrinolysis, while both prostacyclin and nitric oxide will potentiate each other in reducing platelet activity [167, 176, 182].

Table 3. Strategies for prevention of OKT3 nephrotoxicity.

1. Avoid prophylactic OKT3 therapy in patients at risk for thrombosis: a. Patients with lupus anticoagulant or hemolytic-uremic syndrome as primary disease b. Recipients of kidneys from pediatric donors or adult donors with significant vascular lesions
2. Administer 4 to 8 mg/kg steroid pretreatment before the first OKT3 dose*.
3. Avoid hypovolemia and administer a calcium channel blocker for the prevention of postoperative acute tubular necrosis.

**Lower doses do not adequately control the systemic side effects of OKT3 and are associated with increased incidence of acute tubular necrosis while higher doses might promote intragraft thromboses*

Concluding remarks

The initial lymphocyte and monocyte activation induced by the OKT3 mAb is responsible for first dose reactions, which include a transient nephrotoxic effect in kidney, transplant recipients. Moreover, activation of the common pathway of coagulation occurs at the initiation of OKT3 therapy. When additional predisposing factors are present, irreversible intragraft thromboses may ensue. Our present strategy to prevent OKT3 nephrotoxicity is summarized in Table 3. While appropriate clinical management can efficiently prevent OKT3 nephrotoxicity, extrarenal reactions related to the release of cytokines still occur in most patients receiving OKT3. This justifies the efforts made to develop new mAbs for immunosuppression including non-activating anti-CD3 mAbs.

References

1. Luster MI, Simeonova PP, Gallucci R, Matheson J. Tumor necrosis factor α and toxicology. Crit Rev Toxicol 1999; 29(5): 491-511.
2. Vassalli P. The pathophysiology of tumor necrosis factor. Annu Rev Immunol 1992; 10: 411-452.
3. Meldrum DR, Donnahoo KK. Role of TNF in mediating renal insufficiency following cardiac surgery: evidence of a post bypass cardiorenal syndrome. J Surg Res 1999; 85(2): 185-199.
4. Deasley D, Dinarello CA, Cannon JG. Interleukin-1 induces natriuresis in conscious rats: Role of renal prostaglandins. Kidney Int 1987; 33:1059-1065.
5. Bertani T, Abbate M, Zoja C, Corna D, Perico N, Ghezzi P, Remuzzi G. Tumor necrosis factor induces glomerular damage in the rabbit. Am J Pathol 1989; 134: 419-430.
6. Giroir BP, Johnson JH, Allen GL, Beutler B. The tissue distribution of tumor necrosis factor biosynthesis during endotoxemia. J Clin Invest 1992; 90:693-698.
7. Dohan DE. Role of endothelin and tumor necrosis factor in the renal response to sepsis. Nephrol Dial Transplant 1994; 9: 7377.
8. Wang J, Dunn MJ. Platelet-activating factor mediates endotoxin-induced acute renal insufficiency in rats. Am J Physiol 1987; 253: F1283-F1289.
9. Sedor JR. Interleukin-1: a master cytokine in the renal response to injury. In: Molecular Nephrology: Kidney Function in Health and Disease. Marcel Dekker, New York 1995; p. 631-652.
10. Tesch GH, Lan HY, Atkins RC, Nikolic-Paterson DJ. Role of interleukin-1 mesangial cell proliferation and matrix deposition in experimental mesangioproliferative nephritis. Am J Pathol 1997; 151(1): 141-150.
11. Tesch GH, Yang N, Yu H, Lan HY, Foti R, Chadban SJ, Atkins RC, Nikolic-Paterson DJ. Intrinsic renal cells are the major source of interleukin-1 β synthesis in normal and disease. Nephrol Dial Transplant 1997; 12(6): 1109-1115.
12. Ostendorf T, Burg M, Floege J. Cytokines and glomerular injury. Kidney Blood Press Res 1996; 19: 281-289.
13. Al-Wakeel J, Mitwalli A, Tarif N, Al-Mohaya S, Malik G, Khalil M. Role of interferon-a in the treatment of primary glomerulonephritis. Am J Kidney Dis 1999; 33(6): 1142-1146.
14. Maestroni GJ. The photoperiod transducer melatonin and the immune-hematopoietic system. J Photoch Photobiol 1998; 43A(3): 186-192.
15. Stein GM, Berg PA. Mistletoe extract-induced effects on immunocompetent cells: *in vitro* studies. Anticancer Drugs 1997; 8(Suppl 1): S39-S42.
16. Stein GM, Berg PA. Modulation of cellular and humoral immune responses during exposure of healthy individuals to an aqueous mistletoe extract. Eur J Med Research 1998; 3(6): 307-314.
17. Stein GM, Berg PA. Characterization of immunological reactivity of patients with adverse effects during therapy with an aqueous mistletoe extract. Eur J Med Research 1999; 4(5): 169-177.
18. van Welly M, Stoss M, Gorter RW. Toxicity of a standardized mistletoe extract in immunocompromised and healthy individuals. Am J Therap 1999; 6(1): 37-43.
19. Kunze E, Schulz H, Gabius HJ. Inability of galactoside-specific mistletoe lectin to inhibit N-methyl-N-nitrosurea-induced tumor development in the urinary bladder of rats and to mediate a local cellular immune response after long-term administration. J Cancer Res Clin Oncol 1998; 124(2): 73-87.
20. Davis ID. An overview of cancer immunotherapy. Immunol Cell Biol 2000; 78(3): 179-195.

21. Pinilla-Ibarz, J, Cathcart K, Korontsvit T, Soignet S, Bocchia M, Caggiano J, Lai L, Jimenez J, Kolitz J, Scheinberg DA. Vaccination of patients with chronic myelogenous leukemia with bcr-abl oncogene breakpoint fusion peptides generates specific immune responses. Blood 2000; 95(5): 1781-1787.
22. Jayne DR, Chapel H, Adu D, Misbah S, O'Donoghue D, Scott D, Lockwood CM. Intravenous immunoglobin for ANCA-associated systemic vasculitis with persistent disease activity. QJM 2000; 93(7): 433-439.
23. Tan E, Hajinazarian M, Bay W, Neff J, Mendell, JR. Acute renal failure resulting from intravenous immunoglobin therapy. Arch Neurol 1993; 50: 137-139.
24. Kobosko J, Nicol P. Renal toxicity of intravenous immunoglobin. Clin Nephrol 1992; 37: 216-217.
25. Barton JC, Herrera GA, Galla JH, Bertoli LF, Work J, Koopman WJ. Acute cryoglobulinemic renal failure after intravenous infusion of γ globulin. Am J Med 1987; 82: 624-629.
26. Diluydy MS, Delclaux C, Deprecigout V, Haramburu F, Roger I, Deminiere C, Mercie P, Pellegrin JL, Aparicio M. Acute renal failure after polyvalent immunoglobulin therapy. Presse Medicale 2000; 29(17): 942-943.
27. Green MC, Murray JL, Hortobagy GN. Monoclonal antibody therapy for solid tumors. Cancer Treat Rev 2000; 26(4): 269-286.
28. Stebbing J, Copson E, O'Reilly S. Herceptin in advanced breast cancer. Cancer Treat Rev 2000; 26: 287-290.
29. Gopal AK, Press OW. Clinical applications of anti-CD-20 antibodies. J Lab Clin Med 1999; 134(5): 445-450.
30. Nguyen DT, Amess JA, Doughty H, Hendry L, Diamond LW. IDEC-C2B8 anti-CD20 (rituximab) immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoproliferative disorders: evaluation of response in 48 patients. Eur J Haematol 1999; 62(2): 76-82.
31. Van Deventer SJ. Immunotherapy of Crohn's disease. Scand J Immunol 2000; 51(1): 18-22.
32. Issacs JD, Burrows N, Wing M, Keogan MT, Rebello PR, Watts RA, Pye RJ, Norris P, Hazelman BL, Hale G, Waldmann H. Humanized anti-CD4 monoclonal antibody therapy of autoimmune and inflammatory disease. Clin Exp Immunol 1997; 110(2):158-66.
33. Berard JL, Velez RL, Freeman RB, Tsunoda SM. A review of interleukin-2 receptor antagonists in solid organ transplantation. Pharmacotherapy 1999; 19(10): 1127-1137.
34. Belldegrun A, Webb D, Austin H, Steinberg S, White D, Lineham W, Rosenberg S. Effects of Interleukin-2 on renal function in patients receiving immunotherapy for advanced cancer. Ann Intern Med 1987; 106: 817-822.
35. Rosenberg S, Lotze M, Muul L, Chang A, Avis F, Leitman S, Linehan W, Robertson C, Lee R, Rubin J, Seipp C, Simpson C, White D. A progress report on the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukins-2 or high dose interleukins-2 alone. N Engl J Med 1987; 316: 888-897.
36. Lotze M, Matory Y, Rayner A, Ettinghausen S, Vetto J, Seipp C, Rosenberg S. Clinical Effects and toxicity of interleukin-2 in patients with cancer. Cancer 1986; 58: 2764-2772.
37. Margolin K, Rayner A, Hawkins M, Atkins M, Dutcher J, Fisher R, Weiss G, Doroshow J, Jaffe H, Roper M, Parkinson D, Wiernik P, Creekmore S, boldt D. Interleukin-2 and lymphokine-activated killer cell therapy of solid tumors: analysis of toxicity and management guidelines. J Clin Oncol 1989; 7: 486-498.
38. Rosenstein M, Ettinghausen S, Rosenberg S. Extravasation of intravascular fluid mediated by the stemic administration of recombinant interleukins 2. J Immunol 1986; 137: 1735-1742.
39. Schomburg A, Kirchner H, Atzpodien J. Renal, metabolic, and hemodynamic side-effects of itnerleukin-2 and/or interferon α: evidence of a risk/benefit advantage of subcutaneous therapy. J Cancer Res Clin Oncol 1993; 199(12): 745-755.
40. Ponce P, Cruz J, Travassos J, Moreira P, Oliveira J, Melo-Gomes E, Gouveia J. Renal toxicity mediated by continuous infusion of recombinant interleukin-2. Nephron 1993; 64(1): 114-118.
41. Memoli B, De Nicola L, Libetta C, Scialo A, Pacchiano G, Romano P, Palmieri G, Morabito A, Lauria R, Conte G et al. Interleukin-2 induced renal dysfunction in cancer patients is reversed by low-dose dopamine infusion. Am J Kidney Dis 1995; 26(1): 27-33.
42. Palmieri G, Morabito A, Lauria R, Montesarchio V, Matano E, Memoli B, Libetta C, Rea A, Merola C, Correale P. Low-dose dopamine induces early recovery of recombinant interleukin-2 impaired renal function. Eur J Cancer 1993; 29A(8): 1119-1122.
43. Rafi-Janajreh AQ, Chen D, Schmits R, Mak TW, Grayson RL, Sponeberg DP, Nagarkatti M, Nagarkatti PS. Evidence for the involvement of CD44 in endothelial cell injury and induction of vascular leak syndrome by IL-2. J Immunol 1999; 163(3): 1619-1627.
44. Marroquin DE, White DE, Steinberg SM, Rosenberg SA, Schwartzentruber DJ. Decreased tolerance to interleukin-2 with repeated courses of therapy in patients with metastatic melanoma or renal cell cancer. J Immunother 2000; 23(3): 387-392.
45. Textor S, Margolin K, Blayney D, Carlson J, Doroshow J. Renal, volume, and hormonal changes during therapeutic administration of recombinant interleukins-2 in man. Am J Med 1987; 83: 1055-1061.
46. Christiansen J, Skubitz K, Nath K, Ochoa A, Kennedy B. Nephrotoxicity of continuous intravenous infusion of recombinant interluekin-2. Am J Med 1988; 84; 1072-1075.

47. Kozeny G, Nicholas J, Creekmore S, Sticklin L, Hano J, Risher R. Effects of interleukins-2 immunotherapy on renal function. J Clin Onc 1988; 6(7): 1770-1776.
48. Shalmi C, Dutcher J, Feinfeld D, Chun K, Saleemi K, Freeman L, Lynn R, Wiernik P. Acute renal dysfunction during interleukin-2 treatment: suggestion of an intrinsic renal lesion. J Clin Onc 1990; 8(11):1839-1846.
49. Hall T, James P, Cambridge G. Interleukin-2 nephrotoxicity assessed *in vitro*. Res Comm Chem Path Pharm 1991; 73(3): 375-378.
50. Vlasveld LT, van de Wiel-van Kemenade E, de Boer AJ, Sein JJ, Gallee MP, Krediet RT, Mellief CJ, Rankin EM, Hekman A, Figdor CG. Possible role for cytotoxic lymphocytes in the pathogenesis of acute interstitial nephritis after recombinant interleukin-2 treatment for renal cell cancer. Cancer Immunol Immunother 1993; 36(3): 210-213.
51. Dutcher JP, Fisher RI, Weiss G, Aronson F, Margolin K, Louie A, Mier J, Caliendo G, Sosman JA, Eckardt JR, Ernest ML, Doroshow J, Atkins M. Outpatient subcutaneous interleukin-2 and interferon-α for metastatic renal cell cancer: five year follow-up of the Cytokine Working Group Study. Cancer J Sci Am 1997; 3(3): 157-162.
52. Carson WE, Yu H, Dierksheide J, Pfeffer K, Bouchard P, Clark R, Durbin J, Baldwin AS, Peschon J, Johnson PR, Ku G, Baumann H, Caligiuri MA. A fatal cytokine-induced systemic inflammatory response reveals a critical role for NK cells. J Immunol 1999; 162(8):4943-4951.
53. Phillips TM. Interferon-α induces renal dysfunction and injury. Curr Opin Nephrol Hypertens 1996; 5(4): 380-383.
54. Gutterman J, Fine S, Quesada J, Horning S, Levine J, Alexanian R, Bernhardt L, Kramer M, Spiegel H, Colburn W, Trown P, Merigan T, Dziewanowski Z. Recombinant leukocyte A interferon: pharmacokinetics, single-dose tolerance, and biologic effects in cancer patients. Ann Intern Med 1982; 96(5): 549-556.
55. Abdulhay G, DiSaia P, Blessing J, Creasman W. Human lymphoblastoid interferon in the treatment of advanced epithelial ovarian malignancies: a gynecologic oncology group study. Am J Obstet Gynecol 1985; 152: 418-423.
56. Sherwin S, Knost J, Fein S. A multiple-dose phase I trial of recombinant leukocyte A interferon in cancer patients. JAMA 1982; 248: 2461-2466.
57. Quesada J, Hawkins M, Horning S, Alexanian R, Borden E, Merigan T, Adams F, Gutterman J. Collaborative Phase I-II study of recombinant DNA-produced leukocyte interferon (clone A) in metastatic breast cancer, malignant lymphoma, and multiple myeloma. Am J Med 1984; 77: 427-432.
58. Quesada J, Talpaz M, Rios A, Kurzrock R, Gutterman J. Clinical toxicity of interferons in cancer patients: a review. J Clin Onc 1986; 4(2): 234-243.
59. Ferri C, Marzo E, Longombardo G, Lombardini F, La Civita L, Vanacore R, Liberati A, Gerli R, Greco F, Moretti A, Monti M, Gentilini P, Bombardieri S, Zignego A. Interferon-α in mixed cryoglobulinemia patients: a randomized, crossover-controlled trial. Blood 1993; 81(5): 1132-1136.
60. Dhib M, Bakhache E, Postec E, Vanrenterghem B, LeRoy F, Azar R, Francois A, Boldron A, Amaria R, Ozenne G, Hermelin A. Nephrotic syndrome complicating treatment with interferon α (Original title: Syndrome nephrotique compliquant un traitment par interferon α). Press Medicale 1996; 25(23): 1066-1068.
61. Dimitrov Y, Heibel F, Marcellin L, Chantrel F, Moulin B, Hannedouche T. Acute renal failure and nephrotic syndrome with α interferon therapy. Nephrol Dial Transplant 1997; 12(1): 200-203.
62. Gresser I, Maury C, Tovey M. Progressive glomerulonephritis in mice treated with interferon preparations at birth. Nature 1976; 263: 420-422.
63. Morel-Maroger L, Sloper J, Vinter J, Woodrow D, Gresser I. An ultrastructural study of the development of nephritis in mice treated with interferon in the neonatal period. Lab Invest 1978; 39: 513-522.
64. Lederer E, Truong L. Unusual glomerular lesion in a patient receiving long-term interferon α. Am J Kidney Dis 1992; 20(5): 516-518.
65. Parker MG, Atkins MB, Ucci AA, Levey AS. Rapidly progressive glomerulonephritis after immunotherapy for cancer. J Am Soc Nephrol 1995; 5(10):1740-1744.
66. Selby P, Kohn J, Raymond J, Judson I, McElwain T. Nephrotic syndrome during treatment with interferon. Brit Med J 1985; 290: 1180-1181.
67. Herrman J, Gabriel F. Membranoproliferative glomerulonephritis in a patient with hairy-cell leukemia treated with α-II interferon. N Engl J Med 1987; 316(2): 112-113.
68. Averbuch S, Austin H, Sherwin S, Antonovych T, Bunn P, Longo D. N Engl J Med 1984; 310(1): 32-35.
69. Traynor A, Kuzel T, Samuelson E, Kanwar Y. Minimal change glomerulopathy and glomerular visceral epithelial hyperplasia associated with α-interferon therapy for cutaneous T-cell lymphoma. Nephron 1994; 67(1): 94-100.
70. Rettmar K, Kienast J, van de Loo J. Minimal change glomerulonephritis with reversible proteinuria during interferon α 2a therapy for chronic myeloid leukemia. Am J Hematol 1995; 49(4): 355-356.
71. Shah M, Jenis EH, Mookerjee BK, Schriber JR, Baer MR, Herzig GP, Wetzler M. Interferon-α associated focal segmental glomerulosclerosis with massive proteinuria in patients with chronic myeloid leukemia following high dose chemotherapy. Cancer 1998; 83(9): 1938-1946.

72. Ravandi-Kashani F, Cortes J, Talpaz M, Kantarjian HM. Thrombotic microangiopathy associated with interferon therapy for patients with chronic myelogenous leukemia: coincidence or true side effect ? Cancer 1999; 85(12): 2583-2588.
73. Harvey M, Rosenfeld D, Davies D, Hall BM. Recombinant interferon α and hemolytic uremic syndrome: cause or coincidence [letter]. Am J Hematol 1994; 46(2): 152-153.
74. Coronoes E, Petrusevska G, Varghese F, Truong LD. Focal segmental glomerulosclerosis with acute renal failure associated with α-interferon therapy. Am J Kidney Dis 1996; 28(6): 888-892.
75. Bellaiche G, Guettier C, Duboust A, Ley G, Slama JL. Nephrotic syndrome with acute renal failure due to focal segmental glomerulosclerosis in a patient treated with α-interferon for chronic hepatitis C. Gastroenterol Clin Biol 2000; 24(4): 465.
76. Gordon AC, Edgar JD, Finch RG. Acute exacerbation of vasculitis during interferon-α therapy for hepatitis C-associated cryoglobinaemia. J Infect 1998; 36(2): 229-230.
77. Ohta S, Yokoyama H, Wada T, Sakai N, Shimizu M, Kato T, Furuichi K, Segawa C, Hisada Y, Kobayashi K. Exacerbation of glomerulonephritis in subjects with chronic hepatitis C virus infection after interferon therapy. Am J Kidney Dis 1999; 33(6): 1040-1048.
78. Yamabe H, Johnson RJ, Gretch DR, Osawa H, Inuma H, Sasaki T, Kaizuka M, Tamura N, Tsunoda S, Fujita Y et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection responsive to interferon-α. Am J Kidney Dis 1995; 25(1): 67-69.
79. Sarac E, Bastacky S, Johnson JP. Response to high-dose interferon α after failure of standard therapy in MPGN associated with hepatitis C virus infection. Am J Kidney Dis 1997; 30(1): 113-115.
80. Johnson RJ, Gretch DR, Couser WG, Alpers CE, Wilson J, Chung M, Hart J, Willson R. Heptatitis C virus associated glomerulonephritis. Effect of α interferon therapy. Kidney Int 1994; 46(6): 1700-1704.
81. Rostaing L, Modesto A, Baron E, Cisterne JM, Chabannier MH, Tkaczuk J, Durand D, Suc JM. Acute renal insufficiency in renal transplants treated with interferon-α for chronic hepatitis C (Original title: Insuffisance renale aigue chez des transplantes renaus traits par interferon-α pour une hepatatite chronique C. Néphrologie 1996; 17(4): 247-254.
82. Rostaing L, Izopet J, Baron E, Duffaut M, Puel J, Durand D, Suc JM. Preliminary results of treatment of chronic hepatitis C with recombinant interferon α in renal transplant patients. Nephrol Dial Transplant 1995; 10 (Suppl 6):93-96.
83. Rostaing L, Modesto A, Baron E, Cisterne JM, Chabannier MH, Durand D. Acute renal failure in kidney transplant patients treated with interferon α 2b for chronic hepatitis C. Nephron 1996; 74(3): 512-516.
84. Schiller J, Storer B, Willson J, Borden E. Phase I trial of combinations of recombinant interferons β and γ in patients with advanced malignancy. Cancer Treat Rep 1987; 71(10): 945-952.
85. Schiller J, Storer B, Witt P, Nelson B, Brown R, Horisberger M, Grossberg S, Border E. Biological and clinical effects of the combination of β and γ interferons administered as a 5 day continuous infusion. Cancer Res 1990; 50: 4588-4594.
86. Takei Y, Sims TN, Urmson J, Halloran PF. Central role for interferon γ receptor in the regulation of renal MHC expression. J Am Soc Nephrol 2000; 11(2): 250-261.
87. Thompson JA, Cox WW, Lindgren CG, Collins C, Neras KA, Bonnem EM, Fefer A. Subcutaneous recombinant γ interferon in cancer patients: toxicity, pharmacokinetics, and immunomodulatory effects. Cancer Immunol Immunother 1987; 25: 47-53.
88. Sridkandan K, Garner P, Watkinson K, Pettingale K, Brinkley D, Calman F, Tee D. A toxicity study of recombinant interferon-γ given by intravenous infusion to patients with advanced cancer. Cancer Chemother Pharmacol 1986; 18: 63-68.
89. Ault B, Stapleton F, Gaber L, Martin A, Roy S, Murphy S. Acute renal failure during therapy with recombinant human γ interferon. N Engl J Med 1988; 319(21): 1397-1400.
90. Acuto, O. and E.L. Reinherz. The human T-cell receptor. Structure and function. N Engl J Med 1985: 312(17): 1100-1111.
91. Ortho Multicenter Transplant Study Group. A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. N Engl J Med 1985; 313(6): 337-342.
92. Abramowicz D, Wissing M, Broeders N. Induction with anti-CD3 monoclonal antibodies. Current Opin Organ Transplant 1999; 4: 312-317.
93. Goldstein G. Overview of the development of Orthoclone OKT3: monoclonal antibody for therapeutic use in transplantation. Transplant Proc 1987: 19(2 Suppl 1): 1-6.
94. Van Wauwe J, De Mey J Goossens J. OKT3: a monoclonal antibody with potent mitogenic properties. J Immunol 1980; 124: 2708-2713.
95. Abramowicz, D., L. Schandene, M. Goldman, A. Crusiaux, P. Vereerstraeten, L. De Pauw, J. Wybran, P. Kinnaert, E. Dupont and C. Toussaint. Release of tumor necrosis factor, interleukin-2, and γ-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. Transplantation 1989: 47(4): 606-608.
96. Chatenoud L, Ferran C, Reuter A, Legendre C, Gevaert Y, Kreis H, Franchimont P, Bach JF. Systemic reaction to the anti-T-cell monoclonal antibody OKT3 in relation to serum levels of tumor necrosis factor and interferon-γ. N Engl J Med 1989: 320(21): 1420-1421.
97. Chatenoud L, Ferran C, Legendre C, Thouard I, Merite S, Reuter A, Gevaert Y, Kreis H, Franchimont P, Bach JF. *In vivo* cell activation following OKT3 administration. Transplantation 1990: 49(4): 697-702.

98. Bloemena E, ten Berge IJM, Surachno J, Wilmink JM. Kinetics of interleukin 6 during OKT3 treatment in renal allograft recipients. Transplantation 1990: 50(2): 330-331.
99. Gaston RS, Deierhoi MH, Patterson T, Prasthofer E, Julian BA, Barber WH, Laskow DA, Diethelm AG, Curtis JJ. OKT3 first-dose reaction: association with T cell subsets and cytokine release. Kidney Int 1991: 39: 141-148.
100. Goldman M, Gérard C, Abramowicz D, Schandené L, Durez P, De Pauw L, Kinnaert P, Vereerstraeten P, Velu T. Induction of interleukin-6 and interleukin-10 by the OKT3 monoclonal antibody: possible relevance to posttransplant lymphoproliferative disorders. Clin Transplant 1992: 6: 265-268.
101. Ferran, C., F. Dautry, S. Merite, K. Sheehan, R. Schreiber, G. Grau, J.F. Bach and L. Chatenoud. Anti-tumor necrosis factor modulates anti-CD3-triggered T cell cytokine gene expression *in vivo*. J Clin Invest 1994: 93(5): 2189-2196.
102. Alegre M, Vandenabeele P, Flamand V, Moser M, Leo O, Abramowicz D, Urbain J, Fiers W, Goldman M. Hypothermia and hypoglycemia induced by anti-CD3 monoclonal antibody in mice: role of tumor necrosis factor. Eur J Immunol 1990; 20(3): 707-710.
103. Ferran C, Dy M, Sheehan K, Schreiber R, Grau G, Bluestone J, Bach JF, Chatenoud L. Cascade modulation by anti-tumor necrosis factor monoclonal antibody of interferon-γ, interleukin 3 and interleukin 6 release after triggering of the CD3/T cell receptor activation pathway. Eur J Immunol 1991; 21(10): 2349-2353.
104. Matthys P, Dillen C, Proost P, Heremans H, Van DJ, Billiau A. Modification of the anti-CD3-induced cytokine release syndrome by anti-interferon-γ or anti-interleukin-6 antibody treatment: protective effects and biphasic changes in blood cytokine levels. Eur J Immunol 1993: 23(9): 2209-2216.
105. Raasveld MH, Bemelman FJ, Schellekens PT, van Diepen FN, van Dongen A, van Royen EA, Hack CE, ten Berge IJ. Complement activation during OKT3 treatment: a possible explanation for respiratory side effects. Kidney Int 1993; 43(5): 1140-1149.
106. Vallhonrat H, Williams WW, Cosimi AB, Tolkoff-Rubin N, Ginns LC, Wain JC, Preffer F, Olszak I, Wee S, Delmonico FL, Pascual M. *In vivo* generation of C4d, Bb, iC3b, and SC5b-9 after OKT3 administration in kidney and lung transplant recipients. Transplantation 1999; 67(2): 253-258.
107. Bûsing M, Mellert J, Greger B, Hopt U. Acute pulmonary insufficiency due to OKT3 therapy. Transplant Proc 1990: 22: 1779.
108. Mackie JD, Pankewycz OG, Bastos MG, Kelley VE, Strom TB. Dose-related mechanisms of immunosuppression mediated by murine anti-CD3 monoclonal antibody in pancreatic islet cell transplantation and delayed-type hypersensitivity. Transplantation 1990: 49(6): 1150-1154.
109. Waid TH, Lucas BA, Thompson JA, Brown SA, Munch L, Prebeck RJ, Jezek D. Treatment of acute cellular rejection with T10B9.1A-31 or OKT3 in renal allograft recipients. Transplantation 1992; 53(1): 80-86.
110. Thistlethwaite J, Stuart JK, Mayes JT, Gaber AO, Woodle S, Buckingham MR, Stuart FP. Complications and monitoring of OKT3 therapy. Am J Kidney Dis 1988; 11(2): 112-119.
111. Kreis H. Adverse events associated with OKT3 immunosuppression in the prevention or treatment of allograft rejection. Clin Transplantation 1993: 7: 431-446.
112. Simpson MA, Madras PN, Cornaby AJ, Etienne T, Dempsey RA, Clowes GH, Monaco AP. Sequential determinations of urinary cytology and plasma and urinary lymphokines in the management of renal allograft recipients. Transplantation 1989: 47(2): 218-223.
113. Goldman, M., J.L. Van Laethem, D. Abramowicz, L. De Pauw, P. Kinnaert and P. Vereerstraeten. Evolution of renal function during treatment of kidney graft rejection with OKT3 monoclonal antibody. Transplantation 1990: 50(1): 158-159.
114. Batiuk TD, Bennett WM, Norman DJ. Cytokine nephropathy during antilymphocyte therapy. Transplant Proc 1993; 25(2 Suppl 1): 27-30.
115. Waid TH, Lucas BA, Thompson JS, McKeown JW, Brown S, Kryscio R, Skeeters LJ. Treatment of renal allograft rejection with T10B9.1A31 or OKT3: final analysis of a phase II clinical trial. Transplantation 1997; 64(2): 274-281.
116. Woodle ES, Xu D, Zivin RA, Auger J, Charette J, O'Laughlin R, Peace D, Jollife LK, Haverty T, Bluestone JA, Thistlethwaite JR Jr. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3γ1(Ala-Ala) in the treatment of acute renal allograft rejection. Transplantation 1999; 68(5): 608-616.
117. Norman DJ, Kahana L, Stuart FP Jr, Thistlethwaite JR Jr, Shield CF 3rd, Monaco A, Dehlinger J, Wu SC, Van Horn A, Haverty TP. A randomized clinical trial of induction therapy with OKT3 in kidney transplantation. Transplantation 1993; 55(1): 44-50.
118. Opelz, G for The Collaborative Transplant Study. Efficacy of rejection prophylaxis with OKT3 in renal transplantation. Transplantation 1995; 60(11): 1220-1224.
119. Abramowicz D, Norman D, Vereerstraeten P, Goldman M, De Pauw L, Vanherweghem JL, Kinnaert P, Kahana L, Thistlethwaite JR, Shield CF, Monaco AP, Shu-Chen Wu, Haverty T. OKT3 prophylaxis in renal grafts with prolonged ischemia times: association with improvement in long-term survival. Kidney Int 1996; 49: 768-772.
120. Szczech LA, Berlin JA, Feldman HI. The effect of antilymphocyte induction therapy on renal allograft survival. A meta-analysis of individual patient-level data. Anti-Lymphocyte Antibody Induction Therapy Study Group. Ann Intern Med 1998; 128(10): 817-826.

121. Abramowicz D, Goldman M, De Pauw L, Kinnaert P, Vanherweghem Jl, Vereerstraeten P. Long-term effects of prophylactic OKT3 in cadaveric renal transplantation: a single-center, prospective, randomized study. Transplantation 1992; 54: 433-437.
122. Toussaint C, De Pauw L, Vereerstraeten P, Kinnaert P, Abramowicz D, Goldman M. Possible nephrotoxicity of the prophylactic use of OKT3 monoclonal antibody after cadaveric renal transplantation. Transplantation 1989; 48(3): 524-526.
123. Abramowicz D, Pradier O, Marchant A, Florquin S, De Pauw L, Vereerstraeten P, Kinnaert P, Vanherweghem JL, Goldman M. Induction of thromboses within renal grafts by high-dose prophylactic OKT3. Lancet 1992; 339(8796): 777-778.
124. Abramowicz D, Pradier O, De Pauw L, Kinnaert P, Mat O, Surquin S, Doutrelepont JM, Vanherweghem JL, Capel P, Vereerstraeten P, Goldman M. High-dose glucocorticosteroids increase the procoagulant effects of OKT3. Kidney Int 1994; 46: 1596-1602.
125. Gomez E, Aguado S, Gago E, Escalada P, Alvarez-Grande J. Main graft vessels thromboses due to conventional-dose OKT3 in renal transplantation. Lancet 1992; 339(8809): 1612-1613.
126. Noel C, Hazzan M, Coppin MC, Pruvot FR, Bridoux F, Lelievre G. Renal transplant artery thrombosis following treatment of allograft rejection with monoclonal anti-CD3 antibodies (OKT3). Transplant Proc 1995; 27(4): 2438-2439.
127. Shankar R, Bastani B, Salinas-Madrigal L, Sudarshan B. Acute thrombosis of the renal transplant artery after a single dose of OKT3. Am J Nephrol 2001; 21(2): 141-144.
128. Heptinstall RH. Pathology of the kidney (3rd ed.). Little, Brown and Co; Boston 1983.
129. Dussol B, Brunet P, Vacher-Coponat H, Saingra Y, Casanova P, Berland Y. Haemolytic uraemic syndrome in a renal transplant recipient during OKT3 therapy. Nephrol Dial Transplant 1994; 9(8): 1191-1193.
130. Morris-Stiff G, Evans M, Baboolal K, Balaji V, Moore R, Jurewicz A, Lord R. Haemolytic uraemic syndrome associated with OKT3. Transpl Int 1996; 9(5): 522-523.
131. Palleschi J, Novick AC, Braun WE, Magnusson MO. Vascular complications of renal transplantation. Urology 1980: 16(1): 61-67.
132. Groggel GC. Acute thrombosis of the renal transplant artery: a case report and review of the literature. Clin Nephrol 1991: 36(1): 42-45.
133. Abramowicz D, De Pauw L, Le Moine A, Sermon F, Surquin M, Doutrelepont JM, Ickx B, Depierreux M, Vanherweghem JL, Kinnaert P, Goldman M, Vereerstraeten P. Prevention of OKT3 nephrotoxicity after kidney transplantation. Kidney Int 1996; 49: S39-S43.
134. Belldegrun A, Webb DE, Austin HA 3rd, Steinberg SM, White DE, Linehan WM, Rosenberg SA. Effects of interleukin-2 on renal function in patients receiving immunotherapy for advanced cancer. Ann Intern Med 1987;106(6): 817-822.
135. Shalmi CL, Dutcher JP, Feinfeld DA, Chun KJ, Saleemi KR, Freeman LM, Lynn RI, Wiernik PH. Acute renal dysfunction during interleukin-2 treatment: suggestion of an intrinsic renal lesion. J Clin Oncol 1990: 8(11): 1839-1846.
136. Gresser I, Maury C, Tovey M, Morel-Maroger L, Pontillon F. Progressive Glomerulonephritis in mice treated with interferon preparations at birth. Nature 1976; 263: 420-422.
137. Ault BH, Stapleton FB, Gaber L, Martin A, Roy S 3rd, Murphy SB. Acute renal failure during therapy with recombinant human γ interferon. N Engl J Med 1988; 319(21): 1397-1400.
138. Spriggs DR, Sherman ML, Michie H, Arthur KA, Imamura K, Wilmore D, Frei E, Kufe DW. Recombinant human tumor necrosis factor administered as a 24-hour intravenous infusion. A phase I and pharmacologic study. J Natl Cancer Inst 1988: 80(13): 1039-1044.
139. Breisblatt WM, Schulman DS, Stein K, Wolfe CJ, Whiteside T, Kormos R, Hardesty RL. Hemodynamic response to OKT3 in orthotopic heart transplant recipients: evidence for reversible myocardial dysfunction. J Heart Lung Transplant 1991; 10(3): 359-365.
140. Ognibene FP, Rosenberg SA, Lotze M, Skibber J, Parker MM, Shelhamer JH, Parrillo JE. Interleukin-2 administration causes reversible hemodynamic changes and left ventricular dysfunction similar to those seen in septic shock. Chest 1988; 94(4): 750-754.
141. Natanson C, Eichenholz PW, Danner RL, Eichacker PQ, Hoffman WD, Kuo GC, Banks SM, MacVittie TJ, Parrillo JE. Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. J Exp Med 1989: 169(3): 823-832.
142. Quezado ZM, Natanson C. Systemic hemodynamic abnormalities and vasopressor therapy in sepsis and septic shock. Am J Kidney Dis 1992; 20(3): 214-222.
143. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report [see comments]. N Engl J Med 1988: 319(25): 1676-1680.
144. Rosenstein M, Ettinghausen SE, Rosenberg SA. Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2. J Immunol 1986; 137(5): 1735-1742.
145. Thervet E, Martinez F, Legendre C, Dechaux M, Kreis H. First dose OKT3-induced release of endothelin in renal transplant recipients [letter]. Nephrol Dial Transplant 1993; 8(3): 287.

146. Clozel M, Clozel JP. Effects of endothelin on regional blood flows in squirrel monkeys. J Pharmacol Exp Ther 1989; 250(3): 1125-1131.
147. Maessen JG, Buurman WA, Kootstra G. Direct cytotoxic effect of cytokines in kidney parenchyma: a possible mechanism of allograft destruction. Transplant Proc 1989; 21: 309-310.
148. Damle NK, Doyle LV, Bender JR, Bradley EC. Interleukin 2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. J Immunol 1987; 138(6): 1779-1785.
149. Linas SL, Shanley PF, Whittenburg D, Berger E, Repine JE. Neutrophils accentuate ischemia-reperfusion injury in isolated perfused rat kidneys. Am J Physiol 1988; 255: F728-F735.
150. Varani J, Ginsburg I, Schuger L, Gibbs DF, Bromberg J, Johnson KJ, Ryan US, Ward PA. Endothelial cell killing by neutrophils. Synergistic interaction of oxygen products and proteases. Am J Pathol 1989; 135(3): 435-438.
151. Schmaldienst S, Bohmig G, Pohanka E, Kovarik J, Zlabinger GJ. Evidence for granulocyte activation by OKT3. Transplant Proc 1992; 24(6): 2600-2601.
152. Linas SL, Whittenburg D, Parsons PE, Repine JE. Mild renal ischemia activates primed neutrophils to cause acute renal failure. Kidney Int 1992; 42(3): 610-616.
153. Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA. Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc Natl Acad Sci USA 1987; 84(5): 1374-1378.
154. Hirsch R, Eckhaus M, Auchincloss HJ, Sachs DH, Bluestone JA. Effects of *in vivo* administration of anti-T3 monoclonal antibody on T cell function in mice. I. Immunosuppression of transplantation responses. J Immunol 1988; 140(11): 3766-3772.
155. Ferran C, Sheehan K, Dy M, Schreiber R, Merite S, Landais P, Noel LH, Grau G, Bluestone J, Bach JF et al. Cytokine-related syndrome following injection of anti-CD3 monoclonal antibody: further evidence for transient *in vivo* T cell activation. Eur J Immunol 1990; 20(3): 509-515.
156. Alegre ML, Vandenabeele P, Depierreux M, Florquin S, Deschodt Lanckman M, Flamand V, Moser M, Leo O, Urbain J, Fiers W, Goldman M. Cytokine release syndrome induced by the 145-2C11 anti-CD3 monoclonal antibody in mice: prevention by high doses of methylprednisolone. J Immunol 1991; 146(4): 1184-1191.
157. Heemann UW, Tullius SG, Kupiec-Weglinski JW, Tilney NL. Early events in acute allograft rejection: leukocyte/endothelial cell interactions. Clin Transplantation 1993; 7: 82-89.
158. Buysmann S, van Diepen FN, Surachno S, Pals ST, ten Berge RJ. Increased dermal expression of ICAM-1 and VCAM-1 after administration of OKT3 in man. Clin Nephrol 1996; 46(2): 84-91.
159. Bergese SD, Pelletier RP, Ohye RG, Vallera DA, Orosz CG. Treatment of mice with anti-CD3 mAb induces endothelial vascular cell adhesion molecule-1 expression. Transplantation 1994; 57(5): 711-717.
160. Bemelman FJ, Buysmann S, Yong SL, van Diepen FN, Schellekens PT, ten Berge RJ. Biphasic granulocytopenia after administration of the first dose of OKT3. Lab Clin Med 1995; 126(6): 571-579.
161. Pradier O, Surquin M, Stordeur P, De Pauw L, Kinnaert P, Vereerstraeten P, Capel P, Goldman M, Abramowicz D. Monocyte procoagulant activity induced by *in vivo* administration of the OKT3 monoclonal antibody. Blood 1996; 87: 3768-3774
162. Mannucci PM, Giangrande PL. Detection of the prethrombotic state due to procoagulant imbalance. Eur J Haematol 1992; 48(2): 65-69.
163. Pradier O, Marchant A, Abramowicz D, De Pauw L, Vereerstraeten P, Kinnaert P, Vanherweghem JL, Capel P, Goldman M. Procoagulant effect of the OKT3 monoclonal antibody: involvement of tumor necrosis factor. Kidney Int 1992; 42(5): 1124-1129.
164. Raasveld MH, Hack CE, ten Berge I. Activation of coagulation and fibrinolysis following OKT3 administration to renal transplant recipients: association with distinct mediators. Thromb Haemost 1992; 68(3): 264-267.
165. Deira J, Alberca I, Lerma JL, Martin B, Tabernero JM. Changes in coagulation and fibrinolysis in the postoperative period immediately after kidney transplantation in patients receiving OKT3 or cyclosporine A as induction therapy. Am J Kidney Dis 1998; 32(4): 575-581.
166. Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 1986; 163(3): 740-745.
167. Wu KK. Endothelial cells in hemostasis, thrombosis, and inflammation. Hosp Pract Off Ed 1992; 27(4): 145-150.
168. Itaka M, Iwatani Y, Row VV, Volpe R. Induction of monocyte procoagulant activity with OKT3 antibody. J Immunol 1987; 139: 1617-1623.
169. Pradier O, Abramowicz D, Capel P, Goldman M. Procoagulant properties of OKT3 at the monocyte level: inhibition by pentoxifylline. Transplant Proc 1993; 25: 39-40.
170. Ryan J, Geczy CL. Characterization and purification of mouse macrophage procoagulant-inducing factor. J Immunol 1986; 137(9): 2864-2870.
171. Zuckerman SH, Suprenant YM. Induction of endothelial cell/macrophage procoagulant activity: synergistic stimulation by γ interferon and granulocyte-macrophage colony stimulating factor. Thromb Haemost 1989; 61(2): 178-182.

172. Carlsen E, Flatmark A, Prydz H. Cytokine-induced procoagulant activity in monocytes and endothelial cells. Further enhancement by cyclosporine. Transplantation 1988; 46(4): 575-580.
173. Fan ST, Edgington TS. Clonal analysis of mechanisms of murine T helper cell collaboration with effector cells of macrophage lineage. J Immunol 1988; 141(6): 1819-1827.
174. Pradier O, Surquin M, Stordeur P, De Pauw L, Kinnaert P, Vereerstraeten P, Capel P, Goldman M, Abramowicz D. Monocyte procoagulant activity induced by *in vivo* administration of the OKT3 monoclonal antibody. Blood 1996; 87: 3768-3774.
175. Nawroth PP, Handley DA, Esmon CT, Stern DM. Interleukin 1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. Proc Natl Acad Sci USA 1986; 83(10): 3460-3464.
176. Cotran RS, Pober JS. Effects of cytokines on vascular endothelium: their role in vascular and immune injury. Kidney Int 1989; 35(4): 969-975.
177. Emeis JJ, Kooistra T. Interleukin 1 and lipopolysaccharide induce an inhibitor of tissue-type plasminogen activator *in vivo* and in cultured endothelial cells. J Exp Med 1986; 163(5): 1260-1266.
178. van der Poll T, Levi M, Buller HR, van Deventer SJ, de Boer JP, Hack CE, ten Cate JW. Fibrinolytic response to tumor necrosis factor in healthy subjects. J Exp Med 1991; 174(3): 729-732.
179. Zavoico GB, Ewenstein BM, Schafer AI, Pober JS. IL-1 and related cytokines enhance thrombin-stimulated PGI2 production in cultured endothelial cells without affecting thrombin-stimulated von Willebrand factor secretion or platelet-activating factor biosynthesis. J Immunol 1989; 142(11): 3993-3999.
180. Lamas S, Michel T, Brenner BM, Marsden PA. Nitric oxide synthesis in endothelial cells: evidence for a pathway inducible by TNF-α. Am J Physiol 1991; 261: 634-641.
181. Beasley D, Schwartz JH, Brenner BM. Interleukin 1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. J Clin Invest 1991; 87(2): 602-608.
182. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990; 323(1): 27-36.
183. Konkle BA, Schuster SJ, Kelly MD, Harjes K, Hassett DE, Bohrer M, Tavassoli M. Plasminogen activator inhibitor-1 messenger RNA expression is induced in rat hepatocytes *in vivo* by dexamethasone. Blood 1992: 79(10): 2636-2642.
184. Vassalli JD, Hamilton J, Reich E. Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors, and cyclic nucleotides. Cell 1976; 8: 271-281.
185. Blajchman MA, Senyi AF, Hirsh J, Surya Y, Buchanan M. Shortening of bleeding time in rabbits by hydrocortisone caused by inhibition of prostacyclin generation by the vessel wall. J Clin Invest 1979; 63: 1026-1035.
186. Lewis GD, Campbell WB, Johnson AR. Inhibition of prostaglandin synthesis by glucocorticoids in human endothelial cells. Endocrinology 1986; 119(1): 62-69.
187. Knowles RG, Salter M, Brooks SL, Moncada S. Anti-inflammatory glucocorticoids inhibit the induction by endotoxin of nitric oxide synthase in the lung, liver and aorta of the rat. Biochem Biophys Res Commun 1990; 172(3): 1042-1048.
188. Geller, D.A., A.K. Nussler, S.M. Di, C.J. Lowenstein, R.A. Shapiro, S.C. Wang, R.L. Simmons and T.R. Billiar. Cytokines, endotoxin, and glucocorticoids regulate the expression of inducible nitric oxide synthase in hepatocytes. Proc Natl Acad Sci USA 1993: 90(2): 522-6.
189. Shen SMC, Rapaport SI. Increased fibrinogen consumption following endotoxin injection in cortisone-treated rabbits. Proc Soc Exp Biol Med 1974; 145: 851-854.
190. Ueda Y, Ito S, Saito K, Lee KH, Niesen N, Brentjens JR, Gans R. Divergent effects of cortisone on monocyte procoagulant activity and tumor necrosis factor release in the generalized Schwartzman reaction (GSR) in rabbits. J Am Soc Nephrol 1992; 2: 620.

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Radiocontrast agents

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Introduction

The use of iodinated contrast media (CM) continues to be a common cause of hospital-acquired acute renal failure (ARF) and its development increases the in-hospital mortality significantly [1, 2, 2a, 2b] as well as increasing the length of hospital stay [2c]. Contrast media-induced nephropathy (CMIN) is defined as an otherwise unexplained acute deterioration of renal function after intravascular administration of iodinated CM. Although the clinical features and the histopathological findings of CMIN have been well described [3-6], its pathogenesis, prevention and best treatment modality remain uncertain.

Definition

Most authors define CMIN by an increase of serum creatinine of more than 1 mg/dl 2-3 days after CM exposure. Other reasons for an acute deterioration of renal function have to be excluded. Some investigators even believe that a lower increase of serum creatinine (0.5 mg/dl 2-4 days after CM) also should be classified as CMIN. It would also be prudent to look for a fall of GFR (general > 25% from baseline) with more sensitive methods (i.e. inulin clearance, iothalmate clearance, iohexol clearance [7]. Next to changes in GFR or serum creatinine levels an increase in urinary enzyme excretion seems to also be a sensitive marker of tubular damage after CM exposure [7, 8]. However, no conclusive relationship has been demonstrated between the detection of enzymes in urine and the fall in GFR [8-11].

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Clinical findings and histopathology

In most cases, the increase in serum creatinine starts 24 to 48 hours after CM exposure, will peak after 3-5 days and return to baseline 7-10 days after exposure. Except for patients with a profound degree of renal function impairment, CMIN presents as a non-oliguric form of ARF. Temporary or continuous dialysis is rarely required. Although the majority of patients will show only minor and transient effects on renal function after CM exposure, a recent study showed that an increase of serum creatinine that did not require dialysis was associated with a higher in-hospital mortality rate compared to patients without CMIN [1, 2]. For post-angiographic patients who develop CMIN, 10% will require long-term dialysis [11a].

Morphological changes have been observed mainly as vacuolar changes in the proximal convoluted tubular cells [6, 12, 13]. These morphological changes parallel the increases in urinary enzyme excretion [6, 14], but a strong relationship to renal function impairment after CM has not been demonstrated [15].

Incidence

The incidence of CMIN in the literature ranges from less than 1% to over 70% [16-19a]. This discrepancy results from the lack of a single reliable definition, different methods of investigation, different types of radiological procedures, use of high or low osmolar contrast media and the presence or absence of risk factors. In patients without any risk factor the incidence is less than 1% despite the use of up to 800 ml of contrast media [20]. In patients at high risk the frequency of CMIN has been reported to increase in the last few years, which seems to be related to the wider use of diagnostic and therapeutic interventions in elderly and critically ill patients [21].

Risk Factors

A preexistent impairment of renal function is commonly regarded as the most important risk factor [23]. Consistent with multivariate regression analyses, diabetes mellitus is frequently citied next to renal insufficiency as an independent risk factor for CMIN [2, 3, 4, 23, 24]. However, in controlled studies diabetic patients without renal function impairment have not been shown to be at higher risk for developing CMIN [25, 26]. Because diabetic patients suffer from multiple vascular abnormalities, especially endothelial, which contribute to renal damage, the vascular contribution still has to be clarified. After having developed renal insufficiency, diabetics are at significantly higher risk for CMIN compared to patients with other forms of renal failure [27]. In distinction to other patients given contrast media, diabetic often develop an oliguric form of CMIN and subsequently require dialysis. Contrary to previous reports [24, 28, 29], the volume of CM is only a risk factor in azotemic, diabetic patients [19, 30, 31]. Based on a laboratory demonstration that CM addition caused intratubular precipitations of Bence-Jones protein, multiple myeloma has long been held out as an independent risk factor; however, a recent retrospective analysis [32] concluded that patients with multiple myeloma where not at increased risk for developing CMIN (table 1).

Pathogenesis

In general, CM attenuates both renal hemodynamics and renal tubular function [33]. After injection of CM there is a transient increase, followed by a more prolonged decrease in renal blood flow (RBF) [34-36]. A variety of vasoactive substances may modulate the CM-induced vasoconstriction, including prostaglan-

Table 1. Risk factors for contrast media-induced nephropathy.

Confirmed	Suspected	Disproved
Chronic renal failure	Hypertension	Myeloma
Diabetic nephropathy	Generalized atherosclerosis	Diabetes without nephropathy
Severe congestive heart failure	Abnormal liver function tests	
Amount and frequency of contrast media	Hyperuricemia	
Volume depletion/hypotension	Proteinuria	

dins, ANF, adenosine, endothelin, vasopressin, noradrenaline and angiotensin [37]. Of particular interest has been the possible role of superoxide radicals in the pathogenesis of CMIN. Not only do they induce renal vasoconstriction, they also cause direct renal cell injury. Superoxide dismutase prevents the fall in GFR associated with CM , while in a dehydrated animal model, renal levels of superoxide dismutase is diminished which may account for the demonstrated increased susceptibility to CMIN [2]. By sequentially measuring these substances before and after CM exposure and by using antagonists of these vasoactive substances (misoprostol, bosentan, ace-inhibitors, α-blockers, etc.) [36, 38-42] the degree of involvement for each of these potential mediators in the process of developing CMIN has been investigated. To date, only endothelin and adenosine have been shown to play a role as important mediators in CMIN [40, 41, 43].

CM-induces intrarenal hypoxia, possibly related to the hemodynamic changes and/or increased tubular energy expenditure in response to osmotic stress [33]. It has been proposed that increased renal adenosine levels arising from enhanced ATP hydrolysis may be a major contributor to development of acute renal failure after CM application (Figure 1). This is corroborated by the finding that application of CM increases urinary adenosine excretion [44, 45] and the observation that dipyridamol, a nucleoside uptake blocker, magnifies the renal hemodynamic effects of CM [44, 45]. In addition, there are many similarities between CM-induced nephrotoxicity and the renal hemodynamic changes induced by adenosine. Sodium depletion potentate both adenosine action in the kidney [46, 47], and augments the nephrotoxicity of CM [35, 39]. Blockade of the production of vasodilatory prostaglandins by indomethacin potentates both the adenosine effect in the kidney [48], as well as the vasoconstriction induced by CM [15, 49, 50]. Pre-existing renal ischemia prior to application of CM increases the severity of toxicity [51] and renal ischemia is associated with enhanced adenosine generation inducing renal vasoconstriction [46, 52, 53]. CM and adenosine both showed disparate effects regarding regional blood flow of the kidney with cortical vasoconstriction and medullary vasodilation [50, 54]. An additional role for adenosine comes from evidence that the diabetic kidney, due to attenuation of nitric oxide mediated renal vasodilation, has increased sensitivity to adenosine-induced vasoconstriction possible through up-regulation of adenosine A1 receptors [54a].

Experimental studies in a variety of animal models of acute renal failure reveal a consistent nephroprotective effect of adenosine antagonism [55-62]. Theophylline, for instance, acts as a non-specific adenosine receptor antagonist. Studies in both dogs and rats show a nephroprotective effect of theophylline after application of CM [44]. Our own group showed that rats subjected to chronic NO-blockade are highly sensitive to CM damage and when given adenosine antagonists (theophylline and DPCPX) demonstrate favorable ef-

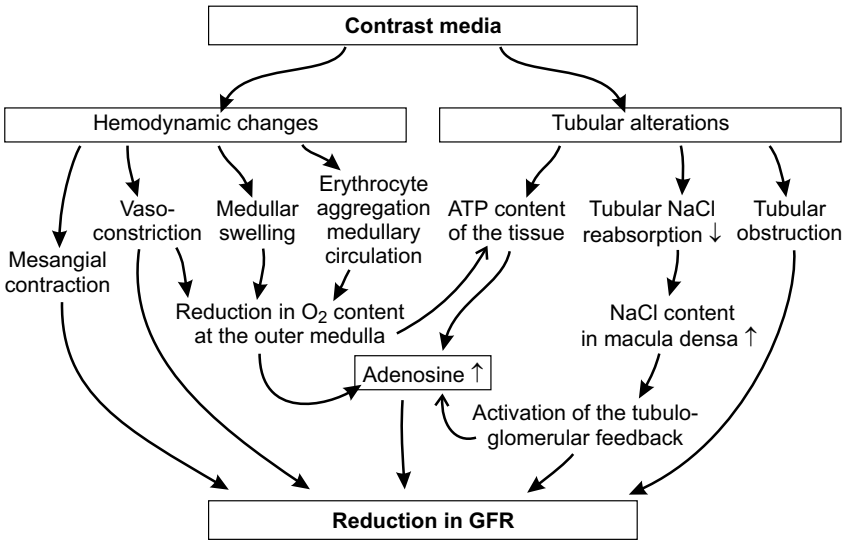

Figure 1. *Pathogenesis of CMIN.*

fects concerning the prevention of a decline in GFR and RBF (Figure 2) in this animal model of CMIN [63].

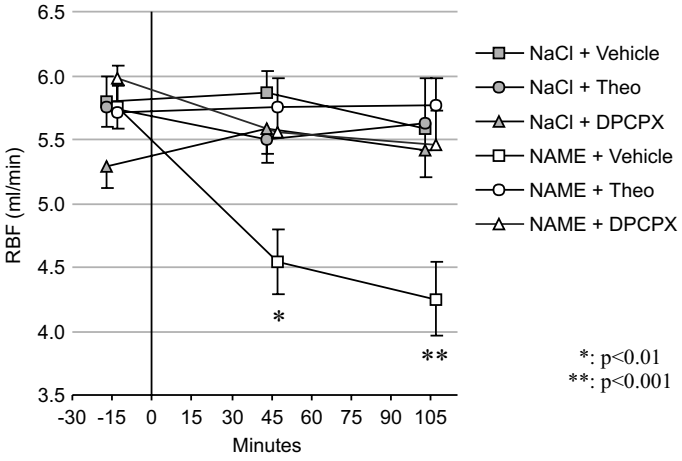

Figure 2. *Decline in renal blood flow (RBF) in rats with hypertension and renal vasoconstriction due to chronic NO-inhibition by L-NAME compared to controls after CM-application.* Data obtained after the administration of adenosine antagonists (theophylline and DPCPX) are also shown [63].

Prevention/treatment

Choice of contrast media

Studies performed after the introduction of new low osmolar (nonionic) contrast media have failed to consistently demonstrate that these more costly substances reduce the incidence of CMIN when compared to high osmolar (ionic) CM [4, 64-67]. The largest trial involving a substantial number of azotemic patients (509 out of a total of 1, 196 patients), including 213 diabetics, also found a negligible incidence of CMIN with either low osmolar or high osmolar CM [24]. Patients suffering from renal function impairment due to diabetic nephropathy had a twofold incidence of CMIN when high osmolar CM was used as compared to low osmolar CM [24]. A recent published meta-analysis concluded that low osmolar CM may be beneficial for patients with azotemia [68], although the difference was very small and the higher price of low osmolar CM must be taken into consideration when choosing contrast agents. One has to keep in mind that the definition of "low osmolar" still involves CM's with an osmolarity of around 600 mOsmol/kg (compared to high osmolar CM with an osmolarity of around 1, 400 mOsmol/kg). Possibly, the development of iso-osmolar CM (i.e. iodixanol) may further reduce the incidence CMIN [37, 69, 70]. An increasing number of studies are choosing magnetic resonance CM as alternative CM in hopes of preventing CMIN in azotemic patients undergoing CT-scanning or angiography [71-73]. However, the only proven benefit of these substances in conventional radiology (despite magnetic resonance) is the lack of iodine exposure in highly allergic patients. Disadvantages of the magnetic resonance CM are their high viscosity and osmolarity. This physical characteristic theoretically also limits their use for CMIN prevention (especially because the volume of contrast agents needed for computed tomography or angiography is much higher than for a magnetic resonance examination, 100 ml versus 15 ml). Controlled studies in azotemic patients treated with either iso-osmolar CM or with magnetic resonance CM are not available.

Another promising imaging agent alternative to conventional X-ray CM is carbon dioxide, which is used to provide a negative contrast. Studies performed so far showed less nephrotoxicity [74, 75], but an image that lasts a short time. However, this agent induces ischemia of the infused organs. This effect on vascular perfusion prevents carbon dioxide from being used for the investigation of cerebral vessels or for detecting smaller vessels. Although not available at the present time, future development of alternative contrast media or alternative investigations could supplant imaging investigations using iodinated CM.

Hydration/mannitol/diuretics

From a theoretical point of view, pre-hydrating patients prior to contrast imaging studies would have the following beneficial effects on the kidney:

- decreased activity of the renin-angiotensin-system,
- down-regulation of the tubulo-glomerular feed back,
- augmentation of diuresis and sodium excretion,

- dilution of the contrast media and thus minimizing renal cortical vasoconstriction,
- reduced pre-constriction of the vessels,
- avoidance of tubular obstruction, and
- reduction of endothelin and other intrarenal vasoconstrictive mediators (e.g. vasopressin).

Most studies involve either saline hydration in the role of mannitol or the value of vasodilators such as dopamine, atrial natriuretic peptide, Ca-antagonists or ACE-inhibitors with regard to protecting the kidney from contrast media damage. The authors found that hydration alone was as equal to or more effective than the additional administration of hypertonic mannitol or the administration of one of the vasodilative agents. Other investigators compared results in patients submitted to special hydration protocols with historical control groups [80, 81] or data reported in the literature [82-84]. Pre-study hydration was the only preventative maneuver consistently associated with a lower incidence of acute renal failure. So far, only one controlled, randomized study compared saline administration alone (0.45% saline over 24 hours, starting 12 hours before administration of radiocontrast dye) with mannitol (25 g of mannitol given 60 minutes before administration of radiocontrast dye) or furosemide (80 mg i.v.) [76]. In this study administration of saline alone was the most successful strategy. In numerous studies dealing with the nephroprotective effect of non-ionic contrast media prehydration of the patients was included in the protocol [24, 67], but patients with cardiac failure, liver cirrhosis or edema mostly have been excluded from the studies in order to avoid overhydration.

Which fluid and when to start ?

Most investigators administer 0.45% saline in combination with 5% dextrose intravenously in various amounts (around 1000-1500 ml starting 12 hours before administration of radiocontrast agents). There is no controlled study that assessed oral hydration in these patients. Until now, there has been no investigation as to how long hydration should be continued. From a theoretical point of view the use of hyperosmolar fluids (such as 15% mannitol) in addition to the administration of the hyperosmolar contrast media may have adverse effects. Therefore, it is not surprising that most studies failed to observe a beneficial effect of mannitol in this setting [27, 76, 80]. In accordance with the reported investigational data, humans have been significantly protected against the development of CMIN when hydrated prior to and up to 12 hours after contrast media exposure [76, 80, 83]. Only a minor protective effect could be demonstrated when fluid was administered during the procedure [81, 84].

Use of diuretics?

No conclusive evidence is available to support a protective role of loop-active diuretics with regard to the prevention of CMIN. It has been claimed that a reduction of the workload of the tubular cells of the thick ascending limb of Henle, by decreasing the rate of sodium reabsorption, might be tubuloprotective. Additionally, there might be a dilution effect by an increment of diuresis after furosemide. Most studies that have investigated this application showed either no beneficial effect or sometimes even worse results in case of furosemide application [76, 85, 86]. The negative effect of furosemide could be due to the reduction of cortical resistance inducing a redistribution of renal perfusion with reduced perfusion of the medulla. In combination with the contrast media-induced vasoconstriction, the oxygen content could be reduced to a critical point and thereby contributing to further deterioration of renal ischemia. Furosemide should be used with caution because there is always the fear of dehydration, which would enhance the nephrotoxicity of contrast agents.

Use of vasoactive substances as prophylaxis

Many of the therapeutic approaches to the prevention of CMIN involve the use of vasodilator agents. The lack of efficacy of such approaches may be contained in a recent concept articulated by a group from Mayo Clinic [54a]. Starting with the evidence that the diabetic kidney has a diminished vasodilatory capacity, they reason that CM released vasoconstrictor, such as endothelin, would inhibit the production and release of nitric oxide and further compromise an already diminished endothelial mediated vasodilation. Thus, they suggest that administration of vasoconstrictor antagonist may be more effective in preventing or minimizing the vasoconstriction induced by CM, rather than applying a direct vasodilator to vessels with dysfunctional capacity. While the concept is most applicable

to diabetics, there is evidence that hypertensive patients share a diminished capacity for generation of nitric oxide in response to renal vasoconstriction [54b].

Calcium channel blockers

Due to their vasodilating effect and the hope to prevent calcium overload in the tubular cells [35], calcium channel blockers have been used in both experimental [57] and clinical studies [44, 87, 88]. Despite early promising results large prospective trials failed to observe a beneficial effect regarding the decline in GFR after CM exposure [89-91]. Taken together a prophylactic value of calcium channel blockers (either short or long acting) has not been proven.

Dopamine

Dopamine, given in the so-called "renal doses" of around 2 μg/kg/min is widely used to prevent and to treat an acute renal failure induced under various circumstances. So far, most prospective studies have failed to demonstrate any real benefit of dopamine in the setting of CMIN [78, 92]. An interesting point seems to be the observation, that a prophylactic treatment with dopamine in 497 patients with ARF increased the mortality rate, which could be due to the pro-arrhythmic effect of this substance [93]. Fenoldopam, a selective dopamine type 1 receptor agonist has shown promise in the prevention of CMIN. In a retrospective evaluation of CRF patients given CM and fenoldopam, Madyoon et al [93a] reported a CMIN incidence of only 13% that was significantly less that the 38% derived from historic controls. In a prospective trial of CRF patients undergoing coronary angiography, Kini et al [93b] found significantly fewer patients had elevated serum creatinine values post procedure than would have been predicted from previously reported incidences. Obviously, in order to confirm a beneficial effect of fenoldopam, a prospective, randomized control trial will be required.

Atrial natriuretic factor (ANF)

Because of its natriuretic and vasodilative activity, in addition to its effect on the intracellular ATP-concentration [94], ANF seems to be a good candidate for the prevention of CMIN. To date no conclusive beneficial results have been obtained in clinical studies [27, 77, 95]. This may be due to the route of application (i.v. versus i.a. in experimental studies) and to intrarenal hemodynamic changes caused by ANF with induction of an arterial-steal phenomenon.

Adenosine Antagonists

In preliminary studies in both animals and humans a nephroprotective effect of theophylline (an unspecific adenosine antagonist) indicated a modification of the reduction of GFR after application of CM [45, 96]. However, a large, double blind, placebo-controlled study performed in patients with chronic renal failure failed to show a benefit of theophylline when given to otherwise stable and well hydrated mildly azotemic patients [9]. Thus, it can be argued that the prolonged tubular exposure to CM because of low tubular flow rates in dehydrated patients in combination with a stimulation of the renin-angiotensin system is the main reason for a fall in GFR after CM and that adenosine role in developing CMIN involving vasoconstriction [43]. Patients with heart failure or inability to be tolerate hydrated due to other conditions and a higher degree of renal insufficiency have been excluded from prospective trials, which investigated the effects of theophylline. Clinical trials involving patients with contraindications for hydration should be carried out in order to clearly evaluate the value of theophylline in the prevention of CM-induced nephropathy. Preliminary results obtained through a retrospective study showed that theophylline administration on an intensive care unit showed good results regarding the incidence of CMIN in patients with cardiac insufficiency (incidence of acute renal failure without theophylline: 15%, with theophylline: 7%) [97].

Antioxidant agents

Reactive oxygen species may have a role in renal damage caused by contrast agents. Acetylcysteine, a thiol-containing antioxidant has been used to treat a variety of pulmonary diseases and to treat acute acetaminophen poisoning. Recently, however, it has been used successfully to ameliorate the toxic effects of a variety of experimentally or clinically induced ischemia-reperfusion syndromes of the heart, kidney, lung, and liver. In each of these syndromes, it is thought that the activity of acetylcysteine is related to its action as a free-radical scavenger, or as a reactive sulphydryl compound that increases the reductive capacity of the cell. Tepel et al. recently published the first clinical trial using acetylcysteine (1200 mg of acetylcysteine per day,

given orally in divided doses on the day before and on the day of the administration of the radiocontrast agent) in order to prevent the decline in renal function in patients with moderate renal insufficiency, who were undergoing computed tomography [98]. On closer evaluation of the data they do not really show a conclusive benefit of acetylcysteine since the placebo comparison group did not show a significant decline in renal function after contrast media exposure and the significant difference between the groups resulted from an unexplained decline in creatinine levels in the acetylcysteine group compared to stable creatinine levels in the placebo group. However, prospective studies involving acetylcysteine have been reported in patients undergoing cardiac catheterization using CM. In a prospective, randomized study of 54 patients, Diaz-Sandoval et al [98a] significantly decreased the incidence of >25% increase in serum creatinine during the first 48 hours following catheterization. In addition at the 51st ACC annual session in 2002, two abstracts were presented [98b,c] suggesting a beneficial effect of acetylcysteine in preventing CM induced increase in serum creatinine. However, Briguori et al [98d] reported a randomized control trial involving 183 patients with impaired renal function undergoing angiography. They compared acetylcysteine plus saline infusion to saline alone using a >25% increase in serum creatinine as an endpoint. After multivariate analysis they concluded that the amount of contrast rather than the prophylactic treatment was the predictor of post-procedure deterioration of renal function. Therefore these data should be confirmed in a larger number of patients and in patients with a more seriously compromised renal function before acetylcysteine can be considered a potentially useful drug for prevention of CMIN.

Endothelin antagonists

Endothelin as a potent vasoconstrictor has been implicated in the pathogenesis of CMIN. Although animal studies showed promising results by application of endothelin antagonists [99] the first clinical study using an endothelin receptor antagonist showed a negative result with an exacerbation [100].

Hemodialysis after CM exposure

Some nephrologists have used hemodialysis in azotemic patients to enhance the elimination rate of CM from the body. From a pathophysiological point of view hemodialysis, which was normally initiated around 30-120 minutes after CM application, cannot prevent the effects on the renal hemodynamic situation. The only prospective study done so far by Lehnert et al. [101] showed no benefit regarding the development of ARF in dialyzed patients compared to controls. In our own institute we performed a controlled study with azotemic patients. Fifteen patients with an impaired renal function (mean serum creatinine concentration 2.7 ± 0.2 mg/dl) were randomly assigned to receive either a hemodialysis procedure for 2-3 hours, started as early as possible after administration of CM (106 ± 25 minutes), or a conservative treatment. The course and absolute changes in serum creatinine over the entire observation period was not different in either groups. The percentage increase of serum creatinine the day after CM application was higher in the group that underwent hemodialysis [102]. The rate of CMIN (defined as serum creatinine increase of greater than or equal to 0.5 mg/dl within 48 h after administration of CM) was significantly higher in the dialyzed group (43% in the hemodialysis group and 13% in the group with conservative treatment, respectively). The serum iodine concentration declined earlier in the dialyzed group. In summary, dialysis has no proven benefit in regard to a prevention of CMIN in azotemic patients.

Conclusions

Table 2 summarizes the clinical maneuvers that, based on current evidence, can be implemented to reduce the chance of CMIN. Before undertaking an imaging study, a risk/benefit analysis is needed. Will the information obtained lead to a change in treatment or is it simply to confirm a clinical suspicion? If the latter, then a compelling need for such confirmation must exist. By following the six recommendations on Table 2, the risk for CMIN can be significantly reduced.

Table 2. Clinical maneuvers that may prove beneficial in reducing the risk of CMIN.

1. Hydrate patients with intravenous saline and sustain in the immediate post-procedure interval.
2. Minimize the amount of CM used, sufficient to insure a interpretable study.
3. Perform diagnostic angiographic studies on separate days.
4. Discontinue any drugs with nephrotoxic potential.
5. Use low-osmolar CM in patients with renal insufficiency, especially diabetics.
6. Consider the use of prophylactic acetylcysteine in patients with renal insufficiency.

References

1. Levy EM, Viscoli CM, Horwitz RI. The effect of acute renal failure on mortality: a cohort analysis. JAMA 1996; 275: 1489-1494.
2. Porter GA. Contrast-associated nephropathy: presentation, pathophysiology and management. Mineral&Electrolyte Metabolism 1994; 20: 232-243.

2a. Kurnik BR, AllgrenRI, GenterFC, Solomon RJ, Bates ER, Weisberg LS: Prospective study of atrial natriuretic peptide for the prevention of radiocontrast-induced nephropathy. Am J Kid Dis. 1998;31:674-80

2b. Gruberg L., Mintz GS, Mehran R, Gangas G, Lansky AJ, Kent KM, Pichard AD, Satler LF, Leon MB: The prognostic implications of further renal function deterioration within 48 h of interventional coronaryprocedures in patients with pre-existing chronic renal insufficiency. J Am Coll Cardiol. 2000;36:1542-8.

2c. Abizaid AS, Clark CE, Mintz GS, Dosa S, Popma JJ, Pichard AD, Satler LF, Harvey M, Kent KM, Leon MB: Effects of dopamine and aminophylline on contrast-induced acute renal failure after coronary antioplasty in patients with preexisiting renal insufficiency. Am J. Cardiol. 1999; 83;260-3.

3. Berns AS. Nephrotoxicity of contrast media (clinical conference). Kidney Int 1989; 36: 730-740.
4. Barrett BJ, Parfrey PS, Vavasour HM, McDonald J, Kent G, Hefferton D, O'Dea F, Stone E, Reddy R, McManamon PJ. Contrast nephropathy in patients with impaired renal function: high versus low osmolar media. Kidney Int 1992; 41: 1274-1279.
5. Rudnick MR, Berns JS, Cohen RM, Goldfarb S. Contrast media-associated nephrotoxicity. Curr Opin Nephrol Hypert 1996; 5: 127-133.
6. Moreau JF, Droz D, Sabto J, Jungers P, Kleinknecht D, Hinglais N, Michel JR. Osmotic nephrosis induced by water soluble triiodinated contrast media in man. Radiology 1975; 115: 329-336.
7. Bettmann MA. The evaluation of contrast-related renal failure (comment). AJR Am J Roentgenol 1991; 157: 66-68.
8. Hunter JV, Kind PRN. Nonionic iodinated contrast media: potential renal damage assessed with enzymuria. Radiology 1992; 183: 101-104.
9. Erley CM, Duda SH, Rehfuss D, Scholtes B, Bock J, Müller C, Osswald H. Prevention of radiocontrast-media-induced nephropathy in patients with preexisting renal insufficiency by hydration in combination with the adenosine antagonist theophylline. Nephrol Dial Transplant 1999; 14: 1146-1149.
10. Donadio C, Tramonti G, Giordani R, Lucchetti A, Calderazzi A, Sbragia P, Bianchi C. Glomerular and tubular effects of ionic and nonionic contrast media (diatrizoate and iopamidol). Contrib Nephrol 1988; 68: 212-219.
11. Porter GA. Urinary biomarkers and nephrotoxicity. Mineral&Electrolyte Metabolism 1994; 20: 181-186.

11a. Scanlon PJ, Faxon DP, Audet AM, Carabello, B, Dehmer GJ, Eagle KA, Legako RD, Leon DF, Murray JD, Nissen SE, Pepine CJ, Watson, RM, Ritchie JL, Gibbons RJ, Cheitlin MD, Gardner TJ, Garson A, Russell RO, Ryan TJ, Smith SC: ACC/AHA guidelines for coronary angiography: a report of the American College of Cardiology/American Heart Associateion Task force on Practic Guidelines (Committee on Coronary Angiography). J Am Coll Cardiol. 1999;33:1756-824.

12. Tervahartiala P, Kivisaari L, Kivisaari R, Vehmas T, Virtanen I. Structural changes in the renal proximal tubular cells induced by iodinated contrast media. Nephron 1997; 76: 96-102.
13. Dobrota M, Powell CJ, Holtz E, Wallin A, Vik H. Biochemical and morphological effects of contrast media on the kidney. Acta Radiol (Suppl) 1995; 399: 196-203.
14. Battenfeld R, Khater AE, Drommer W, Guenzel P, Kaup FJ. Ioxaglate-induced light and electron microscopic alterations in the renal proximal tubular epithelium of rats (see comments). Invest Radiol 1991; 26: 35-39.
15. Heyman SN, Brezis M, Reubinoff CA, Greenfeld Z, Lechene C, Epstein FH, Rosen S. Acute renal failure with selective medullary injury in the rat. J Clin Invest 1988; 82: 401-412.
16. Rich MW, Crecelius CA. Incidence, risk factors, and clinical course of acute renal insufficiency after cardiac catheterization in patients 70 years of age or older. A prospective study. Arch Intern Med 1990; 150: 1237-1242.

17. Davidson CJ, Hlatky M, Morris KG, Pieper K, Skelton TN, Schwab SJ, Bashore TM. Cardiovascular and renal toxicity of a nonionic radiographic contrast agent after cardiac catheterization. A prospective trial. Ann Intern Med 1989; 110: 119-124.
18. D'Elia JA, Gleason RE, Alday M, Malarick C, Godley K, Warram J, Kaldany A, Weinrauch LA. Nephrotoxicity from angiographic contrast material. A prospective study. Am J Med 1982; 72: 719-725.
19. Manske CL, Sprafka JM, Strony JT, Wang Y. Contrast nephropathy in azotemic diabetic patients undergoing coronary angiography. Am J Med 1990; 89: 615-620.
19a. Agrawal M, Stouffer GA: Contrast induced nephropathy after angiography. Am J Med Sci. 2002;323:252-58.
20. Rosovsky MA, Rusinek H, Berenstein A, Basak S, Setton A, Nelson PK. High-dose administration of nonionic contrast media: a retrospective review. Radiology 1996; 200: 119-122.
21. Rudnicki M, Berns JS, Cohen RM, Goldfarb S. Contrast media-associated nephrotoxicity. Curr Opin Nephrol Hypertens 1996; 5: 127-133.
22. Vanzee BE, Hoy WE, Talley TE, Jaenike JR. Renal injury associated with intravenous pyelography in nondiabetic and diabetic patients. Ann Intern Med 1978; 89: 51-54.
23. Moore RD, Steinberg EP, Powe NR, Brinker JA, Fishman EK, Graziano S, Gopalan R. Nephrotoxicity of high-osmolality versus low-osmolality contrast media: randomized clinical trial. Radiology 1992; 182: 649-655.
24. Rudnick MR, Goldfarb S, Wexler L, Ludbrook PA, Murphy MJ, Halpern EF, Hill JA, Winneford M, Cohen MB, Vanfossen DB. Nephrotoxicity of ionic and nonionic contrast media in 1196 patients: a randomized trial. Kidney Int 1995; 47: 254-261.
25. Parfrey PS, Griffiths SM, Barrett BJ, Paul MD, Genge M, Withers J, Farid N, McManamon PJ. Contrast material-induced renal failure in patients with diabetes mellitus, renal insufficiency, or both. A prospective controlled study. N Engl J Med 1989; 320: 143-149.
26. Lautin EM, Freeman NJ, Schoenfeld AH, Bakal CW, Haramati N, Friedman AC, Lautin JL, Braha S, Kadish EG, Sprayregen S. Radiocontrast-associated renal dysfunction: incidence and risk factors (see comments). AJR Am J Roentgenol 1991; 157: 49-58.
27. Weisberg LS, Kurnik PB, Kurnik BR. Risk of radiocontrast nephroathy in patients with and without diabetes mellitus. Kidney Int 1994; 45: 259-265.
28. Gomes AS, Baker JD, Martin-Paredero V, Dixon SM, Takiff H, Machleder HI, Moore WS. Acute renal dysfunction after major arteriography. AJR Am J Roentgenol 1985; 145(6): 1249-1253.
29. Taliercio CP, McCallister SH, Holmes DR, Ilstrup DM, Vlietstra RE. Nephrotoxicity of nonionic contrast media after cardiac angiography. Am J Cardiol 1989; 64: 815-816.
30. Rudnick MR, Berns JS, Cohen RM, Goldfarb S. Contrast media-associated nephrotoxicity. Sem Nephrol 1997; 17: 15-26.
31. Cigarroa RG, Lange RA, Williams RH, Hillis LD. Dosing of contrast material to prevent contrast nephropathy in patients with renal disease. Am J Med 1989; 86: 649-652.
32. McCarthy CS, Becker JA. Multiple myeloma and contrast media. Radiology 1992; 183: 519-521.
33. Heyman SN, Rosen S, Brezis M. Radiocontrast nephropathy: a paradigm for the synergism between toxic and hypoxic insults in the kidney. Exp Nephrol 1994; 2: 153-157.
34. Katzberg RW, Morris TW, Burgener FA, Kamm DE, Fischer HW. Renal renin and hemodynamic responses to selective renal artery catheterization and angiography. Invest Radiol 1977; 12: 381-388.
35. Bakris GL, Burnett JCJ. A role for calcium in radiocontrast-induced reductions in renal hemodynamics. Kidney Int 1985; 27: 465-468.
36. Caldicott WJ, Hollenberg NK, Abrams HL. Characteristics of response of renal vascular bed to contrast media. Evidence for vasoconstriction induced by renin-angiotensin system. Invest Radiol 1970; 5: 539-547.
37. Katzberg RW. Urography into the 21st century: new contrast media, renal handling, imaging characteristics, and nephrotoxicity. Radiology 1997; 204: 297-312.
38. Workman RJ, Shaff MI, Jackson RV, Diggs J, Frazer MG, Briscoe C. Relationship of renal hemodynamic and functional changes following intravascular contrast to the renin-angiotensin system and renal prostacyclin in the dog. Invest Radiol 1983; 18: 160-166.
39. Larson TS, Hudson K, Mertz JI, Romero JC, Knox FG. Renal vasoconstrictive response to contrast medium. The role of sodium balance and the renin-angiotensin system. J Lab Clin Med 1983; 101: 385-391.
40. Heyman SN, Clark BA, Kaiser N, Spokes K, Rosen S, Brezis M, Epstein FH. Radiocontrast agents induce endothelin release *in vivo* and *in vitro*. J Am Soc Nephrol 1992; 3: 58-65.
41. Oldroyd SD, Haylor JL, Morcos SK. Bosentan, an orally active endothelin antagonist: effect on the renal response to contrast media. Radiology 1995; 196: 661-665.
42. Russo D, Minutolo R, Cianciaruso B, Memoli B, Conte G, De Nicola L. Early effects of contrast media on renal hemodynamics and tubular function in chronic renal failure. J Am Soc Nephrol 1995; 6: 1451-1458.
43. Erley CM, Osswald H. Prevention of contrast media-induced renal impairment by adenosine antagonists in humans. Drug Dev Res 1998; 45: 172-175.

44. Arend LJ, Bakris GL, Burnett JCJ, Megerian C, Spielman WS. Role for intrarenal adenosine in the renal hemodynamic response to contrast media. J Lab Clin Med 1987; 110: 406-411.
45. Katholi RE, Taylor GJ, McCann WP, Woods WT, Womack KA, McCoy CD, Katholi CR, Moses HW, Mishkel GJ, Lucore CL, Holloway RM, Miller BD, Woodruff RC, Dove JT, Mikell FL, Schneider JA. Nephrotoxicity from contrast media: attenuation with theophylline. Radiology 1995; 195:17-22.
46. Osswald H, Schmitz HJ, Heidenreich O. Adenosine response of the rat kidney after saline loading, sodium restriction and hemorrhagia. Pflugers Arch 1975; 357: 323-333.
47. Hall JE, Granger JP. Adenosine alters glomerular filtration control by angiotensin II. Am J Physiol 1986; 250: F917-F923.
48. Haas JA, Osswald H. Adenosine induced fall in glomerular capillary pressure: effect of ureteral obstruction and aortic constriction in the Munich- Wistar rat kidney. Naunyn Schmiedebergs Arch Pharmacol 1981; 317: 86-89.
49. Cantley LG, Spokes K, Clark B, McMahon EG, Carter J, Epstein FH. Role of endothelin and prostaglandins in radiocontrast-induced renal artery constriction. Kidney Int 1993; 44: 1217-1223.
50. Heyman SN, Brezis M, Epstein FH, Spokes K, Silva P, Rosen S. Early renal medullary hypoxic injury from radiocontrast and indomethacin. Kidney Int 1991; 40: 632-642.
51. Cederholm C, Almen T, Bergqvist D, Golman K, Takolander R. Acute renal failure in rats. Interaction between contrast media and temporary renal arterial occlusion. Acta Radiol 1989; 30: 321-326.
52. Deray G, Dubois M, Martinez F, Baumelou B, Beaufils H, Bourbouze R, Baumelou A, Jacobs C. Renal effects of radiocontrast agents in rats: a new model of acute renal failure. Am J Nephrol 1990; 10: 507-513.
53. Osswald H, Schmidt HJ, Kemper R. Tissue content of adenosine, inosine and hypoxanthine in the rat kidney after ischemia and postischemic recirculation. Pflügers Arch 1977; 371: 45-49.
54. Agmon Y, Peleg H, Greenfeld Z, Rosen S, Brezis M. Nitric oxide and prostanoids protect the renal outer medulla from radiocontrast toxicity in the rat. J Clin Invest 1994; 94: 1069-1075.
54a. Pflueger A, Larson, TS, Nath KA, King BF, Gross JM, Knox, FG: Role of adenosine in constrast media-induced acute renal failure in diabetes mellitus. Mayo Clin Proc. 2000; 75:1275-83.
54b. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A: Cyclooxygenase inhibition restores nitric oxide activity in essential hypertension. Hyperten. 1997;29(1Pt2):274-9.
55. Osswald H, Helminger I, Jendralski A, Abrar B. Improvement of renal function by theophylline in the acute renal failure of the rat. Naunyn-Schmiedeberg´s Arch Pharmacol 1979; 307: R47 (abstract).
56. Gouyon JB, Guignard JP. Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits. Kidney Int 1988; 33: 1078-1083.
57. Deray G, Martinez F, Cacoub P, Baumelou B, Baumelou A, Jacobs C. A role for adenosine calcium and ischemia in radiocontrast-induced intrarenal vasoconstriction. Am J Nephrol 1990; 10: 316-322.
58. Rossi N, Ellis V, Kontry T, Gunther S, Churchill P, Bidani A. The role of adenosine in HgCl2-induced acute renal failure in rats. Am J Physiol 1990; 258: F1554-F1560.
59. Lin JJ, Churchill PC, Bidani AK. Theophylline in rats during maintenance phase of post-ischemic acute renal failure. Kidney Int 1988; 33: 24-28.
60. Lin JJ, Churchill PC, Bidani AK. Effect of theophylline on the initiation phase of postischemic acute renal failure in rats. J Lab Clin Med 1986; 108: 150-154.
61. Bidani AK, Churchill PC. Aminophylline ameliorates glycerol-induced acute renal failure in rats. Can J Physiol Pharmacol 1983; 61: 567-571.
62. Bidani AK, Churchill PC, Packer W. Theophylline-induced protection in myoglobinuric acute renal failure: Further chracterization. Can J Physiol Pharmacol 1987 ; 65: 42-45.
63. Erley CM, Heyne N, Burgert K, Langanke J, Risler T, Osswald H. Prevention of radiocontrast induced nephropathy by adenosine antagonists in rats with chronic nitric oxide deficiency. J Am Soc Nephrol 1997; 8: 1125-1132.
64. Barrett BJ, Parfrey PS, McDonald JR, Hefferton DM, Reddy ER, McManamon PJ. Nonionic low-osmolality versus ionic high-osmolality contrast material for intravenous use in patients perceived to be at high risk: randomized trial (see comments). Radiology 1992; 183: 105-110.
65. Deray G, Jacobs C. Radiocontrast nephrotoxicity. A review. Invest Radiol 1995; 30: 221-225.
66. Katholi RE, Taylor GJ, Woods WT, Womack KA, Katholi CR, McCann WP, Moses HW, Dove JT, Mikell FL, Woodruff RC. Nephrotoxicity of nonionic low-osmolality versus ionic high- osmolality contrast media: a prospective double-blind randomized comparison in human beings. Radiology 1993; 186: 183-187.
67. Schwab SJ, Hlatky MA, Pieper KS, Davidson CJ, Morris KG, Skelton TN, Bashore TM. Contrast nephrotoxicity: a randomized controlled trial of a nonionic and an ionic radiographic contrast agent. N Engl J Med 1989; 320: 149-153.
68. Barrett BJ, Carlisle EJ. Metaanalysis of the relative nephrotoxicity of high- and low-osmolality iodinated contrast media. Radiology 1993; 188: 171-178.

69. Jakobsen JA. Renal effects of iodixanol in healthy volunteers and patients with severe renal failure. Acta Radiol (Suppl) 1995; 399: 191-195.
70. Jakobsen JA. Renal experience with Visipaque. Eur Radiol 1996; 6 (Suppl 2): S16-S19.
71. Weissleder R. Can gadolinium be safely given in renal failure? AJR Am J Roentgenol 1996; 167: 278-279.
72. Kaufman JA, Geller SC, Waltman AC. Renal insufficiency: gadopentetate dimeglumine as a radiographic contrast agent during peripheral vascular interventional procedures. Radiology 1996; 198: 579-581.
73. Hatrick AG, Jarosz JM, Irvine AT. Gadopentate dimeglumine as an alternative contrast agent for use in interventional procedures. Clin Radiology 1997; 52: 948-952.
74. Seeger JM, Self S, Harward TR, Flynn TC, Hawkins IF Jr. Carbon dioxide gas as an arterial contrast agent. Ann Surgery 1993; 217: 688-697.
75. Hawkins IF Jr, Wilcox CS, Kerns SR, Sabatelli FW. CO_2 digital angiography: a safer contrast agent for renal vascular imaging ? Am J Kidney Dis 1994; 24: 685-694.
76. Solomon R, Werner C, Mann D, D'Elia J, Silva P. Effects of saline, mannitol, and furosemide on acute decreases in renal function induced by radiocontrast agents. N Engl J Med 1994; 331: 1416-1420.
77. Kurnik BR, Weisberg LS, Cuttler IM, Kurnik PB. Effects of atrial natriuretic peptide versus mannitol on renal blood flow during radiocontrast infusion in chronic renal failure. J Lab Clin Med 1990; 116: 27-36.
78. Weisberg LS, Kurnik PB, Kurnik BR. Dopamine and renal blood flow in radiocontrast-induced nephropathy in humans. Renal Failure 1993; 15: 61-68.
79. Weisberg LS, Kurnik PB, Kurnik BR. Radiocontrast-induced nephropathy in humans: role of renal vasoconstriction. Kidney Int 1992; 41: 1408-1415.
80. Anto HR, Chou SY, Porush JG, Shapiro WB. Infusion intravenous pyelography and renal function. Effect of hypertonic mannitol in patients with chronic renal insufficiency. Arch Intern Med 1981; 141: 1652-1656.
81. Eisenberg RL, Bank WO, Hedgock MW. Renal failure after major angiography can be avoided with hydration. Am J Radiol 1981; 136: 859-861.
82. Carraro M, Stacul F, Collari P, Toson D, Zucconi F, Torre R, Faccini L, Dalla Palma L. Contrast media nephrotoxicity: urinary protein and enzyme pattern in patients with or without saline infusion during digital subtracting angiography. Contrib Nephrol 1993; 101: 251-254.
83. Kerstein MD, Puyau FA. Value of periangiography hydration. Surgery 1984; 96: 919-922.
84. Louis BM, Hoch BS, Hernandez C, Namboodiri N, Neiderman G, Nissenbaum A, Foti FP, Magno A, Banayat G, Fata F, Manohar NL, Lipner HI. Protection from the nephrotoxicity of contrast dye. Renal Failure 1996; 18: 639-646.
85. Weinstein JM, Heyman S, Brezis M. Potential deleterious effect of furosemide in radiocontrast nephropathy. Nephron 1992; 62: 413-415.
86. Golman K, Cederholm C. Contrast medium-induced acute renal failure. Can it be prevented ? Invest. Radiol. 1990; 25(Suppl 1): S127-S128.
87. Neumayer HH, Gellert J, Luft FC. Calcium antagonists and renal protection. Renal Failure 1993; 15: 353-358.
88. Russo D, Testa A, Volpe LD, Sansone G. Randomised propspective study on renal effects of two different contrast media in humans: protective role of a calcium channel blocker. Nephron 1990; 55: 254-257.
89. Carraro M, Mancini W, Artero M, Stacul F, Grotto M, Cova M, Faccini L. Dose effect of nitrendipine on urinary enzymes and microproteins following non-ionic radiocontrast administration. Nephrol Dial Transplant 1996; 11: 444-448.
90. Spangberg Viklund B, Berglund J, Nikonoff T, Nyberg P, Skau T, Larsson R. Does prophylactic treatment with felodipine, a calcium antagonist, prevent low-osmolar contrast-induced renal dysfunction in hydrated diabetic and nondiabetic patients with normal or moderately reduced renal function? Scand J Urol Nephrol 1996; 30: 63-68.
91. Khoury Z, Schlicht JR, Como J, Karschner JK, Shapiro AP, Mook WJ, Weber RJ. The effect of prophylactic nifedipine on renal function in patients administered contrast media. Pharmacother 1995; 15: 59-65.
92. Hall KA, Wong RW, Hunter GC, Camazine BM, Rappaport WA, Smyth SH, Bull DA, McIntyre KE, Bernhard VM, Misiorowski RL. Contrast-induced nephrotoxicity: the effects of vasodilator therapy. J Surg Res 1992; 53: 317-320.
93. Chertow GM, Sayegh MH, Allgren RL, Lazarus JM. Is the administration of dopamine associated with adverse or favorable outcomes in acute renal failure? Auriculin Anaritide Acute Renal Failure Study Group. Am J Med 1996; 101: 49-53.
93a. Madyoon H, Croushore L, Weaver D, Mathur V: Use of fenoldopam to prevent radiocontrast nephropathy in high-risk patients. Cather Cardiovasc. Intervent 2001;53;341-5.
93b. Kini A, Sharma S: Managing the high-risk patients: Experience with fenoldopam, a selective dopamine receptor agonist, in prevention of radiocontrast nephropathy during percutaneous coronary intervention. Rev Cardiovas Med. 2001;2(s1):S19-25.
94. Wambach G, Winkert T. Nierenfunktionsstörungen nach Röntgenkontrastmittelgabe: Prophylaxe durch das atriale natriuretische Peptid? Nieren- und Hochdruckkrankheiten 1990; 19: 312-317.

95. Kurnik BR, Allgren RL, Genter FC, Solomon RJ, Bates ER, Weisberg LS. Prospective study of atrial natriuretic peptide for the prevention of radiocontrast-induced nephropathy. Am J Kidney Dis 1998; 31: 674-680.
96. Erley CM, Duda SH, Schlepckow S, Koehler J, Huppert PE, Strohmaier WL, Bohle A, Risler T, Osswald H. Adenosine antagonist theophylline prevents the reduction of glomerular filtration rate after contrast media application. Kidney Int 1994; 45: 1425-1431.
97. Erley CM. Prävention des kontrastmittelbedingten Nierenversagens. Intensivmed 1997; 34: 769-777.
98. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. New Engl J MED 2000; 343: 180-184.
98a. Diaz-Sandoval LJ, Kosowsky BD, Losordo DW: Acetylcysteine to prevent angiography-related renal tissue injury (The APART Trial). Am J Cardiol. 2002;89:356-8.
98b. Adamian MG, Mousssa I, Mehran R: the role of mucomyst administration prior to percutaneous intervention on renal function in patients with chronic renal failure. J Am Coll Cardiol. 2002;39(sA):1A.
98c. Mouhayar EN, Tadros G, Akinwande AAO: Prevention of contrast-induced renal dysfunction with acetylcysteine in patients undergoing conronary angiography. J Am Coll Cardiol. 2002:39(sA):1A.
98d. Briguori C, Manganelli F, Scarpato P, Elia PP, Golia B, Riviezzo G, Lepore S, Librera M, Villari B, Colombo A, Ricciardelli B. Acetylcysteine and contrast agent-associated nephrotoxicity. J Am Coll Cardiol. 2002;40:298-303.
99. Oldroyd S, Slee SJ, Haylor J, Morcos SK, Wilson C. Role for endothelin in the renal responses to radiocontrast media in the rat. Clin Sci Colch 1994; 87: 427-434.
100. Wang A, Holcslaw T, Bashore TM, Freed MI, Miller D, Rudnick MR, Szerlip H, Thames MD, Davidson CJ, Shusterman N, Schwab SJ. Exacerbation of radiocontrast nephrotoxicity by endothelin receptor antagonism. Kidney Int 2000; 57: 1675-1680.
101. Lehnert T, Keller E, Gondolf K, Schaffner T, Pavenstandt H, Schollmeyer P. Effect of haemodialysis after contrast medium administration in patients with renal insufficiency. Nephrol Dial Transplant 1998; 13: 358-362.
102. Berger ED, Bader BD, Bosker J, Risler T, Erley CM. Kontrastmittelinduziertes Nierenversagen läßt sich durch Hämodialyse nicht verhindern. (Contrast media-induced kidney failure cannot be prevented by hemodialysis). Dtsch Med Wochenschr 2001; 16; 126(7): 162-166 (German).

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Lead nephropathy

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Introduction

Because of its stability and malleability, lead has been used by man for millennia. Although lead may be found in remnants from primitive societies, it is present in greater concentrations in industrial societies. It is widely distributed throughout the earth and accumulates in most tissues and plants.

Lead has no essential biological role and, in sufficient concentrations, is potentially toxic to many cells and tissues, including the kidney. That lead absorption could be associated with clinical symptoms has been recognized since the days of Hippocrates and the contribution of chronic lead exposure to the later development of granular contracted kidneys has been recognized since Lanceraux [1]. However, the precise factors that determine the development of toxic effects after exposure to lead have been difficult to define and its metabolism and toxicity have only been defined this century. Adverse cellular effects of lead have been described, including an effect on mitochondrial structure and the inhibition of susceptible enzymes, particularly delta-amino levulinic acid dehydratase, the development of abnormal porphyrin and heme synthesis and its binding to phosphatidyl choline membranes. With the widespread use of lead in industrialized societies, potential hazards from lead continue to be important.

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Handling of lead in the human body

Exposure, absorption, elimination and toxicity

These terms are often used loosely but each has a specific meaning, which should be used with precision.

Exposure. This reflects the amount of lead in the environment to which the particular individual is exposed. Any effects will depend upon the amount of lead absorbed, which will depend upon the intensity and duration of exposure.

Absorption. Lead can be absorbed either from the air or from ingested food or fluid [2]. The amount of lead absorbed by inhalation increases with increasing particle size. Much lead, however, is absorbed from the alimentary tract where food and water may contain lead or be contaminated with lead-containing soil or dust. The lead content of food will depend upon the environment in which it is grown. Most ingested lead traverses the alimentary tract and is excreted in the faeces, the amount absorbed depending upon the amount ingested.

Storage. Absorbed lead is stored in all tissues and is present in most bodily secretions. In the blood, it is principally found in red cells but some is also present in the serum. The skeleton and teeth constitute a major site of storage and lead can readily be found in most tissues including the brain and the hair.

Although it is likely that there are a large number of physiological pools, stored lead is chiefly found in three body compartments [3]. The first rapidly exchangeable pool comprises lead in the blood and other tissues in rapid equilibrium with the blood. Ingested lead first enters this pool and lead excreted in the urine comes principally from this pool. It contains less than 1% of the total body lead and has a mean life of 35 days. The second compartment, the intermediate exchange compartment, generally comprises the soft tissues and the actively exchanging portions of the skeleton, including the lead in hair, nails and bodily secretions and sweat. It too comprises less than 1% of the body lead content and has a mean life of about 40 days. The third compartment comprises the slow exchange pool and includes most of the skeleton. It includes the vast proportion (greater than 98%) of the body lead and has an extremely slow turnover, usually quoted as 30 years. Thus, stored lead has an enormous affinity for bone, with the precise concentration at any site depending upon the rate of turnover of the bone. These three compartments are in equilibrium with each other, with definable transfer coefficients between them. The lead content of bone increases when the skeleton is relatively inactive, unless it is mobilized by an illness, which mobilizes bone. Secondary hyperparathyroidism, or the osteodystrophy of chronic renal failure, can mobilize lead from the skeletal compartment and return it to the soft tissue and blood compartments where it may have toxic manifestations. The lead concentration of blood can be readily measured, although most of the lead is attached to the red cells and the lead content of bone can be measured either at biopsy, autopsy or by *in vivo* X-ray fluorescence.

Elimination. Apart from that lost in bodily secretions, most lead is excreted in the urine. Faecal excretion may reflect current exposure more than that which has been absorbed and stored. Urinary excretion of ingested lead is rapid at first but gradually diminishes as more of the absorbed lead is stored rather than excreted. The rate of loss is more related to the duration of exposure than the quantity accumulated. In a balanced state, elimination of lead is equal to that absorbed. Chronic renal failure does not cause accumulation of lead in the body [4].

Intoxication. This indicates that the absorbed lead is having adverse metabolic effects upon a body tissue. The toxicity is proportional to the concentration of lead at the site. Defining minimal manifestations of toxicity has extended with our increasing ability to develop sensitive indices of toxicity. Inhibition of delta-amino levulinic acid dehydratase with an increase in urinary amino-levulinic acid is a relatively sensitive index of toxicity in intoxication, indicating that the lead is having an adverse effect on porphyrin metabolism [5]. Lead binding proteins are also being recognized [6], although they are less well defined that those which bind cadmium. Increasingly sensitive indices of systemic intoxication are being recognized. Characteristic inclusion bodies may be seen in the proximal tubular lining cells of the kidney, consisting of a lead-protein complex [7]. Lead also modifies many aspects of bone cell function [8].

Modifying factors

Lead can pass across the placenta to affect the foetus, and children appear to be more susceptible to the

toxic effects of lead than are adults. Thus, the age and size of an individual may modify the metabolism and toxicity of absorbed lead. A low dietary calcium increases lead absorption and storage and a high dietary calcium will reduce lead absorption. This is the reason usually given for the higher skeletal lead concentration in cities with soft water supplies. A lower intake of vitamin D will also promote lead absorption, although it is uncertain whether this is because of an associated reduced dietary calcium or because of an impairment by lead of the renal biosynthesis of 1, 25 dihydroxycholecalciferol. Iron deficiency is also said to enhance lead retention and toxicity. Although these have been identified, there are probably many other unrecognized systemic and endocrine effects, which can modify the effect of lead on cellular processes.

Acute lead nephropathy

Acute childhood lead poisoning arising from chewing on lead-painted toys and presenting as the "dry gripes" was first described by John Fothergill in 1775 [9]. In modern times, pica for lead paint has been recognized as the primary cause of acute lead poisoning in children, which is characterized by abdominal colic, encephalopathy and anemia.

In children with lead encephalopathy, a proximal tubule defect for glucose reabsorption (glycosuria without hyperglycemia) was first noted by McKhann in 1926 and aminoaciduria was described by Wilson et al. in 1953 [10, 11]. The Fanconi syndrome (aminoaciduria, phosphaturia, and glycosuria) has been observed [12, 13] in the presence of blood lead levels usually in excess of 150 µg/dl. Loghman-Adham found that a selective Fanconi syndrome, which correlated with the blood lead concentration, persisted for up to 13 years after childhood lead poisoning. Increased uricosuria was not part of the proximal tubule reabsorptive defect, which may help explain the association of gout with lead-poisoning in adults [14]. The lead-induced Fanconi syndrome is rapidly reversed by chelation therapy designed to treat the far more dangerous encephalopathy [15, 16]. The Fanconi syndrome has been induced experimentally in rats fed dietary lead [17]. In both children and experimental animals, acute lead nephropathy is consistently associated with acid-fast intranuclear inclusions in proximal tubule epithelial cells [18, 19]. The intranuclear inclusion bodies consist of a lead-protein complex and may be seen in tubular epithelial cells in the urinary sediment during acute poisoning [20]. Lead-containing intranuclear inclusions have been observed in liver, neural tissue, and osteoclasts as well as in kidney.

Chronic renal disease due to lead

Whereas, in an acute intoxication, there is a clear temporal relationship between the exposure to the toxin and the toxic effect, and the proximal tubule reabsorptive defect is regularly reproduced during acute lead intoxication, there is a much greater problem in establishing an etiological relationship between chronic exposure to a toxin and the subsequent delayed and infrequent development of toxic effects on the kidneys. This has reflected the difficulty in establishing chronic renal disease as a sequel to prolonged, previous but remote, absorption of lead. While an association can be established relatively easily, an etiological relationship between the exposure and a subsequent lesion is difficult when these are separated in time by many years, particularly in relation to chronic renal disease. This is complicated by the relative insensitivity of tests of early renal dysfunction and the fact that plasma urea and creatinine concentrations do not detect early degrees of renal insufficiency.

Several independent lines of evidence provide strong support for an etiological relationship between acute lead intoxication and the later development of renal disease. These are provided principally from the follow-up of childhood lead poisoning in Queensland, Australia, from the studies of "moonshine" (illicitly distilled liquor) drinkers in the southern States of USA and from studies of workers industrially exposed to excessive amounts of lead. At the same time as our epidemiological techniques are becoming increasingly refined, high level industrial lead exposure is being greatly reduced, while low level exposure of the population to lead is increasing. It is therefore difficult to extrapolate the extent of toxicity from chronic low level environmental lead exposure from the more gross complications caused by higher levels of exposure. It is, moreover, difficult to document the extent of lead exposure because sometimes excessive amounts of lead may be absorbed without producing symptoms and susceptibility to the adverse effects of lead may vary at different ages and states of health.

There are, however, several distinctive clinical situations where excessive and prolonged exposure to lead has resulted in chronic renal disease and these have established beyond doubt that an appropriate degree of lead intoxication can result in chronic renal disease.

Chronic lead nephropathy in Queensland

During the decade after 1890, there were numerous reports of acute lead poisoning in children in Queensland (Australia). The symptoms were usually classical and appeared maximally in the sixth year of life. Ultimately, the source of the lead was identified as coming from lead paint, which, used on verandas in the tropical climate, would often powder and flake and come off on children's hands. Children who sucked their thumbs, licked their fingers or licked the raindrops that would develop on the veranda railings could ingest significant amounts of lead over a prolonged period. The common design of housing was of a wooden house which was elevated 2 meters above the ground on blocks, with open verandas on three sides. This was in part for coolness and in part to provide a clear and dry area under the house in wet weather.

Within 10 years of the recognition of this outbreak of acute childhood lead poisoning, an increase in mortality from chronic renal failure was noted in this community and a strong belief grew up that this was a sequel to the acute childhood plumbism. By 1922, the greatly increased mortality from kidney disease throughout Queensland had been established and the Queensland branch of the British Medical Association [21] at that time concluded that this high prevalence of chronic nephritis was likely to be a sequel to the childhood lead poisoning. They were able to promote the enactment of legislation to prohibit the use of lead paint in any part of a house, which was accessible to children, the first place in the world to enact such legislation. Belief in the durability of lead paint was strong, however, and implementation of the legislation was slow and many houses continued to be painted with lead paint. However, the style of architecture gradually changed and fewer open verandas were used and the frequency of childhood lead poisoning steadily fell in the 1930's. At that time, the common pattern of chronic lead nephropathy was of an adolescent who had never been robust or healthy; hypertension and uremia were features, the hypertension sometimes being severe and malignant and at other times being moderate or indolent [22]. Sibling involvement appeared to be consecutive rather than developing from a single episode of exposure. Several studies at that time [23-25] confirmed the high incidence of renal failure as a sequel to childhood lead poisoning. Nye [23] found 29 of 34 children with plumbism to had developed chronic renal disease. An extensive study in 1954 [26] of 401 children who had suffered from childhood lead poisoning between 1915 and 1935 showed that, of 352 who could be traced, two-thirds had died from renal or hypertensive vascular disease.

Later epidemiological studies [27] established a relative as well as an absolute increase in deaths from renal failure in Queensland in comparison with other Australian States and showed that this was consistent with the action of a nephrotoxin which had been operative between 1870 and 1920, which had then started to decline and which resulted in the development of chronic renal disease some 10-40 years later. Pathological study of the kidneys of these patients dying with renal failure revealed two groups, one readily diagnosable as a definable form of chronic renal disease and the other two-thirds who could not be classified into any of these standard varieties. Henderson and Inglis [28] showed that the lead content of bone was comparable with that of the rest of the community in those with diagnosable renal disease whereas it was significantly increased in those whose renal disease could not be so classified. There was thus a clear correlation between the frequency of a high bone lead and excess mortality from chronic renal disease in Queensland. Henderson concluded that these studies left no room for doubt that the excess mortality from chronic renal disease in Queensland was due solely to lead absorption in childhood. There has been no comparable degree and duration of exposure of children to similar amounts of lead elsewhere, particularly in cold climates where lead poisoning is principally a summer disease. Other contributory factors in Queensland were the design of housing and the frequency with which children would play on the open lead-painted verandas.

By the 1960's, the pattern of patients with chronic lead nephropathy had changed from being principally an adolescent condition (as in the 1930's) so that most of the patients were in their 40's. At that time, criteria for diagnosis were established which consisted of long-standing chronic renal disease which was only slowly

progressive and which resulted in equal and usually severe contraction of both kidneys. Any alternative cause for the renal disease needed to be excluded and there needed to be clear evidence of excessive lead absorption, either from a history of acute lead poisoning in childhood in the patient or in a sibling or from the demonstration of an increased lead content in bone. In order to study the condition more clearly in life, Emmerson introduced the EDTA test [29] which provided confirmation of excessive lead stores in these subjects with chronic renal disease and enabled a lead etiology to be established even when there was no other clear evidence of excessive past lead absorption. In these subjects, the amount of lead excreted after 1 g of EDTA correlated well with the degree of renal failure, as reflected in the serum creatinine or serum bicarbonate and this suggested that the associated renal osteodystrophy was contributing to the mobilization of skeletal lead. None of the patients, however, demonstrated any of the biochemical features of lead intoxication at the time, including excretion of coproporphyrins or amino-levulinic acid. This EDTA test then provided objective confirmation of excessive past lead absorption and storage, whether or not a history of this could be obtained [30]. Nonetheless, by itself, the EDTA test cannot prove that the excessive past lead absorption is etiologically related to another disease in that subject, such as chronic renal disease.

These studies also established that chronic renal failure of itself does not retain lead within the organism or result in an increase in lead excretion after EDTA. By facilitating diagnosis in life, the EDTA test made possible the ability to study the renal function of these patients, particularly in relation to abnormalities of handling of urate. Study of the pathology at this time [31] showed granular contracted kidneys with a reduced number of functioning nephrons with tubular atrophy and dilatation and arteriolar and interstitial fibrosis (Figure 1).

Of particular interest was the fact that the cohort surviving into the 1960's had developed a high prevalence of gouty arthritis in comparison with patients with chronic nephritis due to causes other than lead. There was also a disproportionate hyperuricemia in these patients with chronic lead nephropathy, which was caused by a significantly lower urate clearance for any particular degree of renal insufficiency [32]. Studies of discrete tubular functions suggest that this was due to excessive reabsorption of filtered urate [33].

Moonshine

Observations in Birmingham, Alabama in the 1960's indicated a high prevalence of chronic lead poisoning due to illegal alcohol consumption and an association with unexplained renal failure [34]. The alcohol that was consumed had been prepared in illicit stills using lead condensers (often prepared at night by moonlight - hence "moonshine") and it contained variable but generally large amounts of lead, often exceeding 1 mg/

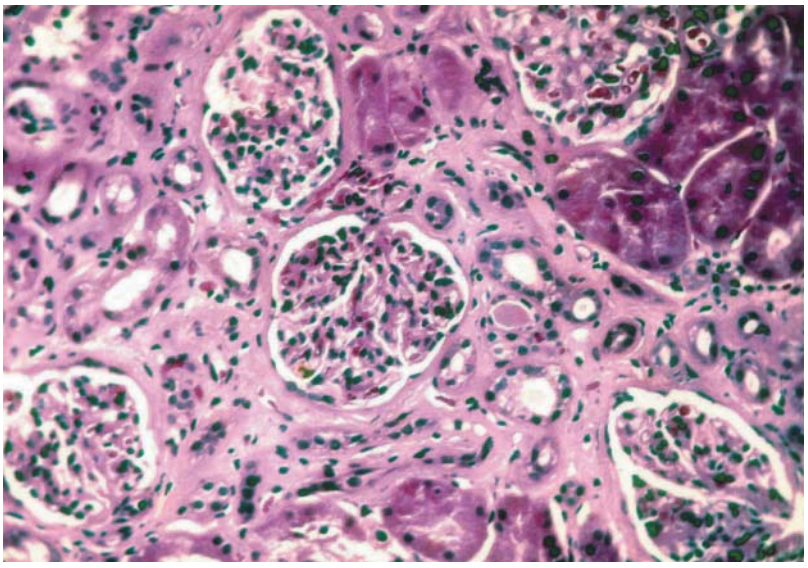

Figure 1. *Tubular atrophy and interstitial fibrosis in a case of chronic lead nephropathy. H&E staining, orig. magn. x300.*

L. Many of the subjects with excessive lead absorption showed the continuing toxic effects on hemopoiesis with anemia and stippling of red cells, together with impairment of renal function. There were clear signs of intranuclear inclusion bodies in these renal tubular cells, implicating lead in the etiology of the renal lesion. This situation was quite different from that in Queensland where the acute lead intoxication had occurred decades before and there were no continuing signs of lead intoxication except for the storage of lead in the skeleton. In the moonshine drinkers, thus, the renal lesion was consistent with a lead etiology with signs of an acute intoxication superimposed on a chronic renal lesion. Gout and hypertension again were features of this syndrome and studies of the pathogenesis of the hyperuricemia indicated a renal cause for the hyperuricemia, sufficient to explain the development of gout [35].

Association with gout

An association between lead absorption and gout has long been recognized. Garrod [36], who in 1854, had recognized uric acid crystals in the serum of gouty patients had recognized by 1876 [37] a high incidence of lead intoxication in patients with gout and there have been intermittent reports of saturnine gout since that time [38]. The recognition that half of the patients with Queensland chronic lead nephropathy suffered from gout and that it was a common feature in moonshine drinkers has raised the question of how many patients suffering from gout have an unrecognized underlying lead nephropathy. Although hyperuricemia invariably accompanies azotemia, gout is rare in patients with renal failure except in those with lead nephropathy. Half of uremic patients with lead nephropathy have clinical gout [30, 39], but in the absence of renal failure, gout cannot usually be attributed to lead despite coexisting hypertension [40].

It is difficult on clinical grounds to separate unequivocally three groups of patients: (i) those with renal disease due to lead; (ii) those with renal disease due to gout; and (iii) those with gout due to primary renal disease, and such a differentiation is necessary if one is to define a lead etiology.

Clinical features, which can enable lead gout to be differentiated from primary gout, have been described [41] and include an involvement of a higher proportion of women, a greater incidence of renal disease in kindred and of hypertension in siblings. The number of acute attacks of gout was less in the lead gout group but renal disease was invariably present and antedated the gouty arthritis. Lead gout also tends to occur in early adult life, to affect principally the lower limbs, and to have no family predisposition in succeeding generations. By contrast, the primary gout group were obese, consumed alcohol regularly and often suffered from renal calculi. In this group, all patients with lead gout had evidence of renal disease prior to the development of gout and all patients with primary gout had gout prior to the development of renal disease.

Studies of patients with gout and/or renal disease in USA have shown a clear association of an increased EDTA-induced lead excretion with gout and renal disease. There was a greater increase in lead excretion after EDTA in patients with gout and renal disease than in patients with gout with normal renal function [42]. Thus, an association has clearly been established, although only an epidemiological study can conclusively establish an etiological basis for the association. Nonetheless, the basic mechanism for lead to produce a renal lesion, which reduces renal excretion of urate, leading to a disproportionate hyperuricemia and gout clearly has been established as a pathogenetic mechanism for lead-induced gout.

Association with hypertension

There is also clear evidence that the sequel of lead inducing a chronic nephropathy with hypertension can occur. At times, this hypertension may be sufficiently severe to be malignant and may precipitate an early demise [22]. In more chronic cases, the hypertension may be of moderate degree and not be sufficient to cause progressive deterioration of renal function [43]. However, when confronted with a patient with hypertension and mild renal damage, it can be difficult to determine which came first and particularly difficult to determine whether lead was a contributor to the renal damage that caused the hypertension. In such cases, the hypertensive mechanism would be the same as those associated with other varieties of chronic renal disease. By contrast, many patients with chronic lead nephropathy have demonstrated suppressed plasma renin concentrations indicative of a hyporeninemic hypoaldosteronism [44].

A role for lead in hypertension gains further credence from epidemiologic studies of low-level lead exposure (i.e., exposure too low in intensity to produce the classic symptoms of acute lead poisoning). The Second National Health and Nutrition Examination Survey performed between 1976 and 1980 included blood lead and blood pressure measurements in almost 10, 000 non-institutionalized Americans aged 6 months to 74 years. The correlation between blood lead and blood pressure was robust even when both measurements were within the accepted "normal" range [45, 46, 46a]. Similar conclusions have been drawn from studies performed throughout the world [47], although contradictory findings in small studies have also been reported. Longitudinal studies of bone lead, blood lead, and blood pressure in the normative aging study in Boston demonstrate that low-level lead absorption correlates positively with blood pressure [47a]. Low-level lead absorption has also been implicated as a cause of renal dysfunction by the demonstration that chelation therapy reverses the progression of renal failure in patients without excessive lead absorption [47b-d]. These studies suggest that environmental exposure to lead may contribute to the decline in renal function and rise in blood pressure associated with the aging process in the general population. Although some doubts have been raised about the magnitude of the dose-response relationship, there is a growing consensus that lead contributes to hypertension, particularly in the presence of renal dysfunction. A meta-analysis of reports on the relationship of blood lead to blood pressure through 1992 concluded that a decrease in blood lead from 10 to 5 μg/dl is associated with a mean decrease in systolic pressure of 1.25 mm Hg [48]. The implications of these findings for public health remain controversial [49, 50].

Alternative explanations have been considered to determine whether lead can induce hypertension in the absence of chronic renal disease [47, 51]. One postulated mechanism involves an alteration in intracellular calcium concentration by lead so as to cause an increased tonic contraction of arterioles leading to hypertension. Others have suggested a direct effect of lead on juxtaglomerular cells leading to an increase in renin secretion. Others have suggested alterations in renal ion transport, particularly relating to an effect of lead on sodium potassium ATPase.

Correlations between blood pressure and blood lead reflect the many factors other than lead that are involved in determining the blood pressure. Thus, an important question is the extent to which chronic lower level lead exposure can lead to hypertension; a review of the evidence is suggestive of a possible causal relationship, although much more data is needed.

Occupational lead nephropathy

Occupational lead nephropathy has developed after a little as 3 years of intense exposure [52]. Analysis of death certificates of 601 men employed at the Bunker Hill Lead Mine and Smelter in Kellogg, Idaho, up to 1977 indicated a two fold increased risk of dying from chronic renal disease [57]. The increased risk approached fourfold after 20 years of occupational exposure. Chronic interstitial nephritis due to lead has also been seen among American workmen (lead burners, firing range- and smelter workers), whose exposure was never severe enough to produce acute symptoms of lead poisoning [52, 57], and in US Armed Service veterans suffering from renal failure attributed to gout or essential hypertension [38, 39]. In the veterans, the diagnosis was only established by the $CaNa_2EDTA$ lead-mobilization test after renal failure was apparent. Medical histories were often misleading; patient recall frequently contradicted the objective evidence of chelation testing. In these occupationally exposed individuals, minimal (about 30%) reductions in glomerular filtration rate were restored to normal by long-term, low-dose chelation therapy (1 g of $CaNa_2EDTA$ with local anesthetic thrice weekly until the chelation test returned to normal). However, this therapeutic response in pre-azotemic lead nephropathy may reflect reversal of functional impairment rather than reversal of established interstitial nephritis. Renal biopsies in lead workers with chronic lead nephropathy show nonspecific tubular atrophy and interstitial fibrosis with minimal inflammatory response as well as mitochondrial swelling, loss of cristae, and increased lysosomal dense bodies within proximal tubule cells [52, 56]. Arteriolar changes indistinguishable from nephrosclerosis are found, often in the absence of clinical hypertension. Intranuclear inclusion bodies are often absent when the renal disease is long standing or following the administration of chelating agents. Clumped chromatin, and nuclear invaginations of cytoplasmic contents may be found even in the absence of intranuclear

inclusions. Morphologic alterations are minimal in glomeruli until the reduction in glomerular filtration rate is advanced. Animal models of chronic lead intoxication have revealed similar tubular findings and have highlighted the importance of disease in small and medium sized arteries of the kidney [55a]. Sanchez-Fructuoso et al. [56] performed EDTA lead mobilization tests in 296 Spanish patients selected because of the absence of known excessive exposure to lead. The mean blood lead concentration was about 16 μg/dl in normal controls, hypertensives without renal failure, and in patients with renal disease of known non-lead etiology. Compared to the mean blood leads levels of < 3 μg/dl in the US population in the same time period, these blood leads suggest considerable absorption from the environment in the absence of occupational exposure in Spain. This study supported the notion that occult lead absorption in Spain contributes to hypertension associated with gout and renal failure. The absence of increased chelatable lead in patients with renal failure of non-lead etiology confirmed the observation that renal failure per se does not cause increased mobilizable lead. Similarly, bone lead determined by transiliac biopsy in 12 Spanish patients with increased mobilizable lead levels demonstrated a good correlation between bone lead and both mobilizable lead and the serum creatinine concentration.

Mortality data show that death from hypertensive cardiovascular disease *was* more frequent among lead workers than among the general population [53, 57, 58]. There is some suggestion that this has disappeared with the introduction of stricter standards.

The functional changes in chronic lead nephropathy appear to be less specific than those observed in acute poisoning. As in other forms of interstitial nephritis, proteinuria and glycosuria are initially absent. In contrast to cadmium nephropathy, the excretion of urinary marker proteins such as human intestinal alkaline phosphatase, total nonspecific alkaline phosphatase, Tamm Horsfall glycoprotein, retinol binding protein, lysozyme, and β2-microglobulin [59-63] is not increased in the absence of a reduced glomerular filtration rate. However, the urinary excretion of the proximal tubular lysosomal enzyme N-acetyl-β-D-glucosaminidase, increases with increasing blood lead, an effect that is independent of bone lead concentrations [64-66]. The increase in urinary N-acetyl-β-D-glucosaminidase with increasing blood lead levels may reflect the Fanconi syndrome of acute lead poisoning rather than the chronic interstitial nephritis associated with occupational lead exposure [67]. In contrast to the predictive ability for progressive renal failure in following exposure to cadmium, enzymuria does not appear to predict progression to renal failure following lead absorption. Nevertheless, observations in men in the normative aging study conducted in Boston since 1961 indicates that even low-level lead exposure has a deleterious effect on renal function as reflected by the serum creatinine concentration [68].

Exhibiting a pattern of eicosanoid excretion noted in essential hypertension, lead-exposed workers showed an increase in TxB_2 and a decrease in PGE_2 and 6-keto-$PGF_{1\alpha}$ in the urine [63]. In contrast to the reabsorptive defect of acute lead nephropathy, saturnine gout is characterized by renal retention of uric acid. The clearance and maximal secretion rate for para-aminohippurate have been found to be variable in patients with occupational lead nephropathy. A reduced maximal reabsorptive rate for glucose has been reported, but simultaneous, matched controls were not obtained [69].

Assessing the body burden

The EDTA test is performed in adults by parenteral administration of 1 to 3 g of $CaNa_2EDTA$ over 4 to 12 hours with subsequent collection of 24-hour urine samples over 1 to 4 days. A dose of 20 to 30 mg EDTA/kg is generally used in children. Adults without undue prior lead absorption excrete up to 650 μg of lead-chelate in the urine. Neither the dose (1 to 3 g) nor the route of administration (intravenous or intramuscular) appears to critically modify the normal response to chelation testing [70, 71], but in the presence of renal failure (serum creatinine greater than 1.5 mg/dl) urine collections should be extended to at least 3 days. The adequacy of collection can be checked by simultaneous measurement of creatinine excretion (1.3 g of creatinine/day is an acceptable lower limit in normal adult males).

Since lead in bone has a biologic half-life measured in decades, compared to a biologic half-life of lead in blood of only 2-4 weeks [72], the bone more closely reflects cumulative body lead stores. Chelatable lead correlates well with bone lead [4, 31]. The decrease in bone lead stores can be monitored by *in vivo* tibial K x-

ray fluorescence, a new, non-invasive technique that is both safe and accurate at bone lead concentrations associated with interstitial nephritis due to lead [73-75].

Treatment

Although chelation therapy effectively reverses acute lead nephropathy and the preclinical renal dysfunction of occupational lead nephropathy, there is no evidence that such therapy reverses established interstitial nephritis due to lead. The partial remissions achieved among moonshiners and symptomatic lead workers may represent reversal of acute poisoning superimposed on chronic lead nephropathy. No improvement in renal function can be expected once advanced interstitial nephritis is present and the steady-state serum creatinine concentration exceeds about 3 mg/dl. Chronic volume depletion and hyporeninemic hypoaldosteronism may contribute to the reversible component of renal dysfunction [76].

Although the EDTA test has been shown to be safe even in the presence of renal failure [77], the cumulative nephrotoxicity of prolonged EDTA therapy in patients with markedly reduced glomerular filtration rates is unknown. Reports that $CaNa_2EDTA$ therapy has been followed by deterioration of renal function warrant careful follow-up of treated patients [78]. Despite these caveats, it may be appropriate to perform EDTA lead-mobilization tests in individuals with gout or hypertension and renal failure or interstitial nephritis of unknown etiology since a positive test may provide the best available indication of etiology.

References

1. Lancereaux E. Saturnisme chronique avec acces de goutte et arthrities uratiques. Compt Rend Soc de Biol (Paris) 1872; 2: 99-106.
2. Rabinowitz MB, Wetherill GW, Kopple JD. Magnitude of lead intake from respiration by normal man. J Lab Clin Med 1977; 90: 238-248.
3. Rabinowitz MB, Wetherhill GW, Kopple JD. Kinetic analysis of lead metabolism in healthy humans. J Clin Invest 1976; 58: 260-270.
4. Van de Vyver FL, D'Haese PC, Visser WJ, Elseviers MM, Knippenberg LJ, Lamberts LV, Wedeen RP, De Broe ME. Bone lead in dialysis patients. Kidney Int 1988; 33: 601-607.
5. Vallee BL, Ulmer DD. Biochemical effects of mercury, cadmium and lead. Ann Rev Biochem 1972; 41: 91-128.
6. Fowler BA, Duval G. Effects of lead on the kidney: roles of high-affinity lead-binding proteins. Environ Health Perspect 1991; 91: 77-80.
7. Goyer RA. Mechanisms of lead and cadmium nephrotoxicity. Toxicol Lett 1989; 46: 153-162.
8. Pounds JB, Long GJ, Rosen JF. Cellular and molecular toxicity of lead in bone. Environ Health Perspect 1991; 91: 17-32.
9. Fothergill J. Observations on disorders to which painters in water colours are exposed. Medical Observations and Inquiries 1775; 5: 393-405.
10. McKhann CF. Lead poisoning in children with notes on therapy. Am. J. Dis Child 1926; 32: 386-392.
11. Wilson V, Thomson ML, and Dent, CE. Amino-aciduria in lead poisoning. A case in childhood. Lancet 1953; 66-68.
12. Chisolm JJ, Harrison HC, Eberlern WR, Harrison HE. Aminoaciduria, hypophosphatemia and rickets in lead poisoning. Am J Dis Child 1955; 89: 159-168.
13. Chisolm JJ Jr, Mellits ED, Barrett MB. Interrelationships among blood lead concentration, quantitative daily ALA-U and urinary lead output following calcium EDTA. In: Effects and dose-response relationships of toxic metals. Nordberg GF (editor). Elsevier, Amsterdam 1976; p 416-433.
14. Loghman-Adham M. Aminoaciduria and glycosuria following severe childhood lead poisoning. Pediatr Nephrol 1998; 12: 218-221.
15. Agency for Toxic Substances and Disease Registry. Public Health Service. US Department of Health and Human Services. The nature and extent of lead poisoning in children in the United States. A report to Congress. Atlanta: US Department of Health and Human Services, Public Health Service, 1988.
16. National Research Council. Airborne lead in perspective. National Academy of Sciences, Washington 1972.
17. Goyer RA, Leonard DL, Bream PR, Irons TG. Aminoaciduria in experimental lead poisoning. Proc Soc Exp Biol Med 1970; 135: 767-771.
18. Goyer RA, Leonard DL, Moore JF, Rhyne B, Kingman MR. Lead dosage and the role of the intranuclear inclusion body: an experimental study. Arch Environ Health 1970; 20: 705-711.

19. Goyer RA, Wilson MH. Lead-induced inclusion bodies. Results of ethylenediaminetetraacetic acid treatment. Lab Invest 1975; 32: 149-156.
20. Schumann GB, Lerner SI, Weiss MA, Gawronski L, Lohiya GK. Inclusion-bearing cells in industrial workers exposed to lead. Am J Clin Pathol 1980; 74: 192-196.
21. Council of the Queensland Branch of the British Medical Association. An historical account of the occurrence and causation of lead poisoning among Queensland children. Med J Aust 1922; 1: 148-152.
22. Nye LJJ. Chronic nephritis and lead poisoning. Angus and Robertson, Sydney 1933.
23. Nye LJJ. An investigation of extraordinary incidence of chronic nephritis in young people in Queensland. Med J Aust 1929; 2: 145-159.
24. Fairley KD. A review of the evidence relating to lead as an etiological agent in chronic nephritis in Queensland. Med J Aust 1934; 1: 600-606.
25. Murray RE. Plumbism and chronic nephritis in young people in Queensland. Commonwealth of Australia, Department of Health Service Publication, 1939, no 2.
26. Henderson DA. A follow-up of cases of plumbism in children. Austr Ann Med 1954; 3: 219-224.
27. Henderson DA. Chronic nephritis in Queensland. Austr Ann Med 1955; 4: 163-177.
28. Henderson DA, Inglis JA. The lead content of bone in chronic Bright's disease. Austr Ann Med 1957; 6: 145-154.
29. Emmerson BT. Chronic lead nephropathy: the diagnostic use of calcium EDTA and the association with gout. Aust Ann Med 1963; 12: 310-324.
30. Emmerson BT. Chronic lead nephropathy. Kidney Int 1973; 4: 1-5.
31. Inglis JA, Henderson DA, Emmerson BT. The pathology and pathogenesis of chronic lead nephropathy occurring in Queensland. J Path 1978; 124: 65-76.
32. Emmerson BT. The renal excretion of urate in chronic lead nephropathy. Austr Ann Med 1965; 14: 295-303.
33. Emmerson BT, Mirosch W, Douglas JB. The relative contributions of tubular reabsorption and secretion to urate excretion in lead nephropathy. Aust NZ J Med 1971; 4: 353-362.
34. Morgan JM, Hartley MW, Miller RW. Nephropathy in chronic lead poisoning. Arch Int Med 1966; 118: 17-29.
35. Ball GV, Sorensen LB. Pathogenesis of hyperuricemia in saturnine gout. N Engl J Med 1969; 280: 1199-1202.
36. Garrod AB. Second communication on the blood and effused fluids in gout, rheumatism and Brights' disease. Med Chir Tr (London) 1854; 37: 49-61.
37. Garrod AB. A treatise on gout and rheumatic gout, 3rd ed. Longmans, Green & Co, London 1876.
38. Wedeen RP. Poison in the pot: the legacy of lead. Southern Illinois University Press, Carbondale, Ill 1984.
39. Craswell PW, Price J, Boyle PD, Heazle wood VJ, Baddeley H, Lloyd HM, Thomas BJ, Thomas BW. Chronic renal failure with gout: a marker of chronic lead poisoning. Kidney Int 1984; 26: 319-323.
40. Peitzman SJ, Bodison W, Ellis I. Moonshine drinking among hypertensive veterans in Philadelphia. Arch Intern Med 1985; 145: 632-634.
41. Emmerson BT. The clinical differentiation of lead gout from primary gout. Arthritis Rheum 1968; 11: 623-624.
42. Batuman V, Maesaka JK, Haddad B, Tepper E, Landy E, Wedeen RP. The role of lead in gout nephropathy. N Engl J Med 1981; 304: 520-523.
43. Batuman V, Landy E, Maesaka JK, Wedeen RP. Contribution of lead to hypertension with renal impairment. N Engl J Med 1983; 309: 17-21.
44. Sandstead HH, Michelakis AM, Temple TE. Lead intoxication: its effect on the renin-aldosterone response to sodium deprivation. Arch Environ Health 1970; 20: 356-363.
45. Harlan WR. The relationship of blood lead levels to blood pressure in the US population. Environ Health Persp 1988; 78: 9-13.
46. Pirkle JL, Schwartz J, Landis JR, Harlan WR. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. Am J Epidemiol 1985; 121: 246-258.
46a. Muntner P, He J, Vupputuri S, Coresh J, Batuman V. Blood lead and chronic kidney disease in the general United States population: Results from NHANES III. Kidney Int 2003; 63: 1044-1050.
47. Sharp DS, Becker CE, Smith AH. Chronic low level lead exposure - its role in the pathogenesis of hypertension. Med Toxicol 1987; 2: 210-232.
47a. Cheng Y, Schwartz J, Sparrow D, Aro A, Weiss ST, Hu H. Bone lead and blood lead levels in relation to baseline blood pressure and prospective development of hypertension: The normative aging study. Am J Epidemiology. 2001; 153: 164-171.
47b. Sánchez-Fructuoso AI, Montserrat C, Arroyo M, Fernández C, Prats D, Barrientos A. Lead mobilization during calcium disodium ethylenediaminetetraacetate chelation therapy in treatment of chronic lead poisoning. Am J Kidney Dis 2002; 40: 51-58.
47c. Lin J-A, Ho H-H, Yu C-C. Chelation therapy for patients with elevated body lead burden and progressive renal insufficiency.. Ann Intern Med 1999; 130: 7-13.

47d. Lin J-A, Lin-Tan D-T, Hsu K-H, Yu C-C. Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. New Eng J Med 2003; 384: 277-286.
48. Schwartz J. Lead, blood pressure, and cardiovascular disease in men. Arch Environ Health 1995; 50: 31-36.
49. Korrick SA, Hunter DJ, Rotnitzky A, Hu H, Speizer FE. Lead and hypertension in a sample of middle-aged women. Am J Public Health 1999; 89: 330-335.
50. Staessen JA, Roels H, Fagard R. Lead exposure and conventional and ambulatory blood pressure. A prosepective population study. JAMA 1996: 275; 1563-1570.
51. Ritz E, Mann J, Stoeppler M. Lead and the kidney. Adv Nephrol 1988; 17: 241-274.
52. Wedeen RP, Maesaka JK, Weiner B, Lipat GA, Lyons MM, Vitale LF, Joselow NM. Occupational lead nephropathy. Am J Med 1975; 59: 630-641.
53. Selevan SG, Landrigan PJ, Stern FB, Jones JH. Mortality of lead smelter workers. Am J Epidemiol 1985; 122: 673-683.
54. Wedeen RP, Mallik DK, Batuman V. Detection and treatment of occupational lead nephropathy. Arch Intern Med 1979; 139: 53-57.
55. Cramer K, Goyer RA, Jagenburg R, Marion H. Renal ultrastructure, renal function, and parameters of lead toxicity in workers with different periods of lead exposure. Brit J Ind Med 1974; 31: 113-127.
55a. Sánchez-Fructuoso AI, Blanco J, Montserrat C, Ortega L, Arroyo M, Fernández C, Prats D, Barrientos A. Experimental lead nephropathy: treatment with calcium disodium ethylenediaminetetraacetate. Am J Kidney Dis 2002; 40: 59-67.
56. Sánchez-Fructuoso AI, Toralbo A, Arroyo M, Lunque M, Ruilope LM, Santos JL, Cruceyra A, Barrientos A. Occult lead intoxication as a cause of hypertension and renal failure. Nephrol Dial Transplant 1996; 11: 1775-1780.
57. Fanning D. A mortality study of lead workers - 1926-1985. Arch Environ Health 1988; 43: 247-251.
58. Gerhardsson L, Chettle DR, Englyst V, Nordberg GF, Nyhlin H, Scott MC, Todo AC, Vesterberg O. Kidney effects in long term exposed lead smelters workers. Brit J Ind Med 1992; 49: 186-192.
59. Buchet J-P, Roels H, Bernard A, Lauwerys R. Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury vapor. Am J Occup Med 1980; 22: 741-750.
60. Vacca C. Heavy metal nephrotoxicity: lead differentiated from cadmium and mercury. Am J Clin Pathol 1980; 73: 308.
61. Omae K, Sakurai H, Higashi T, Muto T, Ichikawa M, Sasaki N. No adverse effects of lead on renal function in lead-exposed workers. Ind Health 1990; 28: 77-83.
62. Mueller PW, Paschal DC, Hammel RR, Klincewicz SL, Macneil ML, Spierto B, Steinberg KK. Chronic effects in three studies of men and women occupationally exposed to cadmium. Arch Environ Cont Toxicol 1992; 23: 125-136.
63. Cardenas A, Roels H, Bernard AM, Barbon R, Buchet JP, Lauwerys RR, Rosello J, Ramis I, Mutti A, Franchini I, Fels LM, Stolte H, De Broe ME, Nuyts GD, Taylor SA, Price RG. Markers of early renal changes induced by industrial pollutants. II. Application to workers exposed to lead. Brit J Ind Med 1993; 50: 28-36.
64. Kumar BD, Krishnaswammy K. Detection of sub-clinical lead toxicity in monocasters. Bull Environ Contam Toxicol 1995; 54: 863-869.
65. Verberk MM, DeWolff FA. Environmental lead and renal effects in children. Arch Environ Health 1996; 51: 83-87.
66. Wedeen RP, Udasin I, Fiedler N, D'Haesse P, De Broe M, Gelpi E, Jones KW, Gochfeld M. Urinary biomarkers as indicators of renal disease. Renal Failure 1999; 3-4: 241-249.
67. Endo G, Horiguchi S, Kiyota I. Urinary N-acetyl-β-D-glucosaminidase activity in lead-exposed workers. J Appl Toxicol 1990; 10: 235-238.
68. Kim R, Rotnitzky A, Sparrow D, Weiss ST, Wager C, Hu H. A longtitudinal study of low-level lead exposure and impairment of renal function. The normative aging study. JAMA 1996; 275: 1177-1181.
69. Hong CD, Hanenson IG, Lerner S, Hammond PB, Pesce AJ, Pollak VE. Occupational exposure to lead: effects on renal function. Kidney Int 1980; 18: 489-494.
70. Leckie WJH, Tomsett SL. The diagnostic and therapeutic use of edathamil calcium disodium (EDTA Versene) in excessive inorganic lead absorption. Q J Med 1958; 27: 65-82.
71. Wedeen RP. Occupational and environmental renal diseases. Curr Nephrol 1988; 11: 65-105.
72. Chamberlain AC. Prediction of response of blood lead to airborne and dietary lead from volunteer experiments with lead isotopes. Proc R Soc Lond [Biol] 1985; 244: 149-182.
73. Ahlgren L, Mattsson S. An x-ray fluorescence technique for *in vivo* determination of lead concentration in a bone matrix. Phys Med Biol 1979; 24: 136-145.
74. Craswell PW, Price J, Boyle PD, Heazlewood VJ, Baddeley H, Lloyd HM, Thomas BJ, Thomas BW, Williams GM. Chronic lead nephropathy in Queensland: alternative methods of diagnosis. Aust NZ J Med 1986; 16: 11-19.
75. Wedeen RP. *In vivo* tibial XRF measurement of bone lead. Arch Environ Health 1990; 45: 69-71.
76. Ashouri OS. Hyperkalemic distal tubular acidosis and selective aldosterone deficiency: combination in a patient with lead nephropathy. Arch Intern Med 1985; 145: 1306-1307.

77. Wedeen RP, Batuman V, Landy E. The safety of the EDTA lead-mobilization test. Environ Res 1983; 30: 58-62.
78. Germain MJ, Braden GL, Fitzgibbons JR. Failure of chelation therapy in lead nephropathy. Arch Intern Med 1984; 144: 2419-2420.

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Cadmium-induced renal effects

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Introduction

The first report on the adverse health effects of cadmium (Cd) exposure was published by Friberg in 1950 [1]. He found a high prevalence of proteinuria (65% using the nitric acid test and 81% using the trichloroacetic acid test) in Cd-exposed workers.

In Japan, an unusual disease named "itai-itai byo", meaning "ouch-ouch disease" was reported in 1955 [2]. This disease is characterized clinically by bone and kidney damage. In 1968, the Japanese Ministry of Health and Welfare concluded that itai-itai disease was caused by chronic Cd poisoning [3].

The kidneys are particularly affected by Cd following long-term exposure [4]. Studies of workers chronically exposed to Cd in air report renal effects as well as respiratory effects. Therefore, the kidneys are considered the critical target organ for Cd in the general population as well as occupationally exposed population.

Exposure

Low concentrations of the element Cd occur naturally in the environment. Human exposure in the general environment occurs mainly from ingested foods. Concentrations of Cd in food items from areas with-

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out industrial contamination are summarized in Table 1.

For basic food items such as rice, potatoes and wheat, Cd concentrations usually are lower than 0.1 mg/kg, while higher concentrations occur naturally in certain meats or shellfish. The daily dietary intake of Cd has been estimated to be 10-20 μg in several countries of the European Union and in several studies from the USA [3, 5]. In areas contaminated by emissions from industrial activities much higher oral intakes may occur with amounts up to 200-1800 μg in people living in Japan and China [3, 6, 7].

Cadmium can also occur as an aerosol in air. While inhalation of ambient air usually does not contribute significantly to the daily intake of Cd, cigarette smoking does. The content of Cd often is 1-2 μg per cigarette. Based on data concerning the Cd content of cigarettes, it has been estimated that smoking of 20 cigarettes per day results in a daily inhalation of 2-4 μg [3]. Since approximately 50% may be absorbed, this can result in an uptake of 1-2 μg of Cd per day.

Occupational exposure in the Cd-related industries can be associated with the inhalation of considerable amounts of Cd. In the 1950's, before the health hazards of Cd were recognized, Cd concentrations in the air of the working environment were sometimes high, i.e. in the order of milligrams per m^3. In recent years, concentrations in industrial air have been reduced to 5-50 $\mu g/m^3$, with higher values being reported in some exceptional cases. Examples of Cd-related industrial activities include: manufacturing of alkaline (nickel-Cd) batteries, smelting operations involving copper/zinc-Cd ores or alloys, soldering with silver-Cd containing solder and welding in Cd-containing materials. In some countries (e.g. Sweden) certain uses of Cd such as its use in pigments, in electroplating and in soldering have been banned.

Table 1. Concentrations of cadmium in different foodstuffs*.

Food	Mean mg/kg wet weight
Beef meat	0.005-0.02
Beef kidney	0.2-1.3
Fish meat (other than crab)	0.004-0.1
Oysters	0.1-4.7
Wheat grains	0.005-0.08
Rice (non-contaminated areas)	0.008-0.13
Milk	0.00017-0.002
Potatoes	0.01-0.06

**From Friberg et al. [5].*

Toxicokinetics

Uptake

Inhalation of airborne Cd leads to variable uptake depending on size and solubility of particles. The systemic uptake of aerosolized Cd with a particle size of 10 μm has been estimated to be about 7%, while the uptake following inhalation of a particle size of 0.1 μm may be as high as 50% [8].

After oral ingestion, systemic uptake has been reported to be 1-6% in animal experiments. Factors that have been shown to influence oral uptake are dose and composition of the diet. In humans, the systemic uptake usually varies between 3 and 7% of the oral intake. In individuals with depleted body iron stores, uptake may be as high as 20% [8].

Transport and distribution

Figure 1 represents the uptake and transfer of Cd to the kidney. Following uptake, Cd is primarily bound in serum to albumin, the form in which it is transported to the various body pools. Cadmium bound to albumin (which is the dominating form in plasma shortly after uptake) is taken up primarily by the liver where it accumulates, and is dissociated. Released Cd-ions induce the synthesis of metallothionein which results in an increasing proportion of liver Cd being bound to metallothionein. The uptake of albumin-bound Cd by liver cells may be mediated by albumin receptors on the sinusoidal surfaces of hepatocytes [9]. In long-term chronic exposure a slow release of Cd-metallothionein from liver to blood occurs. During the phase, when plasma Cd is bound to albumin, there is only limited uptake of Cd in the kidney. A latent effect of a single exposure or long-term chronic exposure is concerned with the fact that a considerable proportion of plasma Cd is bound to metallothionein. The Cd-metallothionein complex, because of its small molecular size, is filtered by the glomerular membrane and is efficiently taken up by renal tubular cells. Moreover, metallothionein-bound Cd is taken up more efficiently by renal cells of Cd exposed animals than by cells from non-

exposed animals [10, 11]. After entering renal tubule cells via pinocytosis [12], the Cd-metallothionein compound is catabolized in lysosomes releasing Cd ions. Thus any Cd remaining in the kidney bound to metallothionein results from de novo synthesis (Figure 1). This process may account for the long biological half-life of Cd in the kidney where the element may be retained 10-20 years [8]. Such a long biological half-life explains why Cd continues to accumulate in humans up to 50 years of age, reflecting the historical intake from the environment.

Excretion of cadmium

The daily elimination of Cd (0.01-0.02% of the body burden per day) via urine and feces is trivial as would be expected from the element's long biological half-life [8]. This implies that there is an age-related accumulation of Cd in the body and the increased urinary excretion of Cd with age is due to the increasing body burden. While this interrelationship has been documented in humans on a group basis, there exists a large variation among individuals. Cadmium is also excreted in the feces, but the majority of fecal Cd consists of the unabsorbed fraction of the metal passing through the alimentary tract. The fecal content is often a good indicator of dietary Cd intake since about 95% of the ingested amount is unabsorbed and eliminated via feces. True fecal elimination of the body burden of Cd is difficult to study in humans due to the preponderance of unabsorbed Cd. Data from animal experiments indicate that fecal elimination is dependent both on dose and body burden. Thus, in long-term low-level exposures, the fecal excretion may be largely related to body burden [13]. The daily fecal content of Cd in persons of which the exposure limited to the general environment is approximately 50 times higher than the urinary excretion.

Mathematical models of cadmium toxicokinetics

A mathematical model of long-term toxicokinetics in humans has been developed [14, 15]. Subsequently, a more detailed description of Cd toxicokinetics was formulated considering additional events that modify the behavior of Cd in humans [16, 17]. The kidney and particularly the cortex, is considered the critical target tissue for Cd and its accumulation is of decisive importance for risk assessment. In long-term exposures (life-long) either a simple one-compartment model or a more multi-compartment model predicts that 1/3 to 1/2 of the total body burden accumulates in the kidney and that the concentration of Cd in the kidney cortex is 1.25 times higher than the average concentration in the whole kidney [8].

Toxic effects of cadmium

Acute toxicity

Acute effects of excess Cd in the diets of humans (more than 15 mg Cd/kg) involve vomiting and diarrhea [18]. Acute inhalation of high concentrations of Cd (about 5 mg/m^3 or higher) causes pneumonitis and may be lethal [3].

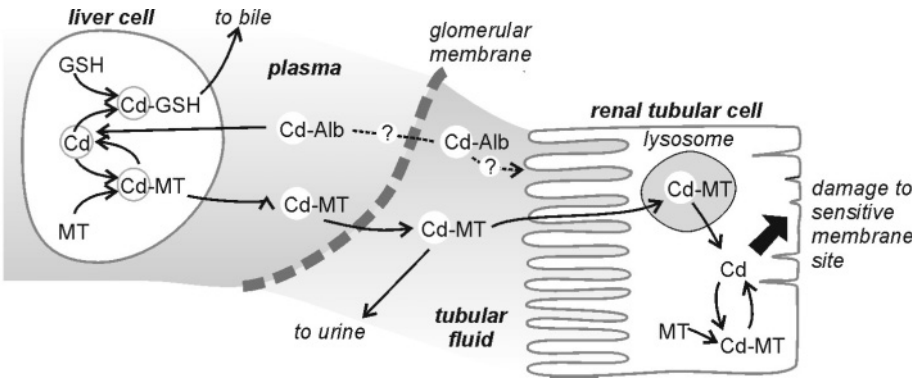

Figure 1. *Pathways of cadmium uptake and interaction with target sites in the kidney.*

Long-term exposure

Pulmonary toxicity may occur after long-term exposure to inhaled Cd. In such situations emphysema and other chronic pulmonary effects have been observed both in animals and in humans. Respiratory effects of Cd have not been recorded in the general population [3].

Reproductive toxicity

It is well known that the injection of Cd in experimental animals induces testicular placental necrosis in males and pregnant females respectively. Whether such effects may also occur after long-term dietary or environmental exposure in animals or humans is still a matter of discussion [19, 20]. A protective role of metallothionein in both human placenta and pregnant rats exposed to Cd may explain the lack of an effect on birth weights of children from Cd-exposed female Cd battery workers [20, 21].

Carcinogenicity

Cadmium has been reported to induce cancer in animals at the site of injection. Respiratory cancers may occur after inhalation of Cd compounds [22]. There is also epidemiological evidence of an association between Cd exposure and cancer in occupational groups such as smelter and battery workers. Both prostate and lung cancers have been reported to occur in increased frequency. The International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence supporting the carcinogenicity of Cd, although methodological problems in the interpretation of the studies have been recognized [23, 24]. Some studies performed after the IARC assessment have not given support for carcinogenicity of Cd [25].

The overall standardized mortality and incidence ratios of all malignant neoplasms among persons previously exposed to environmental Cd, were not significantly increased [26, 27].

Experimental nephrotoxicity

It has long been recognized that Cd exposure either after inhalation or ingestion, can give rise to nephrotoxicity in humans and that this effect is usually considered to be the earliest and most important health effect [28].

In this regard, the dominating effect was recognized early and consisted primarily of injury to the renal tubules inducing a proteinuria characterized by the excretion of low molecular weight (LMW) plasma proteins. As noted previously, in long-term exposures to Cd, both in experimental animals and in humans there is continuous accumulation of Cd in liver and kidneys. Nephrotoxicity in animal experiments usually does not develop until the concentration of Cd in the renal cortex is in the range of 100-400 mg/kg wet weight. Increased concentrations of urinary LMW proteins were found in ±10% of a study population of industrial workers, having a Cd concentration in the kidney cortex ≥ 200 mg/kg as assessed by *in vivo* neutron activation analysis [29, 30]. Recent reports from Belgium indicate that, in workers with a urinary Cd excretion lower than 10 µg/g creatinine, renal effects may occur, whilst concentrations of urinary Cd as low as 2 to 4 µg/g creatinine have been associated with an increased prevalence of various indicators of renal dysfunction in the general population [31, 32]. This concentration in urine corresponds to a renal cortical concentration varying between 50-100 mg/kg wet weight [25].

Although renal cortical concentrations greater than 50 mg/kg wet weight may be accompanied with mild effects on the renal tubules in humans who have been exposed to Cd for a long time, it has been demonstrated in animal models that renal tubular injury can occur following injection of Cd-metallothionein at concentrations as low as 10-20 mg/kg wet weight [33, 34], whereas in animals with long-term exposure concentrations of 100 mg/kg wet weight or higher are required [3]. The explanation for this discrepancy is most likely due to differences in metallothionein induction that may occur in these two situations. Indeed, in the long-term exposure situation, ample time will be available for the induction of a protective level of metallothionein synthesis, whereas this will not be the case of acute exposure after Cd-metallothionein injection. The acute injection delivers a bolus dose of this complex to the renal tubule where it is metabolized in the lysosomes and toxic Cd ions are released, which will interact with cellular targets, principally the plasma membrane (Figure 1) [35]. Recent studies indicate that in addition to the protective effect of metallothionein, stress proteins may also participate in this protection

[36-38].

Cadmium is the most potent inducer of metallothionein synthesis among others. The mechanisms underlying the induction and regulation of metallothionein synthesis remain to be elucidated, but the metallothionein genes have been identified [39]. Reports concerning metallothionein in plasma and urine of Cd-exposed persons are limited [40-42]. This is at least in part due to the fact that the accurate measurement of Cd and metallothionein levels in plasma appears to be difficult [43]. The concentration of metallothionein in urine and blood has to be measured using the Onosaka saturation method, radio immunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). The detection limits in human serum and urine for metallothionein by RIA is 1 pg [43]. For ELISA the detection limits are higher. Normal values range between 0.01-1 ng/ml for serum and between 1-10 ng/ml for urine. Metallothionein concentrations in Cd-exposed workers are reported to vary between 2-11 ng/ml in plasma and 2-155 ng/ml in urine [40].

Human nephrotoxicity by cadmium exposure

Sweden

As mentioned in the introduction to this chapter, chronic Cd poisoning with proteinuria resulting from occupational exposure was identified in Sweden in the late 1940's by Friberg [1]. Subsequently, it was shown that the proteinuria is of the tubular type and that the LMW proteins that were plasma proteins were not reabsorbed because of tubular damage [44, 45]. As a result of the discovery of the role of metallothionein in the toxicology of Cd [40, 46, 47] and the recognition of metallothionein binding as an explanation of the long biological half-life of Cd, interest was focused on the long-term intake of Cd via food. Kjellström et al. [48] assessed the temporal evolution of Cd in Swedish wheat, sampled from 1880 to 1970, and found a statistically significant time-dependent increase. The possibility of a risk for renal dysfunction and disease as a result of long-term dietary Cd intake was recognized and the relationship between occupational Cd exposure, renal accumulation of the element and tubular proteinuria was established [49]. Based on animal and human studies investigating long-term exposure to Cd from food and inhalation, a critical concentration of Cd in renal cortex was related to the risk of developing renal effects. These estimates were published in extensive reviews and evaluation volumes [5, 13-16, 49, 50], confirmed and/or partly revised according to epidemiological data from Japan, Belgium, and China (see the respective separate sections) and later on summarized in a review by Järup et al. [25]. It was concluded that a small increase (less than one percent above background) in the prevalence of tubular dysfunction is expected to occur at renal cortical Cd concentrations exceeding 50 mg/kg. The corresponding level of urinary Cd was estimated at 2.5 μg/g creatinine. In a recent epidemiological study reporting data from subjects previously exposed to Cd in Sweden [51] a relationship between current urinary Cd levels and increased excretion of tubular proteins was demonstrated. Relationships between urinary Cd and occurrence of osteoporosis have also been established [52]. Since the slightly increased urinary Cd levels observed in these studies resulted from past exposures whilst recent exposure to Cd most probably was considerably lower, it is difficult to estimate from these data at what degree of cumulative exposures and urinary Cd values, one might expect an increased proteinuria. In general, these studies give support to the notion that tubular proteinuria might already be induced at relatively low cumulative exposures.

Japan

Clinical features of itai-itai disease

The main features of itai-itai disease are osteomalacia and osteoporosis [2]. The patients usually have several fractures that are caused by events as trivial as coughing. They suffer from severe pain when sleeping or even breathing. Compression fractures in the spine resulting in skeletal deformity and eventually shortening of the stature may occur. Patients also develop a duck-like gait and progressive difficulties in walking. While most of the itai-itai patients are postmenopausal women with several pregnancies no hereditary factors have been identified. X-ray findings include marked decalcification and the presence of 'Looser's zones' localized at areas where pressure causes pain. In severe cases, multiple pathological fractures are found. Skeletal deformities are frequently observed in pelvic bones, costae, and thoracic and lum-

bar vertebrae. Blood chemistry showed an increase in serum alkaline phosphatase and decreases in serum inorganic phosphorus and calcium, while urinalysis revealed proteinuria, glucosuria, and aminoaciduria. The urinary protein excretion is characterized by the so-called 'tubular protein pattern' consisting of mainly LMW proteins such as β_2-microglobulin, retinol binding protein, and lysosomal proteins. The aminoaciduria of the patient is of the "generalized aminoaciduria" type. The Cd content in urine is remarkably high. Increased excretion of calcium is also noticed. The principal pathological changes in bones are similar to the combined findings of osteomalacia and osteoporosis. Nearly 60% of 75 autopsied itai-itai disease patients had some degree of osteomalacia. All of them had severe to extreme osteoporosis [53]. Although the kidney is contracted, there is no obvious change in the glomeruli. The tubuli however, show a marked atrophy and degeneration. By the end of 1999, 183 inhabitants living in the Jinzu River basin had been diagnosed with itai-itai disease and 6 were still alive [54].

Renal effects by cadmium exposure

The typical Cd-induced proteinuria reported by Butler and Flynn resembles that of acquired Fanconi syndrome [55] and mainly consists of LMW proteins derived from the plasma [56].

β_2-microglobulin excretion is considered as one of the best indicators of early Cd-induced nephropathy since serum concentrations are stable and analysis of β_2-microglobulin using radio-, latex-, or ELISA's is sensitive and accurate [57].

Urinary α_1–microglobulin is stable at pH down to 4.5 [14]. It can be analyzed using commercially available ELISA's. A significant correlation has been reported between α_1–microglobulin and β_2-microglobulin in the urine of Cd-exposed subjects [14, 58].

The urinary excretion of metallothionein parallels urinary Cd and evidence of early renal dysfunction as indicated by increased excretion of either β_2-microglobulin and α1–microglobulin. Based on these results, the urinary excretion of metallothionein reflects not only the level of Cd exposure but also any renal dysfunction caused by long-term Cd exposure [15, 25].

Enzymes of higher molecular weight (HMW), which preclude filtration, enter the urine from renal proximal tubuli. They are also indicators of Cd-induced renal damage, which confirm renal tubular damage even in clinical states where the overproduction of LMW proteins in blood occurs.

Of all the urinary enzymes, N-acetyl-β-D-glucosaminidase is the most widely studied and used indicator of renal tubular damage. An increased urinary N-acetyl-β-D-glucosaminidase activity has been documented in Cd-exposed subjects [59]. However, the N-acetyl-β-D-glucosaminidase activity in urine of itai-itai patients was less than twice that of the controls, while β_2-microglobulin levels were more than 100-fold those of the controls [60, 61]. This suggests that urinary N-acetyl-β-D-glucosaminidase activity decreases when renal tubular epithelia destruction becomes so severe that the cells can no longer excrete the enzyme into the urine. N-acetyl-β-D-glucosaminidase is probably a better marker for the acute effects or initial stage of chronic effects.

Urinary trehalase activity in inhabitants of Cd-polluted areas was significantly higher than in the reference area [62].

Intestinal alkaline phosphatase is specifically located in the S3-segment of the proximal tubuli [63]. Urinary intestinal alkaline phosphatase activity is significantly higher in the Cd-exposed subjects than in the non-exposed subjects [64]. The relationship between β_2-microglobulin and intestinal alkaline phosphatase can be fit to a fourth-order mathematical function. The β_2-microglobulin level corresponding to the inflexion point of intestinal alkaline phosphatase activity is smaller than that for N-acetyl-β-D-glucosaminidase. This result supports the contention that intestinal alkaline phosphatase is more useful for detecting renal tubular damage in the early stage of Cd exposure.

Higher molecular weight proteins such as albumin or mucoproteins are also excreted by the Cd-exposed subjects [65].

Urinary levels of various indicators of Cd exposure assessed in subjects living near the Kakehashi River basin (one of the Cd-polluted areas in Japan) and non-exposed subjects are compared in Table 2.

Some causal relations among various urinary indices were identified using path analysis method. Cadmium-induced renal dysfunction develops in the following order: Cd exposure ➜ increased β_2-microglobulin and/or metallothionein ➜ increased excretion of amino-nitrogen and/or total protein ➜ increased excretion of glucose [66].

Table 2. Proteinuria and urinary cadmium in cadmium-exposed and non-exposed subjects.

		Cadmium-exposed subjects			Non-exposed subjects		
	Sex	N	Mean	S.D.	N	mean	S.D.
β2-microglobulin	M	67	7116	6.38**	26	141	387
(μg/g creatinine)	F	102	10934	5.11**	55	174	3.47
α1–microglobulin (μg/g creatinine)	M/F	27	18880	5.5**	10	352	4.2
N-acetyl-β-D-glucosaminidase	M	39	51.1	2.45**	22	25.3	1.60
(U/g creatinine)	F	36	43.9	2.21*	26	27.2	1.88
Human intestinal alkaline	M	18	4.62	2.07**	18	1.26	1.75
phosphatase (IU/g creatinine)	F	22	4.74	2.99**	22	1.82	2.28
Mucoprotein	M	67	228.6	1.82**	26	75.9	1.75
(mg/g creatinine)	F	102	309.2	1.84**	55	81.5	1.90
Albumin	M	67	93.6	4.79**	26	29.3	2.47
(mg/g creatinine)	F	102	140.0	3.60**	55	31.7	2.80
Total protein	M	67	185.6	3.62**	26	68.3	1.81
(mg/g creatinine)	F	102	251.7	2.73**	55	73.4	2.01
Cadmium	M	67	7.5	1.82**	26	2.5	1.58
(μg/g creatinine)	F	102	10.1	1.74**	55	4.0	1.45

*Mean S.D.: Geometric mean and geometric standard deviation.**: Significant difference from control ($p < 0.05$).**: Significant difference from control ($p < 0.01$).*

A decline of the creatinine clearance was also evident during the early stage of renal dysfunction and a significant correlation between tubular reabsorption of phosphate and glomerular filtration rate was reported in subjects exposed to Cd [67, 68]. These results provide evidence of Cd-induced glomerular dysfunction. However, histopathological examination revealed that while the glomeruli were relatively well maintained in number and size, renal tubuli were markedly damaged, resulting in obstruction of the lumen [69]. The mechanism responsible for the changes in glomerular function following Cd exposure is still uncertain. It has been proposed that Cd exerts a direct effect on the glomeruli [70]. It has also been suggested that Cd-induced tubular damage leads to a certain degree of interstitial nephritis, which in turn results in a decreased glomerular filtration rate [56].

As renal tubular damage progresses, the concentration of serum creatinine increases. One of the most severe cases in the Cd-polluted Kakehashi River basin had a serum creatinine value of 4.4 mg/100 ml. Progression to renal failure was evidenced by high blood nitrogen, severe anemia, acidosis, hyponatremia, hyperphosphatemia and hypocalcemia [71]. It was reported that four out of six itai-itai disease patients died of uremia [72].

Epidemiological studies

In 1967 and 1968, data of an extensive epidemiological investigation involving 13,183 inhabitants (6, 155 men and 7,028 women) aged 30 years and older living in the district where itai-itai disease occurred and adjacent districts were reported [73]. The prevalence of proteinuria and glucosuria in the endemic area was found to be markedly higher than that in the non-endemic district.

Using the Cd concentration in rice as an index of exposure and the element's urinary excretion as an index of health effect, a significant dose-response relationship was demonstrated between the two indices. The allowable values of Cd concentration in rice were estimated to be in the range of 0.05-0.20 mg/kg, representing values lower than the 0.4 mg/kg provisionally adopted by the Japanese government [74].

A large number of epidemiological studies were subsequently performed in 10 Cd-polluted areas using urinary protein and glucose levels as indicators of renal damage [75]. However, statistically significant differences in the prevalence of proteinuria and glucosuria could not be demonstrated in any of the studies

Table 3. Prevalence (%) of abnormal urinary findings in cadmium-exposed and non-exposed subjects.

	Cadmium-exposed subjects					Non-exposed subjects				
Age:	50-59	60-69	70-79	80-	Total	50-59	60-69	70-79	80-	Total
Male										
N	600	494	265	65	1424	62	38	26	7	133
Glucose ≥20 mg/dl with protein ≥5 mg/dl	1.3	2.6	4.2	7.7	2.6	4.8	0	0	0	2.3
Amino acids ≥300 mg/g creatinine	0.0	1.6	3.0	9.2	1.8	1.6	2.6	0	0	1.5
β2-microglobulin ≥1000 μg/g creatinine	4.8	13.0**	28.7	52.3	14.3**	0	0	26.9	14.3	6.0
Metallothionein ≥638 μg/g creatinine	1.5	6.5	7.5	6.2	4.6	4.9	0	0	0	2.3
Female										
N	713	591	340	110	1754	64	49	34	14	161
Glucose ≥20 mg/dl with protein ≥5mg/dl	0.6	1.9	7.1	20.0	3.5	0	4.1	0	7.1	1.9
Amino acids ≥300 mg/g creatinine	5.9	7.8	10.6	23.6*	8.6**	1.6	2.0	2.9	0	1.9
β2-microglobulin ≥1000 μg/g creatinine	4.9*	17.1*	36.5**	61.8*	18.7**	0	6.1	5.9	21.4	5.0
Metallothionein ≥693 μg/g creatinine	4.5	10.2	10.9	16.5	8.4*	0	10.2	0	0	3.1

*: *Significant difference from control ($p<0.05$).*

**: *Significant difference from control ($p<0.01$).*

suggesting that this indicator is rather insensitive to detect early renal effects. It should be noted that the level of Cd exposure in these area's was generally lower than that in the itai-itai disease endemic district.

The LMW protein, β_2-microglobulin, which is considered to be a more sensitive indicator of Cd-induced renal tubular dysfunction was measured in an epidemiological study in 3, 178 inhabitants over 50 years of age and living in the Kakehashi River basin [76]. The prevalence of β_2-microglobulinuria (β_2-microglobulin ≥ 1000 μg/g creatinine) was significantly higher in Cd-exposed subjects than in the non-exposed subjects although no significant difference was noted in the concurrent prevalence of proteinuria and glucosuria, as shown in Table 3.

Ten years after cessation of Cd exposure, urinary Cd concentrations in men ≥ 40 years and in women ≥ 30 years old were significantly higher than those of younger ages whilst levels of ≤ 50 years were significantly lower than those of subjects aged ≥ 60 years [77].

The epidemiological study reported by the Japan Environment Agency in 1989 failed to detect any renal tubular dysfunction among 7,196 persons in the Cd non-polluted areas, while in 202 persons among 13,570 (1.5%) of the Cd-polluted areas, proximal renal tubular dysfunction was seen [78].

A follow-up survey on 2,101 inhabitants (1,566 men and 535 women), who participated in a 1967-health survey and had resided in their actual rural community since birth, was conducted to determine the influence of environmental Cd exposure on the mortality of the general population in the Jinzu River basin. The Cox hazard ratio's for males and females exposed to Cd concentration in rice ≥ 0.30 ppm, were 1.42 and 1.10, respectively. Especially, this value is statistically significant in men. Since the mean Cd concentration in rice was closely related to the development of renal injury, the Cd-induced renal injury is believed to be the factor underlying the increased mortality observed [79].

Relationship between cadmium-induced renal and bone effects

Itai-itai disease is considered the most advanced stage of chronic Cd intoxication. Cadmium-induced bone effects are also suggested to occur in the more advanced stage. Originally, attention was focused on osteomalacia in the diagnosis of this disease. Recent studies, however, showed that osteopenia, a main characteristic of osteoporosis, can be detected in the early stage of chronic Cd intoxication.

Bone density was analyzed in 28 women with itai-

itai disease, 92 men and 114 women with Cd-induced renal dysfunction and 44 men and 66 women living in non-polluted areas using a microdensitometer [80]. To assess the degree of bone density by microdensitometry, an X-ray of the hands along with an aluminum step-wedge was obtained and the bone density was measured at the middle site of the metacarpal bone 11 [81]. The values of indices for both cortical width and bone mineral content were significantly lower in itai-itai disease patients than the Cd-exposed subjects. The Cd-exposed women also showed a decrease in bone density compared with the non-exposed subjects. A significant decrease in bone density was also observed between Cd-exposed men and the non-exposed subjects, although the difference was not as distinct as in women. In other Cd-polluted areas such as the Jinzu River basin or Tsushima Island, a decrease in bone density in Cd-exposed subjects has also been reported using the same method [82, 83].

The relationship between the bone density and renal dysfunction was studied in 85 female inhabitants of the Cd-polluted Jinzu River basin aged 55 to 71 years who had various concentrations of β_2-microglobulin in urine [82]. A significant negative correlation between the urinary β_2-microglobulin level and indicators of microdensitometry was found.

In a study involving 203 Cd-exposed subjects with renal dysfunction and 80 non-exposed subjects an association was observed between Cd-induced renal dysfunction and osteopenia [84]. The relationship between biological parameters such as urinary β_2-microglobulin and serum creatinine, calcium, and phosphorus and microdensitometric indices were analyzed using multivariate analysis. Age, urinary β_2-microglobulin, and serum creatinine were significantly associated with indices of osteopenia in Cd-exposed men. In contrast, age showed the most significant association with the microdensitometric parameters in women of both groups. However, only in Cd-exposed women did urinary β_2-microglobulin levels significantly correlate with indices of microdensitometry.

More recently, using ultrasonic equipment, bone density was measured in 35 Cd-exposed and 68 non-exposed subjects [85]. The bone density was significantly decreased in Cd-exposed subjects as compared to the non-exposed subjects. Values obtained with this method (which is considered to be safer since it lacks radiation exposure) showed a significant correlation with those measured by microdensitometry.

Bone-G1a protein (osteocalcin) is rapidly emerging as a clinically important diagnostic parameter of bone pathology since bone-G1a protein appears to be a highly specific marker of osteoblast function and is expressed during bone formation. Serum levels of bone-G1a protein were evaluated in 76 Cd-exposed subjects with renal tubular dysfunction and 133 non-exposed subjects [86]. Serum bone-G1a protein levels were higher in Cd-exposed subjects than in the non-exposed subjects. In 29 Cd-exposed men, bone-G1a protein, % tubular reabsorption of phosphorus (TRP) and base excess were found to show significant associations with the microdensitometry indicators. In 42 Cd-exposed women, parathyroid hormone, age, blood Cd and bone-G1a protein significantly correlated with the microdensitometric indicators. Only serum bone-G1a protein showed a significant correlation in both sexes of the Cd-exposed subjects, and a sex difference was found in the relationship between bone metabolic markers and osteopenia.

Out of these results, one may deduce that itai-itai disease only represent the tip of the iceberg. Indeed, in the earlier stage of chronic Cd exposure, the presence of Cd-induced bone effects such as osteopenia may be reflected by both microdensitometry and biochemical indices of bone turn-over. The degree of bone damage closely parallels the degree of renal damage.

To investigate the mechanism of bone disease caused by exposure to Cd, 1α, 25-dihydroxyvitamin D, parathyroid hormone, β_2-microglobulin, calcium and inorganic phosphorus were assessed in serum samples of 5 itai-itai disease patients, 36 Cd-exposed residents with renal tubular damage and 17 non-exposed individuals [87]. Measurements of %TRP were performed only on the Cd-exposed subjects. Serum 1α, 25-dihydroxyvitamin D levels were lower in the itai-itai disease patients and Cd-exposed subjects with renal damage than in non-exposed subjects. Parathyroid hormone and serum β_2-microglobulin concentrations were higher in the Cd-exposed subjects [87, 88]. Decreases in serum lα, 25-dihydroxyvitamin D levels were closely related to serum concentrations of parathyroid hormone, β_2-microglobulin and %TRP. This study suggests that Cd-induced bone effects were mainly due to a disturbance in vitamin D and parathyroid hormone metabolism, which most probably resulted from the Cd-induced kidney damage.

In a further study, serum concentrations of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D and 1α,25-dihydroxyvitamin D were measured in 10 Cd-exposed subjects and 5 non-exposed subjects [89]. The Cd-exposed subjects were divided into two groups according to serum 1α,25-dihydroxyvitamin D levels. No significant differences for 25-hydroxyvitamin D were found between the Cd-exposed group with low or normal serum 1α,25-dihydroxyvitamin D and the non-exposed group. The concentrations of 24,25-dihydroxyvitamin D were lowest in the Cd-exposed group with low serum 1α,25-dihydroxyvitamin D, highest in the non-exposed group, and significantly lower in the Cd-exposed group with normal serum 1α,25-dihydroxyvitamin D than in the non-exposed group. Renal function was substantially worse in the Cd-exposed group with low serum 1α,25-dihydroxyvitamin D than in the group with normal serum 1α,25-dihydroxyvitamin D. These findings suggest that Cd initially disturbs hydroxylation from 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D and then disturbs hydroxylation from 25-hydroxyvitamin D to 1α,25-dihydroxyvitamin D. The decrease of serum 24,25-dihydroxyvitamin D and 1α,25-dihydroxyvitamin D in Cd-exposed subjects most probably is not due to a decrease of the serum 25-hydroxyvitamin D level.

Based on the current knowledge obtained from both experimental and human studies, three different mechanisms might be active in development of Cd-induced bone effects.

Firstly, Cd causes renal damage with effects principally on renal tubular cells, i.e. the site of 1α,25-dihydroxyvitamin D synthesis resulting in an intrinsic vitamin D deficiency. This will impair the gastrointestinal absorption of calcium, reduce the calcium incorporation in bone and ultimately result in the development of osteomalacia. It is well known that 1α,25-dihydroxyvitamin D is the biologically active metabolite of vitamin D. As there is a sequential relationship between the synthesis of 1α,25-dihydroxyvitamin D in the kidney and cyclic-adenosine monophosphate, adenylcyclase, parathyroid hormone, a direct interference of Cd with any of these steps cannot be excluded.

Secondly, Cd directly interferes with the gastrointestinal calcium absorption leading to the bone decalcification found in osteoporosis.

Finally, Cd may directly affect bone collagen formation as indicated by a reduction in lysyl-oxidase activity.

To date, however, no clear-cut data are available that inevitably present evidence for a particular mechanism underlying the development of Cd-induced bone effects in human subjects.

Dose-response relationship between cadmium exposure and renal effects

It is assumed that urinary Cd reflects the body burden of Cd at low exposure (environmental pollution), whilst it might be a valuable index of current exposure when exposure is high (industrial situation) [90].

In an epidemiological study involving 1,815 Cd-exposed and 240 non-exposed inhabitants of the Kakehashi River, the significance of the urinary Cd concentration as an indicator of the internal dose for subjects living in a Cd-polluted environment was investigated [91]. The mean urinary Cd concentration increased in a dose-related manner as assessed by classifying subjects according to the average Cd concentration in their rice and to their residence period in the polluted area. A strong direct correlation was found ($r=0.93$ in men and $r=0.88$ in women) between the total Cd intake and urinary Cd excretion. This made the authors conclude that, on a group basis, urinary Cd is a useful indicator of the internal dose resulting from environmental Cd exposure.

In another study investigating the dose-effect and dose-response relationship between the Cd concentration in rice and urinary concentrations/prevalence of abnormal levels of markers of renal dysfunction, significant correlations between Cd concentration in rice and concentrations as well as prevalence rates of abnormal urinary β_2-microglobulin, metallothionein, glucose and amino-nitrogen levels were found. The highest maximum allowable concentration of Cd in rice calculated for these indicators was 0.34 mg/kg when the uncorrected value was used and 0.29 mg/kg when the creatinine corrected value was used. Both values are lower than 0.4 mg/kg, the tentative limit prescribed by the Japanese government [92].

Reversibility of renal effects

The reversibility of β_2-microglobulinuria, glucosuria and aminoaciduria was evaluated in 74 inhabitants over the age of 50 who lived in the Cd-polluted Kakehashi River basin [93]. The initiation of the ex-

aminations coincided with the cessation of Cd exposure after which patients were followed-up during 5 years. The geometric mean concentrations of β_2-microglobulinuria, glucosuria and aminoaciduria indicated significant increases in excretion during the 5-year follow-up period. In cases where the initial level of β_2-microglobulin in urine exceeded 1000 μg/g creatinine, almost all individuals showed a further increase of β_2-microglobulinuria, whereas in the cases in which the urinary excretion of β_2-microglobulin was less than 1000 μg/g creatinine, no progression was observed. A 15-year follow-up study in the Jinzu River basin and a 10-year follow-up study in Nagasaki also confirmed the irreversibility of β_2-microglobulinuria when the initial urinary level of β_2-microglobulin was over 1000 μg/g creatinine.

In 21 Cd-exposed subjects who had renal tubular dysfunction, serum creatinine and arterial blood pH were measured annually during 9-14 years [71]. During this time period, mean serum creatinine increased significantly, from 1.19±1.28 mg/100 ml to 1.68±1.56 mg/100 ml. Even after cessation of Cd exposure, a progressive deterioration of glomerular filtration was seen. The mean arterial blood pH values decreased significantly in all subjects (from 7.40±0.02 to 7.36±0.03), which, in the absence of respiratory disease, was ascribed to metabolic acidosis resulting from the severe renal tubular dysfunction. In Nagasaki, serum creatinine levels were followed in 15 inhabitants living in the Cd-polluted area for 15 years [94]. Although most of the serum creatinine levels were below 2 mg/100 ml, a gradual increase was noted recently.

Prognosis of cadmium-induced renal effects

Despite the fact that a number of studies on the influence of environmental Cd exposure on the mortality of inhabitants of Cd-polluted areas have been conducted, no consensus has been reached so far. Shigematsu et al. investigated the outcome of residents of Cd-polluted areas in Akita, Miyagi, Nagasaki, and Toyama Prefectures and reported lower standardized mortality rates in these polluted areas as compared to non-polluted areas with even greater decreases in the standardized mortality ratios in the most severely polluted areas [95].

However, in contrast herewith, in a 20-year follow-up study in which (i) patients diagnosed as having itai-itai disease, (ii) subjects who were suspected of having the disease, and (iii) controls were included (95 subjects per category matched for age, sex, and residential area) [96], the cumulative survival rate of the patients who had a definite diagnosis of itai-itai disease was significantly lower than that of the control group from a > 3 year follow-up period on. Moreover, the cumulative survival rate of the subjects who were suspected of having itai-itai disease with evidence of severe renal dysfunction due to Cd pollution was significantly lower than that of the control group.

In another 9-year follow-up study of 3,178 persons living in a Cd-polluted area, the standardized mortality rates of the urinary β2-microglobulin positive subjects (≥ 1000 μg/g creatinine) of both sexes were higher than those of the general Japanese population, whereas the cumulative survival curves were lower than those of the urinary β2-microglobulin negative group [97]. A significant association was also found between urinary β_2-microglobulin and mortality, using a Cox's proportional hazards model.

In multiple comparisons using four indices of renal dysfunction (i.e. urinary β_2-microglobulin, protein, glucose and amino acid), urinary protein and β_2-microglobulin in women and urinary protein in men were the most contributive factors to the mortality rates [98].

Data from a 7-year follow-up study in another Cd-polluted area (Nagasaki) showed that, in both men and women, serum β_2-microglobulin and creatinine, as well as urinary total protein and β_2-microglobulin were significantly related to mortality independent of age as assessed by the Cox's proportional hazards model [99]. In advanced cases, the excess mortality of subjects with Cd-induced renal tubular dysfunction is, to some extent, might be ascribed to a reduction in GFR.

In conclusion, these results suggest that the prognosis of subjects with Cd-induced renal dysfunction is unfavorable. The mortality rate tended to become higher as the severity of renal dysfunction progressed. Moreover, isolated increase in urinary β_2-microglobulin is an important factor in assessing the prognosis of persons with mild proximal tubular dysfunction.

Belgium

Cadmium is an important occupational and environmental pollutant in Belgium. This is mainly due to the long-standing commercial production of this metal

as a "by-product" of the mining and refining of zinc ores, which contain minor quantities of Cd (0.1–0.3 %). Because of the presence of zinc/lead ores, non-ferrous metallurgy workshops developed in the Meuse–Vesdre Valley near Liège as early as the 18th century. After Canon Jean-Jacques Dony discovered in Liège a coal-based thermic process to extract zinc from zinc blende (ZnS), an industrial revolution occurred in the zinc metallurgy from the 1850's on. This industry expanded rapidly in the Liège area concomitantly with coal mining and iron and steel works. After the 1st world war, increasing amounts of imported zinc ores were refined using the DONY-process, which in 1972 was replaced by electrolytic zinc refinery. Dust and waste from the primary zinc industry constituted the bulk of the basic material for the production of Cd using thermic refinery processes. After the 2nd world war, however, the heavy industrial activity in the Meuse Valley basin declined which resulted in a progressive shut down of non-ferrous industries of which all activity ceased in the early 1980's.

In 1888, a similar non-ferrous metallurgical activity developed in the northeast of Belgium, a rural region near the Dutch border (Noorderkempen), where during the 20th century several primary zinc smelters and Cd refineries were in operation. Thermic processes, such as the horizontal retort zinc furnace for reduction of zinc calcine with coal at 1100-1300°C (DONY-process), were widely used in zinc refineries. As the boiling point of Cd (765°C) is much lower and the technology to recover Cd fume/dust from zinc furnaces was not very efficient, thermic processes were one of the main causes of the large scale dispersion of Cd in this rural area comprising about 200 km^2. For instance, the Lommel-Overpelt smelters refined 250 metric tonnes of Cd in 1950 whereby 340 kg Cd/day were emitted in the air. Technological improvements raised the Cd production to 300 metric tonnes in 1970 while the atmospheric Cd losses, though still high, had dropped to 200 kg Cd/day. By 1974, the ore-roasting/electrolysis-based zinc refining process had replaced the zinc furnaces and in the 1980's the electrolytic Cd refinery process, used since 1935 in the Balen smelter, was generalized and the re-melting and casting ovens were modernized. Hence, for 600 metric tonnes of Cd produced in 1989 only 0.35 kg Cd/day was lost in the air. In 1992, the two remaining smelters in the Noorderkempen produced 1000 metric tonnes of Cd whereby barely 0.04 kg Cd/day escaped in the atmosphere [100]. Belgium has always been a major Cd producer in Europe and by 1997 its share of the European Union Cd production was 20%. To date, one high-performing big zinc smelter (Umicore-Balen) remains and there are plans to shut down its Cd refinery facility.

Although cases of acute Cd intoxication were first recorded in 1858 in Belgium (domestic servants polishing silverware with Cd carbonate) [101], it should be pointed out that before 1970 systematic epidemiological studies had never been conducted in Belgium to assess health risks of Cd exposure in the industrial setting or the general population. For historical reasons, however, it is interesting to mention the 1953-report of the occupational physician of the Vieille Montagne plant (Balen) dealing with clinical observations made in a group of 30 workers who were exposed to fume and dust of Cd in the Cd production facility of this plant [102]. In 10 workers with less than 2 years of exposure, a slight reticulocytosis was seen and the urinary Cd concentrations ranged 10-20 µg Cd/L as assessed by the dithizon method. In 8 workers with 2-8 years of exposure, the characteristic yellow dental Cd line was noticed together with a reticulocytosis exceeding 2%, a mild hyperchromic anemia, and a urinary Cd varying from 20 to 90 µg Cd/L. The same observations were made in twelve workers with 8 to 30 years of exposure and urinary Cd levels that usually amounted to 60 µg Cd/L. Interestingly in 7 of them a proteinuria was found which displayed the same LMW protein characteristics as the Cd proteinuria already described by Friberg in 1948 [103].

Occupational exposure to cadmium in Belgium

Critical cadmium concentration in kidney and urine

In the early 1970's, Lauwerys et al. conducted the first cross-sectional epidemiological survey ever in Belgian factories. Here workers (31 women, 49 men) were exposed to Cd dust and fume, in an electronic workshop, a nickel-Cd storage battery factory, and a Cd producing plant [104]. At the time of the study, the average airborne Cd ranged from 31 to 134 µg Cd/m^3 (total dust), which was below the American Conference of Governmental Industrial Hygienists' (ACGIH) threshold limit value (TLV) being 200 µg Cd/m^3 in 1972. The kidney was found more sensitive to Cd exposure than the lung. Proteinuria showed abnormal

electrophoretic patterns of LMW and/or HMW proteins in 4/27 male workers with less than 20 years of exposure and in 15/22 with more than 20 years of exposure. Moreover, on the basis of the correlation between total proteinuria and cadmium concentration in urine (CdU), it was suggested that the risk of renal damage would be low when CdU is kept below 15 μg Cd/g creatinine [104]. In addition, blood Cd was found to reflect current exposure to Cd, whereas Cd in urine would reflect body burden of Cd when industrial exposure is low to moderate, but it would reflect current exposure when industrial exposure is high [104].

Subsequent studies in male workers of two Cd refineries confirmed previous findings of other investigators [105-107], in that prolonged Cd exposure is usually characterized by microproteinuria due to impairment of the tubular reabsorption of plasma-derived LMW proteins, e.g. β_2-microglobulin and retinol binding protein [108]. An isolated glomerular effect with increased permeability of HMW proteins, e.g. albumin and transferrin, was less commonly found [108, 109]. To obtain a reliable and direct estimate of the critical body burden of Cd in relation to Cd nephropathy, the Cd concentrations of liver and left kidney were determined in 1978 in about 300 male workers from two Cd refineries using *in vivo* neutron activation analysis; the urinary β_2-microglobulinuria concentration was measured as well. A dose-response relation between liver Cd and prevalence of increased β_2-microglobulinuria was found, indicating an increased prevalence (>5%) of abnormal β_2-microglobulinuria when hepatic Cd was exceeding 30 μg Cd/g wet weight (Table 4). Unlike liver Cd, renal Cd was found to drop in workers with abnormal urinary β_2-microglobulinuria concentrations and a concomitant steep rise in urinary Cd excretion may be seen. This study established that abnormal β_2-microglobulinuria is likely to occur when Cd in the renal cortex or in the urine exceeds the critical concentrations of 216 mg Cd/g wet weight and 10.8 mg Cd/g creatinine respectively [30, 110].

Predictive significance of tubular proteinuria

Further research on occupational Cd nephropathy aimed at a better understanding of the predictive value of Cd-induced microproteinuria and explored underlying features of early glomerular impairment seen in a few Cd-exposed workers. A retrospective examination of serum creatinine, total proteinuria, aminoaciduria, albuminuria, retinol binding proteinuria, and β_2-microglobulinuria was carried out in a group of nineteen workers (40 to 60 years old) with 16 to 42 years of occupational Cd exposure. These renal markers were measured on average 1.2 years before and 4.2 years after removal from exposure and showed in this group of workers that the Cd-induced nephropathy was not reversible when exposure ceased, that the microproteinuria in particular exacerbated, and that serum creatinine tended to increase [111]. A few workers turned into end-stage renal insufficiency (unpublished data). To better assess the health significance of Cd-induced microproteinuria in male Cd workers three studies were carried out.

Table 4. Dose-response relation between cadmium concentration in liver and abnormal β2-microglobulinuria in a group of 148 workers from two zinc/cadmium smelters in Belgium.

Cadmium in liver[a] (μg/g)	Number of workers	Prevalence of abnormal β2-microglobulinuria[b] n	%	Mean β2-microglobulinuria in workers with abnormal values (mg/g creatinine)
10-19	54	0	0	
20-29	27	1	4	7.30
30-39	28	3	11	0.28
40-49	18	3	17	1.42
50-59	8	2	25	7.00
60-69	5	2	40	4.89
70-160	8	8	100	6.45

Adapted from Roels et al. [30].
[a]*Cadmium concentration measured in vivo by neutron activation analysis.*
[b]*β2-microglobulinuria was considered abnormal when exceeding 0.20 mg/g creatinine.*

The first study was a 5-year prospective study conducted in 23 Cd workers removed from exposure because of the discovery of microproteinuria [112]. They were exposed for 25 years on average and at the time of the first examination the mean age of the group was 59 (46-68 years). The mean ± SEM CdU in the subjects amounted to 22.2±2.9 mg Cd/L, the geometric means of urinary retinol binding and β_2-microglobulin were 1.57 and 1.77 mg/L respectively whilst serum creatinine was normal (<1.4 mg/100 ml) in all subjects, except in two (2.0 and 2.2 mg/100 ml). This longitudinal study corroborated not only the irreversibility of Cd-induced microproteinuria (about 30 and 50% increase in urinary retinol binding proteinuria and β_2-microglobulin respectively at the end), but unequivocally showed that creatinine and β_2-microglobulin in serum significantly increased with time indicating a progressive reduction of the GFR. The estimated GFR was found to decrease five times more rapidly than what could be expected due to aging alone. Elevated microproteinuria predicts thus an exacerbation of the age-related decline of GFR and, hence, it should be regarded as an early adverse health effect in occupational exposure to Cd. This finding raised the question whether a CdU threshold value of 10 mg Cd/g creatinine would not only prevent the occurrence of microproteinuria, but also the loss of nephron mass. In other words, does an increased CdU not yet sufficient to modify the urinary excretion of plasma-derived proteins, impair the renal filtration reserve capacity ?

The second study addressed this point by investigating the GFR in Cd workers without (n=31) or with (n=12) increased microproteinuria, i.e. urinary β_2-microglobulin or retinol binding proteinuria > 0.30 mg/g creatinine, and whose geometric mean (range) of CdU was 4.7 (2.1-8.8) and 11.1 (5.8-21.7) µg Cd/g creatinine respectively. The subjects in both groups aged on average 55 years (50 to 64 years), all had a normal serum creatinine (< 1.4 mg/100 ml) [113]. GFR was estimated as the creatinine clearance under baseline conditions and after an acute oral protein load to assess the hyperfiltration capacity of the kidney. The baseline creatinine clearance was normal in both groups (mean 116 ml/min). The creatinine clearance after protein load, however, failed to rise in the group with microproteinuria (mean 114 ml/min) and remained significantly lower than that in the group without microproteinuria (mean 124 ml/min). The mean creatinine clearance after protein load in the latter group was similar to that of an age-matched control group (n=35; CdU < 2 µg Cd/g creatinine). This study showed that the filtration reserve capacity of the kidney is lost when elevated microproteinuria is present, but that there was no functional impairment at a renal Cd burden not yet causing microproteinuria. Implicitly, it validated the proposal of a CdU of 10 µg Cd/g creatinine as biological exposure limit to prevent microproteinuria in male Cd workers.

The third study was prospective (observation periods 1980-84 and 1990-92) and aimed at an evaluation of Cd-induced microproteinuria by assessing its evolution in 32 Cd workers as a function of CdU and severity of the microproteinuria at the time exposure was substantially reduced or had ceased [114]. The finding that 15/24 workers with historical CdU > 10 µg Cd/g creatinine had an elevated β_2-microglobulinuria (> 0.30 mg/g creatinine) corroborated our earlier finding, namely that the risk of abnormal microproteinuria may dramatically increase when CdU regularly exceeds 10 µg Cd/g creatinine. However, when reduction of Cd exposure took place at the time β_2-microglobulinuria did not exceed 0.30 mg/g creatinine, the risk of developing tubular dysfunction at a later stage was low, even in cases with historical CdU values occasionally > 10 but always < 20 µg Cd/g creatinine. There was also indication that the tubulotoxic effect of Cd may be reversible, provided that the historical CdU values never exceeded 20 µg Cd/g creatinine and the β_2-microglobulinuria was mild (< 1.5 mg/g creatinine) at the time the Cd exposure was reduced. When a β_2-microglobulinuria > 1.5 mg/g creatinine was found in combination with historical CdU values above 20 µg Cd/g creatinine, Cd-induced tubular dysfunction exacerbated in spite of reduction or cessation of Cd exposure. The latter condition was present in 10 of the 15 above-mentioned workers who compared well with the Cd-exposed workers of our previous studies [111, 112, 115]. Their past Cd exposure was characterized by a mean CdU_{max} of 35 µg Cd/g creatinine (range: 19-83) and in 1980-84 a frank microproteinuria was diagnosed which in the observation period 1990-92 exacerbated for β_2-microglobulin from 8.96 to 18.1 mg/g creatinine (mean values) and for retinol binding proteinuria from 4.28 to 6.81 mg/g creatinine. The severe microproteinuria was thus irreversible and still progressive 15 years after removal from exposure. That only 5 workers were

identified with a reversible microproteinuria was after all not surprising, because in the 1980's technological improvements, industrial hygiene controls, and systematic health surveillance were implemented in the Cd-producing plants to reduce overexposure and to prevent health risks. It should also be noted, that the exact point in time at which the first sign of a Cd-induced microproteinuria would appear in a subject is yet unpredictable and that the accumulation of Cd in the kidney and the ability to develop a concomitant tubulopathy are time-dependent processes likely to display inter-individual variability [30]. Hence, in ongoing Cd exposure variable time windows would exist within which abnormal microproteinuria would show up and remain "reversible" before it would turn into a progressive exacerbation. Interestingly, the five Cd workers with reversible microproteinuria had a mean historical CdU_{max} = 16.6 µg Cd/g creatinine (range: (mean values) 14-19) which was half that of the workers with irreversible and more severe microproteinuria. Mean age (57 vs. 61) and mean duration of exposure (26 vs 28 years) did not differ significantly between both subgroups. Thus, age and the years of exposure did not seem to play a role, but it would rather be the cumulative exposure of the past (body burden of Cd) in combination with the severity of the tubulopathy at the time exposure was reduced or ceased that were the determining factors. The past Cd exposure of the 5 workers with reversible microproteinuria apparently did not result in a renal cortex Cd level sufficient to induce progressive tubulopathy, 7-12 years after cessation of exposure.

Target-segment nephrotoxicity of cadmium

One of the characteristics of the kidney is its ability to compensate for renal damage and for this reason classical function tests, for example serum creatinine or endogenous creatinine clearance, are insensitive since they only deviate late in the cascade of damage events when there is a large reduction in nephron mass. The last decade has seen a lot of effort in developing diagnostic tests that are sensitive enough to assess changes in renal integrity at an early stage that is before clinical manifestations. In the frame of a large collaborative network-project, involving laboratories of 5 countries of the European Union, the diagnostic potential of more than 25 urine or serum markers of kidney function/integrity was evaluated in a cohort of male Cd workers (n=37; CdU=2-16 mg Cd/g creatinine) and an age-matched control group (n=43; CdU < 2 µg Cd/g creatinine). The aim was to assess whether one or more of the anatomical regions of the nephron segments (glomerulus, proximal or distal tubule, loop of Henle, and interstitium) may be targets of Cd toxicity. The tests comprised functional markers (e.g., creatinine and β_2-microglobulin in serum; LMW and HMW proteins in urine), cytotoxicity markers (e.g., tubular antigens or enzymes in urine), and biochemical markers (e.g., glycosaminoglycans, kallikrein, sialic acids, and eicosanoids in urine) [116]. The results demonstrated a target-segment nephrotoxicity and the first Cd-induced alterations were at the proximal tubule as evidenced by increased urinary excretion of brush-border antigens and lysosomal as well as brush-border enzymes such as N-acetyl-β–D-glucosaminidase and intestinal-type alkaline phosphatase, a specific and sensitive marker of the S3 segment of the proximal tubule [63]. Cadmium exposure was found to have an early effect on the urinary excretion of 6-keto-prostaglandin $F_{1\alpha}$ of which the glomerulus is the principal site of synthesis. Nearly half of the subjects had abnormally increased urinary values of this eicosanoid. As long as the biological significance and implication(s) of this marker in Cd nephropathy are not elucidated, it would be premature to propose a biological exposure threshold based on this highly sensitive marker. An increased urinary excretion of HMW proteins (transferrin and albumin) occurring at a Cd body burden where the protein reabsorption capacity of tubular cells does not yet seem to be impaired was shown as well, which would indicate a slight loss of the glomerular barrier function. This glomerular-type proteinuria may be ascribed to a depletion of the glomerular polyanion charge [117, 118] and suggests that in some subjects subtle alterations of the glomerular filter may precede the onset of tubular-type proteinuria. Traditional markers indicative of tubular damage, i.e. a rise in urinary excretion of plasma-derived LMW proteins like β_2-microglobulin and retinol binding proteinuria, are likely to be affected at a higher Cd body burden. As a cumulative nephrotoxin, Cd can thus produce a broad spectrum of site-specific dose-related effects on the nephron over a wide range of Cd body burden, as estimated on the basis of CdU. Logistic regressions showed significant dose-response rate (CdU-%) curves for renal marker values (cut-off: 95th centile of values ob-

Table 5. Thresholds of urinary cadmium concentration for abnormal values of urinary markers of renal effects found in male workers with chronic occupational cadmium exposure.

Urinary markers	Cut-off values[a]	Threshold effect concentration of urinary cadmium (μg Cd/g creatinine)
Biochemical alterations		
6-keto-prostaglandin $F_{1\alpha}$	280 ng/L	2.3
Sialic acid	501 mg/L	2.4
Cytotoxic effects/enzymuria		
Brush border antigen BBA	6.70 U/L	3.7
N-acetyl-β-*D*-glucosaminidase	2.19 U/L	4.0
Intestinal-type alkaline phosphatase	2.72 U/L	4.1
Renal function changes		
Glomerulus		
Transferrin	0.90 mg/L	3.6
Albumin	19 mg/L	4.1
Proximal tubule		
Retinol binding protein	0.19 mg/L	10.4
β_2-microglobulin	0.32 mg/L	11.5

Adapted from Roels et al. [116].
[a]Upper limit of normal, defined as the 95th centile of the values in control workers with a urinary cadmium concentration < 1 μg Cd/g creatinine.
BBA: Brush border antigen

served in control group) and allowed to determine marker-specific CdU threshold values which were arbitrarily set at a response rate twice the background of elevated values (Table 5). Three main groups of CdU thresholds could be identified: one around 2 mg Cd/g creatinine associated with biochemical alterations (6-keto-$PGF_{1\alpha}$ and sialic acids in urine), a second around 4 mg Cd/g creatinine associated with cytotoxic effects (renal brush-border antigen, intestinal alkaline phosphatase, and N-acetyl-β-D-glucosaminidase in urine) or glomerular barrier dysfunction (albumin and transferrin in urine), and a third around 10 mg Cd/g creatinine associated with tubular reabsorption dysfunction (microproteinuria), or changes in other markers (e.g. glycosaminoglycans in urine). In this study, a few other renal markers were tested as well, including the structural protein fibronectin in urine as a marker of the integrity of the extracellular matrix of the glomerulus, the renal kallikrein activity in urine as a structure/function marker of the distal tubule, and anti-glomerular basement membrane antibodies in serum as a marker of dysfunction of the glomerular basement membrane. None of these last three markers was disturbed in the present group who got a rather moderate Cd exposure and whose current CdU averaged 5.4 mg Cd/g creatinine (range: 2.1-16.4). Previous studies in male workers with much higher Cd exposure, however, showed a significantly decreased urinary kallikrein activity [119] in a group with a mean CdU of 10.4 mg Cd/g creatinine and in another group a significantly increased prevalence of circulating anti-laminin antibodies [120] was found at CdU > 20 mg Cd/g creatinine.

Conclusion

The studies in the occupational setting of Cd-exposed male workers in Belgium carried out in the period 1970-2000 validated that the critical Cd concentration in the renal cortex lies around 200 μg/g which is associated with an increased risk of microproteinuria and a CdU threshold of 10 mg Cd/g creatinine. So far, Cd-induced LMW proteinuria is the only renal effect of Cd with documented health risk significance. Indeed, ongoing overexposure to Cd may lead to irreversible renal damage predictive of a severe exacerbation of the age-related decline in GFR and a decrease in the filtration reserve capacity. However, for several reasons like the long biological half-life of Cd, the safety margin, the occurrence of other renal effects of which the health significance is still unknown, and the fact that no treatment to remove Cd from its storage sites is presently available, several investigators proposed to revise the health-based limit for CdU of 10 mg Cd/g creatinine recommended by the World Health Organization [121] for occupational exposure to Cd. The

adoption by the ACGIH of 5 mg Cd/g creatinine as biological exposure index for Cd seems for the time-being justified [122]. Studies on the predictive value of renal changes other than microproteinuria are needed to assess the validity of this biological exposure index. It is interesting to note that recently suggestive evidence of an excess of neurotoxic complaints has been shown in Cd smelter workers before signs of Cd-induced microproteinuria occurred [123].

Environmental exposure to cadmium in Belgium

First, it should be pointed out that the severe renal and skeletal outcomes of the endemic Cd exposure in Japanese people [78] had never been reported in the Belgian general population. Nevertheless, at the end of the 1970's a few pilot surveys were conducted in the Liège area, the first epidemiological studies ever done in groups of the Belgian general population environmentally exposed to Cd. In the wake of the experience with exposure to Cd in Belgian industries a crucial question emerged, whether the health conservation strategies, as applied to 20-55 year-old men in the industry to avoid nephrotoxic effects of Cd exposure, could be extrapolated to the general population with long-term low-level exposure to historical Cd contamination of their environment ? In other words are more stringent exposure guidelines justified for the general population, for example as to the urinary Cd excretion, and which are the critical adverse effects to be taken into account ?

Pilot studies in the Liège area

Aged women who had lived in the contaminated Liège area (n=60) were compared with a group from a 'control' industrial area (Charleroi, n=70) who were matched for age and socioeconomic characteristics. Only women of Belgian nationality were recruited. They were non-smokers, not bedridden, more than 60 years old (mean about 80), and resided in the respective areas more than 25 years (mean about 70). They were not suffering from diabetes mellitus or clinically confirmed renal disease and had not been treated in the past for renal diseases (e.g. glomerulonephritis, pyelonephritis, ...) [124, 125]. The median values for the estimated urinary excretion of Cd were 2.02 µg/24h (range: 0.36-8.76) in the Liège group against 0.79 µg/24h (range 0.05-3.74) in the Charleroi group, and for the urinary excretion of β_2-microglobulinuria it was 0.18 against 0.12 mg/24h respectively. The prevalence of women with a β_2-microglobulinuria exceeding 1.2 mg/24h was 18% in Liège and 7% in Charleroi. This study suggested that living in the Liège area might significantly increase the body burden of Cd, though the CdU values were much lower than in Japan. The higher β_2-microglobulinuria in Liège suggested further that the critical level of urinary Cd (thus also in the renal cortex) might be much lower in groups of the general population than in middle-aged male workers. A retrospective mortality study in the same two areas showed that in both males and females the standardized mortality ratios from nephritis and nephrosis (ICD 580 and 584) for the years 1969-1976 were twice as high in Liège compared to Charleroi [126]. This finding, which did not seem to be confounded by a difference in analgesic consumption between the two areas [127], supported the hypothesis that Cd may be a contributing environmental factor of renal insufficiency. Postmortem analysis of Cd in liver and kidney cortex of Belgian citizens corroborated that people who had lived in the industrial area of Liège had accumulated significantly more Cd in their tissues than those who resided in Brussels or the southern provinces of Belgium. In the age group 40-49 years, the Cd concentration in the renal cortex of nonsmoker women was about 40 µg Cd/g wet weight in the Liège area against 14 µg Cd/g wet weight in the other areas [128].

Cadmibel study

The outcomes of these three pilot studies provided the incentive to carry out a large scale cross-sectional population-based investigation in Belgium. Hence, from 1985 to 1989 the Cadmibel study was conducted in about 2,300 subjects to assess the extent of exposure and health effects associated with low-level environmental Cd pollution. Citizens of Belgian nationality were randomly recruited from four areas: Liège (urban) and Noorderkempen (rural), two areas documented with records of environmental Cd pollution due to the activities of various zinc/Cd smelters in the past, and Charleroi (urban) and Hechtel-Eksel (rural), two areas without such industries [129]. Some selection criteria were applied so that the statistical analysis pertaining to renal effects was carried out on about 1,700 adults (males and females aged 20-80 years) who had never been occupationally exposed to heavy met-

Table 6. Thresholds of urinary cadmium concentration for abnormal values of urinary markers of renal tubular effects found in the Cadmibel study population with long-term low-level environmental cadmium exposure.

Urinary markers	Cut-off values[a]	Threshold effect urinary cadmium excretion (μg /24h)
Calciuria	9.8 mmol/24h	1.9
N-acetyl-β-D-glucosaminidase	3.6 U/24h	2.7
Retinol binding protein	0.34 mg/24h	2.9
β2-microglobulin	0.28 mg/24h	3.1
Aminoaciduria	357 mg α–N/24h	4.3

Adapted from Buchet et al [31].
[a]Upper limit of normal, defined as the 95th centile for an "internal control group" of Cadmibel participants without diabetes or urinary tract diseases and who did not regularly took analgesics.

als and who resided the last 8 years in the respective areas. After allowing for major co-variates like age and smoking habits, the 24-hour urinary Cd excretion averaged 25% higher in women than in men and was found 20 to 60% higher in both genders living in the polluted areas Liège or Noorderkempen [130]. Stepwise multiple regression analysis showed that only markers of tubulotoxicity (e.g. 24-hour urinary excretion of calcium, N-acetyl-β-D-glucosaminidase, retinol binding proteinuria, β_2-microglobulin, and amino acids) were significantly and positively associated with CdU. In logistic regression, the likelihood of 10% abnormal values for these tubular effect markers (cut-off: 95th centile of values observed in an "internal control group") would occur at urinary Cd excretion values different from those seen in male Cd workers (Table 6) [31]. For example, the threshold effect level of urinary Cd approximates 3 μg Cd/g creatinine for microproteinuria (retinol binding proteinuria and β_2-microglobulin) in the general population, whereas it is 10 μg Cd/g creatinine in adult male Cd workers. Järup et al. [130a] also reported that renal tubular damage due to exposure to Cd develops at lower levels of Cd body burden than previously anticipated. A 5-year follow-up of a subcohort from the Cadmibel study (about 1100 subjects from the rural area) suggested that the subclinical renal effects seen at baseline were not progressive and that tubular effects were not necessarily associated with a subsequent deterioration in glomerular function [131].

A striking and unexpected outcome of the Cadmibel study was the clear-cut interference of the low-level Cd exposure with calcium metabolism. For example, when urinary Cd excretion increased twofold, serum alkaline phosphatase activity and urinary calcium excretion rose by 3-4% and 0.25 mmol/24h respectively [132]. The dose (CdU)-response rate of increased calciuria (> 9.8 mmol/24h) suggested a 10% prevalence of hypercalciuria when CdU exceeded 1.9 μg Cd/24h [31]. Hypercalciuria should be considered an early adverse tubulotoxic effect, because it may exacerbate the development of osteoporosis, especially in the elderly. A prospective study from 1992-1995 (median follow-up of 6.6 years) in the above-mentioned Cadmibel subcohort from the rural area showed for a two-fold increase in urinary Cd a significant ($p<0.02$) decrease of 0.01 g/cm^2 in forearm bone density in post-menopausal women. In addition, the relative risks associated with doubled urinary Cd were 1.73 (95% CI 1.16-2.57; $p=0.007$) for fractures in women and 1.60 (0.94-2.72; $p=0.08$) for height loss in men. Cadmium excretion in the four districts near the smelters was 22.8% higher ($p=0.001$) than in the six other districts, with fracture rates of 16.0 and 10.3 cases per 1000 person-year, respectively, and a population-attributable risk of 35% [133]. Low-level environmental exposure to Cd may thus promote skeletal demineralization, which may lead to increased bone fragility and raised risk of fractures. Therefore it has been proposed that a CdU value of 2 μg Cd/g creatinine should be regarded as the maximum tolerable internal Cd dose for individuals from the general population. Hence, one may assume that in the early 1990's about 10% of the general population in Belgium exceeded this threshold value and that it amounted to 20% in the rural area with historical pollution by Cd from non-ferrous smelters. In this area, a clear-cut impact of a preventive action to decrease the Cd transfer from the environment to the inhabitants was observed, because the Cd concentration

had decreased by about 30% in blood and 15% in urine; the decrease was, however, less pronounced in subjects living closer to the smelters and in pre-menopausal women [134].

Conclusion

The results of the various epidemiological investigations performed since the 1970's by the Unit of Industrial Toxicology and Occupational Medicine, Université Catholique de Louvain, Brussels, indicate that in Belgium the efforts made by industries and the health authorities to reduce occupational and environmental exposure to Cd are fully justified. As practical guidelines to control kidney effects at an early stage in occupational or environmental exposure to Cd, the urinary Cd concentration should not exceed 5 μg Cd/g creatinine in male workers and 2 μg Cd/g creatinine in the general population.

Other countries

A relationship between urine Cd levels and increased excretion of tubular proteins in long-term occupationally exposed workers has been reported from USA [135]. In Germany, Jung et al. [136] reported increased prevalence of elevated urinary excretion of N-acetyl-β-D-glucosaminidase and retinol binding proteinuria both in occupationally and non- occupationally Cd exposed groups. In occupationally Cd exposed workers in Singapore [137] elevated levels of β2-microglobulin, N-acetyl-β-D-glucosaminidase and α_1–microglobulin were reported. The latter two indicators were positive at urinary Cd levels of only a few μg per gram creatinine.

In China, increased occurrence of tubular proteinuria has been reported in Cd contaminated areas. In an area in southeast China, high dietary exposure via the intake of high cadmium containing rice, contaminated by the effluents of a smelter, an increased excretion of tubular proteinuria was reported that for β2-microglobulin and albumin correlated in a dose-related pattern with the urinary Cd excretion [7]. Urinary β_2-microglobulinuria and N-acetyl-β-D-glucosaminidase excretion was evident also at relatively low urinary Cd concentrations [138].

In Chinese Cd-exposed workers it was demonstrated that those with a high ability to induce metallothionein suffered less tubular damage than those with a low ability to induce metallothionein in peripheral blood lymphocytes [139]. This observation gives support for an important role of metallothionein induction in humans and that such induction in peripheral blood lymphocytes can be used as a sensitive biomarker of renal damage.

The observations of dose-response relationships between cumulative absorbed dose of Cd, urinary Cd levels, and occurrence of tubular proteinuria in other countries, support the relationships observed in Sweden, Japan, and Belgium and thus give further support to the conclusions given in the respective sections of the present chapter.

References

1. Friberg L. Health hazards in the manufacture of alkaline accumulators with special reference to chronic cadmium poisoning. Act Med Scand 1950; 138 (suppl 240): 1-124.
2. Nogawa K. Itai-itai disease and follow-up studies. In: Nriagu JO, editor. Cadmium in the environment. Part II: Health effects. John Wiley & Sons, New York 1981; p. 1-37.
3. World Health Organization. Cadmium (environment health criteria; 134). World Health Organization, Geneva 1992; p. 174-188.
4. Kjellström T. Critical organs, critical concentrations, and whole body dose-response relationships. In: Cadmium and Health: a toxicological and epidemiological appraisal. Volume II: effects and response. Friberg L, Elinder C-G, Kjellström T, Nordberg GF (editors). CRC Press, Boca Raton, Florida 1986; p. 231-246.
5. Friberg L, Elinder C-G, Kjellström T, Nordberg GF (editors). Cadmium and health: a toxicological and epidemiological appraisal. CRC Press, Boca Raton, Florida 1986, vol II.
6. Cai SW, Yue L, Hu ZN, Zhong XZ, Ye ZL, Xu HD, Liu YR, Ji RD, Zhang WH, Zhang FY. Cadmium exposure and health effects among residents in an irrigation area with ore dressing wastewater. Sci Total Environ 1990; 90: 67-73.
7. Nordberg GF, Jin T, Kong Q, Ye T, Cai S, Wang Z, Zuang F, Wu X. Biological monitoring of cadmium exposure and renal effects in a population group residing in a polluted area in China. Sci Total Environ 1997; 119: 111-114.

8. Nordberg GF, Kjellström T, Nordberg M. Kinetics and metabolism. In: Friberg L. Elinder C-G, Kjellström T, Nordberg GF (eds). Cadmium and health: a toxicological and epidemiological appraisal, volume I. CRC Press, Boca Raton Florida 1985; p. 103-178.
9. Berk PD, Stremmel W. Hepatocellular uptake of organic anions. Prog Liver Dis 1986; 8: 125-144.
10. Jin T, Nordberg GF, Nordberg M. Uptake of cadmium in isolated kidney cells - influence of binding forms and *in vivo* pretreatment. J Appl Toxicol 1986; 6: 397-400.
11. Jin T, Nordberg GF. Cadmium toxicity in kidney cells. Resistance induced by short term pretreatment *in vitro* and *in vivo*. Acta Pharmacol Toxicol 1986; 58: 137-143.
12. Fowler BA, Nordberg GF. Renal toxicity of cadmium metallothionein: morphometric and X-ray microanalytical studies. Toxicol Appl Pharmacol 1978; 46: 609-623.
13. Nordberg GF. Metabolism of cadmium. In: Nephrotoxic mechanisms of drugs and environmental toxins. Porter G (editor). Plenum Publishing Co, New York, London, Paris 1982; p. 285-303.
14. Friberg L, Piscator M, Nordberg GF (editors). Cadmium in the environment. CRC Press, Boca Raton, Florida 1971.
15. Friberg L Piscator M, Nordberg GF, Kjellström T (editors). Cadmium in the environment, 2nd edition. CRC Press, Boca Raton, Florida 1974.
16. Kjellström T, Nordberg GF. A kinetic model of cadmium metabolism in the human being. Environ Res 1978; 16: 248-269.
17. Kjellström T, Nordberg GF. Kinetic model of cadmium metabolism. In: Cadmium and health: a toxicological and epidemiological appraisal. Friberg L, Elinder C-G, Kjellström T, Nordberg GF (editors). CRC Press, Boca Raton, Florida 1985; p. 179-197.
18. Nordberg GF, Slorach S, Stenström T. Kadmium-forgiftning orsakad av kalldrycksautomat. Likartidningen 1973; 70: 601-604.
19. Clarkson TW, Nordberg GF, Sager PR. Reproductive and developmental toxicity of metals. Scand J Work Environ Health 1985; 11: 145-154.
20. Goyer RA, Cherian MG. Role of metallothionein in human placenta and rats with cadmium exposure. In: Cadmium in the human environment: toxicity and carcinogenicity. Nordberg G, Herber R, Alessio L (editors). International Agency for Research on Cancer (IARC) Scientific Publications vol 118, Lyon 1992; p. 239-247.
21. Berlin M, Blanks R, Catton M, Kazantzis G, Mottet N, Samiullah Y. Birth weight of children and calcium accumulation in placentas of female nickel-cadmium (long-life) battery workers. In: Cadmium in the human environment: toxicity and carcinogenicity. Nordberg G, Herber R, Alessio L (editors). International Agency for Research on Cancer (IARC) Scientific Publications vol 118, Lyon 1992; p. 257-262.
22. IARC. Beryllium, cadmium, mercury and exposure in the glass manufacturing industry. International Agency for Research on Cancer (IARC) Monographs on the evaluation of carcinogenic risks to humans, vol 58; 1993.
23. Boffetta P. Methodological aspects of the epidemiological association between cadmium and cancer in humans. In: Cadmium in the human environment: toxicity and carcinogenicity. Nordberg G, Herber R, Alessio L (editors). International Agency for Research on Cancer (IARC) Scientific Publications vol 118, Lyon 1992; p. 425-434.
24. Doll R. Cadmium in the human environment: closing remarks. In: Cadmium in the human environment: toxicity and carcinogenicity. Nordberg G, Herber R, Alessio L (editors). International Agency for Research on Cancer (IARC) Scientific Publications vol 118, Lyon 1992; p. 459-464.
25. Järup L, Berglund M, Elinder C G, Nordberg GF, Vahter M. Health effects of cadmium exposure - a review of the literature and a risk estimate. Scand J Work Environ Health 1998; 24 (suppl 1): 1-51.
26. Nakagawa H, Nishijo M, Morikawa Y, Tabata M, Miura K, Takahara H, Okumura Y, Yoshita K, Kawano S, Nishi M, Kido T, Nogawa K. Increased urinary β_2-microglobulin and mortality rate by cause of death in a cadmium polluted area. Environ Health and Preventive Med 1996; 1: 144-148.
27. Arisawa K, Nakano A, Saito H, Liu XJ, Yokoo M, Soda M, Koba T, Takahashi T, Kinoshita K. Mortality and cancer incidence among a population previously exposed to environmental cadmium. Int Arch Occup Environ Health 2001; 74(4): 255-262.
28. Nordberg G. Effects of dose-response relationships of toxic metals. AmElsevier Publishing Co, Amsterdam 1976; p. 111.
29. Ellis KJ, Yuen K, Yasumura S, Cohn SH. Dose-response analysis of cadmium in man: body burden versus dysfunction. Environ Res 1984; 33: 216-229.
30. Roels HA, Lauwerys RR, Buchet JP, Bernard A, Chettle DR, Harvey TC, Al-Haddad IK. *In vivo* measurement of liver and kidney cadmium in workers exposed to this metal: its significance with respect to cadmium in blood and urine. Environ Res 1981; 26(1): 217-240.
31. Buchet JP, Lauwerys R, Roels H, Bernard A, Bruo P, Claeys F, Ducoffre G, De Plaen P, Staessen J, Amery A, Lijnen P, Thijs L, Rondia D, Sator F, Saint-Remy A, Nick L. Renal effects of cadmium body burden of the general population. Lancet 1990; 336: 699-702.
32. Bernard A, Roels H, Buchet JP, Cardenas A, Lauwerys R. Cadmium and health: the Belgian experience. In:. In: Cadmium in the human environment: toxicity and carcinogenicity. Nordberg G, Herber R, Alessio L (editors). International Agency for Research on Cancer (IARC) Scientific Publications vol 118, Lyon 1992; p. 15-33.

33. Nordberg GF, Goyer RA, Nordberg M. Comparative toxicity of cadmium mettalothionein and cadmium chloride on mouse kidney. Arch Pathol 1975; 99: 192-197.
34. Squibb KS, Pritchard JB, Fowler BA. Cadmium metallothionein nephrotoxicity: ultrastructural/biochemical alterations and intracellular cadmium-binding. J Pharmacol Exp Ther 1984; 229: 311-321.
35. Nordberg GF, Jin T, Nordberg M. Subcellular targets of cadmium nephrotoxicity: cadmium binding to renal membrane proteins in animals with or without protective metallothionein synthesis. Environ Health Perspect 1994; 102(suppl 3): 191-194.
36. Fowler BA, Akkerman M. The role of Ca^{++} in cadmium-induced renal tubular cell injury.. In: Cadmium in the human environment: toxicity and carcinogenicity. Nordberg G, Herber R, Alessio L (editors). International Agency for Research on Cancer (IARC) Scientific Publications vol 118, Lyon 1992; p. 271-277.
37. Liu X, Squibb KS, Akkerman M, Nordberg GF, Lipsky M, Fowler BA. Cytotoxicity, zinc protection and stress protein induction in rat proximal tubule cells exposed to cadmium chloride in primary cell culture. Renal Failure 1996; 8: 867-882.
38. Leffler P E, Jin T, Nordberg GF. Differential calcium transport disturbances in renal membrane vesicles after cadmium-metallothionein injection in rats. Toxicology 2000; 143: 227-234.
39. Karin M, Richards RJ. The human metallothionein gene family. Structure and expression. Environ Health Perspect 1984; 54: 111-115.
40. Nordberg GF, Garvey JS, Chang CC. Metallothionein in plasma and urine of cadmium workers. Environ Res 1982; 28: 179-182.
41. Roels HA, Lauwerys R, Buchet JP, Bernard A, Garvey JS, Linton HJ. Significance of urinary metallothionein in workers exposed to cadmium. Int Arch Occup Environ Health 1983; 52: 159-166.
42. Tohyama C, Shaikh ZA, Nogawa K, Kobayashi E, Honda R. Elevated urinary excretion of metallothionein to environmental cadmium. Toxicology 1981; 20: 289-297.
43. Garvey JS. Metallothionein: structure/antigenicity and detection/quantification in normal physiological fluids. Environ Health Perspect 1984; 54: 117-127.
44. Piscator M. Proteinuria in chronic cadmium poisoning. I. An electrophoretic and chemical study of urinary and serum proteins from workers with chronic cadmium poisoning. Arch Environ Health 1962; 4: 607.
45. Piscator M. Proteinuria in chronic cadmium poisoning. II. The applicability of quantitative methods of protein determination for the demonstration of cadmium proteinuria. Arch Environ Health 1962; 5: 325-332.
46. Nordberg GF. Cadmium metabolism and toxicity. Environm Physiol Biochem 1972; 2: 7-36.
47. Nordberg GF. Studies on metallothionein and cadmium. Environ Res 1978; 15: 381-404.
48. Kjellström T, Linnman L, and Elinder C-G. Variation of cadmium concentration in Swedish wheat and barley. An indicator of changes in daily cadmium intake during the 20th century. Arch. Environ Health, 1975; 30: 321-328.
49. Kjellström T, Evrin P-E and Rahnster B. Dose-response analysis of cadmium induced tubular proteinuria. A study of urinary β-2-microglobulin excretion among workers in a battery factory. Environ Res 1977; 13: 303-317.
50. Nordberg GF, Health hazards of environmental cadmium pollution. Ambio 1974; 3: 55-66.
51. Järup L, Hellström L, Alfven T, Carlsson MD, Grubb A, Persson B, Petterson C, Spang G, Schultz A and Elinder C-G. Low level cadmium exposure and early kidney damage – the OSCAR study. Occup Environ Med 2000; 57(10): 668-672.
52. Alfven T, Elinder C-G, Carlsson MD, Grubb A, Hellström L, Persson B. Low level cadmium exposure and osteoporosis. J Bone Mineral Res 2000; 15: 1579-1586.
53. Kitagawa M, Yamashita H. Studies on histopathological findings of osteomalacia of itai-itai disease. Kankyo Hoken Report (in Japanese) 1993; 60: 172-174.
54. Kasuya M. Studies on itai-itai disease. Kankyo Hoken Report (in Japanese) 2000; 67: 5-6.
55. Butler EA, Flynn FV. The proteinuria of renal tubular disorders. Lancet 1958; 2: 978-980.
56. Piscator M. The nephropathy of chronic cadmium poisoning. In: Cadmium. Foulkes EC (editor). Springer-Verlag, Berlin 1986; p. 179-194.
57. Piscator M. Markers of tubular dysfunction. Toxicol Lett 1989; 46: 197-204.
58. Kido T, Honda R, Yamada Y, Tsuritani I, Ishizaki M, Nogawa K. α_1–microglobulin determination in urine for the early detection of renal tubular dysfunctions caused by exposure to cadmium. Toxicol Lett 1985; 24: 195-201.
59. Sugihira N, Saito H. Urinary excretion of N-acetyl-β-D-glucosaminidase and β_2-microglobulin in people living in a cadmium-polluted area. Jpn J Hyg 1986; 41: 665-671.
60. Nogawa K, Yamada Y, Honda R, Tsuritani I, Ishizaki M, Sakamoto M. Urinary N-acetyl-β-D-glucosaminidase and β_2-microglobulin in 'itai-itai' disease. Toxicol Lett 1983; 16: 317-322.
61. Nogawa K, Yamada Y, Kido T, Honda R. Ishizaki M, Tsuritani I, Kobayashi E. Significance of elevated urinary N-acetyl-β-D-glucosaminidase activity in chronic cadmium poisoning. Sci Total Environ 1986; 53: 173-178.
62. Nakano M, Aoshima K, Katoh T, Teranishi H, Kasuya M. Urinary trehalase activity and renal brush-border damage in inhabitants of a cadmium-polluted area (Jinzu River basin). Toxicol Lett 1986; 34: 159-166.
63. Verpooten GF, Nouwen EJ, Hoylaerts MF, Hendrix PG, De Broe ME. Segment-specific localization of intestinal-type alkaline phosphatase in human kidney. Kidney Int 1989; 36: 617-625.

64. Kido T, Hayano M, Kobayashi E, Nogawa K, Nishijo M, Tabata M, Nakagawa H, Tsuritani I. Significance of elevated urinary human intestinal alkaline phosphatase in Japanese people exposed to environmental cadmium. Toxicol Lett 1995; 80: 49-54.
65. Kido T, Honda R, Tsuritani I, Ishizaki M, Yamada Y, Nakagawa H, Nogawa K. Significant increase of urinary mucoprotein in environmental-cadmium-exposed Japanese subjects. Toxicol Lett 1991; 55: 303-309.
66. Abe T, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Shaikh ZA, Nogawa K. Application of path analysis to urinary findings of cadmium-induced renal dysfunction. J Environ Sci Health Part A Tox Hazard Subst Environ Eng 2001; 36(1): 75-87.
67. Nogawa K, Kobayashi E, Honda R, Ishizaki A, Kawano S, Matsuda H. Renal dysfunction of inhabitants in a cadmium-polluted area. Environ Res 1980; 23: 1323.
68. Nogawa K. Biologic indicators of cadmium nephrotoxicity in persons with low-level cadmium exposure, Environ Health Perspect 1984; 54: 163-169.
69. Takebayashi S. First autopsy case, suspicious of cadmium intoxication, from the cadmium-polluted area in Tsushima, Nagasaki Prefecture. In: Cadmium-induced osteopathy. Shigematsu I, Nomiyama K (editors). Japan Public Health Association, Tokyo 1980; p. 124-138.
70. Bernard A, Lauwerys R. Effects of cadmium exposure in humans. In: Cadmium. Foulkes EC (editor). Springer-Verlag, Berlin 1986; p. 150-159.
71. Kido T, Nogawa K, Ishizaki M, Honda R, Tsuritani I, Yamada Y, Nakagawa H, Nishi M. Long-term observation of serum creatinine and arterial blood pH in persons with cadmium-induced renal dysfunction. Arch Environ Health 1990; 45: 35-41.
72. Kajikawa K, Kitagawa M, Nakanishi I, Ueshima H, Katsuda S, Kuroda K. A pathological study of "itai-itai disease". J Juzen Med Soc (in Japanese) 1974; 83: 309-347.
73. Fukushima M. Environmental pollution by cadmium, and its health effects: an epidemiological approach to the "itai-itai" disease. In: New methods in environmental chemistry and toxicology. Coulston F, Koprte F, Goto M (editors). International Academic Printing Co, Tokyo 1973; p. 231-252.
74. Osawa K, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Nogawa K. A retrospective study on the relation between renal dysfunction and cadmium concentration in rice in individual hamlets in the Jinzu River basin. Toyama Prefecture, Japan. Environ Res 2001; 86(1): 51-59.
75. Tsuchiya K. Environmental pollution and health effects. In: Cadmium studies in Japan: a review. Tsuchiya K (editor). Kodansya Ltd, Tokyo 1978; p. 144-253.
76. Kido T, Honda R, Tsuritani I, Yamaya H, Ishizaki M, Yamada Y, Nogawa K. An epidemiological study on renal dysfunction of inhabitants in cadmium-exposed areas in the Kakehashi River basin in Ishikawa Prefecture. Jpn J Hyg (in Japanese) 1987; 42: 964-972.
77. Kido T, Nishijo M, Nakagawa H, Kobayashi E, Nogawa K, Tsuritani I, Honda R, Kito H. Urinary findings of inhabitants in a cadmium-polluted area ten years after cessation of cadmium exposure. Hokuriku J Public Health 2001; 27(2): 81-84.
78. Japan public health association cadmium research committee. Summary report on studies of health effects of cadmium. Japan Public Health Association, Tokyo 1989; p. 53-67.
79. Ishihara T, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Nishijyo M, Nakagawa H, Nogawa K. Association between cadmium concentration in rice and mortality in the Jinzu River basin, Japan. Toxicology 2001; 28; 163(1): 23-28.
80. Kido T, Nogawa K, Yamada Y, Honda R, Tsuritani I, Ishizaki M, Yamaya H. Osteopenia in inhabitants with renal dysfunction induced by exposure to environmental cadmium. Int Arch Occup Environ Health 1989; 61: 271-276.
81. Inoue T, Kusida K, Miyamoto S, Sumi Y. Quantitative assessment of bone density on X-ray picture. J Jpn Orthop Ass 1983; 57: 1923-1936.
82. Aoshima K, Iwata K, Kasuya M. Environmental exposure to cadmium and effects on human health. Part 2. Bone and mineral metabolism in inhabitants of the cadmium-polluted Jinzu River basin in Toyama Prefecture. Jpn J Hyg (in Japanese) 1988; 43: 864-871.
83. Harada T, Matsuo S, Hara K, Yoshimura S. The association between proximal renal tubular dysfunction and osteopenia using microdensitometry in subjects required observation in cadmium-polluted areas. Kankyo-Hoken Report (in Japanese) 1986; 52: 109-113.
84. Kido T, Nogawa K, Honda R, Tsuritani I, Ishizaki M, Yamada Y, Nakagawa H. The association between renal dysfunction and osteopenia in environmental cadmium-exposed subjects. Environ Res 1990; 51: 71-82.
85. Tsuritani I, Honda R, Ishizaki M, Yamada Y, Nishijo M, Nakagawa H. Bone mineral measurement in inhabitants in a cadmium polluted area by ultrasound bone densitometer. Jpn J Hyg (in Japanese) 1994; 49: 125.
86. Kido T, Honda R, Tsuritani I, Ishizaki M, Yamada Y, Nakagawa H, Nogawa K, Dohi Y. Serum levels of bone G1α-protein in inhabitants exposed to environmental cadmium. Arch Environ Health 1991; 46: 43-49.
87. Nogawa K, Tsuritani I, Kido T, Honda R, Yamada Y, Ishizaki M. Mechanism for bone disease found in inhabitants environmentally exposed to cadmium; decreased serum 1α,25-dihydroxyvitamin D level. Int Arch Occup Environ Health 1987; 59: 21-30.
88. Nogawa K, Kobayashi E, Yamada Y, Honda R, Kido T, Tsuritani I. Ishizaki M. Parathyroid hormone concentration in the serum of people with cadmium-induced renal damage. Int Arch Occup Environ Health 1984; 54: 187-193.

89. Nogawa K, Tsuritani I, Kido T, Honda R, Ishizaki M, Yamada Y. Serum vitamin D metabolites in cadmium-exposed persons with renal damage. Int Arch Occup Environ Health 1990; 62: 189-193.
90. Lauwerys RR, Buchet JP, Roels H. The relationship between cadmium exposure or body burden and the concentration of cadmium in blood and urine in man. Int Arch Occup Environ Health 1976; 36: 275-285.
91. Kido T, Nogawa K, Ohmichi M, Honda R, Tsuritani I, Ishizaki M, Yamada Y. Significance of urinary cadmium concentration in a Japanese population environmentally exposed to cadmium. Arch Environ Health 1992; 47: 196-202.
92. Nakashima K, Kobayashi E, Nogawa K, Kido T, Honda R. Concentration of cadmium in rice and urinary indicators of renal dysfunction. Occup Environ Med 1997; 54(10): 750-755.
93. Kido T, Honda R, Tsuritani I, Yamaya H, Ishizaki M, Yamada Y, Nogawa K. Progress of renal dysfunction in inhabitants environmentally exposed to cadmium. Arch Environ Health 1988; 43: 213-217.
94. Harada T, Hara K, Ogata T, Nishimura K, Shigeno N, Shichijo T, Kuwazuka M, Sato S. Changes of main biochemical findings of subjects required observation in cadmium-polluted areas in Tsushima, Nagasaki Prefecture. Kankyo Hoken Report (in Japanese) 1991; 58: 205-211.
95. Shigematsu I, Takeuchi J, Minowa M. Nagai M, Usui T, Fukushima M. A retrospective mortality study on cadmium-exposed population in Japan. Kankyo Hoken Report (in Japanese) 1980; 46 (part 2): 1-71.
96. Nakagawa H, Tabata M, Morikawa Y, Senma M, Kitagawa Y, Kawano S, Kido T. High mortality and shortened life-span in patients with itai-itai disease and subjects with suspected disease. Arch Environ Health 1990; 45: 283-287.
97. Nakagawa H. Nishijo M, Morikawa Y, Tabata M, Senma M, Kitagawa Y, Kawano S. Ishizaki M, Sugita N, Nishi M, Kido T, Nogawa K. Urinary β_2-microglobulin concentration and mortality in a cadmium-polluted area. Arch Environ Health 1993; 48: 428-434.
98. Nishijo M, Nakagawa H, Morikawa Y, Tabata M, Senma M. Kitagawa Y, Kawano S. Ishizaki M, Sugita N, Nishi M, Kido T, Nogawa K. Prognostic factors of renal dysfunction induced by environmental cadmium pollution. Environ Res 1994; 64: 112-121.
99. Iwata K, Saito H, Nakano A. Association between cadmium-induced renal dysfunction and mortality: further evidence. Tohoku J Exp Med 1991; 164: 319-330.
100. Staessen JA, Roels H, Vangronsveld J, Clijsters, H, De Schrijver K, De Temmerman L, Dondeyne F, Van Hulle S, Wildemeersch D, Wilms L. Preventiemaatregelen voor bodemverontreiniging met cadmium. Tijdschr Geneesk 1995; 51: 1387-1395.
101. Sovet. Empoisonnement par une poudre servant à écurer l'argenterie; symptômes graves; gastro-entérite suraigue; accidents dyssentériques; antidote; antiphlogistiques; ipécacuana; analyse chimique. Press Méd Belge 1858; 10: 69-70.
102. Leduc V. Pathologie du cadmium. Report to the Vieille Montagne plant management, September 10, 1953.
103. Friberg L. Proteinuria and kidney injury among workmen exposed to cadmium and nickel dust. J Ind Hyg Toxicol 1948; 30: 32-36.
104. Lauwerys RR, Buchet JP, Roels HA, Brouwers J, Stanescu D. Epidemiological survey of workers exposed to cadmium.-Effects on lung, kidney, and several biological indices. Arch Environ Health 1974; 28: 145-148.
105. Butler EA, Flynn FV, Harris H, Robson EB. A study of urine proteins by two dimensional electrophoresis with special reference to the proteinuria of renal tubular disorders. Clin Chim Acta 1962; 7: 172-178.
106. Kazantzis G, Flynn FV, Spowage JS, Trott DG. Renal tubular malfunction and emphysema in cadmium pigment workers. Quart J Med 1963; 32: 165-192.
107. Piscator M. Proteinuria in chronic cadmium poisoning. III. Electrophoretic and immuno-electrophoretic studies on urinary proteins from cadmium workers, with special reference to the excretion of low molecular weight proteins. Arch Environ Health 1966; 12: 335-344.
108. Lauwerys RR, Roels HA, Buchet JP, Bernard A, Stanescu D. Investigations on lung and kidney function in workers exposed to cadmium. Environ Health Perspect 1979; 28: 137-145.
109. Bernard A, Buchet JP, Roels H, Masson P, Lauwerys R. Renal excretion of proteins and enzymes in workers exposed to cadmium. Eur J Clin Invest 1979; 9: 11-22.
110. Roels H, Lauwerys R, Dardenne AN. The critical level of cadmium in human renal cortex: a re-evaluation. Toxicol Lett 1983; 15: 357-360.
111. Roels H, Djubgang J, Buchet JP, Bernard A, Lauwerys R. Evolution of cadmium-induced renal dysfunction in workers removed from exposure. Scand J Work Environ Health 1982; 8: 191-200.
112. Roels HA, Lauwerys RR, Buchet JP, Bernard AM, Vos A, Oversteyns M. Health significance of cadmium induced renal dysfunction: a five year follow up. Br J Ind Med 1989; 46: 755-764.
113. Roels HA, Lauwerys RR, Bernard AM, Buchet JP, Vos A, Oversteyns M. Assessment of the filtration reserve capacity of the kidney in workers exposed to cadmium. Br J Ind Med 1991; 48: 365-374.
114. Roels HA, Van Assche FJ, Oversteyns M, De Groof M, Lauwerys RR, Lison D. Reversibility of microproteinuria in cadmium workers with incipient tubular dysfunction after reduction of exposure. Am J Ind Med 1997; 31: 645-652.

115. Buchet JP, Roels H, Bernard A, Lauwerys R. Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury vapor. J Occup Med 1980; 22: 741-750.
116. Roels H, Bernard AM, Cardenas A, Buchet JP, Lauwerys RR, Hotter G, Ramis I, Mutti A, Franchini I, Bundschuh I, Stolte H, De Broe ME, Nuyts GD, Taylor SA, Price RG. Markers of early renal changes induced by industrial pollutants. III. Application to workers exposed to cadmium. Br J Ind Med 1993; 50: 37-48.
117. Bernard AM, Ouled Amor A, Lauwerys RR. Decrease of erythrocyte and glomerular membrane negative charges in chronic cadmium poisoning. Br J Ind Med 1988; 45: 112-115.
118. Cardenas A, Bernard AM, Lauwerys R. Disturbance of sialic acid metabolism by chronic cadmium exposure and its relation to proteinuria. Toxicol Appl Pharmacol 1991; 108: 547-558.
119. Roels HA, Lauwerys RR, Buchet JP, Bernard AM, Lijnen P, Van Houte G. Urinary kallikrein activity in workers exposed to cadmium, lead, or mercury vapour. Br J Ind Med 1990; 47: 331-337.
120. Bernard AM, Roels HA, Foidart JM, Lauwerys RR. Search for anti-laminin antibodies in the serum of workers exposed to cadmium, mercury vapour or lead. Int Arch Occup Environ Health 1987; 59: 303-309.
121. World Health Organization (WHO). Recommended health-based limits in occupational exposure to heavy metals. Report of a study group. WHO Technical Report Series No. 647. Geneva, 1980.
122. American Conference of Governmental Industrial Hygienists (ACGIH). Supplement: Cadmium and inorganic compounds. Biological Exposure Indices. Cincinnati, Ohio 1996.
123. Viaene MK, Masschelein R, Leenders J, De Groof M, Swerts LJVC, Roels HA. Neurobehavioral effects of occupational exposure to cadmium: a cross sectional epidemiological study. Occup Environ Med 2000; 57: 19-27.
124. Lauwerys R, Roels H, Bernard A, Buchet JP. Renal response to cadmium in a population living in a nonferrous smelter area in Belgium. Int Arch Occup Environ Health 1980; 45: 271-274.
125. Roels HA, Lauwerys RR, Buchet JP, Bernard A. Environmental exposure to cadmium and renal function of aged women in three areas of Belgium. Environ Res 1981; 24: 117-130.
126. Lauwerys R, de Wals Ph. Environmental pollution by cadmium and mortality from renal diseases. Lancet 1981; 1: 383.
127. Vanherweghem JL, Even-Adin D. Epidemiology of analgesic nephropathy in Belgium. Clin Nephrol 1982; 17: 129-133.
128. Lauwerys R, Hardy R, Job M, Buchet JP, Roels H, Bruaux P, Rondia D. Environmental pollution by cadmium and cadmium body burden: an autopsy study. Toxicol Lett 1984; 23: 287-289.
129. Lauwerys R, Amery A, Bernard A, Bruaux P, Buchet JP, Claeys F, De Plaen P, Ducoffre G, Fagard R, Lijnen P, Nick L, Roels H, Rondia D, Saint-Remy A, Sartor F, Staessen J. Health effects of environmental exposure to cadmium: objectives, design and organization of the Cadmibel study: a cross-sectional morbidity study carried out in Belgium from 1985 to 1989. Environ Health Perspect 1990; 87: 283-289.
130. Sartor FA, Rondia DJ, Claeys FD, Staessen JA, Lauwerys RR, Bernard AM, Buchet JP, Roels HA, Bruaux PJ, Ducoffre GM, Lijnen PJ, Thijs LB, Amery AK. Impact of environmental cadmium pollution on cadmium exposure and body burden. Arch Environ Health 1992; 47: 347-353.
130a. Järup L, Hellström L, Alfrén T, Carlsson MD, Grubb A, Persson B, Petterson C, Spång G, Schütz A, Elinder C-G. Low level exposure to cadmium and early kidney damage: the OSCAR Study. Occup Environ Med 2000; 57: 668-672.
131. Hotz P, Buchet JP, Bernard A, Lison D, Lauwerys R. Renal effects of low-level environmental cadmium exposure: 5-year follow-up of a subcohort from the Cadmibel study. Lancet 1999; 354: 1508-1513.
132. Staessen J, Amery A, Bernard A, Bruaux P, Buchet JP, Claeys F, De Plaen P, Ducoffre G, Fagard R, Lauwerys RR, Lijnen P, Nick L, Saint-Remy A, Roels H, Rondia D, Sartor F, Thijs L. Effects of exposure to cadmium on calcium metabolism : a population study. Br J Ind Med 1991; 45: 710-714.
133. Staessen JA, Roels HA, Emelianov D, Kuznetsova T, Thijs L, Vangronsveld J, Fagard R. Environmental exposure to cadmium, forearm bone density, and risk of fractures: a prospective population study. Lancet 1999; 353: 1140-1144.
134. Staessen JA, Kuznetsova T, Roels HA, Emelianov D, Fagard R. Exposure to cadmium and conventional and ambulatory blood pressures in a prospective population study. Am J Hypertens 2000; 13: 146-156.
135. Mueller PW, Smith SJ, Steinberg KK, Thun MJ. Chronic renal tubular effects in relation to urine cadmium levels. Nephron 1989; 52(1): 45-54.
136. Jung K, Pergande M, Graubaum H C, Fels LM, Endl U and Stolte H. Urinary proteins and enzymes as early indicator of renal dysfunction in chronic exposure to cadmium. Clin Chem 1993; 39(5): 757-765.
137. Chia KS, Tan AL, Chia SE, Ong CN Jeyaratnam J. Renal tubular function of cadmium exposed workers. Ann Acad Med Singapore 1992; 21: 756-759.
138. Jin T, Nordberg GF, Wu X, Ye T, Kong Q, Wang Z, Zhuang F and Cai S. Urinary N-Acetyl-β-D-glucosaminidase isoenzymes as biomarker of renal dysfunction caused by cadmium in a general population. Environ Res Sect A 1999; 81: 167-173.
139. Lu J, Jin T, Nordberg GF, Nordberg M. Metallothionein gene expression in peripheral lymphocytes from cadmium exposed workers. Cell Stress Chaperones 2001; 6(2): 97-104.

26

Mercury-induced renal effects

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Physical and chemical properties of mercury

Mercury (Hg, CAS Number 7439-97-6) is a naturally-occurring metal that has an atomic number of 80 and an atomic weight of 200.6. Many different organic and inorganic mercury compounds are found in nature because of mercury's ability to form covalent and ionic bonds with other chemicals. Mercury exists in three forms in three oxidation states (0, +1, +2): elemental mercury (Hg^0), organic mercury (e.g., methyl mercury), and inorganic mercury (e.g., Hg^{1+}, Hg^{2+}). Elemental mercury is a silvery, white liquid at room temperature, and because of this, Aristotle named mercury "quicksilver."

History of human use of mercury

Mercury has been used by humans since antiquity. More than 10, 000 years ago, prehistoric humans used the bright red stone of mercury ore (cinnabar, mercury sulphide, HgS) to color cave drawings. Theofrastos, the disciple of Aristotle, described the production of metallic mercury from cinnabar. Egyptians and Romans identified several of the occupational hazards in mercury mines. During the Roman period, slaves, convicts, and political enemies were sent to the mercury mines in Almadén in Spain where they succumbed to the toxic effects of mercury. In 1700, the Italian physician Ramazzini, regarded as the founder of occupational medicine, in his classical book 'De Morbis Artificum'

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 531-544

(Diseases of Workers) described several signs of mercury poisoning, which he observed not only among miners but also gilders, mirror makers, and syphilis patients given treatment with mercury ointments. It was not uncommon for the doctor who administered mercury ointment to also suffer from mercury poisoning.

The use of mercury in the treatment of diseases such as syphilis, psoriasis, and congestive heart failure began more than two centuries ago. Although mercury's medicinal use has tapered off in recent years, mercury compounds such as thimerosal and phenylmercuric nitrate still have a limited use in human and veterinary medicines to prevent bacterial growth in injection solutions (e.g. vaccines), antiseptics, and skin ointments. The United States Food and Drug Administration [1] estimates that approximately 200 human and veterinary drug products marketed in the U.S. contain mercury as an active or inactive ingredient.

The unique physical properties of metallic mercury led to its widespread industrial use during the 19th century. Because of this, epidemics of occupational poisonings were documented in the mirror and felt hat industries. Symptoms and signs of severe poisoning included pneumonitis, tremor, inflammation of the gums with excessive salivation, and psychiatric symptoms such as excitability, insomnia, irritation, and shyness.

Worldwide production of mercury has declined in recent years. Total world production of mercury was 1, 400 tons in 2001, compared to 2, 750 tons in 1997 [2, 3]. Mercury recovered from primary sources accounts for about 60% of world consumption, with the remainder being supplied from recycled sources [4]. Spain, Kyrgyzstan, and Algeria accounted for approximately 70% of the world's mined mercury in 2001 [2].

Mercury compounds continue to have numerous commercial uses. Besides its use as a preservative, mercury is used in the manufacture of many technical and medical instruments including blood pressure measurement devices, manometers, thermometers, and barometers. Mercury is also used in production of certain types of fluorescent lamps and in the chloralkali industry, where chlorine and caustic soda are produced using brine electrolysis in mercury cells. Metallic mercury is used in the production of precious metals such as gold and silver. As part of the production process, metallic mercury can be used to concentrate gold from crushed ore or sediments. This method, also known as the amalgamation method, results in occupational and environmental exposures to vaporized mercury, posing an immediate health threat. Such a health hazard was a common occurrence in the in the 1850s during the California gold rush. Although phased out by most gold producing countries, the amalgamation method is still used in several countries. It has been estimated that some 500, 000 gold miners in Brazil are exposed to liquid mercury during the concentration of gold from sediments [5, 6].

Mercury has been used for more than 150 years in dental silver amalgams. Dental silver amalgams in tooth fillings are composed of a mixture of 50% metallic mercury and metal powder, usually silver, tin, copper and zinc.

Exposure

Humans can be exposed to mercury compounds via the oral, inhalation, and dermal routes. The primary sources of mercury exposure among the general population are dental amalgams and the diet, with amalgam fillings being the most important source of inorganic mercury, and fish and other seafood (marine mammals, crustaceans) the principle sources of methylated or organic mercury.

The release of mercury from amalgam fillings is proportional to both the number of fillings and the total amalgam surface area. It has been challenging to accurately estimate the release from amalgam fillings, but according to the National Research Council, estimates of average daily mercury intake from dental amalgams range from 3.8-21 μg/day [7]. Measurements of urinary excretion of mercury have revealed that individuals with a habit of tooth grinding or 'Bruxism' release considerably more mercury from their dental fillings compared to persons who do not grind their teeth [8]. Most of the exposure from amalgam fillings probably comes from release of mercury vapor (Hg^0) but uptake from amalgam particles in the gastrointestinal tract, at least after dental treatment, may contribute [8a].

The concentration of mercury in most foodstuffs is generally below the reported limit of detection, which is usually 20 μg/kg fresh weight [9]. Fish and other seafood products are the primary dietary sources of mercury, and scientists have determined that mercury

concentrations in fish and shellfish are approximately 10 to 100 times greater than in other foods, including cereals, potatoes, vegetables, fruits, meats, poultry, eggs, and milk [10]. It only takes a small amount of mercury to pollute aquatic organisms, and render them unfit for consumption. Mercury in fish and seafood is almost completely in the form of methyl mercury, which is a particular health threat to infants and the developing fetus [11]. Certain marine fish, (e.g. shark, swordfish, king mackerel, and tuna), as well as certain freshwater fish (e.g. pike, walleye and bass) may contain high concentrations of mercury. Levels of mercury among these marine fish species range from 0.05-4.54 mg methyl mercury/kg fish (wet weight), while mercury levels in these freshwater fish species range from 0.5 to 2 mg methyl mercury/kg fish (wet weight) [10, 12]. Daily consumption of 100 g of fish possessing an average mercury concentration of 1 mg methyl mercury/kg results in an intake of 100 µg methyl mercury, which exceeds the tolerable limits recommended by the World Health Organization, the United States Environmental Protection Agency, and the United States Food and Drug Administration [13, 14].

Besides dietary and dental amalgam exposure to mercury compounds, accidental exposure to mercury vapors may occur among the general population (e.g., from breakage of a mercury-containing thermometer), or from use of metallic mercury or mercury containing ointments, creams, and drugs.

Occupational exposure to inorganic mercury is quite common, and occurs in the dental and chloralkali industries, as well as in thermometer factories, and in mercury mines. Approximately 70, 000 workers in the United States are regularly exposed to mercury [15]. Measurements of mercury in blood and urine are useful in quantifying the magnitude of exposure (see section about biological monitoring below). In most instances there is a linear relationship between ambient air and urine concentration of mercury, where the urine concentration (µg/L) corresponds to air concentration (µg/m^3) multiplied by 1-2 [16]. In dentistry, ambient mercury vapor concentrations during the1960s-1970s were often around 25 µg/m^3. Due to improved ventilation and handling of amalgam, most dental offices have reduced levels below 5 µg/m^3.

Ambient mercury vapor concentrations of 100 µg/m^3 or higher have been measured during chloralkali production and mercury mining [17]. Adverse health effects were common sequelae from such exposures. During recent years, most countries have reduced mercury's occupational threshold limit value to 50 µg/m^3 or less.

Toxicokinetics

The toxicokinetics (i.e., absorption, distribution, metabolism, and excretion) of mercury is highly dependent on the form of mercury to which an individual has been exposed [14]. This difference in toxicokinetics is illustrated in Figures 1 and 2, where intravenous administration of 10 ml (135 grams) of elemental mercury distributed in the lung, while oral ingestion of 236 ml (3212 grams) of elemental mercury distributed in the large intestine. The subject in Figure 1 attempted suicide by injecting elemental mercury intravenously because elemental mercury is poorly absorbed via other routes, such as the gastrointestinal route, as illustrated in Figure 2.

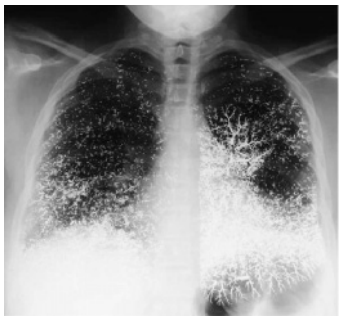

Figure 1. *Elemental mercury embolism to the lung (Reproduced from Gutiérrez and Leon [18], with permission).*

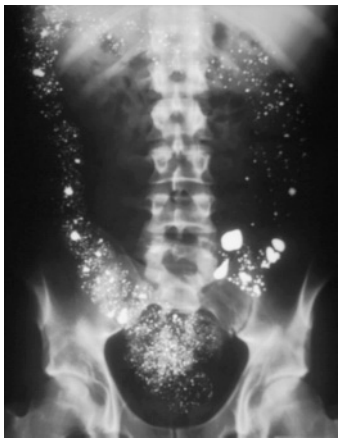

Figure 2. *Elemental mercury distribution in the intestine (reproduced from Diner and Brenner [19], with permission).*

Mercury vapor (Hg°)

While pulmonary absorption of mercury vapor is high (75-85%) [20], this particular chemical form of mercury is poorly absorbed from either the gastrointestinal tract or across the skin. The gastrointestinal absorption of mercury from amalgam powder in humans has been estimated as 0.04% [8a]. The kidney is the major site of mercury deposition following mercury vapor exposure [14]. The half-life of mercury in kidneys following inhalation has been calculated [21] to be approximately 64 days in humans. The half-life of mercury in blood of workers following an acute high dose exposure was reported [22] to be biphasic with a fast phase (3.1 days) and a slow phase (18 days). A significant fraction of the inhaled mercury vapor is eliminated during exhalation with a majority of the absorbed remainder eliminated in the feces.

Ionized inorganic mercury (Hg^{1+}, Hg^{2+})

As with other metals, the pulmonary absorption of Hg^{1+} and Hg^{2+} varies with particle size [23]. Clarkson [24] reports a 40% pulmonary absorption of mercuric chloride in dogs. The gastrointestinal absorption of Hg^{1+} or Hg^{2+} is approximately 15%. The kidney is the major site of deposition following inorganic mercury exposure. Inorganic mercury has a relatively long half-life in the body, and has been estimated to be 40 days [25] and 67 days with a range of 49-96 days [26]. Urinary and fecal elimination are the major routes of removal from the body. Concomitant exposure to selenium results in the formation of Hg-Se intranuclear inclusion bodies in renal proximal tubule cells [27]. The formation of these complexes may temporarily prevent mercury-induced tissue damage as well as delay excretion [14].

Organomercurials

Organomercurials can be found in three forms: aryl compounds (aromatics containing mercury atoms, such as phenyl mercury), and short- and long-chain alkyl compounds (aliphatic compounds containing carbon, hydrogen, and mercury atoms, such as methyl mercury). The absorption of organomercurials from the gastrointestinal tract and skin varies with the nature of the organic moiety and stability of the organomercurial bond. Alkyl mercurials such as methyl mercury are highly absorbed. Humans absorb approximately 95% of methyl mercury contained in contaminated fish [28, 29]. Alkyl mercurials are dealkylated [30]. This dealkylation process is extremely slow, as evidenced by continued inorganic mercury excretion among Macaca fascicularis monkeys administered oral doses of methyl mercury [31] (the inorganic mercury was thought to have been demethylated in the brains of monkeys). Methyl mercury has a relatively long half-life of 70-80 days in the human body [29]. While the kidney is a major site of deposition, the hair and central nervous system are other important sites of deposition. There also exist sex-related differences in the handling of organomercurials by rodents [32, 33]. Methyl mercury is primarily excreted in the feces but dealkylation reactions result in sex-related differences in urinary excretion of Hg^{2+} [34].

Biological monitoring of mercury

Analytical methods are available to measure mercury in blood, urine, tissue, hair, and breast milk [10]. Biological monitoring of mercury is very useful for assessing exposure as well as risk for health effects [16], but complicated by the fact that both organic and inorganic forms of mercury occur in the body and can be identified in blood and urine. Mercury concentration in individuals who are not occupationally exposed, and whose fish intake is moderate or low, varies between 0.1 and 7 μg/L. The lower values are found in urine and the higher in blood. Urinary mercury is thought to indicate most closely the mercury levels present in the kidneys [35]. The concentration of mercury in urine relates primarily to an exposure to metallic mercury vapor or inorganic mercury compounds. There is a relationship between the number of amalgam fillings and the excretion of mercury in urine. However, for people who are not occupationally exposed, the urinary levels are seldom higher than 10 μg/L. In the case of occupational exposure, there is a linear relationship between air and urine concentrations of mercury. Urine concentrations (μg/L) correspond to air concentrations (μg Hg/m^3) multiplied by 1–2. If mercury concentrations in urine exceed 100 μg Hg/g creatinine, the risk of adverse effects in the nervous system becomes significant. Tremor, nervousness, irritation and kidney damage with proteinuria may be observed. At expo-

sure levels between 50 and 100 μg Hg/g creatinine in urine, these symptoms are less pronounced. Some studies indicate that early signs of adverse effects relating to the nervous system or kidneys can be observed even at urinary levels between 25 and 35 μg Hg/g creatinine [36]. There is a general consensus that if 24-hour urine levels of mercury are greater than 50 μg Hg/g creatinine, nephrotoxicity is probable, comprising cytotoxic effects at the proximal tubule (e.g. enzymuria and increase in tubular antigens) and functional changes (e.g. proteinuria, increase in serum β2-microglobulin) [37, 38].

Because methyl mercury freely distributes throughout the body, monitoring of mercury in the blood is usually carried out to identify exposure to methyl mercury. The concentration of total mercury in blood among people who are not occupationally exposed is influenced by their consumption of fish. Heavy consumers of lake fish have higher blood mercury levels than those who eat fish only rarely. People who never eat fish have blood levels of around 2 μg Hg/L, while the mercury concentrations of those who eat fish three times a week may reach close to 10 μg Hg/L.

During long-term constant exposure (several months) to methyl mercury in food, there is a linear relationship between daily intake of methyl mercury and the concentration of mercury in blood. The mercury concentration in blood (μg/L) corresponds to the daily intake of methyl mercury (μg/day) multiplied by 0.5–1. When exposure is continuous, the blood mercury concentration is proportional to the concentration in the brain, the critical organ for methyl mercury toxicity. Because of mercury's short half-life in the blood (2-4 days), evaluation of blood mercury is of limited clinical value if a substantial amount of time has passed since time of exposure [39].

Analyses of methyl mercury in scalp hair can be used to make a retrospective estimation of maternal exposure during pregnancy. It has been found that children born to mothers whose hair mercury concentrations ranged between 70 and 640 μg Hg/g, show a considerably higher prevalence of developmental changes than controls. Scalp hair levels exceeding 6 μg/g are considered elevated and should be confirmed by a 24-hour urine collection.

Studies from New Zealand and the Faroe Island indicate that adverse effects in children can be correlated with maternal hair levels as low as 10–20 μg/g [40].

As identified in Table 1, the most common methods used to determine mercury levels in blood, urine, and hair of humans and animals include atomic absorption spectrometry (AAS), neutron activation analysis (NAA), x-ray fluorescence (XRF), and gas chromatography (GC).

Roels et al. [38] points out that the analytical techniques identified in Table 1 are not easily available and are not well-suited for routine biomonitoring of occupational or environmental exposures. Instead, indirect biomarkers such as urinary enzymes are often used with success to evaluate mercury exposure and injury. Zalups [35] identifies numerous methods used to detect renal tubular injury induced by mercury. These methods monitor the urinary excretion of enzymes that leak from injured and necrotic proximal tubules, including lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and N-acetyl-β-D-glucosaminidase (NAG). Although advocated by Zalups (35) to detect renal tubular injury, Mason et al. (48) questions the practical utility of such biomarkers in occupational surveillance. According to Mason et al., small increases in NAG, leucine

Table 1. Analytical methods for the detection of mercury in biological samples.

Method	Able to distinguish methyl mercury?	Detection limit (ppm)	Reference
NAA	No	0.1	Byrne and Kosta [41] WHO [42]
AAS	No No[a]	2 ppb range	Hatch and Ott [43] Magos and Clarkson [44]
GC-Electron Capture	Yes	0.1	Von Burg et al. [45] Cappon and Smith [46]
XRF	No	"Low ppm"	Marsh et al. [47]

[a]*The Magos and Clarkson method estimates methyl mercury by subtracting the inorganic mercury content from the total mercury content. Adapted from U.S. EPA [14]*

aminopeptidase (LAP), and other markers of renal tubular cell function are of unclear significance in the prediction of clinical renal disease among occupationally-exposed workers.

General human toxicity

In the assessment of patients with possible mercury exposure, the three key determinants of clinical toxicity are the form of mercury, the route of exposure, and the dose [49]. Diagnosis of mercury poisoning is usually made by obtaining a patient's complete history and performing a physical examination. In addition, laboratory tests may demonstrate increased mercury levels [50]. In 1998, there were 3, 861 documented cases of mercury-related poisonings in the United States [51]. Among these cases, 1 death was reported, while 6 and 52 cases of major and minor illnesses were reported, respectively. Toxic effects from mercury exposure can result from mercury inhalation, injection, ingestion, or dermal absorption. Because of the importance of the chemical form of mercury, toxicity is discussed separately for mercury vapor, inorganic, and organic mercury compounds. Adverse renal effects from all forms of mercury are presented in detail in the next section.

Mercury vapor

The inhalation of mercury vapor at concentrations exceeding 1 mg/m^3 produces a severe and sometimes fatal interstitial pneumonitis. At air concentrations between 100 to 1000 μg/m^3, a variety of signs and symptoms occur after mercury vapor exposure. Typically the mercury-poisoned subject displays severe intention tremor involving the fingers and hands, which make handwriting difficult. In the mouth, gums become tender and inflamed. Salivation is excessive and the salivary glands often are swollen. The third hallmark in mercury poisoning comprises personality changes and psychiatric symptoms that include: anxiety, erethism, irritability, excitability, fearfulness, shyness, memory loss, depression, fatigue, weakness, and drowsiness [6, 20, 52].

Recent occupational health studies have focused on detecting early effects from mercury on the central nervous system. A dose-response relationship between subjective symptoms and/or impaired performance on psychological tests has been reported [53-56]. It is now conceded that an increased prevalence of neurotic symptoms may occur following long-term exposure to mercury vapor at concentrations exceeding 25 μg/m^3 [20]. An air concentration of 25 μg/m^3 roughly corresponds to a urinary excretion of 50 μg Hg/L.

Small children who are accidentally exposed to high concentrations of mercury vapor may develop acrodynia, or Pink disease. This is a syndrome characterized by a body rash, swelling and irritation of palms and feet followed by skin desquamation, irritability, photophobia, fever, insomnia, and perfuse sweating [52, 57]. Curtis et al. [58] describes a typical case. A healthy 18-month old boy moved with his family to another house. After one month, he became irritable and anorexic. He developed a cough and dribbled saliva. His hands and feet were swollen. On examination his hands and feet were bright pink with peeling skin. He could not sit up because of profound proximal muscle weakness. Pink disease was suspected and confirmed by measuring mercury in urine and detecting a concentration of 70 μg Hg/L. Subsequent analysis of mercury at the boy's home revealed high air levels, in particular near the floor level (up to 300 μg/m^3). Lifting the carpet displayed droplets of mercury underneath. The former occupant of the house had used metallic mercury when building silver telescopic mirrors. Although it has been suspected and claimed by some there are no hard scientific evidence that mercury released from amalgam fillings may cause significant health effects. In a thorough examination of more than 400 individuals with suspected adverse health effects from release of mercury from amalgam fillings not a single case of toxicity from mercury could be confirmed [58a].

Inorganic mercury compounds

Mercuric mercury compounds are inorganic salts with mercuric ions (Hg^{2+}), e.g. mercuric chloride and mercuric iodide. Mercurous mercury compounds are salts with Hg^{2+} ions having an apparent valence of +1, e.g. calomel (mercurous chloride, Hg_2Cl_2).

Previously, inorganic mercury compounds were used as medicines. For example, calomel was used as a teething powder in small children, and in the treatment of severe congestive heart failure, but today its use is rare. Accidental and suicidal intoxications have occurred. Generally, the ingestion of inorganic mercury compounds is associated with acute toxicity characterized by erosive damage in the gastrointestinal

tract, accompanied by severe abdominal pain, gastrointestinal bleeding, and in severe cases circulatory collapse. Also, kidney lesions with tubular necrosis and oliguria may develop following ingestion of high doses of soluble inorganic mercury [20, 52].

Acrodynia, or Pink disease, discussed above, was common among infants in the UK and USA until the late 1940s when it became evident that the condition was caused by exposure to calomel in teething powders and in antihelmintic preparations. An allergic reaction towards mercury with variable susceptibility is considered to be involved in the pathogenesis of Pink disease because the syndrome develops in only a small proportion of all exposed (less than 1%) [57]. Furthermore, only infants and small children are affected.

Organic mercury compounds

For organic mercury compounds, the mercury is covalently bound to carbon in compounds of the R-Hg^+ and R-Hg-R type where R represents the organic moiety. With regard to human exposure and health effects, methyl mercury is most important. Consumption of methyl mercury-contaminated seafood and grain products (e.g., bread) has been associated with severe epidemics of poisonings in both Japan and Iraq, respectively [13, 52]. Such epidemics were caused by industrial discharge of methyl mercury in Minimata Bay, Japan, and the accidental ingestion of bread baked from methyl mercury-treated grain in Iraq. As is the case with mercury vapor, the central nervous system is affected, albeit the symptoms slightly different. Symptoms of poisoning include paraesthesia, notably around the mouth, malaise, constriction of the visual field, deafness, and ataxia. The fetus is particularly vulnerable to methyl mercury, and may succumb to the neurotoxic effects of methyl mercury even if the pregnant mother shows no signs of toxicity [40].

Although there is experimental evidence of nephrotoxicity from methyl mercury in animals, no reports of human renal toxicity from methyl mercury exposure have been identified [13].

Certain organic mercury compounds, such as phenyl mercury and methoxyalkyl mercury, are metabolized relatively fast in the human body and are excreted in urine. In contrast to methyl mercury, these compounds do not accumulate in the body nor do they cause toxicity in the central nervous system. On the other hand they affect renal function, and mercury-containing diuretics have been used in the management of congestive cardiac failure. Membranous glomerulonephritis with nephrotic syndrome and severe tubular damage complicating the nephrotic syndrome have been reported as side effects during the treatment of heart failure with organic mercurials [59, 60].

Immunotoxicity

Over the past decade there has, as result of experimental studies, been a growing appreciation that mercury may exert an effect on the immune system. As summarized by Silbergeld and Devine [61], mercury has at least two types of effects on the immune system. First, mercury induces autoimmunity to renal basement membrane proteins, causing mercury-induced glomerulonephritis in certain strains of mice and rats. Secondly, mercury exposure impairs cell-mediated and humoral immunity by affecting Th1 and Th2 responses, which in turn impairs the body's ability to effectively respond to antigens or pathogens.

However, most studies on humans occupationally exposed to mercury identify no effects on immunological markers such as serum immunoglobulins and autoantibodies [62, 63], albeit Ellingsen et al. [64] noted subtle elevations in plasma antibodies against myeloid peroxidase (anti-MPO) and proteinase 3 (anti-PR3).

Adverse renal effects

Bioaccumulation of mercuric ions occurs preferentially in the kidney after exposure to inorganic or elemental mercury. At sufficiently high concentrations, profound nephrotoxicity can occur after exposure to inorganic or elemental mercury. Organic mercurials, such as methyl mercury are much less nephrotoxic, although they do have the potential to cause adverse effects, secondary to other effects such as neurotoxicity. As illustrated in Figure 3, the pars recta of the proximal tubule (the S3 segment) is most vulnerable to the toxic effects of mercury, particularly at the junction of the cortex and the outer medulla [35]. This can be explained in part because tubular transport of heavy metals (and subsequent accumulation) is localized primarily in the proximal tubule. The S3 segment of the proximal tubule comprises proteins that contain sulfhydryl groups, which bind readily to heavy metals such as mercury. It is hypothesized that interaction between

protein sulfhydryl groups and mercury may result in cellular dysfunction and death.

Experimental studies

Mercury vapor (Hg°)

Prolonged exposure to Hg° is known to result in renal damage characterized by proteinuria and edema. This effect involves both tubular and immunological mechanisms [66].

Ionized inorganic mercury (Hg^{1+}, Hg^{2+})

For many years, acute administration of either Hg^{1+} or Hg^{2+} has been known to produce necrosis of the third segment of the proximal tubule [67-73]. The mechanisms that lead to these effects appear to involve alterations in intracellular calcium concentrations secondary to membrane damage. In addition, exposure to Hg^{2+} also produces immunological effects in rodents [74-80] with glomerular lesions since proteinuria is composed mainly of albumin. The inducibility of such immune lesions appears to be highly strain dependent.

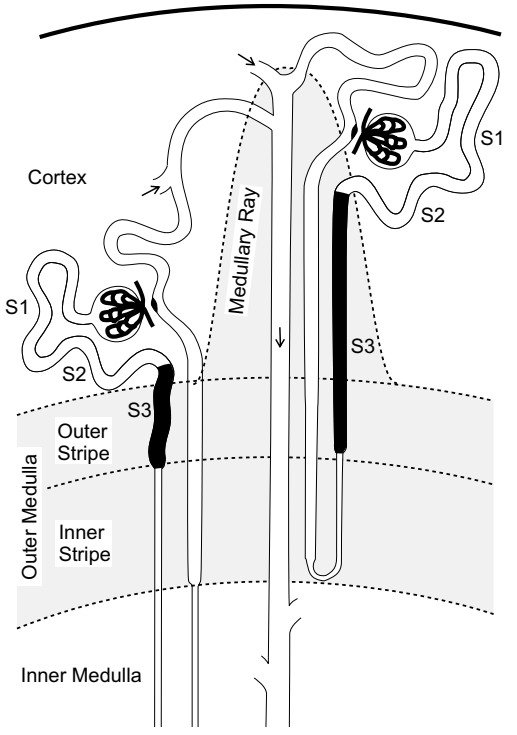

Figure 3. *Illustration of the kidney (reproduced from De Broe [65], with permission).*

Organomercurials

A number of animal studies confirm that high concentrations of mercury accumulate in the kidneys following acute [81] or chronic exposure to methyl mercury [32, 34, 82] and produce renal tubular toxicity. Similar results have been reported using aryl mercury [83]. At present, it is unclear whether these effects are the result of the inorganic mercury yielded by demethylation of methyl mercury in the kidney or the combined action of both organic and inorganic mercurials in renal proximal tubule cells. There are data suggesting that pretreatment with agents that stimulate microsomal drug metabolizing enzyme systems reduce the nephrotoxicity of methyl mercury by increasing urinary excretion of Hg^{2+} [30, 32]. There is also evidence of marked differences in gender sensitivity between male and female animals to methyl mercury nephrotoxicity [32, 33]. Alterations in renal heme biosynthesis following prolonged exposure to methyl mercury cause a relatively specific porphyrinuria pattern [74].

Kidney toxicity – The human experience

Mercury gives rise to different types of renal effects in humans, including acute renal failure, and tubular and glomerular damage with a nephrotic syndrome. In 1818, Blackall documented that mercury caused proteinuria in humans (cited in [60]). A nephrotic syndrome characterized by edema, marked proteinuria and a pronounced decrease in plasma albumin, may develop from mercury exposure and result in a combination of either predominantly tubular or glomerular lesions. The tubular lesions appear early and are dose-related, whereas the glomerular ones may have an immunologic basis. The risk for glomerular damage giving rise to a nephrotic syndrome increases with dose.

Ingestion of large doses of soluble mercuric salts causes acute renal failure with tubular necrosis and possibly coexisting renal vasospasm [52]. In the 1950's, when acute treatment with dialysis was not available, the lethal dose of mercuric bichloride, was estimated to range from 2 to 3.5 g [84]. Long-term ingestion of a laxative containing mercurous chloride by two demented patients resulted in renal impairment with elevated serum urea and creatinine [85]. Microscopic examination of renal biopsy tissue revealed chronic

tubular lesions. Analysis of mercury in tissues confirmed the diagnosis of mercury poisoning with high concentrations of mercury in the kidney. One of the patients also had moderate proteinuria.

In addition to tubular lesions, a classic nephrotic syndrome may develop following mercury exposure [59, 86-88]. Preddy and Russel [89], describe a 54-year old woman who developed severe tubular damage with excessive urinary losses of sodium and a nephrotic syndrome, but with trivial morphological glomerular damage (i.e., minimal change nephropathy) after 68 weeks of treatment with an intravenous mercurial diuretic. Six similar cases of nephrotic syndrome and tubular damage following mercurial diuretics are presented by Burston et al. [87] and Riddle et al. [59]. Williams and Bridge [90] present a 52-year old man with nephrotic syndrome after prolonged use of a mercury containing ointment in the treatment of psoriasis. Diagnosis was confirmed by a urinary mercury excretion of 240 µg Hg/24h. After treatment with a chelating agent, dimercaprol, and withdrawal of the mercury skin ointment, the nephrotic syndrome resolved. Five cases of mercury-induced nephrosis in infants were reported by Wilson et al. [91]. The children had been given mercury containing teething powders or drugs for at least three months, with cumulative mercury doses in the order of several grams. Urinary excretion of mercury was excessive in all cases, in the order of 1000 µg/L. Four of the infants recovered completely, three of them after treatment with dimercaprol.

Nephrotic syndrome with specific histopathological signs of a primarily glomerular damage has also been seen after mercury exposure. Becker et al. [88] report on five cases of biopsy proven membranous glomerulonephritis after exposure to ammoniated mercury ointments (3 cases), mercury paint additive (1 case), and mercury diuretics (1 case). The tubular lesions were not prominent and the authors suggested that mercury induced an autoimmune response that in turn caused the glomerular lesions. Cameron and Trounce [60] present a 64-year old man with heart failure who developed a full blown nephrotic syndrome with urinary excretion of up to 44 g protein daily after receiving injections of organic mercury (chlormerodrin Mersalyl®). The glomerular filtration rate, estimated by creatinine clearance, was 40 ml/min. Postmortem examination of renal tissue revealed a typical membranous glomerulonephritis with no signs of tubular damage. Two cases of membranous nephropathy and a nephrotic syndrome were recently reported from a fluorescent-tube recycling industry in Germany [91a]. Heavy occupational exposure to mercury was evident from markedly elevated urinary excretion of mercury; 118 and 158 µg Hg/L respectively. After withdrawal from exposure and treatment with 2,3 dimercaptopropane-1 sulphonate (DMPS) urinary excretion of mercury and protein in one patient was almost normalized after two years whereas the second patient was also treated but lost for follow.

From Nairobi, nephrotic syndrome has been reported in young females who used mercury containing skin lightning creams. Most of those affected had minimal changes in the kidney (50%) at renal biopsy examination. Urinary excretion of mercury was excessive in most of the nephrotic patients, and it was suspected that the mercury containing cream was involved in the pathogenesis of the nephrotic syndrome [92]. Another case of nephrotic syndrome possibly attributable to the use of a skin lightning cream was reported by Olivera et al. [93]. A 46-year old female developed a membranous glomerulonephritis after using a cream containing 1% mercury. The urinary excretion of mercury was markedly elevated. Although no kidney toxicity was reported, Weldon et al. [94] reported elevated urine mercury concentrations among a predominantly female, Hispanic population living in the Southwestern portion of the United States who used a Mexican mercury chloride-containing creme called "Crema de Belleza-Manning." Median urinary mercury levels were 79 µg/L, with individual values as large as 1, 170 µg/L, providing clear evidence that systemic absorption mercury can occur via topical application of a cosmetic containing mercury.

Pink disease in children may be accompanied by a nephrotic syndrome [95]. Two sisters developed severe proteinuria four days apart a few weeks after that their parents had spilled metallic mercury in the bedroom. The younger girl had typical red-colored palms.

Albuminuria and nephrosis may also follow occupational exposure. Friberg et al. [96] found two such cases in a group of 50 workers exposed to metallic mercury. Both men recovered after the exposure was eliminated. Likewise, Kazantzis et al. [97] describe four cases from two factories where 72 men were exposed to mercury compounds. The urinary excretion of mer-

cury was excessive in most of the workers, ranging from not detectable to more than 1000 μg Hg/L. Recovery from the nephrotic syndrome was complete after removal from exposure.

There are also data showing more subtle effects of mercury on the kidneys after occupational exposure.

Roels et al. [55] and Buchet et al. [98] observed a slightly higher prevalence of elevated urinary excretion of albumin, transferrin, retinol binding protein and the tubular enzyme β-galactosidase in chloralkali workers with a urinary excretion of mercury exceeding 50 μg/g creatinine.

Analysis of the tubular enzyme N-acetyl-β-D-glucosaminidase appears to be particularly effective in detecting early evidence of adverse renal effects from mercury [99, 100]. In an extensive cross sectional examination of chloralkali workers exposed to mercury at air concentrations around 25 $\mu g/m^3$, Langworth et al. [101] noted a significant correlation and dose-response relationship between urinary excretion of mercury and N-acetyl-β-D-glucosaminidase (Figure 4). No significant correlation was evident for other renal parameters: U-albumin, U-orosomucoid, U-β2-microglobulin, U-copper, S-creatinine, and S-β2-microglobulin.

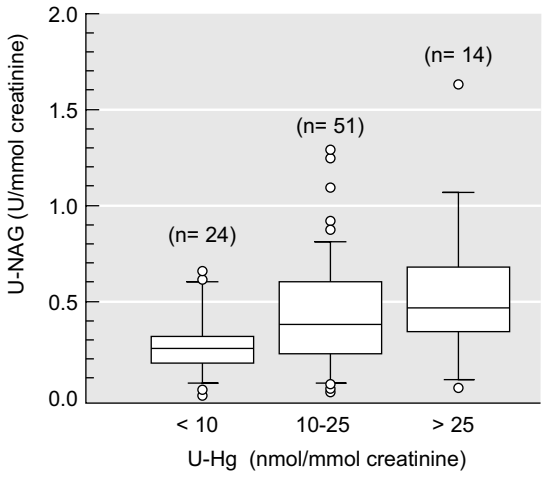

Figure 4. *Box-plots showing the relation between U-Hg and U-NAG in the exposed group (10th, 25th, 50th, 75th, 90th percentiles, and outliers indicated). (Reproduced from Langworth [101], with permission).*

There are no reports of human nephrotoxicity caused by release of mercury from amalgam fillings. This is supported by experimental data from ten humans where standard measurements of renal function (glomerular filtrate rate, urinary albumin excretion, β2-microglobulin, N-acetyl-β-D-glucosaminidase) were monitored before and 60 days after the removal of mercury amalgam fillings [102].

Treatment

Treatments currently available for mercurial poisoning in humans involve the use of thiol-based chelating agents such as British Anti-Lewisite (BAL), penicillamine [52] and more recently, agents such as 2, 3 dimercaptopropane-1 sulphonate (DMPS) [103]. Chelation is the formation of a metal ion complex in which the metal ion is association with a charged or uncharged electron donor. Studies by Bluhm et al. [104] compared the efficacy of D-penicillamine with dimercaptosuccinic acid (DMSA), and demonstrated that DMSA was able to increase the excretion of mercury to a greater extent than D-penicillamine. Studies in humans demonstrate that chelation therapy successfully lowers body burden of mercury and increases urinary mercury levels [105, 106]. However, the impact of chelation on long-term outcome of parenteral mercury exposure remains uncharacterized [107]. Standard dose regimens for the above chelators are as follows: penicillamine given with paradoxen at doses of 500 mg P.O., every 6 hours for 5 days; DMPS given at total a dose of 250 mg I.M. or I.V./4 hours on day 1, 6 hours on day 2, and on 6 to 8 hours on day 3 for the remaining course; DMSA given at 10 mg/kg P.O. every 8 hours for 5 days. Although DMPS has been used in Europe for the past 25 years (under the names Unithiol and Dimaval), it is not widely used in the United States because it is not approved as a drug.

References

1. United States Food and Drug Administration. Mercury compounds in drugs and foods. Center for Biologics Evaluation and Research (CBER). 2001.
2. United States Geological Service (U.S.G.S.). U.S. Geological Survey, Mineral Commodity Summaries (Mercury). 2002.
3. United States Geological Service (U.S.G.S.). U.S. Geological Survey, Mineral Commodity Summaries (Mercury). 1999.
4. Chevalier, P. Mercury. Canadian Minerals Yearbook 1999. Natural Resources Canada. 2000.
5. Byrne L. Brazil's mercury poisoning disaster. Brit Med J 1992; 304: 1397.
6. Schutte NP, Knight AL, Jahn O. Mercury and its compounds. In: Dickerson OB, Horovath EP, Zenz C, editors. Occupational medicine, 3rd ed. St Louis: Mosby-Year Book Inc, 1994: 549-57.
7. National Research Council (NRC). Toxicological effects of methylmercury. 2000.
8. Sällsten G, Barregård L, Österberg T. Tandgnissling hos amalgambärare - en orsak till hög kvicksilverutsöndring ? (Tooth grinding among wearers of amalgam fillings - a cause of high mercury release ?). Läkartidningen (Sweden) 1991; 88: 232-3.

8a. af Geijersstam E, Sandborgh-Englund G, Jonsson F, Ekstrand J. Mercury uptake and kinetics after ingestion of dental amalgam. J Dent Res 2001; 80:1793-6.

9. International Agency for Research on Cancer (IARC). IARC monograph on Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. Volume 58. Lyon, France. 1994: 239-345.
10. United States Environmental Protection Agency (U.S. EPA). Mercury update: impact on fish advisories. 2001. Office of Water.
11. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PJ. Cognitive deficit in 7-year old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 1997; 19: 417-28.
12. United States Food and Drug Administration (U.S. FDA). Methyl mercury levels in commercial seafood species. 2000. Center for Food Safety and Applied Nutrition. Office of Seafood.
13. World Health Organization. Methylmercury. Environmental Health Criteria 101. World Health Organization, Geneva 1990.
14. United States Environmental Protection Agency (U.S. EPA). Mercury study report to Congress. 1997. Office of Air Quality Planning and Standards and Office of Research and Development.
15. Campbell D, Gonzales M, Sullivan J.B. 1992. Mercury. In, Sullivan, J.B. & Krieger, G.R., eds, Hazardous Materials Toxicology. 824-33. Clinical Principles of Environmental Health, Baltimore:Williams and Wilkins.
16. Elinder C-G, Friberg L, Nordberg G F, Kjellström T, Oberdoerster G. Biological monitoring of metals. Chemical safety monographs. International Programme on Chemical Safety. WHO/EHG/94.2 1994: 1-80.
17. Sällsten G, Barregård L, Järvholm B. Mercury in the Swedish chloralkali industri - an evaluation of the exposure and preventive measures over 40 years. Ann Occup Hyg 1990; 34: 205-14.
18. Gutiérrez F, Leon L. 2000. Elemental mercury embolism to the lung. New England Journal of Medicine 342(24): 1791.
19. Diner B, Brenner B. 2001. Toxicity, mercury.
20. World Health Organization. Inorganic mercury. Environmental Health Criteria 118. World Health Organization, Geneva 1991.
21. Hursh JB, Clarkson TW, Cherian MG, Vostal J, Mallie RV. Clearance of mercury (Hg-197 and Hg-203) vapor inhaled by human subjects. Arch Env Health 1976; 31: 302-9.
22. Barregård L, Ställsten G, Schutz A, Attwell R, Skerfving S, Jarvholm B. Kinetics of mercury in blood and urine after brief occupational exposure. Arch Env Health 1992; 47: 176-84.
23. Morrow PE, Gibb FR, Johnson L. Clearance of insoluble dust from the lower respiratory tract. Health Phys 1964; 10: 543-55.
24. Clarkson TW. Human toxicity of mercury. The Journal of Trace Elements in Experimental Medicine 1998; 11: 303-17.
25. Rahola T, Hattula T, Korolainen A, Miettinen JK. Elimination of free and protein-bound ionic mercury in man. Ann Clin Res 1973; 5: 214-19.
26. Hall LL, Allen PV, Fisher HL, et al. 1994. The kinetics of intravenously-administered inorganic mercury in humans. In: Kinetic Models of Trace Elements and Mineral Metabolism During Development, K.M.S. Subramanian and M.E. Wastney, Ed. CRC Press, Boca Raton, FL. p. 1-21.
27. Carmichael NG, Fowler BA. Effects of separate and combined chronic mercuric chloride and sodium selenite administration in rats: histologic, ultrastructural and x-ray microanalytical studies of liver and kidney. J Env Pathol Toxicol 1979; 3: 399-412.
28. Aberg B, Ekman L, Falk R, Greitz U, Persson G, Snihs J. Metabolism of methyl mercury (Hg) compounds in man: Excretion and distribution. Arch Environ. Health 1969; 19: 478-84.
29. Miettinen JK. 1973. Absorption and elimination of dietary (Hg^{++}) and methyl mercury in man. In: Mercury, Mercurial, and Mercaptans, M.W. Miller and T.W. Clarkson, Ed. Springfield, IL. p. 233-43.
30. Fowler BA, Lucier GW, Mushak P. Phenobarbital protection against methylmercury nephrotoxicity. Proc Soc Exp Biol Med 1975; 149: 75-9.
31. Vahter M, Mottet NK, Friberg L, Lind B, Shen DD, Burbacher T. Speciation of mercury in the primate blood and brain following long-term exposure to methyl mercury. Toxicol Appl Pharmacol 1994; 124(2): 221-29.

32. Fowler BA. The morphologic effects of dieldrin and methyl mercuric chloride on pars recta segments of rat kidney proximal tubules. Am J Pathol 1972; 69: 163-74.
33. Yasutake A, Hirayama K, Inouye M. Sex difference in acute renal dysfunction induced by methyl mercury in mice. Renal Failure 1990; 12: 233-40.
34. Fowler BA, Brown HW, Lucier GW, Krigman MR. The effect of chronic oral methylmercury exposure on the lysosome system of rat kidney. Morphometric and biochemical studies. Lab Invest 1975; 32: 313-22.
35. Zalups RK. Molecular interactions with mercury in the kidney. Pharmacological Reviews. 2000; 52(1):113-143.
36. Clarkson TW. Mercury. J. Am. Coll. Toxicol. 1989; 8(7): 1291-96.
37. Hu H. Exposure to metals. Occupational and Environmental Medicine 2000; 27(4):983-96.
38. Roels HA, Hoet P, Lison D. Usefulness of biomarkers of exposure to inorganic mercury, lead, or cadmium in controlling occupational and environmental risks of nephrotoxicity. Renal Failure 1999; 21(3 & 4): 251-62.
39. Satoh H. Occupational and environmental toxicology of mercury and its compounds. Industrial Health 2000; 38: 153-64.
40. Grandjean P, Weihe P, White RF, Debes F: Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. Environ Res 1998; 77(2): 165-72.
41. Byrne AR, Kosta L. Simultaneous neutron-activation determination of selenium and mercury in biological samples by volatilization. Talanta. 1974; 21: 1083-90.
42 WHO (World Health Organization). 1976. Environmental Health Criteria: Mercury. World Health Organization, Geneva, Switzerland, 121.
43. Hatch WR, Ott WL. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Anal Chem 1968; 40(14): 2085-87.
44. Magos L, Clarkson TW. Atomic absorption and determination of total, inorganic, and organic mercury in blood. J AOAC 1972; 55(5): 966-71.
45. Von Burg R, Rustam H. Electrophysiological investigations of methyl mercury intoxication in humans: Evaluation of peripheral nerve by conduction velocity and electromyography. Electroenceph Clin Neurophysiol 1974; 37: 381-92.
46. Cappon CJ, Smith JC. A simple and rapid procedure for the gas-chromatographic determination of methylmercury in biological samples. Bull Environ Contam. Toxicol 1978; 19(5): 600-7.
47. Marsh, DO, Clarkson TW, Cox C, Amin-Zaki L, Al-Tikriti S. Fetal methyl mercury poisoning: Relationship between concentration in single strands of maternal hair and child effects. Arch Neurol 1987; 44: 1017-22.
48. Mason HJ, Hindell P, Williams NR. Biological monitoring and exposure to mercury. Occup Med 2001; 51(1): 2-11.
49. Kulig, K. A tragic reminder about organic mercury. The New England Journal of Medicine. 1998; 338(23): 1692-94.
50. Goldman L, Shannon MW, The Committee on Environmental Health.
Technical Report: Mercury in the Environment: Implications for Pediatricians. Pediatrics 2001; 108(1): 197-205.
51. Litovitz TL, Klein-Schwartz W, White S, Cobaugh DJ, Youniss J, Drab A., Benson BE. The 1999 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance Systems. The American Journal of Emergency Medicine 1999; 18(5): 517-74.
52. Berlin M. Mercury. In: Friberg L, Nordberg GF, Vouk VB, editors. Handbook on the toxicology of metals, Vol II. Amsterdam: Elsevier, 1986: 387-445.
53. Smith RG, Vorwald AJ, Patil LS, Mooney TF. Effects of exposure to mercury in the manufacture of chlorine. Am Ind Hyg Ass J 1970; 31: 687-700.
54. Smith PJ, Langolf GD, Goldberg J. Effects of occupational exposure to elemental mercury on short term memory. Brit J Ind Med 1983; 40: 413-9.
55. Roels H, Gennart JP, Lauwerys R, Buchet JP, Malchaire J, Bernard A. Surveillance of workers exposed to mercury vapour: validation of a previously proposed biological threshold limit value for mercury concentration in urine. Am J Ind Med 1985; 7: 45-71.
56. Langworth S, Almkvist O, Söderman E, Wikström BO. Effects of occupational exposure to mercury vapour on the central nervous system. Brit J Ind Med 1992; 49: 545-55.
57. Clarkson TW. Mercury - an element of mystery. New Eng J Med 1990; 323: 1137-9.
58. Curtis HA, Ferguson SD, Kell RL, Samuel AH. Mercury as a health hazard. Arch Dis Childhood 1987; 62: 293-5.
58a. Langworth S, Bjorkman L, Elinder CG, Jarup L, Savlin P. Multidisciplinary examination of patients with illness attributed to dental fillings. J Oral Rehabil 2002; 29:705-13.
59. Riddle M, Gardner F, Beswick I, Filshie I. The nephrotic syndrome complicating mercurial diuretic therapy. Brit Med J 1958; 1: 1274-7.
60. Cameron JS, Trounce R. Membranous glomerulonephritis and the nephrotic syndrome appearing during mersalyl therapy. Guy's Hosp Report 1965; 114: 101-7.
61. Silbergeld E, Devine PJ. Mercury- are we studying the right endpoints and mechanisms. Fuel Processing Technology 2000; 65-66: 35-42.

62. Langworth S, Elinder CG, Sundqvist KG: Minor effects of low exposure to inorganic mercury on the human immune system. Scand J Work Environ Health 1993; 19(6): 405-13.
63. Langworth S, Elinder CG, Sundquist KG, Vesterberg O: Renal and immunological effects of occupational exposure to inorganic mercury. Br J Ind Med 1992; 49(6): 394-401.
64. Ellingsen DG, Efskind J, Berg KJ, Gaarder PI, Thomassen Y. Renal and immunologic markers for chloralkali workers with low exposure to mercury vapor. Scand J Work Environ Health 2000; 26(5): 427-35.
65. De Broe, M. 2001. Renal toxicity from environmental toxins, drugs, and contrast agents. In: Essential Atlas of Nephrology. Lippincott, Williams & Wilkins.
66. Hua J, Pelletier L., Berlin M, Druet P. Autoimmune glomerulonephritis induced by mercury vapour exposure in the Brown Norway rat. Toxicology 1993; 79: 119-29.
67. Gritzka TL, Trump BF. Renal tubular lesions caused by mercuric chloride: electron microscopic observations. Am J Pathol 1968; 52: 1225-78.
68. Ganote CE, Reimer KA, Jennings RB. Acute mercuric chloride nephrotoxicity: an electron microscopic and metabolic study. Lab Invest 1975; 31: 633-47.
69. Kempson SA, Ellis BG, Price RG. Changes in rat renal cortex, isolated plasma membranes, and urinary enzymes following the injection of mercuric chloride. Chem Biol Interact 1977; 18: 217-34.
70. Trump BF, Berezesky IK, Sato T, Laiho KU, Phelps PC, DeClaris N. Cell calcium, cell injury and cell death. Environ Health Perspect 1984; 54: 281-7.
71. Trump BF, Berezesky IK, Smith MW, Phelps PC, Eliget KA. The relationship between cellular ion deregulation and acute and chronic toxicity. Toxicol Appl Pharmacol 1989; 97: 6-22.
72. Smith MW, Ambudkar IS, Phelps PC, Regec AL, Trump BF. $HgCl_2$-induced changes in cytosolic Ca^{2+} of cultured rabbit renal tubular cells. Biochim Biophys Acta 1987; 931: 130-42.
73. Nielsen JB, Andersen HR, Andersen O, Starklint H. Mercuric chloride-induced kidney damage in mice: time course and effect of dose. J Toxicol Environ Health 1991; 34: 469-83.
74. Woods JS, Fowler BA. Renal porphyrinuria during chronic methyl mercury exposure. J Lab Clin Med 1977; 90: 266-72.
75. Hultman P, Bell LJ, Eneström S, Pollard KM. Murine susceptibility to mercury. I. Autoantibody profiles and systemic immune deposits in inbred, congenic, and intra-H-2 recombinant strains. Clin Immunol Immunopathol 1992; 65: 98-109.
76. Hultman P, Eneström S. Mercury induced antinuclear antibodies in mice: characterization and correlation with renal immune complex deposits. Clin Exp Immunol 1988; 71: 269-74.
77. Hultman P, Eneström S. Mercury induced b-cell activation and antinuclear antibodies in mice. J Clin Lab Immunol 1989; 28: 143-50.
78. Bernard AM, Collette C, Lauwerys R. Renal effects of in utero exposure to mercuric chloride in rats. Arch Toxicol 1992; 66: 508-13.
79. Bellon B, Capron M, Druet E, Verroust M-CV, Sapin C, Girard JF, Foidart JM, Mathieu P, Druet P. Mercuric chloride induced autoimmune disease in Brown-Norway rats: sequential search for anti-basement membrane antibodies and circulating immune complexes. Eur J Clin Invest 1982; 12: 127-33.
80. Esnault VLM, Mathieson PW, Thiru S, Olveira DBG, Lockwood M. Autoantibodies to myeloperoxidase in Brown Norway rats treated with mercuric chloride. Lab Invest 1992; 67: 114-20.
81. Klein R, Herman SP, Bullock BC, Talley FA. Early functional and pathological changes in rat kidney during methyl mercury intoxication. Arch Pathol 1976; 96: 83-90.
82. Fowler BA. Ultrastructural evidence for nephropathy induced by long-term exposure to small amounts of methylmercury. Science 1972; 175: 780-1.
83. Gage JC. The toxicity of alkyl- and arylmercury salts. Biochem Pharmacol 1961; 8: 77.
84. Troen P, Seymour A, Kaufman SA, Katz KH. Mercuric bichloride poisoning. N Engl J Med 1951; 244: 459-63.
85. Wands JR, Weiss SW, Yardley JH, Maddrey WC. Chronic inorganic mercury poisoning due to laxative abuse. Am J Med 1987; 57: 92-101.
86. Munck O, Nissen NI. Development of nephrotic syndrome during treatment with mercurial diuretics. Acta Med Scand 1956; 153: 397-413.
87. Burston J, Darmady EM, Stranack F. Nephrosis due to mercurial diuretics. Brit Med J 1958; 1: 1277-8.
88. Becker CG, Becker EL, Maher JF, Schreiner GE. Nephrotic syndrome after contact with mercury. Arch Intern Med 1962; 83: 178-86.
89. Preedy LRK, Russel DS. Acute salt depletion associated with the nephrotic syndrome. Development during the treatment with a mercurial diuretic. Lancet 1953; 2: 1181-4.
90. Williams NE, Bridge HGT. Nephrotic syndrome after the application of mercury ointment. Lancet 1958; 2: 602.
91. Wilson VK, Thomson ML, Holzel A. Mercury nephrosis in young children. Brit Med J 1952; 1: 358-60.

91a Langworth S, Bjorkman L, Elinder CG, Jarup L, Savlin P. Multidisciplinary examination of patients with illness attributed to dental fillings. J Oral Rehabil 2002; 29:705-13.
92. Barr RD, Rees PH, Cordy PE, Kungu A, Woodger BA, Cameron HM. Nephrotic syndrome in adult African in Nairobi. Brit Med J 1972; 2:131-4.
93. Oliviera DBG, Foster G, Savill J, Syme PD, Taylor A. Membranous nephropathy caused by mercury-containing skin lightning cream. Postgr Med J 1987; 63: 303-4.
94. Weldon MM, Smolinski MS, Maroufi A, Hasty BW, Gilliss DL, Boulanger LL, Balluz LS, Dutton RJ. Mercury poisoning associated with a Mexican beauty cream. West J Med 2000; 173:15-18.
95. Agner E, Jans H. Mercury poisoning and nephrotic syndrome in two siblings. Lancet 1978; 2: 951.
96. Friberg L, Hammarström S, Nyström A. Kidney injury after chronic exposure to inorganic mercury. Arch Ind Hyg Occup Med 1953; 8: 149-53.
97. Kazantzis G, Schiller KFR, Asscher AW, Drew RG. Albuminuria and the nephrotic syndrome following exposure to mercury and its compounds. Quart J Med 1962; 31: 403-19.
98. Buchet JP, Roels H, Bernard A, Lauwerys R. Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury vapor. J Occup Med 1980; 22: 741-50.
99. Stonard MD, Chater BV, Duffield DP, Nevitt AL, O'Sullivan JJ, Steel GT. An evaluation of renal function in workers occupationally exposed to mercury vapor. Int Arch Environ Health 1983; 52: 177-89.
100. Langworth S, Elinder CG, Sundquist KG, Vesterberg O. Renal and immunological effects of occupational exposure to inorganic mercury. Br J Ind Med 1992; 49: 394-401.
101. Langworth S. Early effects of occupational and environmental exposure to inorganic mercury (dissertation). Karolinska Institute Stockholm, Sweden 1992.
102. Sandborgh-Englund G, Nygren, AT, Ekstrand J, Elinder CG. 1996. No evidence of renal toxicity from amalgam fillings. Am J Physiol 271(4 Pt 2):R941-945.
103. Sällsten G, Barregård L, Schutz A. Clearance half-life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on excretion of mercury in urine. Brit J Occupational Environ Med 1994; 51: 337-42.
104. Bluhm RE, Bobbit RG, Welch LW, Wood AJ. Elemental mercury vapour toxicity, treatment and prognosis after acute, intensive exposure in chloralkali plant workers. Part I: History, neuropsychological findings and chelator effects. Human Exp Toxicol 1992; 11: 201-10.
105. Gonzalez-Ramirez, D, Zuniga-Charles M, Narro-Juarez A, Molina-Recio Y, Hurlbut KM, Dart RC, Aposhian HV. DMPS (2, 3-dimercaptopropane-1-sulfonate, dimaval) decreases the body burden of mercury in humans exposed to mercurous chloride. J Pharmacol Exp Ther 1998; 287(1):8-12.
106. Garza-Ocanas L, Torres-Alanis O, Pineyro-Lopez A. Urinary mercury in twelve cases of cutaneous mercurous chloride (calomel) exposure: effect of sodium 2, 3-dimercaptopropane-1-sulfonate (DMPS) therapy. J Toxicol Clin Toxicol 1997; 35(6): 653-5.
107. McFee RB, Caraccio TR. Intravenous mercury injection and ingestion: clinical manifestations and management. J Toxicol Clin Toxicol 2001; 39(7): 733-8.

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Hydrocarbons, silicon-containing compounds and pesticides

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Summary

Environmental/industrial exposure to heavy metals, light hydrocarbons, pesticides and silicon-containing compounds all have been associated with the development and/or progression of renal failure. Occupational exposure to heavy metals, more particularly lead, cadmium and mercury has been linked with the development of acute or chronic renal failure. Indeed, the current literature contains a growing body of information linking solvent exposure with renal injury. To what extent exposure to environmental/occupational contaminants such as pesticides play either a causal or contributive role in the development of chronic renal failure is less clear. Recently reported observations suggest either a primary or secondary role of silicon-containing compounds in the development of anti-neutrophil cytoplasmic antibody-positive rapid progressive glomerulonephritis and Wegener's granulomatosis. Such observations deserve further confirmation as do recent studies suggesting a particular sensitivity of the diabetic kidney for the damaging effects of certain occupational exposures.

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 545-558

Introduction

Despite the overwhelming amount of information concerning the nephrotoxic effects of particular environmental/occupational exposures that have appeared in the literature, to date no data have been reported on the incidence of renal diseases resulting from exposure to particular toxins and chemicals. In view of this it is worth noting that recent data (year 2000) from the Belgian Society of Nephrology finds that the cause of the disease is not known or unclear in up to 22% of cases of end-stage renal disease. This allows us to suggest that the impact of exposure to environmental and occupational toxins on the development of renal disease probably is more important than generally assumed. Hence, better information as to the impact of such exposure is of paramount importance because it can lead to both primary or secondary prevention, a rather exceptional privilege in nephrology. With the exception of lead [1], the failure to demonstrate an etiological role for other potential "environmental/occupational nephrotoxins" in the development or progression of renal disease is due, in fact, to the lack of well elaborated clinical or epidemiological studies.

In the search for a role for such exposure, the following questions need to be answered: (i) does occupational/environmental exposure to a potential nephrotoxic substance play a direct etiological role in the induction of a particular renal disease, (ii) does the exposure correlate with an increased risk for the progression of renal damage already present in patients with glomerulonephritis, diabetic nephropathy, hypertensive renal disease etc. (iii) do both possibilities have to be considered concomitantly or separately?

Some interesting observations have been published recently. While some authors confirm the role of previously identified risk factors others have, based on some experimental evidence, put forward an additional number of potential occupational/environmental nephrotoxins. Also a contributory role for specific occupational exposures, such as organic solvents, on the progression of diabetic nephropathy been suggested. Finally, recent studies reporting a striking association between exposure to silicon containing compounds and the occurrence of Wegener's granulomatosis may contribute better insight to the pathogenesis of this disease.

Organic solvents

Hydrocarbons: *What's in a name?*

The term "hydrocarbon" refers to any aliphatic, alicyclic, aromatic, halogenated and oxygenated hydrocarbons, glycols and organic solvents of either abuse or chemicals used in various industrial processes and household activities. *Halogenated* hydrocarbons (carbon tetrachloride, chloroform) are contained in cleaning agents, insecticides, plastics, degreasers, paint removers, household cleaners. *Aromatic* hydrocarbons are additives in glues and plastics while the *aliphatic* compounds occur in fuels. The *oxygenated* hydrocarbons include alcohols, ketones and ethers and are mostly contained in paint removers, varnishes and glues. Glycols (e.g. ethylene glycol, diethylene glycol, dioxane, glycerol) are used in household and industry. Solvents of abuse are e.g. toluene and xylene.

As can be appreciated, solvents possess a wide variety of chemical and physical properties. Because of this diversity there are many different health effects associated with excessive exposure to solvents. While acute renal failure has been documented following exposure to halogenated hydrocarbons [2], glycols [3] and aromatic hydrocarbons, those attributed to light petroleum hydrocarbon exposure are restricted to isolated clinical case reports [4]. More important, but less well proven, is the role of organic solvents in the development or progression of glomerulonephritis or other types of renal diseases.

Exposure to organic solvents

Exposure to organic solvents can occur either through inhalation, skin and/or mouth contact. For most solvents, inhalation is considered the most important route of exposure. Once inhaled, the solvent vapors directly irritate the upper respiratory tract (nose, throat and bronchial tubes) and the lungs. Solvent vapors are easily absorbed from the lungs into the bloodstream and are distributed to other parts of the body to produce additional toxic effects. Solvents can also be absorbed through the skin and thus be distributed to various organs. Although not a common route of entry, mouth contact with contaminated hands, food and cigarettes may provide solvents entry into the body and the bloodstream via the digestive system.

The route of entry of any solvent depends, to a certain extent, on the chemical group involved. Thus, alcohols enter the body through inhalation, skin absorption, and ingestion. Aromatic hydrocarbons are readily absorbed through the skin, whilst chlorinated hydrocarbons vaporize, presenting an inhalation hazard. Glycols are water-soluble and glycol ethers and several ketones are absorbed through the skin; an exposure route which can be more serious than inhalation.

Nephrotoxicity

Epidemiological studies

Sprecace [5] was the first to suggest an association between gasoline exposure and the pulmonary renal presentation of "idiopathic pulmonary hemosiderosis", more commonly known as Goodpasture's syndrome. Following this observation several *cross-sectional* [6, 7-14] and *case-control* [15-29] studies investigating the relation between renal impairment and occupational hydrocarbon exposure have been published.

Cross-sectional studies [6, 7-14] mainly involve the determination of a few up to 23 urinary markers of early tubular or glomerular changes/dysfunction in individuals chronically exposed to organic solvents with various compositions. In these studies renal effects were defined as early subclinical effects. Overt clinical problems have not been reported. In a critical literature review on cross-sectional epidemiological studies of gasoline associated glomerulonephritis Churchill et al. [30] concluded that based upon study design and execution only the study by Ravnskov et al. [18] made a compelling case for a causal association. Furthermore they judged that neither a cohort analytical study nor randomized clinical trial hold a feasible approach to confirm a suspected association. Here, additional case-control studies are recommended [30].

From 1975 on, fifteen *case-control* studies [15-29] directed at the nephrotoxic effects of occupational exposure to solvents have been reported. Although in general the reported findings are highly suggestive of a relation between hydrocarbon exposure and glomerulonephritis, the applied methodology and statistical power have been criticized. These shortcomings are summarized in two excellent reviews by Churchill et al. [30] and Angell [31] who identify four areas of methodological weaknesses: (i) inappropriate control groups, (ii) use of unblinded interviewers, (iii) no consideration of recall bias and (iv) failure to define a credible measure of the degree and duration of solvent exposure. In addition, epidemiological studies should consider the magnitude of the observed effect and weigh it against the "biological plausibility". It must be noted that experimental models are not available which possess the genetic and/or environmental factors that make specific individuals susceptible to solvent nephropathy.

Based on epidemiological studies the relation between hydrocarbon exposure and glomerulonephritis seems to be well established by both case-control and cross-sectional studies. However, at present it is unclear which solvents are associated with which type of glomerulonephritis. The studies by Stengel et al. [29] and Porro et al. [25] suggest that the risk is highest for IgA nephropathy and that the possible role of oxygenated solvents in the development of this particular renal disease should be further investigated. Yaqoob et al. [26] found risk factors of 15.5, 5.3, 2.0 for respectively aliphatic, halogenated (greasing/degreasing agents) and aromatic and oxygenated (glue/paints) compounds. Furthermore, they demonstrated a direct correlation between the intensity of hydrocarbon exposure and the appearance of (early) markers of renal dysfunction such as serum creatinine, proteinuria, urinary N-acetyl-β-D-glucosaminidase, leucine aminopeptidase, and γ-glutamyl transferase [6].

An accelerated progression of glomerulonephritis has been reported in patients with intense and continued solvent exposure [32, 33]. A recent cohort study has investigated the contributive role of solvent exposure in the progression of primary glomerulonephritis [6]. Yaqoob et al. found that progressive renal failure was associated with a greater exposure to organic solvents when compared to individuals presenting with stable or improving renal function. Moreover patients whose occupational solvent exposure continued following the diagnosis of glomerulonephritis, presented with heavy proteinuria and more severe hypertension. In two recent reviews, Ravnskov [34, 35] considered both the hypothesis of a direct casual effect of solvent exposure and the hypothesis that the exposure worsens renal function separately. Results from 14 cross-sectional, 18 case-control studies, 2 cohort studies and 15 experiments on laboratory animals and 2 on humans, together with many case reports satisfied all but one (lack of specificity) of Hill's criteria for both hypoth-

eses prompting the author to conclude that early elimination of the exposure may prevent the progress of renal failure in many patients.

Aside from glomerulonephritis, the impact of solvent exposure in other renal diagnoses needs to be explored. Indeed, it is of particular note that all these studies are limited to the former type of renal disease while the role of hydrocarbons in the other renal diagnoses such as diabetic nephropathy should be considered [27, 28]. Interestingly in this context is the recent observation by Nuyts et al. [28] in a group of patients with diabetic nephropathy where hydrocarbon exposure was found in 39% of the patients with that particular type of renal disease. This corroborates the results of Yaqoob et al. [27] who found higher exposure scores to hydrocarbons in patients with incipient (odds ratio 4.0) and overt (odds ratio 5.8) diabetic nephropathy as compared to diabetic individuals without clinical evidence of nephropathy.

Pathology and mechanism(s) of solvent induced nephrotoxicity

Whereas acute renal failure has been documented following exposure to halogenated hydrocarbons [2], glycols [3] and aromatic hydrocarbons, episodes attributed to exposure to light hydrocarbons are restricted to isolated clinical case reports [4]. More important, and less well proven is the role of organic solvents in the development or progression of glomerulonephritis or other types of renal diseases.

One of the portals for entry of volatile hydrocarbons is the lung. Lipophilic hydrocarbons rapidly penetrate the lipid membranes thus gaining intracellular access. The link between pulmonary and renal lesions is believed to result from the antigenic similarity shared by the basement membranes of the alveolus and the glomerulus. The immunodominant or epitope is located within the glomerular non-collagenous domain of type IV collagen. It has been proposed that organic solvents or other environmental agents may expose the otherwise cryptic Goodpasture antigen (type IV collagen α 3 chain) to the immune response system in susceptible individuals [36, 37].

The major pathologic presentation of solvent associated nephropathy is that of anti-glomerular basement membrane disease [38] but epimembraneous and subacute proliferative glomerulonephritis have also been demonstrated. In addition, Narvarte et al. [39] reported on a patient with ulcerative colitis in which chronic interstitial nephritis developed that later was attributed to long-term exposure to organic solvents.

The histological severity of tubulointerstitial damage in primary glomerular disorders appears to correlate with severity of renal impairment and can predict the future outcome of renal disease [40]. Recent data correlating solvent exposure with morphological parameters of tubulointerstitial damage in 59 patients with biopsy-proven primary glomerulonephritis showed that solvent exposure correlated significantly with relative interstitial volume and serum creatinine. Solvent exposure, relative interstitial volume, degree of interstitial fibrosis and magnitude of chronic inflammatory cellular infiltrate in the renal cortex at the time of renal biopsy were higher in these glomerulonephritic patients developing progressive renal failure as compared to those presenting a stable or improving renal function [6].

Since, in solvent associated nephropathy, the renal injury is insidious its accurate detection/diagnosis remains an intriguing challenge. Indeed, to be of clinical value methods of detection must be sensitive, quantitative, and correlate with the usual parameters of renal impairment. Measurements of enzymuria, proteinuria and specific tubular antigens have all been proposed. However, until now there is no consensus on their diagnostic sensitivity, specificity and predictive value [12, 41-44]. At present albuminuria, compatible with altered membrane permeability [44], turns out to be the most consistent renal abnormality in solvent-associated nephropathy.

The issue of coexisting solvent-associated tubular damage is more controversial. While a urinary increase in tubular derived enzymes has been reported by some authors [12, 42, 43], others have failed to detect any change using either β2-microglobulin or retinol binding protein excretion [13, 42, 44].

The mechanism underlying solvent-induced glomerulopathy remains speculative. Possible pathways have been proposed Roy et al. [4] (Figure 1). Conceptually it is proposed that when a genetically sensitive individual is exposed to environmental hydrocarbons, any or all three of the pathways could induce a hypersensitive reaction leading to glomerulonephritis. Glomerulonephritis appears to be mainly an immune-mediated disease and some solvents are found to act as immunosuppressants [45-47]. Experimentally, solvent

exposure results in glomerular and tubulointerstitial injury [48] which can be explained since membranous glomerulonephritis can be induced by administration of proximal tubular brush border antigens [49], thus suggesting that solvent exposure may induce a low-grade tubular injury. This tubular injury could provoke local autoimmunity by releasing tubular or basement membrane antigens (antibodies to proximal tubular changes, laminin, Goodpasture's antigens) with activation or damage of the underlying endothelium resulting in the induction of glomerulonephritis. Alternatively potential glomerulotoxic immune factors may arise independently of solvent exposure. Also, the immunosuppressant action of solvents may facilitate the deposition of these mediators of immune damage in renal tissue.

Experimental studies

Several animal models have been used for studying the nephrotoxic effects of solvent exposure. Using rats exposed to petroleum vapors Klavis and Drommer [50] demonstrated renal lesions similar to those noted in Goodpasture's syndrome. In another study 60% of rats fed N,N'-diacetylbenzidine [51] had an increased blood urea nitrogen level. The N,N'-diacetylbenzidine induced glomerulonephritis was characterized by rapid crescent formation, fragmentation of the capsular basement membrane and early obliterative glomerulosclerosis. The site of action of N,N'-diacetylbenzidine appeared to be localized at Bowman's capsule and was not dependent on either deposition of fibrin or coagulative mechanisms [52]. Zimmerman and Norbach [53] demonstrated mesangial proliferative glomerulonephritis with focal glomerulosclerosis after long-term administration of carbon tetrachloride to rats. Although the pathogenesis of the glomerular lesion was not clear, glomerular deposits of antigen-antibody complexes did not accompany the observed lesions. In addition to the glomerular lesions the same workers also noted tubulointerstitial damage in a similar experiment [53].

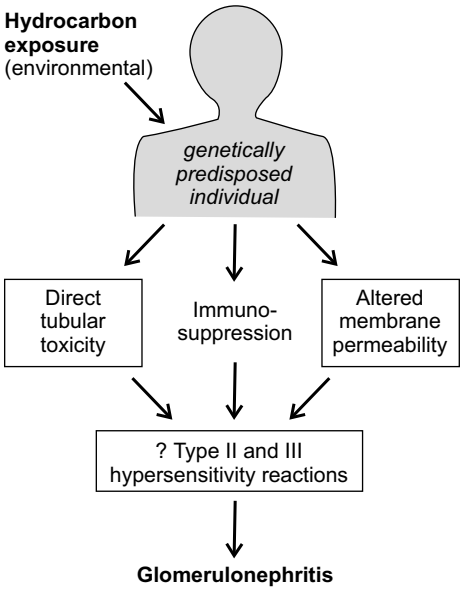

Figure 1. *Possible mechanism of hydrocarbon associated glomerulonephritis (adapted with permission from Roy et al. [4]).*

Silicon containing compounds

Silicon: Occurrence, uses and essential chemistry

Silicon (Si) is the second most abundant element in the earth's crust, contributing around 28%. Silicon acts as a nonmetal in its chemical behavior but its electrical and physical properties are those of a semimetal. Crystalline silicon is a gray, lustrous solid. The chemistry of silicon is dominated by compounds that contain the silicon-oxygen (Si-O) linkage.

The element is used in ceramic industries and for the fabrication of semiconductors. Silicon-based polymers (*silicones*: polymeric chains containing alternately linked silicon and oxygen atoms) have wide application in industry as well as for clinical and pharmaceutical purposes.

In the literature the nomenclature used to describe the various silicon containing compounds is confusing. In nature silicon does not occur as the free element; rather it is either found as silicon dioxide (SiO_2), the so-called *silica,* in an enormous variety of *silicates* or in its carbide form i.e. carborundum (SiC). The soil water or the so-called 'soil solution' [54] contains silicon as silicic acid (H_4SiO_4). In the form of silicic acid silicon is readily absorbed by plants and all soil grown plants contain it as an appreciable but variable fraction of the dry matter [54]. Particularly the hulls of grains and the macrohairs of a number of grasses may contain high concentrations of the element (up to 10% of the plant's dry weight).

Exposure to silicon containing compounds

Due to the element's abundance in nature, human beings are exposed to relatively large but variable amounts of silicon through food, drinking water and dust. In the human body, however, the element is only present in trace amounts. The prolonged inhalation of crystalline silica dust is associated with silicosis. Amorphous silica is considered much less pathogenic than crystalline forms. In occupational settings, the main concern regarding exposure to silicon containing compounds with inhalation of silica and silicate-containing mineral dust. Since silica is such an abundant mineral, there are multiple industries in which exposure may occur. However, in modern, industrialized societies, due to the extensive enforcement of occupational health standards, exposure from well recognized sources such as mining and quarrying activities, sandblasting, stonecutting, ceramics, glass, abrasives etc. … is well controlled, while other sources such as cosmetics, electrical and electronic machinery, grain dust and cotton or wool textiles are less well recognized.

Nephrotoxicity of silicon containing compounds

Epidemiology

Silicon toxicity is virtually limited to occupational exposure to silicon compounds e.g. miners, sandblasters, bricklayers, pottery workers in which inhalation of the compounds has been associated with the diseases of the lung. The later being evidenced by nodule formation and acute silicosis, mixed dust fibrosis and diatomite pneumoconiosis.

Knowledge regarding renal injury and the development of anti-neutrophil cytoplasmic antibody (ANCA) positivity associated with silica exposure is rudimentary. Moreover, information about the health significance of the occupational exposure to other silicon containing compounds apart from silica and crystalline silicates is lacking.

During the past decade a number of case reports have described the occurrence of different forms of renal disease in patients exposed to silica [55-63]. However, only a few reports concerned subjects exposed to silica but without silicosis. Most of the cases demonstrated renal lesions compatible with rapidly progressive glomerulonephritis with a necrotizing component present in most cases. Crescent formation was described in a patient with proliferative glomerulonephritis [56] and three individuals with IgA nephropathy [57].

In recent reports renal lesions observed after silica exposure were associated with ANCA positivity suggesting a pathogenetic role of ANCA [61-63]. Other autoimmune manifestations have been reported in a cohort of 50 workers after occupational exposure to a scouring powder mainly containing silica [64]. Systemic symptoms were present in 32 of these subjects including Sjögren's syndrome (n=6), systemic lupus erythematosus (n=3), "overlap" syndrome (n=5) and with undifferentiated findings (n=13) not meeting the criteria for a defined disease (Table 1). In most patients renal disease occurred after a long latency period. In the reports where the information is available, renal symptoms occurred 3 to 27 years after silica exposure. Data of a recent retrospective cohort study including 2412 white male gold miners which had been working underground for at least 1 year between 1940 and 1965 showed an elevated relative risk for non-systemic end-stage renal disease (i.e. glomerulonephritis or interstitial nephritis) of 4.22 (95% CI: 1.54-9.19) increasing to 7.70 (95% CI: 1.59-22.48) among workers with 10 or more years of employment [65] when compared to the incidence of end-stage renal disease in the US population. Recent observations revealed increased levels of early markers of renal dysfunction even in currently exposed workers [66, 67]. The cross-sectional observations in workers exposed to silica confirmed signs of renal impairment in patients with silicosis [66, 68] as well as in workers exposed to silica dust for less than 2 years and without lung injury [69].

In a cross-sectional study by Boujemaa et al. [67], who evaluated early indicators of renal dysfunction in silicotic workers (n=116), recorded a delay after cessation of exposure up to 30 years (mean 23 years). The silicotic subjects excreted, on average, slightly higher amounts of albumin, retinol binding protein and N-acetyl-β-D-glucosaminidase [66, 67, 69].

A survey of the literature [28, 55-62, 66, 67, 69-74] indicates that the most frequent exposure to silicon involves exposure to silica and silicates mainly in their crystalline forms. Health risks associated with the exposure to other silicon containing compounds were reported in the mortality study of 16.661 man-made mineral fiber workers employed during 1945 to 1963 at one of 17 U.S. manufacturing plants [70]. Fiber exposure

Table 1. Observations in silica exposed workers.

CROSS SECTIONAL STUDIES

Reference	Exposed workers	Non-exposed workers	Lung	Early markers of renal dysfunction: increased compared to controls
Ng et al. 1992 [66]	33 drillers/crushers in granite quarries current exposure	19 age-matched non-exposed workers	Silicosis (7)	Albumin α1-microglobulin β-N-acetyl-glucosaminidase
Hotz et al. 1995 [69]	86 workers in quartzite rock quarry current exposure	86 age-matched non-exposed workers	No silicosis	Albumin Transferrin Retinol-binding protein β-N-acetyl-glucosaminidase

MORTALITY STUDIES

Reference	Exposed workers	Non-exposed workers	Disease	Standardized mortality ratio
Marsh et al. 1985 [70]	16661 man-made mineral fiber workers	/	Malignant neoplasms Respiratory cancer	108.3 112.1
Goldsmith 1993 [71]	Man-made mineral fiber workers	/	Renal disease	/
Steenland et al. 2001 [75]	4626 workers in sand industry	US population	Renal disease	161.0

COHORT STUDIES

Reference	Exposed workers	Non-exposed workers	Disease
Prospective Sanchez-Roman 1993 [64]	50 scouring powder producing factory	/	Systemic illness (32) - Sjörgen (6) - Systemic sclerosis (5) - Overlap syndrome (5) - Systemic lupus erythematosus (3) - Undifferentiated findings (13)
Retrospective Calvert et al. 1997 [65]	2412 silica exposed gold miners	US population	Non-systemic end-stage renal disease - Glomerulonephritis or interstitial nephritis Standard incidence ratio 4.22 (1.54-9.19)

CASE CONTROL STUDIES

Reference	Cases	Controls	Occupational exposure	OR (95% CI)
Steenland et al. 1990 [70]	325 end-stage renal failure patients	325 age-matched general population	Silica Brick and foundry	1.67 (1.02-2.74) 1.92 (1.06-3.46)
Gregorini et al. 1993 [73]	16 patients with ANCA-positive rapidly progressive glomerulonephritis	32 age-matched other renal failure patients	Silica dust	14.0 (1.7-113.8)
Nuyts et al. 1995 [28]	272 renal failure patients	272 age-matched general population	Silicon containing compounds Grain dust	2.51 (1.37-4.60) 2.96 (1.24-7.04)
Nuyts et al. 1995 [74]	16 patients with Wegener's granulomatosis	32 age-matched general population	Silica Silicon containing compounds	5.0 (1.4-11.6) 6.5 (1.3-13.5)
Stratta et al. 2001 [76]	31 cases of biopsy proven vasculitis*	58 age/sex residence-matched controls	Silica	2.4 (p=0.04)
Hogan et al. 2001 [76a]	65 cases with ANCA-associated small vessel vasculitis**	65 age/sex-matched other renal failure patients	Silica dust	4.4 (1.36-13.4)

OR = Odds ratio ANCA = anti-neutrophil cytoplasmic antibody CI = Confidence interval GN = glomerulonephritis
**18 pauci-immune crescentic glomerulonephritis, 9 microscopic polyangitis, 4 Wegener's granulomatosis*
***all patients had pauci-immune necrotizing glomerulonephritis*
(Adapted with permission from De Broe et al. [106])

in the plants producing fibrous glass or mineral wool, or both, was associated with increased standardized mortality ratios for overall mortality as well as for mortality from nephritis and nephrosis. Further evidence of the nephrotoxic role of these and other kinds of silicon containing compounds was reported by Goldsmith and Goldsmith [71]. They argued that in California an increased mortality from diseases of the urinary system was observed for farmers and farm workers. More recently, Steenland et al. [75] examined renal disease morbidity and mortality as well as arthritis mortality in a cohort of 4.626 silica-exposed workers in the industrial sand industry (an industry previously unstudied). Comparison of the cohort with the US population revealed an excess mortality ratio from chronic renal disease of 1.61 [95% CI = 1.13-2.22]. Linking of the cohort with the US registry of end-stage renal disease for the years 1977-1996 demonstrated an excess of end-stage renal disease incidence (standardized incidence ratio: 1.97, 95% CI: 1.25-2.96), which was highest for glomerulonephritis (3.85, 95% CI: 1.55-7.93) and increased with increasing cumulative exposure.

The most firmly based epidemiological observations are derived from recently published case-control studies [72-74]. Two studies, based on a large sample size, retrospectively examined occupational exposures of renal failure patients. Amongst others, an increased odds ratio's for silicon-containing compounds was also observed [28, 72]. Nuyts et al. [28] were the first to demonstrate an increased risk for the exposure to grain dust that potentially may contain high amounts of silicon. Other studies only [73, 74] focused on a small sample of patients with rapidly progressive glomerulonephritis and the specific exposure to silicon containing compounds. Gregorini et al. [73] selected only ANCA positive patients and Nuyts et al. [74] investigated patients with Wegener granulomatosis, 80% of who were ANCA positive. Studying a group of 31 cases of biopsy proven vasculitis (18 pauci-immune crescentic glomerulonephritis, 9 microscopic polyangitis, 4 Wegener granulomatosis) Stratta et al. [76] also found an increased odds ratio (2.4) for exposure to silica whilst no other significant association with a series of other exposures could be found. More recently, Hogan et al. [76a] studying 65 patients with ANCA-associated small-vessel vasculitis (all of them having biopsy-proven pauci-immune crescentic glomerulonephritis) also demonstrated the odd's ratio of silica dust exposure in the development of the disease to be 4.4 times greater as compared to control subjects. In contrast to an increased risk for the development of ANCA-associated small-vessel vasculitis, exposure to silica could not be associated with systemic lupus erythematosus [76a] .

Pathology and mechanism(s) of silicon-induced nephrotoxicity

Data presented above are highly indicative for an association between silica and renal disease. The underlying pathophysiological mechanisms, however, are far from clear. At least two mechanisms have been proposed. A direct nephrotoxic effect of silicon has been suggested by Hauglustaine et al. [77]. Recently, Hotz et al. [69] reported on subclinical renal effects as indicated by an increased excretion of albumin, transferrin, retinol binding protein and N-acetyl-β-D-glucosaminidase following short time (less then 2 years) exposure to silica in non-silicotic workers. In a recent review on the association between renal disease and silica exposure Kallenberg [78] suggested that the tubular dysfunction observed in silica workers resulted from a direct nephrotoxic effect of the silicon compound. Experimentally, the nephrotoxic potential of silica has been demonstrated in the dog [79].

The exact mechanism responsible for the nephrotoxic effect of silicon remains to be elucidated although membrane damage possibly related to oxidant generation [78] or inhibition of superoxide dismutase activity [80] might be rational explanations. Based on reports on lung toxicity related to the chemical, morphological and surface characteristics of the various silicon compounds, it is not known yet to which extent these exhibit direct toxic effects at the level of the kidney [79, 81].

A second possible mechanism consists in the interaction of the inhaled silicon compounds with the cell membrane particularly that of macrophages. Once engulfed a series of events may ensue resulting in an important inflammatory reaction at the alveolar level. In addition silica particles have been shown to induce rupture of phagosomes of macrophages [82] with the release of lysosomal enzymes such as proteinase 3 or myeloperoxidase the antigens of ANCA into the microenvironment that in turn may be followed by the generation of the autoantibodies. Recently, increasing interest has been raised in the role of apoptosis in the

induction of autoimmunity [83]. There is growing evidence that apoptotic antigens are the natural targets for many autoantibodies [84]. The possibility that silica, *in vitro*, may induce apoptosis of monocytes or macrophages and possibly neutrophils may represent an alternative mechanism that is operative in the induction of ANCA-associated vasculitis [84, 85]. Surface expression of ANCA antigens proteinase 3 and myeloperoxidase have been demonstrated during apoptosis of neutrophils [84, 86]. Therefore, ANCA's may bind to their target antigen on apoptotic cells and via an Fc-dependent bridging, the antibodies may amplify the release of cytokines, oxygen radicals, and lysosomal enzymes.

To which extent the generated ANCA's are responsible for initiating vasculitis, or may increase or even perpetuate vasculitis remains to be determined. Since ANCA (i) may directly activate neutrophils *in vitro*, (ii) may damage endothelial cells expressing the proteinase-3 antigen, (iii) are capable of inducing *in vitro* adherence of neutrophils to endothelial cells, (iv) block (c-ANCA) the inactivation of proteinase-3 by α-1 antitrypsin, a pathophysiological role may be suggested.

Based on experimental studies it has been suggested that silicates may stimulate lymphocytes via a T-cell receptor Vβ-specific T-cell activation pathway resulting in the production of autoantibodies or autoimmune diseases [83, 87, 88]. In this context it must be noted that not only ANCA's but also other autoantibodies such as antinuclear antibodies and rheumatoid factors frequently occur in workers heavily exposed to silicon-containing compounds [64, 75].

An intriguing observation made from case-control studies remains the controversy that exists between silica exposure and the development of a particular renal disease. Indeed, in a recent case-control study on occupational risk factors for chronic renal failure, Nuyts et al. [28] demonstrated exposure to silicon containing compounds to be related to the development of virtually all diagnostic groups of chronic renal failure. On the other hand, in two other studies [73, 74] silicon exposure was linked to a significantly higher relative risk for the development of ANCA-associated rapidly progressive glomerulonephritis or Wegener disease. These observations might indicate that silicon-containing compounds may act as a contributive as well as a causative factor in the development of renal disease. A similar observation has also been made in subjects taking analgesics. Here, besides the development of the so-called analgesic nephropathy identifiable with high accuracy by the visualization of renal papillary necrosis [89], analgesic abuse also seems to hold an increased risk for the development of the other types of renal diseases [90]. In a recent study it was demonstrated that acetaminophen and aspirin exhibit exacerbating effects on the development of all types of chronic renal failure [91].

Pesticides

The information linking environmental/occupational exposure to pesticides (including herbicides/fungicides/insecticides) is confined to some case reports and an occasional retrospective review on occupational exposure and acute renal failure [92]. Serious exposure to pesticides is usually accidental although suicidal ingestion's have occurred [92, 93]. Since many of these compounds have both commercially and domestic application exposures usually occur when proper protective precautions are ignored. Usually the acute renal failure following pesticide poisoning turns out to be multifactorial. For example, poisoning by the now banned pesticide Lindane® caused both acute volume depletion [94] and rhabdomyolysis [95], either of which could account for the subsequent acute renal failure. Other examples of multifactorial causes of acute renal failure due to pesticide exposure are reviewed by Abuelo [92].

Due to the increased litigation based on premise of product liability a renewed interest in the renal effects of herbicides, fungicides, pesticides, and insecticides has been noted during the last years. However, because of the lack of a valuable experimental animal model the current knowledge of the pathophysiologic mechanisms of the pesticide-induced renal injury is highly limited. The possibility that these pesticides act similar to that of light hydrocarbons is worthwhile to be considered, however, at the present is at is still highly speculative.

Little is also known of the long term renal effects of chronic low dose exposure to pesticides. Chronic exposure to the now banned dichlorodiphenyltrichloroethane (DDT), a lipophilic compound with prolonged body fat retention, has been associated with renal injury [94].

Insights in the renal handling of 2,4-dichlorophe-

noxyacetic acid have contributed to a better knowledge of the extent of occupational exposure to this widely used herbicide [96-100]. Recently, Kancir et al. [101] reported on a case of oliguric acute renal failure complicated by profound and recurrent hypocalcemia, severe hyperphosphatemia, and inappropriately high urinary sodium concentrations following exposure to this compound. In the studies of Manninen et al. [100] the peak herbicide concentration which was noted during the first 12 hours post exposure turned out to be associated with an increased excretion of both sodium and potassium. In *in-vitro* experiments the uptake of either 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid via a proximal tubule organic acid transport system was demonstrated in both rat and rabbit renal cortical slices [102]. Based on these experiments it was suggested that once 2,4-dichlorophenoxyacetic acid is secreted into the proximal tubule, it is probably non-reabsorbable an acts to bind intraluminal sodium and potassium. This, in turn, induces electrolyte depletion which could cause the rhabdomyolysis and severe hypocalcemia and hyperphosphatemia observed by Kancir et al. in the above mentioned study [101]. Lindane® [95], diquat® [93], copper sulphate [103] and paraphenylene diamine [104, see also chapter 32] all have been reported to induce rhabdomyolysis and acute renal failure. Recently, Talbot et al. [105] reported the poisoning of 93 patients with the glyphosphate-surfactant herbicide (Round-up®). In ten patients (14%) manifest renal abnormalities were noted which was accompanied by a nearly uniform increase in serum creatinine (>180 μM/L) and oliguria in 3 patients. Based on their own investigations and those of Japanese workers, the authors concluded [105] that in 50% of the cases in which exposure to glyphosphate-surfactant herbicide was reported renal failure resulted.

Conclusion

Recent literature clearly points to a role for exposure to solvents in the development or progression, or both, of chronic renal failure. With regard to long-term exposure to pesticides no clear-cut evidence for a linkage with renal disease has been presented to date. Furthermore, a number of observations of the past two years suggest a primary or secondary role of substances such as silicon-containing compounds in the development of ANCA-associated rapidly progressive glomerulonephritis or Wegener's granulomatosis as well as an increased susceptibility of the diabetic kidney to the toxic effects of particular occupational pollutants. Further experimental studies are required to gain insight in the underlying mechanisms by which the environmental/occupational contaminants exert their toxic action at the level of the kidney.

References

1. Croll DG. The incidence of chronic nephritis amongst young people in Queensland. Med J Aust 1929; 2: 144-145.
2. Pederen LM. Biological studies in human exposure to and poisoning with organic solvents. Pharmacol Toxicol 1987; 3: 1-38.
3. Porter GA. The treatment of ethylene glycol poisoning simplified. N Eng J Med 1988; 319(2): 109-110.
4. Roy AT, Brautbar N, Lee DBN. Hydrocarbons and renal failure. Nephron 1991; 58: 385-392.
5. Sprecace GA. Idiopathic pulmonary hemosiderosis. Am Rev Resp Dis 1963; 8: 330-337.
6. Yaqoob M, Bell GM, Stevenson A, Mason H, Percy DF. Renal impairment with hydrocarbon exposure. Quart J Med 1993; 86: 165-174.
7. Krusell L, Nielsen HK, Baelum J, Lundqvist G, Omland O, Vaeth M, Husted SE, Mogensen CE, Geday E. Renal effects of chronic exposure to organic solvents: a clinically controlled trial. Acta Med Scand 1985; 218: 323-327.
8. Enarson DA, Chan-Yeung M, Embree V, Wang R, Schulzer M. Occupational exposure to chlorophenates. Scand J Work Environ Health 1986; 12: 144-148.
9. Ng TP, Ong SG, Lam WK, Jones MG, Cheung CK, Ond CN. Urinary levels of proteins and metabolites in workers exposed to toluene. Int Arch Occup Environ Health 1990; 62: 43-46.

10. Hotz P, Pilliod J, Bernard A, Berode M, Rey F, Mazzocato C, Guillemin M, Boillat MA. Hydrocarbon exposure, hypertension and kidney function tests. Int Arch Occup Environ Health 1990; 62: 501-508.
11. Lauwerys R, Herbrand J, Buchet JP, Bernard A, Gaussin J. Health surveillance of workers exposed to tetrachloroethylene in dry-cleaning shops. Int Arch Occup Environ Health 1983; 52: 69-77.
12. Mutti A, Lucertini S, Falzoi M, Cavatorta A, Franchini I. Organic solvents and chronic glomerulonephritis: a cross-sectional study with negative findings for aliphatic and alicyclic C5-C7 hydrocarbons. J Appl Toxicol 1981; 1(4): 224-226.
13. Viau C, Bernard A, Lauwerys R, Buchet JP, Quaeghebeur L, Cornu L, Cornu M, Phillips SC, Mutti A, Lucertini S, Francini I. A cross-sectional survey of kidney function in refinery employees. Am J Ind Med 1987; 11: 177-187.
14. Mutti A, Alinovi R, Bergamaschi E, Giagini C, Cavazinni S, Franchini I, Lauwerys RR, Bernard AM, Roels H, Gelpi E, Rosello J, Ramis I, Price RG, Taylor SA, De Broe ME, Nuyts GD, Stolte H, Fels LM, Herbort C. Nephropathies and exposure to perchloroethylene in dry cleaners. Lancet 1992; 340: 189-193.
15. Zimmerman SW, Groehler K, Beirne GJ. Hydrocarbon exposure and chronic glomerulonephritis. Lancet 1975; 2: 199-201.
16. Lagrue G, Kamalodine T, Hirbec G et al. Rôle de l'inhalation de substances toxiques dans la genèse des glomérulonéphritis. Nouv Press Med 1977; 6: 3609-3613.
17. Ravnskov U. Exposure to organic solvents - a missing link in poststreptococcal glomerulonephritis ? Acta Med Scand 1978; 203: 351-356.
18. Ravnskov U, Forsberg B, Skerfving S. Glomerulonephritis and exposure to organic solvents: a case control study. Acta Med Scan 1979; 205: 575-579.
19. Finn R, Fennerty AG, Ahmad R. Hydrocarbon exposure and glomerulonephritis. Clin Nephrol 1980; 14(4): 173-175.
20. Van der Laan G. Chronic glomerulonephritis and organic solvents: a case control study. Int Arch Occup Environ Health 1980; 47: 1-8.
21. Ravnskov U, Lundström S, Nordén A. Hydrocarbon exposure and glomerulonephritis evidence from patients' occupations. Lancet 1983; 2: 1214-1216.
22. Bell GM, Gordon ACH, Lee P, Doig A, MacDonald MK, Thomson D, Anderton JL, Robson JS. Proliferative glomerulonephritis and exposure to organic solvents. Nephron 1985; 40: 161-165.
23. Harrison DJ, Thomson D, MacDonald MK. Membranous glomerulonephritis. J Clin Pathol 1986; 39: 167-171.
24. Harrington JM, Whitby H, Gray CN, Reid FJ, Aw TC, Waterhouse JA. Renal disease and occupational exposure to organic solvents: a case referent approach. Brit J Ind Med 1989; 46: 643-650.
25. Porro A, Lomonte C, Coratelli P, Passavanti G, Maria Ferri G, Assennato G. Chronic glomerulonephritis and exposure to solvents: a case referent study. Brit J Ind Med 1992; 49: 738-742.
26. Yaqoob M, Bell GM, Percy D, Finn R. Primary glomerulonephritis and hydrocarbon exposure: a case control study and literature review. Quart J Med 1992; 301: 409-418.
27. Yaqoob M, Patrick AW, McClelland P, Stevenson A, Mason H, Percy DF, White MC, Bell GM. Occupational hydrocarbon exposure in diabetic nephropathy. Diabetic Med 1994; 11(8): 789-793.
28. Nuyts GD, Van Vlem E, Thys J, De Leersnijder D, D'Haese PC, Elseviers MM, De Broe ME. New occupational risk factors for chronic renal failure. Lancet 1995; 346: 7-11.
29. Stengel B, Cénée S, Limasset JC, Protois JC, Marcelli A, Brochard P, Hémon D. Organic solvent exposure may increase the risk of glomerular nephropathies with chronic renal failure. Int J Epidem 1995; 24: 427-434.
30. Churchill DN, Fine A, Gault MH. Association between hydrocarbon exposure and glomerulonephritis. An appraisal of the evidence. Nephron 1983; 33: 169-172.
31. Angell M. The interpretation of epidemiologic studies. N Engl J Med 1990; 323: 823-825.
32. Bell GM, Doig D, Thompson D, Anderton JL, Robson JS. End-stage renal disease associated with occupational exposure into organic solvents. Proc EDTA-ERA 1985; 22: 725-729.
33. Ravnskov U. Influence of hydrocarbon exposure on the course of glomerulonephritis. Nephron 1986; 42: 156-160.
34. Ravnskov U. Hydrocarbon exposure may cause glomerulonephritis and worsen renal function: evidence based on Hill's criteria for causality. Q J Med 2000; 93: 551-556.
35. Ravnskov U. Hydrocarbons may worsen renal function in glomerulonephritis: A meta-analysis of the case-control studies. Am J Ind Med 2000; 37: 599-506.
36. Salant DJ. Immunopathogenesis of crescentic glomerulonephritis and lung purpura. Kidney Int 1987; 32: 408-425.
37. Kalluri R, Gattone VH 2nd, Noelken ME, Hudson BG. The α 3 chain of type IV collagen induces autoimmune Goodpasture syndrome. Proc Natl Acad Sci USA 1994; 91(13): 6201-6205.
38. Daniell WE, Couser WG, Rosenstock L. Occupational solvent exposure and glomerulonephritis. A case report and review of the literature. JAMA 1988; 259: 2280-2283.
39. Narvarte J, Sabe SR, Ramirez G. Occupational exposure to organic solvents causing chronic tubulointerstitial nephritis. Arch Intern Med 1989; 149: 154-158.
40. Cameron JS. Tubular and interstitial factors in the progression of glomerulonephritis. Pediatr Nephrol 1992; 6: 292-303.

41. Meyer BR, Fischbein A, Rosenman K, Lerman Y, Drayer DE, Reidenberg MM. Increased urinary enzyme excretion in workers exposed to nephrotoxic chemicals. Am J Med 1984; 76: 989-998.
42. Askergen A. Studies in kidney function in subjects exposed to organic solvents. III. Excretion of cells in the urine. Acta Med Scand 1981; 210: 103-108.
43. Francini I, Cavatorta A, Folzoi M Lucertini S, Mutti A. Early indicators of renal damage in workers exposed to organic solvents. Int Arch Occup Environ Health 1983; 52: 1-9.
44. Lauwerys R, Bernard A, Viau C, Buchet JP. Kidney disorders and hemotoxicity from organic solvent exposure. Scan J Work Environ Health 1985; II (suppl 1): 84-90.
45. Ravnskov U. Possible mechanism of hydrocarbon-associated glomerulonephritis. Clin Nephrol 1985; 23: 294-298.
46. Bekesi JG, Holland JF, Anderson HA, Fischbein AS, Rom W, Wolff MS, Selikoff IJ. Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. Science 1978; 199: 1207-1209.
47. Peters DK, Lachmann PJ. Immunity deficiency in the pathogenesis of glomerulonephritis. Lancet 1974; 1: 58-61.
48. Shibita S, Yokoyama M. Nephritogenic glycoproteins. Nephron 1990; 55: 152-158.
49. Glassock RJ, Lachmann PJ, Watson JI, Dixon FJ. Autologous immune complex nephritis induced by renal tubular antigens. J Exp Med 1986; 127: 573-587.
50. Klavis G, Drommer W. Goodpasture's syndrome and the effects of benzene. Arch Toxicol 1970; 26: 40-55.
51. Harman JW, Miller EC, Miller JA. Chronic glomerulonephritis and nephrotic syndrome induced in rats by N, N'-diacetylbenzidine. Am J Pathol 1952; 28: 529.
52. Harman JW. Chronic glomerulonephritis and nephrotic syndrome induced in rats with N, N'-diacetylbenzidine. J Pathol 1970; 104: 119-128.
53. Zimmerman SW, Norbach DH. Nephrotoxic effects of long term carbon tetrachloride administration in rats. Arch Pathol Lab Med 1980; 104: 94-99.
54. Epstein E. The anomaly of silicon in plant biology. Proc Natl Acad Sci 1994; 91: 11-17.
55. Slavin RE, Swedo JL, Brandes D, Gonzalez-Vitale JC, Osornio-Vargas A. Extrapulmonary silicosis: a clinical, morphologic, and ultrastructural study. Hum Pathol 1985; 16: 393-412.
56. Osorio AM, Thun MJ, Novak RF, Van Cura EJ, Avner ED. Silica and glomerulonephritis: case report and review of the literature. Am J Kidney Dis 1987; 9(3): 224-230.
57. Bonnin A, Mousson C, Justrabo E, Tanter Y, Chalopin JM, Rifle G. Silicosis associated with crescentic IgA mesangial nephropathy. Nephron 1987; 47: 229-230.
58. Sherson D, Jorgensen F. Rapidly progressive crescenteric glomerulonephritis in a sandblaster with silicosis. Brit J Ind Med 1989; 46: 675-676.
59. Arnalich F, Lahoz C, Picazo ML, Monerero A, Arribas JR, Martinez Ara J, Vazques JJ. Polyarteritis nodosa and necrotizing glomerulonephritis associated with long-standing silicosis. Nephron 1989; 51: 544-547.
60. Dracon M, Noel C, Wallaert B, Dequiedt P, Lelievre G, Tacquet A. Glomérulonéphrites rapidement progressives chez les mineurs de charbon pneumoconiotiques. Néphrologie 1990; 11: 61-65.
61. Gregorini G, Tira P, Lancini L, Bozzola G, Maiorca R. Silica exposure as a risk factor in the development of necrotising and crescentic glomerulonephritis with anti-mieloperoxidase antibodies. Nephrol Dial Transplant 1991; 6: 773 (abstract).
62. Neyer U, Wöss E, Neuweiler J. Wegener's granulomatosis associated with silicosis. Nephrol Dial Transplant 1994; 9: 559-561.
63. Nakajima H, Miyazaki M, Imai N, Yokokawa T, Yamamoto S. A case of silicosis with MPO-ANCA-associated glomerulonephritis and alveolar hemorrhage. Nippon Jinzo Gakkai Shi 2001; 43: 351-356.
64. Sanchez-Roman J, Wichmann I, Salaberri J, Varela JM, Nunez-Roldan A. Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. Ann Rheum Dis 1993; 52: 534-538.
65. Calvert GM, Steenland K, Palu S. End-stage renal disease among silica-exposed gold miners. A new method for assessing incidence among epidemiologic cohorts. JAMA 1997; 277: 1219-1223.
66. Ng TP, Ng YL, Lee HS, Chia KS, Ong HY. A study of silica nephrotoxicity in exposed silicotic and non-silicotic workers. Brit J Ind Med 1992, 49: 35-37.
67. Boujemaa W, Lauwerys R, Bernard A. Early indicators of renal dysfunction in silicotic workers. Scand J Work Environ Health 1994; 20: 188-191.
68. Rosenman KD, Moore-Fuller M, Reilly MJ. Kidney disease and silicosis.
Nephron 2000; 85: 14-19.
69. Hotz P, Gonzalez-Lorenzo J, Siles E, Trujillano G, Lauwerys R, Bernard A. Subclinical signs of kidney dysfunction following short exposure to silica in the absence of silicosis. Nephron 1995; 70: 438-442.
70. Marsh GM, Enterline PE, Stone RA, Henderson VL. Mortality among a cohort of U.S. manmade mineral fiber workers: 1985 follow-up. J Occup Med 1985; 32: 594-604.
71. Goldsmith JR, Goldsmith DF. Fiberglass or silica exposure and increased nephritis or end-stage renal disease. Am J Ind Med 1993; 23: 873-881.

72. Steenland NK, Thun MJ, Ferguson CW, Port FK. Occupational and other exposures associated with male end-stage renal disease: a case/control study. Am J Public Health 1990; 80: 153-159.
73. Gregorini G, Ferioli A, Donato F, Tira P, Morassi L, Tardanico R, Lancini L, Maiorca R. Association between silica exposure and necrotizing crescentic glomerulonephritis with P-ANCA and anti-MPO antibodies: a hospital-based case-control study. Adv Exp Med Biol 1993; 336: 435-439.
74. Nuyts GD, Van Vlem E, De Vos A, Daelemans RA, Rorive G, Elseviers MM, Schurgers M, Segaert M, D'Haese PC, De Broe ME. Wegener granulomatosis is associated to exposure to silicon compounds: a case-control study. Nephrol Dial Transplant 1995; 10: 1162-1165.
75. Steenland K, Sanderson W, Calvert GM. Kidney disease and arthritis in a cohort study of workers exposed to silica. Epidemiology 2001; 12: 405-412
76. Stratta P, Messuerotti A, Canavese C, Coen M, Luccoli L, Bussolati B, Giordia L, Malavenda P, Cacciabu M, Bugiani M, Bo M, Ventura M, Camussi G, Fubini B. The role of metals in autoimmune vasculitis: epidemiological and pathogenic study. Sci Total Environ 2001; 270: 179-190.
76a. Hogan SL, Satterly KK, Dooley MA, Nachman PH, Jennette JC, Falk RJ. Silica exposure in anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and lupus nephritis. J Am Soc Nephrol 2001; 12: 134-142.
77. Hauglustaine, Van Damme B, Daenens P, Michielsen P. Silicon nephropathy: a possible occupational hazard. Nephron 1980; 26: 219-224.
78. Kallenberg CGM. Renal disease-another effect of silica exposure. Nephrol Dial Transplant 1995; 10: 1117-1119 (Editorial Comment).
79. Warheit DB, Driscoll KE, Oberdoerster G, Walker C, Kuschner M, Hesterberg TW. Symposium overview. Contemporary issues in fiber toxicology. Fund Appl Toxicol 1995; 25: 171-183.
80. Schainkin-Kerstenbaum R, Adler AJ, Berlyne GM. Inhibition of superoxide dismutase activity by silicon. J Trace Elem Electrolytes Health Dis 1990; 4: 97-99.
81. Fubini B, Bolis V, Giamell E, Volante M. Chemical functionalities at the broken fiber surface relatable to free radical production. In: Mechanisms in fibre carcinogenisis. Brown RC, editor. Plenum Press, New York 1991, p. -.
82. Fubini B. The possible role of surface chemistry in the toxicity of inhaled fibres. In: Fiber toxicology. Warheit DB (editor). Academic Press, San Diego 1993; p. 229-257.
83. Tervaert JWC, Stegeman CA, Kallenberg CGM. Silicon exposure and vasculitis. Current Opinion Rheumatol 1998; 10: 12-17.
84. Gilligan HM, Bredy B, Brady HR, Hebert MJ, Slayter HS, Xu Y, Rauch J, Shia MA, Koh JS, Levine JS. Antineutrophil cytoplasmatic autoantibodies interact with primary granule constituents on the surface of apoptotic neutrophils in the absence of neutrophil priming. J Exp Med 1996; 184: 2231-2241.
85. Iyer R, Hamilton RF, Li L, Holian A. Silica-induced apoptosis mediated via scavenger receptor in human alveolar macrophages. Toxicol Appl Pharmacol 1996; 141: 84-92.
86. Yang JJ, Tuttle R, Taylor JG, Jennette JC, Falk RJ. ANCA target antigens are not on the surface of normal neutrophils but are present on both primed and apoptotic neutrophils. J Am Soc Nephrol 1997; 8; 546 (abstract).
87. Ueki A, Yamaguchi M, Ueki H, Watanabe Y, Ohsawa G, Kinuguwa K, Kawakami Y, Hyodoh F. Polyclonal human T-cell activation by silicate *in vitro*. Immunology 1994; 82: 332-335.
88. Shiffenbauer J, Johnson H, Soos J. Superantigens in autoimmunity: their role as etiologic and therapeutic agents. In: Superantigens: Molecular biology, immunology, and relevance to human disease. Leung DYM, Huber BT, Schlievert PM (editors). Marcel Dekker, New York 1997; p. 525-549.
89. Elseviers MM, De Schepper A, Corthouts R, Bosmans JL, Cosyn L, Lins RL, Lornoy W, Matthys E, Roose R, Van Caesbroeck D, Waller I, Horackova M, Schwarz A, Svrcek P, Bonucchi D, Franek E, Morlans M, De Broe ME. High diagnostic performance of CT scan for analgesic nephropathy in patients with incipient to severe renal failure. Kidney Int 1995; 48: 1316-1323.
90. Perneger TV, Whelton PK, Klag MJ. Risk of kidney failure associated with the use of acetaminophen, aspirin and nonsteroidal antiinlammatory drugs. New Engl J Med 1994; 331: 1675-1679.
91. Fored CM, Ejerblad E, Lindblad P, Fryzek JP, Dickman PN, Signorello LB, Lipworth L, Elinder CG, Blot WJ, McLaughlin JK, Zack MM, Nyrén O. N Engl J Med 2001; 345: 1801-1808.
92. Abuelo JG. Renal failure caused by chemicals, foods, plants, animal venoms, and misuse of drugs. Arch Intern Med 1990; 150: 505-510.
93. Vanholder R, Colardyn F, De Reuck J, Praet M, Lameire N, Ringoir S. Diquat intoxication: report of two cases and review of the literature. Am J Med 1981; 70: 1267-1271.
94. Poisoning: toxicology, symptoms, treatments. Arena JM, Drew RH, editors. Charles C Thomas Publ, Springfield Ill 1983; p. 177-179.
95. Munk ZM, Nantel A. Acute lindane poisoning with development of muscle necrosis. Can Med Assoc J 1977; 117: 1050-1052.
96. Nash RG, Kearney PC, Maitlen JC, Sell CR, Fertig SN. Agricultural applicators exposure to 2, 4-Dichlorophenoxyacetic acid. In: Pesticide residues and exposure. Plimmer JR, editor. ACS symposium series #182, 1982, Chap 10: 119-132.

97. Kolmodin-Hedman B, Hoglund S, Akerblom M. Studies on phenoxy acid herbicides. I. Field study. Occupational exposure to phenoxy acid herbicides (MCPA, dichlorprop, mecoprop and 2, 4-D) in agriculture. Arch Toxicol 1983; 54(4): 257-265.
98. Kolmodin-Hedman B, Hoglund S, Swensson A, Akerblom M. Studies on phenoxy acid herbicides. II. Oral and dermal uptake and elimination in urine of MCPA in humans. Arch Toxicol 1983; 54(4): 267-273.
99. Libich S, To JC, Frank R, Sirons GJ. Occupational exposure of herbicide applicators to herbicides used along electric power transmission line right-of-way. Am Ind Hyg Assoc J 1984; 45: 56-62.
100. Manninen A, Kangas J, Klen T, Savolainen H. Exposure of finnish farm workers to phenoxy acid herbicides. Arch Envir Contam Toxicol 1986; 15: 107-111.
101. Kancir CB, Andersen C, Olesen AS. Marked hypocalcemia in a fatal poisoning with chlorinated phenoxy acid derivatives. Clin Toxicol 1988; 26: 257-264.
102. Berndt WO, Koschier F. *In vitro* uptake of 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) by renal cortical tissue of rabbits and rats. Toxicol Appl Pharmacol 1973; 26: 559-570.
103. Chugh KS, Singhal PC, Nath IVS, Pareek SK, Ubroi HS, Sarkar AK. Acute renal failure due to non-traumatic rhabdomyolysis. Postgrad Med J 1979; 55: 386-392.
104. Averbukh Z, Modai D, Leonov V, Weissgarten J. Rhabdomyolysis and acute renal failure induced by paraphenylenediamine. Hum Toxicol 1989; 8: 345-348.
105. Talbot AR, Shiaw MH, Huang JS, Yang SF, Goo TS, Wang SH, Chen CL, Sanford TR. Acute poisoning with a glyphosate-surfactant herbicide ('Round-up'): a review of 93 cases. Hum Exper Toxicol 1991; 10: 1-8.
106. De Broe ME, D'Haese PC, Nuyts GD, Elseviers MM. Occupational renal diseases. Current Opinion Nephrol Hypert 1996; 5: 114-121.

28

Lithium-induced renal effects

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Introduction

Since its introduction several decades ago for the occasional treatment of "psychotic excitement", lithium is still a mainstay in the treatment and prophylaxis of manic-depressive disorders [1]. The biologic basis for the clinical efficacy of lithium is not completely known. Interestingly, the agent relieves both mania and depression, states which appear to be opposites. Its therapeutic range, however, is narrow, and even at the lowest effective dosage, some unwanted side effects may occur [2]. Serum levels above 1.5 mEq/L often result in acute intoxication, which may be severe. The therapeutic range varies, depending on methodology, but it is advisable to target a level of about 0.5 mEq/L, with an upper range at 1.0 mEq/L. Values above this level signify a warning range of impending toxicity.

An equation to predict daily lithium dose has been recently suggested:

daily lithium carbonate dose (mg) =
100.5
+752.7 x desired lithium concentration (mEq/L)
– 3.6 x age (years)
+ 7.2 x weight (kg)
– 13.7 x blood urea nitrogen [BUN] (mg/dl) [3].

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Lithium is one of the smallest elements, between H and Na in the Periodic Table of elements, and is always ionized (Li^+) in watery solutions [1]. In living organisms it has strong pharmacologic and toxic activity. Lithium occurs in two isotopic forms of mass number 6 and 7, with natural abundances of 7.4 and 92.6% respectively [4]. Pharmaceutical lithium is prepared from the isotope mixture [4]. The smaller ^6Li has higher charge to mass and radius ratio. This can produce differences in the isotopes' electrostatic interactions with water molecules and negatively charged membranes. An increase in the rate of ^6Li transport, compared to ^7Li transport, across membranes is expected [4]. Accordingly, elimination or reduction of ^6Li from pharmaceutical preparations may merit further evaluation as a way to develop potentially less nephrotoxic form of lithium [4].

The red blood cell, which is a convenient model, shows a cell-to-plasma lithium ratio of 0.3-0.6, whereas the Nernst equation would predict a 1.6 ratio. When red blood cells are loaded with lithium *in vitro* its extrusion is accomplished by a Na^+/Li^+ countertransporter (SLC), the physiological role of which is unclear, but some believe it represents a mode of operation of the Na^+/H^+ exchanger. Interestingly, a recent paper suggested that red cell SLC may be a marker of the activity of Na^+/H^+ exchanger-3 the isoform expressed in the kidney proximal tubule rather than the ubiquitous Na^+/H^+ exchanger-1 isoform [5].

Because lithium is cleared from the body by the kidneys, its blood level at a given dosage depends critically on renal excretion, which is subject to various physiological and pathological influences. An insight into renal "lithium handling" is a prerequisite for effective prevention of complications and treatment of lithium intoxication when it occurs. Another reason why lithium is of interest to nephrologists is that its clearance has been used as a tool to investigate segmental tubular function [6-9].

With the widespread use of lithium in the treatment of affective disorders, many questions have centered on its long-term effect on the kidneys. Of particular interest is the action of lithium at distal nephron sites where it inhibits water transport, hydrogen secretion, and possibly potassium secretion as well [10, 11]. The most common side effect of chronic lithium therapy is an impairment of renal concentrating ability [11]. Lithium therapy is also associated with side effects related to hormonal alterations and changes in calcium metabolism.

Lithium transport along the nephron

Lithium is freely filtered by the glomeruli, whereas excretion into the urine is 20-30% of that amount [2]. Thus, at least 70% of the filtered load undergoes tubular reabsorption. Lithium clearance closely parallels changes in natrium delivery from the proximal tubule (Figure 1).

a. Proximal tubule. Early micropuncture studies reported Lithium concentration at the end of the convoluted proximal tubule to be close to unity [12]. Subsequent recent studies [13-19] using lower lithium plasma concentrations and more sensitive methods all found filtrate-to-plasma ratios to be definitely higher, the average value being 1.14. This value was not influenced by various manipulations such as natrium depletion, osmotic diuresis, prostaglandin inhibition, or infusion of acetazolamide, furosemide, or angiotensin [13, 14, 16]. Lithium can enter the cells via the Na^+/H^+ exchanger (Na^+/H^+ exchanger-1 isoform) but it is not

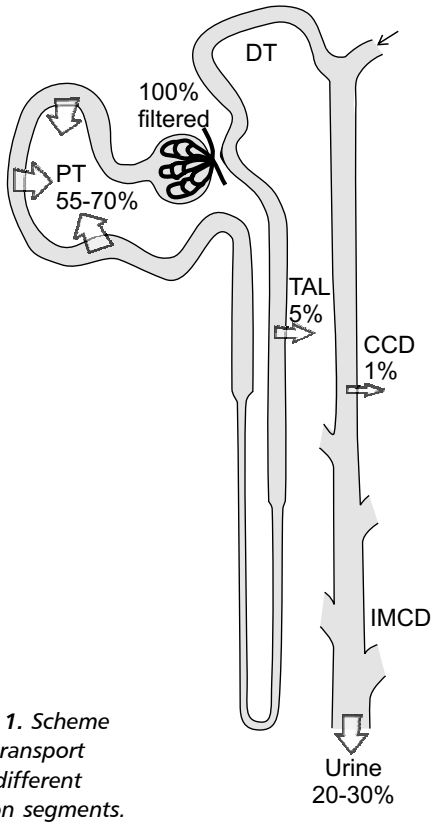

Figure 1. *Scheme of Li^+ transport along different nephron segments.*

clear how it may leave these cells. Although some possibilities in this regard have been suggested, the transcellular transport of lithium if it occurs at all is likely to be much less than that of natrium. Importantly, the remarkable constant fluid to blood ratio for lithium, despite large changes in proximal fluid reabsorption, suggests that lithium delivery from this part of the nephron, while systematically overestimating natrium delivery, can be considered a marker of proximal natrium reabsorption [13, 18]. It is generally believed that this also is true for the straight part of the proximal tubule, because paracellular transport in this part is even more important than in the convoluted tubules. However, because of their inaccessibility to micropuncture no direct evidence exists.

b. Henle's loop. Earlier investigations suggested that the amount of lithium arriving at the early distal tubule was the same as the amount calculated to enter the loop of Henle [12]. Recent studies, however, showed that the difference between the amounts of lithium reaching the late proximal and early distal tubule is about 25% of the filtered load [13-17]. This does not necessarily indicate that lithium is actively transported by the thick ascending limb. In the thick ascending limb of Henle's loop, a $Na^+/K^+/2Cl^-$ cotransporter is responsible for NaCl reabsorption. Lithium may substitute for natrium in isolated cell models including rabbit thick ascending limb cells. It has been shown that lithium can be reabsorbed through a paracellular pathway with an affinity 1.5 that of Cl^- and 65% that of Na^+ [20]. Moreover, some lithium might be reabsorbed through $Na^+/K^+/2Cl^-$ cotransporter and extruded at the basolateral site through a K^+/Cl^- cotransporter [20]. It is likely that transport also occurs in the highly lithium permeable thin descending limb along the osmotic gradient to the hyperosmolar inner medulla [21]. Indeed, studies using loop diuretics suggest that lithium is concentrated in the medulla to the same extent as natrium and this accumulation is largely abolished by such diuretics.

About 5% of the filtered lithium may be actively reabsorbed in the thick ascending limb [19]. This active "reabsorption" can increase to about 15% after prostaglandin inhibition [9, 17]. These observations suggest that lithium clearance cannot be used as a precise marker of proximal fluid reabsorption.

c. Distal tubule. Micropuncture studies have shown that urinary excretion of lithium is almost equal to the amount reaching the early distal tubule, indicating no further reabsorption beyond this point [12]. The permeability and transport characteristics of this "tight" epithelium also suggest no active or passive lithium transport. Thiazide diuretics whose action is confined to the distal tubule did not affect lithium clearance in a recent micropuncture study since identical amounts of lithium were found at the beginning and at the end of this segment [19].

d. Collecting duct. Although this is also a "tight" epithelium with high electrical resistance and low natrium permeability, there is evidence that lithium can be transported under certain conditions. In rats and dogs lithium clearance drops markedly on severe salt restriction (a reflection of enhanced proximal reabsorption) and increases after the diuretic amiloride, which acts in the cortical collecting tubule. This drug, though, does not affect lithium clearance on a normal diet [22, 23]. The limitation in reabsorption of Li^+ during Na^+-replete conditions may be either the cellular influx across the apical membrane or extrusion through the basolateral membrane making the rate of distal tubular Na^+ delivery a crucial determinant for reabsorption of Li^+. Under Na^+-depleted conditions, the apical membrane becomes hyperpolarized due to a low-Na^+ influx, and distal Li^+ reabsorption can occur [23]. However, an acute decrease in urine volume induced by antidiuretic hormone administration causes a definite but limited fall in lithium excretion particularly during a low sodium diet.

This effect likely results from enhanced reabsorption in the cortical collecting tubule.

In normal humans, values for fractional excretion of lithium varying from 19 to 38% have been reported [9]. These marked discrepancies are probably due mainly to variable experimental conditions such as natrium intake and urine flow rate. Of note also are the marked inter-individual differences in lithium clearance documented by Boer et al. [18, 24]. Fortunately, intra-individual variability is small. Thus, lithium clearance is still a sensitive tool to detect changes in tubular sodium handling for a given individual studied on different occasions. The limitations and advantages of this method have been discussed elsewhere [6-8, 14, 18]. The use of lithium clearance as a research tool to analyze segmental tubular sodium reabsorption has been controversial. Recent investigations, while refuting the claim that it is an absolute

measure of fluid delivery from the proximal tubule, have confirmed its value as a directional, non-quantitative marker of proximal fluid and sodium reabsorption [13, 18]. Indeed, in clinical practice it provides information which cannot be obtained by any other non-invasive method [13].

Effects of drugs on lithium excretion

As can be expected, many drugs that interfere with renal function also influence lithium excretion. This and other drug interactions are listed in Table 1.

a. Diuretics. All diuretics cause a negative natrium balance and various degrees of contraction of the extracellular volume. The extent to which this occurs is dependent on the dose of the drug and the level of sodium intake. Extracellular volume contraction caused by thiazide diuretics predictably decreases lithium clearance by increasing proximal reabsorption. For clinical purposes, there is an important risk of causing lithium intoxication when diuretics are administered to patients on maintenance lithium therapy, and they should be given under close control of serum lithium levels or avoided altogether. Loop diuretics (furosemide, ethacrynic acid, torsemide and bumetanide) all have a powerful enhancing effect on lithium excretion. At their usual dosage they double lithium clearance when given acutely [8, 25]. This has been ascribed to the combined effect of increasing glomerular filtration rate and decreasing proximal reabsorption. Their marked effect, however, clearly suggests inhibition of lithium reabsorption in the loop of Henle as well. Recent micropuncture studies [14, 16] indicate that they inhibit 10-12% of filtered lithium reabsorption in this region. If lost salt and water are replaced concurrently these drugs have a role for treating lithium intoxication. Because of their short duration of action, this will be followed by a period of lithium retention which is dependent on the frequency of the dosage. The net effect may be no change in 24-hour lithium excretion.

Acetazolamide, and probably other diuretics which inhibit carbonic anhydrase, cause a strong inhibition of proximal $NaHCO_3$ reabsorption and lithium reabsorption. However, unlike loop diuretics, acetazolamide does not interfere with tubuloglomerular feedback and causes a 20% decrease in glomerular filtration rate. The increase in absolute lithium excretion is somewhat lower than that caused by loop diuretics [22].

Table 1. Drug interactions with lithium.

Drug	Effect on serum lithium concentration
Thiazide diuretics	Increase
Acetazolamide and other carbonic anhydrase inhibitors	Decrease*
Osmotic diuretics	Decrease
K^+ sparing	Minimal decrease or no effect
Methyl xanthine inhibitors	Decrease
Loop diuretics	Decrease*
ACE inhibitors	Increase
NSAIDs:	
Indomethacin	Increase
Ibuprofen	Increase
Mefenamic acid	Increase
Naproxen	Increase
Sulindac	No effect
Asprin	No effect

** when given acutely for lithium intoxication*

Colussi et al. [25] reported the effect of furosemide and acetazolamide to be additive, indicating a dual site of action (i.e., inhibition of lithium reabsorption in both the proximal tubule and the loop of Henle).

Thiazide diuretics predictably increase serum lithium levels. However, they differ among themselves in that they may or may not have a carbonic anhydrase inhibitory action. Those which have such an activity, e.g. chlorothiazide, inhibit proximal reabsorption and thus may increase lithium excretion, at least when given acutely. Those devoid of such an effect (such as bendroflumethiazide) may not change lithium clearance [22]. Potassium-sparing drugs (spironolactone, triamterene and amiloride) have no obvious action on lithium excretion in humans. Amiloride enhances lithium excretion in rats and dogs only during sodium restriction under conditions, where natrium uptake by distal tubule is more abbit. In humans, however, no effect has been reported even when natrium retention was severe [12]. However, small changes in distal and cortical collecting tubule lithium reabsorption could easily be missed by clearance studies. Amiloride seems to prevent lithium uptake in the cortical collecting tubule [26].

b. Antihypertensives. Angiotensin II and noradrenaline infusions reduce lithium excretion [27]. Al-

though no systematic experimental studies or controlled clinical observations are available, lithium is known to activate the renin-angiotensin system through several mechanisms. This effect can be reversed by converting enzyme inhibitors. When given alone, however, converting enzyme inhibitors have little influence on lithium excretion. Anecdotal observations suggest that renal dysfunction may occur when unadjusted doses of angiotensin converting enzyme inhibitors are administered to patients on long-term lithium treatment [27]. Potential mechanisms involved in this are: a) Lithium causing volume loss and activation of RAS by interfering with H_2O reabsorption and to a lesser extent Na^+; b) Effects of lithium on cellular events including reduced specific angiotensin II binding, inhibition of sympathetic transmission and phosphatidylinositol signalling [27]. In addition, direct interactions between lithium and angiotensin II may take place at a cellular level.

Renal function should be closely monitored when patients on lithium treatment are given angiotensin converting enzyme inhibitors. Doses of both drugs should be chosen with caution to avoid serious drug interaction [27].

c. NSAIDs. These drugs, in particular indomethacin, have a depressing effect on lithium clearance which is enhanced by salt restriction [28]. Micropuncture studies showed that an additional 13% reabsorption of filtered lithium is caused by these drugs, probably half of it in the thick ascending limb, the rest in the thin limb of Henle [17]. Therefore, when drugs of this group are given to patients on lithium treatment, close control of blood levels is recommended.

d. Other drugs. Of some practical importance is the finding that cyclosporine A decreases lithium clearance. This likely reflects enhanced proximal fluid reabsorption secondary to vasoconstriction caused by this drug.

Other situations

a. Volume status. Salt intake is an important determinant of lithium excretion. Acute as well as chronic NaCl loading increases absolute as well as fractional lithium clearance, while salt restriction causes a marked decrease. Upright posture and tilt also cause a decrease in absolute as well as fractional lithium excretion, while head-out water immersion increases it.

These investigations of lithium handling by the kidneys have provided information which is useful for prevention and treatment of lithium intoxication. In general, all conditions associated with salt depletion strongly impair renal capacity to eliminate lithium.

Abnormal values of fractional lithium excretion have been reported in a variety of conditions. In hyperthyroidism and Bartter's syndrome fractional lithium clearance is increased. After unilateral nephrectomy, lithium clearance by the remaining kidney increases. After two weeks, fractional lithium clearance returns to normal. Rombola et al. [8] reported markedly increased fractional lithium clearance values in patients with Fanconi syndrome, renal glycosuria, and hypercalciuria.

b. Pregnancy. After investigations in animals suggested the potential of lithium to disrupt embryonic development, questions arose regarding the safety of lithium in human pregnancy [29]. These concerns emerged as data from anecdotal case descriptions and a registry of infants born to women treated with lithium during pregnancy indicated that such treatment might pose a substantial risk of cardiovascular anomalies. More recent controlled epidemiologic investigations demonstrate that most women who are treated with lithium during pregnancy have normal infants and that the risk to the fetus is less than previously believed. This more modest risk estimate may have a dramatic effect on clinical management of women with bipolar disorder, given the morbidity associated with discontinuation of lithium therapy [29].

Hormonal effects of lithium

It has been long recognized that prolonged lithium therapy can cause hypothyroidism. In fact, determination of serum thyroid-stimulating hormone once a year is recommended in all subjects on prolonged lithium therapy [30, 31]. Lithium perturbs receptor-mediated signaling events such as cyclic adenosine monophosphate and inositol phosphate accumulation [32]. These effects likely explain many hormonal side effects of lithium.

Lithium can increase the Ca^{2+} set-point for inhibition of parathyroid hormone secretion during both *in vitro* and *in vivo* studies [33]. The calcium receptor plays a central role in calcium sensing by the parathyroid gland and other organs, including the brain. Chronic

lithium therapy causes a significant alteration in calcium-sensing by the calcium receptor-expressing parathyroid chief cells through an unknown mechanism. As a result of this the parathyroid hormone set-point (the level of calcium that half-maximally suppresses parathyroid hormone secretion) is shifted to the right [33] (Figure 2). In other words, it takes a higher serum calcium concentration to inhibit parathyroid hormone secretion, a phenomenon known as a reset of the "set-point" [33, 34].

A causal relationship between lithium treatment and hyperparathyroidism has been suggested [35]. Lithium seems to induce morphological changes in the parathyroid glands with an increase in parathyroid volume, and an increase in cellular DNA synthesis [35-37], which may explain why its effects may not be completely reversible. It is not rare to find patients with hypercalcemia, usually mild, after discontinuation of prolonged lithium therapy. A number of cases have been reported where hypercalcemia and hypocalciuria persisted even after discontinuation. We also have seen persistence of hypercalcemia and hyperparathyroidism several years after discontinuation of lithium therapy [Batlle et al unpublished, 2000].

Most cases of lithium-induced hyperparathyroidism are mild [38]. Both pre-existing parathyroid abnormalities which may have been unmasked by lithium therapy and *de novo* hypercalcemia and hyperparathyroidism have been documented [39]. Parathyroid hyperplasia [33%] and adenomas [67%] were reported in one series of hypercalcemic patients treated with lithium [40]. Bilateral neck exploration has been proposed as an appropriate management approach because of a relatively high frequency of multi-gland involvement. However, parathyroid resection should be limited to evident disease [41].

Features of lithium-induced hyperparathyroidism include: a) a low urinary calcium excretion and the absence of nephrolithiasis; b) normal urinary cyclic adenosine monophosphate excretion; and c) normal plasma inorganic phosphate [30]. In lithium-induced hypercalcemia, a higher frequency of conduction defects has been noted [42]. Lithium also inhibits parathyroid hormone-mediated renal reabsorption of Ca^{2+} and Mg^{2+} and blunts parathyroid hormone–mediated phosphaturia [43]. Lithium interferes with the formation of renal cyclic adenosine monophosphate, which is regulated by parathyroid hormone. Levels of urinary cyclic adenosine monophosphate are typically normal in patients with elevated levels of parathyroid hormone related to lithium maintenance, rather than high, as in cases of primary hyperparathyroidism [30].

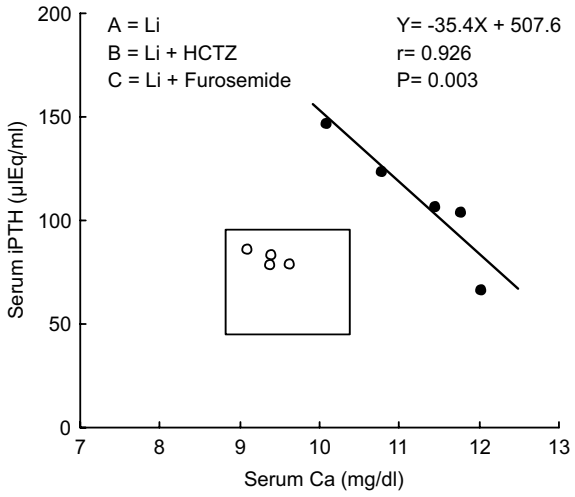

***Figure 2**. Inverse correlation between serum calcium concentration and serum immunoreactive parathyroid hormone (iPTH) level when patient was on lithium (Li) treatment (solid line). The square denotes the normal range for serum calcium and iPTH. Solid circles denote when patient was taking lithium and open circles when lithium had been withdrawn. HCTZ= hydrochlorothiazide. (Reproduced with permission from Shen et al. [33]).*

In lithium treated subjects, there is no evidence of reduced bone mass at any of the measured sites in relation to that of control subjects. The mechanism responsible for the maintenance of bone mass despite biochemical evidence of hyperparathyroidism is not clear [40]. We suspect that it is due to renal calcium retention. Indeed, in dogs lithium administration for only 3 days causes a striking decrease in urinary calcium excretion which is independent of the presence of parathyroid hormone and occurs despite the concurrent development of metabolic acidosis [Batlle D, Arruda J, and Kurtzman NA 1981 unpublished observations].

Effect of lithium on water transport

Overview of experimental studies

Polyuria is a common side effect associated with lithium use, and is often found in patients whose lev-

els are within the therapeutic range [10, 11]. The mechanism whereby lithium causes polyuria has been studied extensively in humans, experimental animals, and epithelial analogs of the mammalian collecting tubule [26, 44-54]. In the aggregate, these studies have provided compelling evidence for a direct inhibitory effect of lithium on arginine vasopressin-mediated water reabsorption by renal tubules.

In the toad urinary bladder, an experimental model of the mammalian collecting tubule, addition of lithium to the mucosal surface (but not to the serosal surface) markedly inhibited both basal and arginine vasopressin-stimulated water flow [45]. The concentration of mucosal lithium used in these studies (10 mEq/L) was comparable to or even lower than that usually found in the urine of patients on well-controlled lithium therapy (that is, 10 to 40 mEq/L) [55]. Fernandez et al. [48] confirmed the inhibitory effect of lithium on water flow in toad urinary bladders exposed only submaximal concentrations of arginine vasopressin. Inhibition of cyclic adenosine monophosphate-stimulated water flow when lithium (2 mEq/L) was applied to the serosal surface of the toad bladder was reported in one study. Such an effect of lithium, when applied to the serosal surface has, to our knowledge, not been found by any other investigators. As herein discussed, the bulk of evidence supports the notion that the action of lithium on water transport is the result of its cell uptake from the luminal (apical) surface of the collecting tubule.

The mechanism of the action of lithium on H_2O transport lies at some point along the arginine vasopressin-mediated transport process, either before or beyond the formation of cyclic adenosine monophosphate. Forrest et al. [46] suggested that lithium interference with the cellular action of arginine vasopressin occurs, in part, at a step beyond the formation of cyclic adenosine monophosphate. This was deduced from their findings that infusions of dibutyryl cyclic adenosine monophosphate to lithium-treated rats had only a marginal effect on urine osmolality. Another study in rats treated with intraperitoneal lithium injections suggested that lithium impairs the action of arginine vasopressin on water transport at steps both proximal and distal to the intracellular formation of cyclic adenosine monophosphate [47].

Cogan and Abramow [50] addressed this issue more directly. These authors showed that addition of lithium to the luminal perfusion solution of isolated cortical collecting tubules reduces the hydro-osmotic action of arginine vasopressin. This effect persisted after removal of lithium from the luminal fluid, which strongly suggests that its inhibitory effect on H_2O transport is exerted only after cell uptake from the luminal site. To determine whether the lithium-arginine vasopressin interaction affected the generation of cyclic adenosine monophosphate or the effect of this second messenger in the cell, these authors investigated the effect of lithium on the hydro-osmotic action of 8-Br-cyclic adenosine monophosphate, a derivative of cyclic adenosine monophosphate resistant to the action of phosphodiesterase. The hydro-osmotic action of 8-Br-cyclic adenosine monophosphate was not diminished by the presence of lithium in the lumen of cortical collecting tubules perfused *in vitro*. This finding strongly suggests that the inhibitory effect of lithium on the hydro-osmotic action of arginine vasopressin occurs at a step preceding the formation of cyclic adenosine monophosphate [50].

These *in vitro* findings were expanded by Christiensen et al. [51] using an experimental model that closely resembles the clinical setting of chronic lithium therapy. In this model, lithium was administered to rats for several weeks in conjunction with a NaCl drinking solution to prevent sodium depletion. At serum lithium levels within the accepted therapeutic range (0.7 to 1.5 mEq/L), marked polyuria and polydipsia developed after four weeks of lithium administration. Cortical collecting tubules isolated from lithium-treated rats displayed decreased ability to generate cyclic adenosine monophosphate *in vitro* in response to arginine vasopressin stimulation. In contrast, tubules from polyuric rats with hypothalamic diabetes insipidus (Brattleboro homozygotes) had intact arginine vasopressin-dependent cyclic adenosine monophosphate generation. The activity of cyclic adenosine monophosphate phosphodiesterase was not affected in lithium-treated rats. Hence, it seems that the main cellular effect of lithium involves impairment of arginine vasopressin-sensitive adenylate cyclase and that this results in impairment of intracellular cyclic adenosine monophosphate formation.

Other studies also support lithium's primary effect at the level of cyclic adenosine monophosphate generation [52, 53]. Though the greatest effect of arginine vasopressin is in the cortical collecting tubule, an argi-

nine vasopressin-mediated cyclic adenosine monophosphate response has been demonstrated throughout the distal nephron. Jackson, Edwards and Dousa [53] evaluated the effects of lithium on the medullary thick ascending limb and medullary collecting tubule. Isolated tubules perfused in a hyper-osmotic medium (800 mOsm) displayed a significant rise in arginine vasopressin-dependent cyclic adenosine monophosphate generation. In contrast, in tubules exposed to lithium there was a significant decrease in arginine vasopressin-dependent cyclic adenosine monophosphate formation in both the medullary ascending limb and medullary collecting tubule segments when compared to controls [53]. This study also showed a dose-dependent decrease in cyclic adenosine monophosphate levels with increasing lithium concentrations [53]. Thus, reduced arginine vasopressin-sensitive adenyl cyclase activity in the medullary collecting tubule of lithium-treated polyuric rats may contribute to the observed reduction in concentrating ability. In other work, micro-dissected medullary collecting tubules from rats chronically treated with lithium responded to pertussis toxin, an inhibitor of inhibitory GTP binding protein, with an increase in arginine vasopressin dependent cyclic adenosine monophosphate production similar to that seen in control rats [54]. This finding suggests that lithium may inhibit arginine vasopressin dependent cyclic adenosine monophosphate formation by activation of inhibitory GTP binding protein [54].

Renal urinary concentration is associated with enhanced expression of rBSC1, a rat sodium cotransporter, in the thick ascending limb of Henle. In two recent studies by Kwon et al [54a] and Michimata et al [54b] dehydration or high plasma AVP resulted in an enhanced expression of rBSC1 in rats with lithium induced nephrogenic diabetes insipidus . rBSC1 expression was closely associated with the adverse effects of Li ions on collecting duct function [54a, 54b].

Role of aquaporin-2

In recent years our understanding of the mechanism of H_2O transport and concentrating ability has widened with the discovery of aquaporin-2. The gene for aquaporin-2 [AQP2], a member of the aquaporin family of water channels, has been identified and sequenced [55-57]. Several studies have shown that AQP2 is the predominant vasopressin-sensitive water channel of the renal collecting duct [55-57]. AQP2 is selectively localized in the collecting duct principal cells, mainly in the apical plasma membrane and intracellular vesicles. Vasopressin acutely increases the water permeability of the collecting ducts by stimulating insertion of AQP2 water channels from intracellular vesicles into the apical plasma membrane [58]. Normally, the expression of AQP2 was increased by dehydration or chronic vasopressin administration, providing a long-term regulatory mechanism in antidiuresis. Mutations of AQP2 can result in severe nephrogenic diabetes insipidus, thus demonstrating that AQP2 is necessary for urinary concentrating ability [58].

Chronic lithium administration to rats results in marked downregulation of AQP2 expression in medullary collecting ducts, parallel with the development of severe polyuria [58]. This effect of lithium therapy on AQP2 expression is only partially reversed by thirsting, intravenous 1-desamino-8-D-arginine-vasopressin [dDAVP], or return to a lithium-free diet [58]. After a one-week cessation of lithium administration, AQP2 was only partly reversed, consistent with the clinical findings of slow recovery of concentrating ability after lithium therapy [58].

In contrast to the process of spontaneous recovery after downregulation of AQP2 expression, which appears to be a slow process, upregulation in response to a stimulus such as thirsting occurs within 1-2 days despite continued lithium treatment [58]. The level of AQP2 expression was still below control levels, presumably reflecting a continued effect of lithium. It is to be noted that thirsting induces a considerably greater increase in expression of AQP2 than with dDAVP, but fails to induce significant redistribution of AQP2. This raises the possibility of a differing mechanism involved in water channel delivery to the plasma membrane during thirsting and dDAVP administration [56, 58] (Figure 3).

In rats treated with lithium, dDAVP, a specific V2 agonist, produced an increase in urine osmolality associated with increased apical plasma membrane AQP2 labeling. The action of dDAVP was likely due, at least in part, to its ability to overcome the block of adenylate cyclase caused by lithium. This may cause relocation of AQP2 to the apical part of the cell, and presumably induction of water channel insertion into the plasma membrane may be restored [55, 58]. The stimulatory effect of dDVAP in the presence of lithium, to

our knowledge, has not been shown to be clinically useful for treating the polyuria associated with long-term lithium therapy.

Clinical data

An early study by Forrest et al. [46] demonstrated a significant decline in maximal urinary osmolality (from 1,110 to 854 mOsm/kg H_2O) in ten patients studied before and after only eight weeks of lithium therapy. Since urine osmolalities around 800 mOsm/kg H_2O after fluid deprivation are in the low range of normal, this investigation also demonstrated the importance of having information on urinary osmolality prior to lithium use. A subtle but significant decrement in concentrating ability caused by lithium could otherwise go unnoticed. This early and mild impairment in concentrating ability appears to be, at least in part, a functional defect caused by the temporal exposure of distal tubular cells to lithium [10].

The prevalence of polyuria among unselected lithium-treated patients has been difficult to ascertain [11]. Polyuria, as defined by a 24-hour urine output exceeding 3 L, varies considerably among patients on chronic lithium therapy [59-72]. In a review of a total of 841 unselected patients evaluated for 24-hour urine volume, we found that 160 (or 19%) had polyuria as defined by these criteria [11]. It was found that 85% of lithium treated patients have a normal glomerular filtration rate and that the remaining 15% have only mild reductions in renal function [11]. After fluid deprivation of approximately 24-hours duration, normal individuals should be able to raise urinary osmolality above 800 mOsm/kg H_2O. In a survey of a total of 1,105 lithium-treated patients [61-66], we found that at least 602 (or 54%) had a subnormal concentrating ability de-

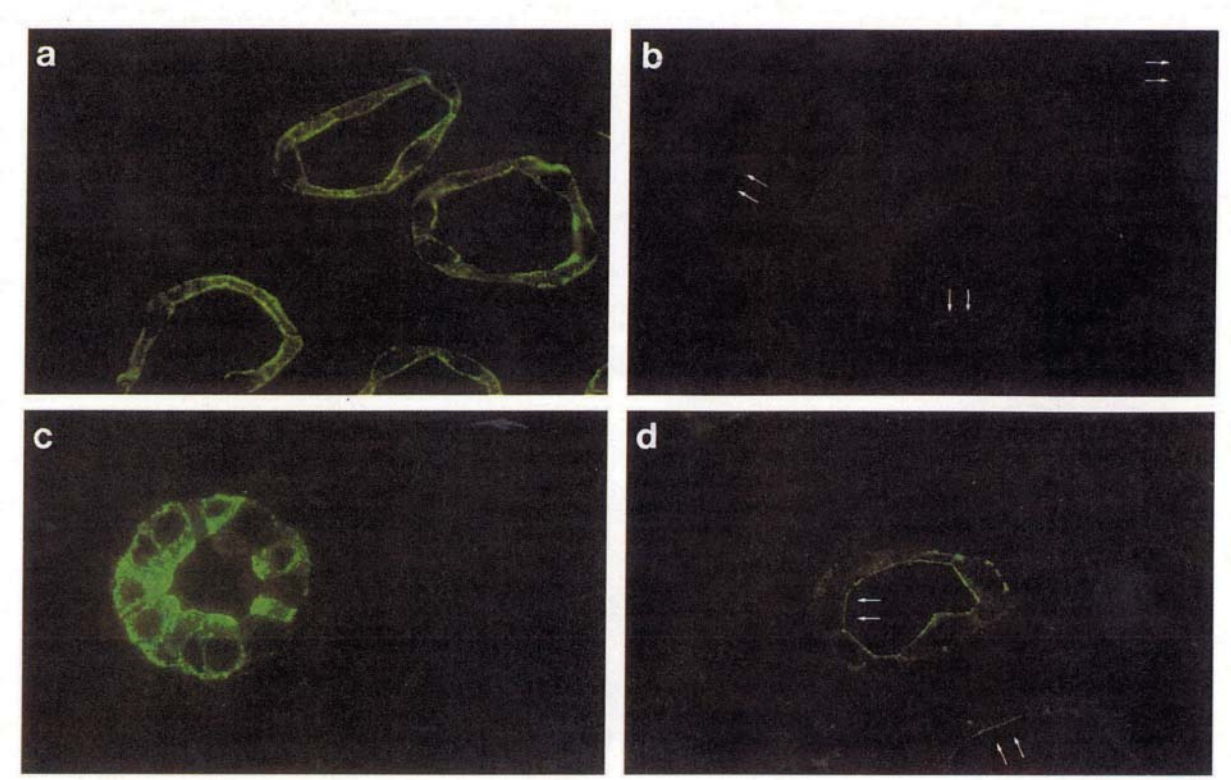

***Figure 3**. Immunofluorescent localization of AQP2 in cryosections of kidney inner medulla. (**a**) In control rats, collecting ducts labeled prominently with antibodies against AQP2. No other structures were labeled. (**b**) After lithium treatment, only traces of labeling remained. (**c**) Thirsting for 48 h in the continued presence of lithium increased expression relative to lithium alone, but levels are still lower than seen in controls. Labeling was widely distributed throughout the cells. (**d**) After 7d of dDAVP treatment in the continued presence of lithium, there was some increase in labeling, which was concentrated very close to the apical surface of the cells. x840. (reproduced with permission from Nielsen* et al. *[56]).*

fined by this criterion [11].

The impairment of concentrating ability was reported to be mild or moderate in many studies [64, 66-69]. However, maximal urine osmolalities below 400 mOsm/kg H_2O were not infrequent [64]. Difficulties in completing 24-hour urine collections may also explain the relatively low prevalence of urine outputs exceeding 3 L/24-hours in relation to the high prevalence of reduced concentrating ability disclosed by urine osmolality measurements. Nocturia, an indirect but useful marker of polyuria, is a frequent complaint among lithium-treated patients [11]. For instance, of 153 lithium-treated patients, 105 (or 68%) reported at least one urination per night. Of these 105 subjects, 50 reported one urination, 35 reported two urinations, and the remaining 20 reported more than two urinations per night [62]. Several studies have shown that impairment of concentrating ability directly correlates with the duration of lithium therapy [59, 60, 64-67].

Persistence of a concentrating defect despite the discontinuation of lithium therapy is common [73-78]. In a study including 87 patients, maximal urine osmolality measured 8 weeks after discontinuation of lithium increased only slightly (from 517 ± 197 to 658 ± 203 mOsm/kg H_2O) [79]. These investigators also described a persistent defect in concentrating ability (urinary osmolality 800 mOsm/kg H_2O) in 17 of 27 patients who were studied one year after discontinuation of lithium. Although concentrating ability improved significantly during the first two months after lithium was stopped, there was no further improvement thereafter [79]. Persistence of nephrogenic diabetes insipidus following the discontinuation of lithium has been associated with renal biopsy findings consistent with chronic interstitial nephritis. The impairment in concentrating ability which is evident shortly after initiation of lithium therapy is usually mild and reversible [11]. Over the course of long-term therapy, the impairment in concentrating ability may be progressive and non-reversible as it is caused by structural tubulo-interstitial damage [11].

Treatment of lithium-induced polyuria

The management of lithium-induced polyuria includes the use of either thiazide diuretics alone, amiloride alone, or a combination of both. The use of thiazide diuretics to treat the polyuria induced by lithium therapy has had the problems of potentiating overt lithium toxicity by contracting the extracellular space, thereby causing compensatory proximal reabsorption of sodium and lithium. In the toad urinary bladder, an epithelium that transports water in a manner analogous to that of the mammalian collecting duct, amiloride blocks the entry of lithium across the apical surface, much as it does that of sodium [45]. Importantly, the addition of this agent to the mucosal side of this membrane markedly diminishes the inhibitory effect of lithium on arginine vasopressin-mediated water transport.

We utilized the above principle to treat lithium-induced polyuria [26]. This reduction in urine output could not be ascribed to increased proximal fluid reabsorption and decreased delivery of fluid to the distal nephron as a result of the volume contraction caused by amiloride. Fractional lithium excretion, a marker of proximal sodium reabsorption, did not fall during amiloride treatment, arguing against volume contraction induced by amiloride as possible mechanism [26]. In lithium treated patients, urinary osmolality increased when treated with amiloride (Figure 4). Amiloride at-

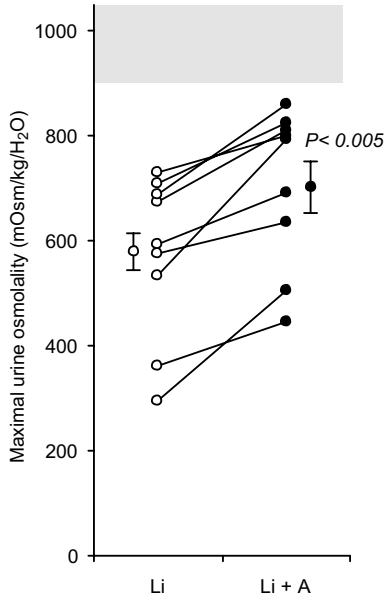

***Figure 4**. Maximal urine osmolality after fluid deprivation and vasopressin administration during (closed circles) and before (open circles) amiloride administration. The shaded area shows the range of urine osmolality in normal subjects tested after fluid deprivation and vasopressin administration in our laboratory. Li denotes lithium and Li +A lithium plus amiloride (reproduced from Batlle et al [26]).*

tenuates the inhibitory effect of lithium on vasopressin-mediated water reabsorption [26].

In cases where sufficient tubulointerstitial damage causing impaired concentrating ability has occurred, amiloride is less effective; still, it can be used in combination with a thiazide diuretic to reduce polyuria [2]. Moreover, hypokalemia, a common side effect of thiazides, is not observed with amiloride [26]. Amiloride obviates the need for potassium supplementation, which is required when thiazide diuretics are used to treat polyuria and, in addition, is less likely to cause lithium intoxication. Although both lithium and amiloride interfere with distal urinary acidification, the development of metabolic acidosis is uncommon with therapeutic dosages of either drug. Further, no significant change in plasma bicarbonate levels was noted when given in combination [26]. This suggests that the suppressive effect of these two agents on distal acidification is not additive.

Effect of lithium on distal nephron acidification

The administration of large doses of lithium to experimental animals consistently results in hyperchloremic metabolic acidosis and inability to normally lower urine pH, features that are characteristic of the distal form of renal tubular acidosis [80, 81]. In humans on well-controlled lithium therapy, however, metabolic acidosis usually does not ensue. None of the 14 patients studied by us had metabolic acidosis, and they were all able to lower urine pH below 5.5 after the administration of ammonium chloride for three consecutive days [82]. The capacity of the collecting tubule to secrete hydrogen ions was examined further using the urine PCO_2 in a highly alkaline urine as an index of urinary acidification. Urine PCO_2 examined as a function of urine bicarbonate concentration was found to be reduced in lithium-treated individuals. This defect was apparent in patients with near normal and in those with frankly reduced urine osmolality [25]. The findings of a reduced urine PCO_2 was taken as evidence that lithium administration, at therapeutic doses, causes a mild distal acidification detect. Since metabolic acidosis did not spontaneously develop in these patients, it was proposed that lithium therapy results in a variant of incomplete distal renal tubular acidosis [82].

In the turtle bladder, mucosal lithium inhibits hydrogen ion secretion by reducing the transepithelial electrical potential [83]. Laski and Kurtzman [84] investigated the effect of lithium on bicarbonate transport and transepithelial voltage in both the cortical collecting tubule and medullary collecting tubule. Acidification in the cortical collecting tubule is facilitated by the active reabsorption of sodium, which results in the formation of a transtubular voltage (lumen negative). When sodium reabsorption is inhibited acidification declines, and vice versa. If, however, sodium reabsorption is inhibited while the lumen is kept at a constant negative potential, acidification proceeds. This effect is seen in the cortical collecting tubule but not in the medullary collecting tubule where acidification occurs without active sodium absorption [84]. When lithium is substituted for sodium in the bath of isolated rabbit cortical collecting tubules, the potential difference decreases. This effect is associated with a reduction in the rate of bicarbonate (TCO_2) transport. In contrast, neither an effect on potential difference nor TCO_2 transport is seen in medullary collecting tubules exposed to lithium. Thus, this study demonstrated a nephron segment-specific site of action for lithium within the collecting tubule [84]. In a more recent study, Kurtzman's group has shown that lithium administration to rats also inhibits H^+/K^+ ATPase activity in the cortical but not in the medullary collecting tubule [85].

Potassium balance

The effects of lithium on potassium balance have not been well characterized. Galla et al. [86] suggested that distal potassium secretion is impaired in rats given lithium to produce high serum levels (2 to 5 mEq/L). We found similar results under acute potassium loading conditions [87]. Prior to potassium loading, however, potassium excretion was higher in lithium-treated rats than in controls. These two findings can be explained by postulating that potassium reabsorption in the proximal tubule is inhibited by lithium, while potassium secretion in the distal nephron is impaired. These two effects may offset each other so that plasma potassium does not need to change under ordinary conditions. The inhibitory effect of lithium on distal potassium secretion is likely to occur in the cortical collecting tubule where it decreases Na^+ uptake and the transepithelial voltage (lumen-negative) that normally

favors potassium secretion.

Usually neither hyperkalemia nor hypokalemia pose a problem for the management of lithium treated patients. However, in lithium-treated subjects given thiazide diuretic, hypokalemia often develops [88]. This is due to the diuretic-induced increase in sodium delivery to the collecting tubule combined with the lithium-induced increase in urine flow.

Renal histological findings

Kidney biopsy findings consistent with chronic interstitial nephritis were first described by Hestbech et al. [89] in 14 patients who were selected on the basis of impaired concentrating ability or previous episodes of lithium intoxication. Renal function as judged by plasma creatinine was normal in all but one patient. The abnormalities described in this study included tubular atrophy, cortical and medullary fibrosis, sclerotic glomeruli, tubular dilatation, and cyst formation. Similar lesions were subsequently described by the same investigators in patients selected for biopsy on the basis of either impaired concentrating ability or reduced glomerular filtration rate [90] and confirmed by other investigators [59, 60, 91, 92]. A significant positive correlation between the duration of lithium therapy and the extent of tubular atrophy and interstitial fibrosis has been noted by other investigators [85, 88]. One of the most consistent findings observed in biopsies from lithium-treated patients is microcyst formation and distal tubular dilation [59, 90-94].

An important issue is whether this type of tubulointerstitial lesions develop in individuals not selected on the basis of abnormal clinical findings. In a group of 23 nonselected lithium-treated patients reported by Jorgensen et al. [72], focal interstitial fibrosis and glomerular sclerosis were found. These changes were more pronounced than those found in a ''control'' group of patients who had been biopsied for either acute oliguria or proteinuria. However, tubular atrophy and total fibrosis did not differ between the two groups. Rafaelson et al. [95] performed biopsies in 37 randomly chosen lithium-treated patients. Analysis of their data reveals that in nine of 12 patients who had polyuria (>3 L/d), kidney biopsies disclosed either borderline or advanced tubulointerstitial changes. Renal biopsies were normal in most patients who were not polyuric (18 of the remaining 25 patients). These observations suggest that the impairment of concentrating ability has, at least in part, a structural basis. The impairment in concentrating ability, which is evident shortly after initiation of lithium therapy, is usually mild and probably reversible. Over the course of long-term therapy, the impairment in concentrating ability may be progressive and related to structural tubulointerstitial alterations as discussed below.

In a study of 24 biopsies performed on bipolar disorder patients with lithium–associated chronic tubulointerstitial nephropathy was characterized by tubular atrophy and interstitial fibrosis, typically out of proportion to the extent of glomerular or vascular disease with tubular cysts in 62.5% and lesser degrees of tubular dilatation in additional 33% of biopsies [92]. All these patients had serum creatinine of >2.5 at time of biopsy. Another important but unexplained finding was that lesions of FSGS were present in 50% of their biopsies, with a strong correlation between the FSGS lesions and presence of proteinuria >1.0 g/d [92]. This could indicate a potential direct glomerular toxicity of lithium. The presence of significant foot process fusion in some cases and recurrence of FSGS in the single transplant patient in whom lithium was not withdrawn lend further support to the above hypothesis. By cox regression analysis, it was found that a serum creatinine > 2.5 at the time of biopsy was a reliable predictor of progression to end-stage renal disease, even on discontinuation of lithium in 42% of patients [92]. Immunohistochemical and lectin staining revealed tubular cysts of predominantly distal tubular and collecting duct origin and rare cysts of proximal propagation of the distal portions of the cysts of the nephron [92].

The specificity of the chronic tubulointerstitial changes ascribed to lithium administration has been rightfully questioned because similar lesions have been described in psychiatric patients not receiving lithium [59, 96-98]. Walker et al. [59] compared biopsies from 47 patients treated with lithium for an average duration of 5 years to 32 patients with affective disorders who had never been treated with lithium. Using a semiquantitative analysis, they found no difference in interstitial fibrosis between the two groups even though the lithium group had a significantly lower glomerular filtration rate. Therefore, psychiatric patients, with or without the use of lithium, may develop chronic tubulointerstitial changes as compared to healthy controls. This may explain the reduced concentrating abil-

ity demonstrated in many psychiatric patients not receiving lithium therapy [65, 66, 99, 100].

Of interest is the finding of a lesion confined to the distal nephron that has been described in patients taking lithium but not in psychiatric controls [59, 94-97]. Several studies from Australia have reported this lesion, which appears to involve the distal convoluted tubules and collecting ducts of lithium-treated patients [94-98]. Their findings include cytoplasmic swelling with the accumulation of glycogen deposits, dilated tubules, and microcyst formation. The lesion appears to be specific for lithium in that a similarly localized accumulation of glycogen has not been found in kidney biopsy material obtained from either psychiatric patients who have never taken lithium or from normal subjects donating a kidney for transplant [97]. Walker et al. [93] described a similar lesion in rabbits and speculated that the accumulation of glycogen in the distal nephron might be related to decreased intracellular formation of cyclic adenosine monophosphate by lithium. If this hypothesis were true, one could anticipate that the lesion should be reversible once cyclic adenosine monophosphate formation normalized after removal of lithium from distal tubular cells. Various studies have shown that the distinctive distal tubular lesion ascribed to lithium therapy appears very early after therapy is started and is reversible [97-99]. Burrows et al. [99] observed this lesion in two patients who had been on lithium for less than a year. Renal biopsies from patients who had discontinued lithium for 2 months to 5 years prior to biopsy did not show this type of distal tubular lesion [98].

In a study using rabbits, renal biopsies were performed at 1, 3, 6 and 12 months of lithium administration [93]. Cytoplasmic vacuolization and glycogen accumulation in cells lining distal convoluted tubules and collecting ducts was found. Thus, lithium induces a tubular lesion in rabbits which resembles the lesion described in humans. McAuliffe et al. [100] examined kidney specimens from rats given lithium for 7 weeks whose lithium levels were within the therapeutic range. They found glycogen deposits, cellular edema, and cellular detachment from type I basement membrane in cells lining the collecting tubule. The aggregate of these observations suggests that this specific lesion associated with lithium is manifested functionally by inhibition of H_2O transport in the collecting tubule, appears very early in therapy, and is likely to be reversible.

In a recent study using male Wistar rats fed lithium containing diet for 16 weeks postnatally, a marked decrease in glomerular volume was found [100a]. This was not associated with detectable changes in structural parameters. Moreover, no effect of ACE inhibitor treatment could be demonstrated on glomerular volume [100a].

Effect of chronic lithium therapy on glomerular filtration rate

In rats with lithium-induced tubulo-interstitial damage, a rise in plasma urea levels after 16 weeks of treatment has been demonstrated even though plasma lithium levels were in the accepted therapeutic range for humans with mood disorders [101]. In contrast to this finding in rats, progression of the chronic tubulo-interstitial lesion towards renal insufficiency is unusual in humans.

In our analysis of glomerular filtration rate data available from reports published up to 1986 we found only minor changes in glomerular filtration rate despite prolonged lithium therapy [11]. The majority of studies used endogenous creatinine clearance as a marker of glomerular filtration rate. Of 491 patients investigated using this method, 78 (or 15%) had a somewhat reduced glomerular filtration rate [64, 68, 71, 102-103]. In one study, glomerular filtration rate measured by the EDTA clearance method was found to be reduced in 39 of 179 patients (or 22%) [66]. A study involving 153 patients revealed that 31 patients (20%) had an EDTA clearance below the 95th percentile confidence limits corrected for age and sex [62]. Combined analysis of data from six studies using EDTA clearance showed that glomerular filtration rate was reduced in 92 of 538 patients (17%) [61, 62, 66, 72, 104, 105]. Of a total of 1,172 patients in whom glomerular filtration rate was measured by different methods we found it to be reduced in only 15% [11].

The overall prevalence (15%) of reduced glomerular filtration rate among unselected lithium-treated patients probably overestimates the proportion of patients in whom such reduction can be ascribed to lithium [11]. First, a sizeable number of patients had prior episodes of lithium intoxication [68, 72, 103-105]. A reduced glomerular filtration rate could be related to factors other than lithium, such as the common use of other psychotropic drugs. The latter possibility is suggested

from studies that found tubulo-interstitial damage in psychiatric patients taking drugs other than lithium [87].

Of particular importance is the level of glomerular filtration rate among the patients in whom it was felt to be reduced. The distribution of glomerular filtration rates in the large series of patients studied by Wallin et al. [65] and Lokkegaard et al. [62] revealed that the reduction of glomerular filtration rate in lithium populations, when present, is very moderate. If lithium therapy were to result in lowering of glomerular filtration rate, it would be expected that there would be a progressive decline with the continuation of lithium therapy. A significant correlation between reduced glomerular filtration rate and the duration of therapy has not been found in the majority of studies that addressed this issue [59, 61, 67, 70, 100, 104, 106]. A significant but weak correlation (r= 0.29) between glomerular filtration rate and time on lithium was found among 231 patients on lithium for an average of 6.5 years [66]. Lokkegaard et al. [62] studied 153 patients treated for a mean duration of 10 years, a substantially longer period than all previous studies. A significant but also weak correlation between declining EDTA clearances and the duration of treatment was also found by these authors (r= 0.29).

While a review of the information available up to 1986 argues against a major effect of lithium in reducing glomerular filtration rate in most patients, there are reports of chronic renal impairment occurring after many years of lithium administration [107-109]. Moreover, cases of end stage renal disease presumably secondary to lithium therapy have been reported [110-111]. It seems likely that at least in some susceptible individuals, prolonged lithium administration results in chronic renal failure, although this is relatively rare. In such patients, after discontinuation of lithium therapy the renal function often stabilizes or deteriorates very slowly, consistent with other kinds of chronic interstitial nephritis.

Clinical side-effects of lithium intoxication

Symptoms of lithium toxicity can be expected when serum lithium level increases above 1.5 mEq/L. Most patients receiving lithium have side effects, reflecting the drug's narrow therapeutic index [1]. Many symptoms and signs of toxicity correlate with serum lithium concentrations (Table 2). The amount of lithium inside the cells, however, may be more predictable for lithium toxicity. Equilibration between intra- and extracellular lithium occurs rather slowly. Therefore intoxication develops more easily during chronic therapy, while after an acute high intake symptoms may be less despite higher serum levels.

Typical symptoms of lithium intoxication are summarized in Table 2 [112-117]. The clinical picture of lithium intoxication is dominated by neuromuscular and cerebral symptoms: in mild cases apathy, muscle weakness, tremor, and unsteady gait are seen. In more severe cases speech disturbances, myoclonic twitching, coma and convulsion can occur. Pulse irregularities and circulatory collapse may supervene. Lithium often causes T-wave flattening or inversion on the electrocardiogram, but clinically important cardiovascular effects are rare, with sinus-node dysfunction reported most often [113]. Residual neurological sequelae consisting of cerebellar dysfunction with ataxia, neuropathy and supra-bulbar symptoms are not unusual.

As discussed above, lithium inhibits the synthesis of thyroid hormone and its release from the thyroid, and stimulates the formation of antithyroid antibodies in susceptible subjects [112]. Lithium-induced hypothyroidism responds to thyroxine therapy. Lithium can increase the secretion of parathyroid hormone and therefore can increase serum calcium concentrations, but symptomatic hypercalcemia is rare (see hormonal effects).

Acute renal insufficiency with or without oliguria can occur, usually in association with severe volume depletion in, which case renal function is rapidly restored with appropriate fluid therapy. The picture may resemble that of acute tubular necrosis but prerenal failure seems more likely. Histological biopsy findings show remarkably few abnormalities.

Lithium poisoning can be categorized into two groups:

(1) Acute poisoning, in patients who are not actually being treated for lithium, but have obtained the medication either voluntarily (e.g. suicide attempts) or involuntarily (e.g. accidental childhood mishaps). Usually, these patients have an excellent prognosis as a result of normal baseline renal functioning, hence not interfering with the renal elimination of lithium.

Table 2. Clinical symptomatology associated with lithium poisoning.

Organ System	Acute Poisoning	Chronic Poisoning
Endocrine	None	Hypothyroidism, Hyperparathyroidism
Gastrointestinal	Nausea, vomiting	Minimal
Heart	Prolonged QT interval, ST and T wave changes	Myocarditis
Hematologic	Leukocytosis	Aplastic anemia
Neurologic		
a. Mild	Fine tremor, lightheadedness, weakness	Same
b. Moderate	Apathy, drowsiness, hyper-reflexia, muscle twitching, slurred speech, tinnitus	Same
c. Severe	Choreoathetoid movements, clonus, coma, confusion, muscle irritability, seizures	Memory deficits, Parkinson's disease, pseudo-tumor cerebri, psychosis
Neuromuscular	Myopathy, peripheral neuro-pathy	Same
Renal	Urine concentrating defect	Chronic interstitial nephritis, nephrogenic diabetes insipidus, end stage renal disease[rare]
Skin	None	Dermatitis, localized edema, ulcers

(2) Acute on top of chronic poisoning occurs in individuals who have been on a chronic lithium prescription, and who in one way or another ingest an overdose of the lithium, or are given medications that increase lithium levels. Conditions where sodium conservation is stimulated, such as low salt intake, loss of body fluid by way of vomiting, diarrhea, or use of diuretics which decrease lithium clearance (thiazides) are usually the precipitating factors.

The polyuria which often accompanies lithium treatment is normally compensated for by drinking water, but when consciousness is impaired severe hypernatremia may develop. When any acute illness (particularly if associated with gastrointestinal symptoms) occurs or when new medication is given, lithium blood levels should be closely monitored, and the lithium dose adjusted.

Management of lithium toxicity

Regardless of the manner of presentation, the ini-

Table 3. Management of lithium intoxication.

1. Protect oral airway if consciousness is impaired
2. Volume resuscitation
3. Gastric lavage, whole bowel irrigation with polyethylene glycol to prevent continued absorption of lithium
4. Lithium removal:
   - Serum lithium level >3.5-4 mEq/L: Most patients require hemodialysis.
   - Serum lithium levels 2-4 mEq/L: Unstable patients and patients with severe nephrologic signs of renal insufficieny require hemodialysis.
   - Serum lithium levels 1.5-2.5 mEq/L:
     - o Hemodialysis indicated for patients with renal failure or if patients fail to reach a lithium level below 1 mEq/L.
     - o Fluid therapy or forced diuresis treatment should be recommended in patients with early signs of lithium intoxication and normal renal function, and when it is known that lithium levels have been elevated for only a few days.

tial management is the same. If the patient presents with mental status changes (e.g. decreased consciousness), an oral airway must be secured in the immediate instance. Volume status should be assessed and isotonic saline administered for volume repletion. A serum lithium level and a serum chemistry panel (serum sodium, potassium, chloride, CO_2, BUN and creatinine, and calcium) should be drawn immediately to assess the degree or level of intoxication, as well as underlying renal function.

Volume resuscitation is the cornerstone of management of lithium toxicity (Table 3) [114, 115]. Patients with underlying lithium-induced diabetes insipidus may initially present with volume depletion. It must be borne in mind, however, that hypernatremia [115] is a potential complication, especially in those with underlying diabetes insipidus. Forced saline diuresis is expected to increase lithium clearance by decreasing proximal tubular reabsorption. With normal renal function, lithium can be cleared at a rate of 10-40 mL/min [115]. The excretion of lithium can be further increased acutely by using acetazolamide and/or loop diuretics [114, 115].

Peritoneal dialysis clears only 9-15 mL/min of lithium, and is therefore not recommended for the treatment of acute lithium toxicity [115, 116]. Conventional hemodialysis, on the other hand, decreases serum lithium levels at a rate of 1 mEq/L with every 4 hours of treatment [116]. Several treatment sessions of hemodialysis may be required, and serum lithium levels need to be checked frequently even after hemodialysis, because of the shifting of lithium from inside the cells (lithium rebound phenomenon). In those patients who may have ingested the sustained-release form of lithium, continued absorption from the GI tract may cause a rise in serum lithium levels between hemodialysis sessions [118].

Continuous arteriovenous hemodialysis and continuous venovenous hemodialysis can clear 60-85 L per day of lithium [119]. The main advantage of this treatment is that it decreases chances of lithium rebound. The disadvantages pertain to the fact that such continuous therapies do not reduce levels as quickly as hemodialysis and are often limited by the need for anticoagulation.

References

1. Price LH, Heininger GR. Lithium in the treatment of mood disorders. N Engl J Med 1994; 331: 591-598.
2. Batlle D, Dorhout-Mees EJ. Lithium and the kidney. In: Clinical Nephrotoxins - renal injury from drugs and chemicals. De Broe ME, Porter GA, Bennett WM, Verpooten GA (editors). Kluwer Academic Publ, Dordrecht 1998; p. 383-395.
3. Terao T, Okuno K, Okuno T, Nakano H, Shinkai T, Ohmori O, Yoshimura R, Suzuki T, Abe K. A simpler and more accurate equation to predict daily lithium dose. J Clin Psychopharmacol 1999; 19(4): 336-340.
4. Stoll PM, Stokes PE, Okamoto M. Lithium isotopes: differential effects on renal function and histology. Bipolar Disorders 2001; 3: 174-180.
5. Ng LL, Quinn PA, Paulene A, Baker F, Carr SJ. Red cell Na/Li countertransport and Na/H exchanger isoforms in human proximal tubules. Kidney Int 2000; 58(1): 229-235.
6. Thomsen K, Schou M, Steiness I, Hansen E. Lithium as an indicator of proximal tubular reabsorption. Pflugers Arch 1969; 308: 180-184.
7. Navar LG, Schafer JA. Comments on lithium clearance: a new research area. News Physiol Sci 1987; 2: 34-35.
8. Rombola G, DeFerrari Colussi G ME, Surian M, Malberti F, Minetti L. Clinical evaluation of segmental tubular reabsorption of sodium and fluid in man: lithium vs free water clearance. Nephrol Dial Transplant 1987; 2: 212-218.
9. Boer WH, Koomans HA, Dorhout Mees EJ. Lithium clearance in healthy humans suggesting lithium reabsorption beyond the proximal tubules. Kidney Int 1990; 37 (suppl 28): 39-44.
10. Godinich MJ, Batlle DC. Renal tubular effects of lithium. Kidney Int 1990; 37 (suppl 28): S52-S57.
11. Boron R, Gaviria M, Batlle DC. Prevalence, pathogenesis, and treatment of renal dysfunction associated with chronic lithium therapy. Am J Kidney Dis 1987; 10(5): 329-345.
12. Hayslett IP, Kashgarian M. A micropuncture study of the renal handling of lithium. Plugers Arch 1979; 380: 159-163.
13. Leyssac PP, Holstein-Rathlou NH, Skott P, Alfrey AC. A micropuncture study of proximal tubular transport of lithium during osmotic diuresis. Am J Physiol 1990; 258: F1090-F1095.
14. Shirley DG, Walter SJ, Sampson B. A micropuncture study of renal lithium reabsorption; effects of amiloride and furosemide. Am J Physiol 1992; 263: F1128-F1133.
15. Fransen R, Boer WH, Boer P, Koomans HA. Amiloride sensitive lithium reabsorption in rats; a micropuncture study. J Pharmacol Exp Therapy 1992; 263: 646-650.

16. Fransen R, Boer WH, Boer P, Dorhout Mees EJ, Koomans HA. Effects of furosemide or acetazolamide infusion on renal handling of lithium; a micropuncture study in rats. Am J Physiol 1993; 264: 129-34.
17. Boer WH, Fransen R, Boer P, Roos R de, Koomans HA. Prostaglandin synthesis inhibition stimulates lithium reabsorption in Henle's loop in rats. Kidney Int 1993; 43: 301-306.
18. Koomans HA, Boer WH, Dorhout Mees EJ. Evaluation of lithium clearance as a marker of proximal tubule sodium handling. Kidney Int 1989; 36: 2-12.
19. Kirchner KA. Lithium as a marker for proximal tubular delivery during low salt uptake and diuretic infusion. Am J Physiol 1987; 253: F188-F196.
20. Greger R. Possible sites of lithium transport in the nephron. Kidney Int. 1990; 37 (suppl 28): S26-S30.
21. Imai M, Isozaki T, Yasoshima K, Yoshitomi K. Permeability characteristics and probability of lithium transport in the thin limbs of Henle's loop. Kidney Int 1990; 37 (suppl 28): S31-S35.
22. Atherton JC, Green R, Higgins A. Large A, McNicholas C, Parker D. Pempkowiak L, Rajani K, Smith J. Lithium clearance in healthy humans: effects of sodium intake and diuretics. Kidney Int. 1990; 37 (suppl 28): 36-38.
23. Shalmi M, Jonassen T, Thomsen K, Kibble JD, Bie P, Christensen S. Am J Physiol 1998; 274 (Renal Physiol 43): F445-F452.
24. Boer WH, Koomans HA, Beutler JJ. Gaillard CA, Rabelink AJ, Dorhout Mees EJ. Small intra- and large inter-individual variability in lithium clearance in humans. Kidney Int 1989; 35: 1183-1188.
25. Colussi G, Rombola G, Surian M, DeFerrari ME, Airaghi C. Benazzi E, Malberti F, Minetti L. Lithium clearances in humans: effects of acute administration of acetazolamide and furosemide. Kidney Int 1990; 39 (suppl 28): S63-S66.
26. Batlle DC, Von Riotte AB, Gaviari M, Grupp M. Amelioration of polyuria by amiloride in patients receiving long-term lithium therapy. N Engl J Med 1985; 312: 409-414.
27. Lehmann K, Ritz E. Angiotensin-converting enzyme inhibitors may cause renal dysfunction in patients on long-term lithium treatment. Am J Kidney Dis. 1995; 25: 82-87.
28. Ragheb M. The clinical significance of lithium non-steroidal anti-inflammatory drug interactions. J Clin Psychopharmacol 1990; 10: 350-354.
29. Cohen LS, Friedman JM, Jefferson JW, Johnson EM, Weiner ML. A re-evaluation of risk of in utero exposure to lithium. JAMA 1994; 271: 146-150.
30. Stancer HC, Forbath N. Hyperparathyroidism, hypothyroidism, and impaired renal function after 10 to 20 years of lithium treatment. Arch Intern Med 1989; 149: 1042-1045.
31. Schou M, Dr. H C Mull. Forty years of lithium treatment. Arch Gen Psychol 1997; 54(1): 9-13.
32. Racke F, McHenry C, Wentworth D. Lithium-induced alterations in parathyroid cell function: insight into the pathogenesis of lithium-associated hyperparathyroidism. Am J Surg 1994; 168(5): 462-465.
33. Shen F-H, Sherrad DJ. Lithium-induced hyperparathyroidism: an alteration of the "set-point". Ann Int Med 1982; 96(1): 63-65.
34. Haden ST, Brown EM, Stoll AL, Scott J, El-Hajj F, Ghada. The effect of lithium on calcium-induced changes in adrenocorticotrophin levels. J Clin Endocrin Met 1999; 84(1): 198-200.
35. Bendz H, Sjodin I, Toss G, Berglund K. Hyperparathyroidism and long term effects of lithium therapy - a cross-sectional study and the effect of lithium withdrawal. J Int Med 1996; 240: 357-365.
36. Saxe AW, Gibson G. Lithium increase tritiated thymidine by abnormal human parathyroid tissue. Surgery 1991; 110: 1067-1077.
37. Saxe AW, Gibson G, Silvera E. Effects of long-term lithium infusion on normal parathyroid. Surgery 1995; 117: 577-580.
38. Wolff ME, Moffat M, Dempsey S. Lithium therapy, hypercalcemia, and hyperparathyroidism. Am J Therap 1997; 4: 323-325.
39. McHenry C, Stenger D, Racke F. Investigation of calcium-induced hydrolysis of Phosphoinositedes in normal and lithium treated parathyroid cells. Am J Surgery 1995; 170 (5): 484-487.
40. Nordenstrom J, Elvius M., Zhao B, Torring O. Biochemical hyperparathyroidism and bone mineral status in patients treated long-term with lithium. Metabolism 1994; 43(12): 1563-1567.
41. Abdullah H, Bliss R, Guinea AI, Delbridge L. Pathology and outcome of surgical treatment for lithium-associated hyperparathyroidism. Brit J Surgery 1999; 86(1): 91-93.
42. Wolf EM, Ranade V, Molnar J, Somberg J, Mosnaim A. Hypercalcemia, arrhythmia and mood stabilizers. J Clin Psychopharmacol 2000; 20(2): 260-264.
43. Carney S, Jackson P. Acute effect of lithium administration on renal Ca, Mg, and PO_4 transport in the rat. Kidney Int 1996; 50(4): 1406.
44. Singer I, Rottenberg D, Puschett IB. Lithium-induced nephrogenic diabetes insipidus: *in vivo* and *in vitro* studies. J Clin Invest 1972; 51: 1081-1091.
45. Singer I, Franko EA. Lithium-induced ADH resistance in toad bladders. Kidney Int 1973; 3: 151-159.
46. Forrest J Jr, Cohen AD, Torretti J, Himmelhoch JM, Epstein FH. On the mechanism of lithium-induced diabetes insipidus in man and the rat. J Clin Invest 1974; 53: 115-123.

47. Martinez-Maldonado M, Stravroulaki-Tsapar A, Tsaparas N. Renal effects of lithium administration in rats: alterations in water and electrolyte metabolism and the response to vasopressin and cyclic adenosine monophosphate during prolonged administration. J Lab Clin Med 1975; 86: 445-461.
48. Fernandez-Repollet E, LeFurgey A, Hardy MA, Tisher CC. Structural and functional response of toad urinary bladder of LiCi. Kidney Int 1983; 24: 719-730.
49. Harris CA, Jenner FA. Some aspects of the inhibition of the action of antidiuretic hormone by lithium ions in the rat kidney and bladder of the toad *Bufo marinus*. Brit J Pharmacol 1972; 44: 223-232.
50. Cogan E, Abramow M. Inhibition by lithium of the hydro-osmotic action of vasopressin in the isolated perfused conical collecting tubule of the rabbit. J Clin Invest 1986; 77: 1507-1514.
51. Christiensen S, Kusano E, Yusufi ANK. Pathogenesis of nephrogenic diabetes insipidus due to chronic administration of lithium in rats. J Clin Invest 1985; 75: 1869-1879.
52. Dousa TP, Barnes LD. Lithium-induced diuretic effect of antidiuretic hormone in rats. Am J Physiol 1976; 231: 1754-1759.
53. Jackson BA, Edwards RM, Dousa TR. Lithium-induced polyuria: effect of lithium on adenylate cyclate and adenosine 3'5' monophosphate phosphodisterase in medullary ascending limb of Henle's loop and in medullary collecting tubules. Endocrinol 1980; 107(6): 1693-1698.
54. Yamaki M, Kusano E, Homma S. Cellular mechanisms of lithium induced nephrogenic diabetes insipidus. Am Soc Nephrol 1989; 404A.
54a. Kwon TH, Laursen UH, Marples D. Altered expression of renal AQP2s and Na^+ transporters in rats with lithium induced NDI. Am J Physiol 2000; 279:F552-F564.
54b. Michimata M, Fujita S, Araki T, Mizukami K, Kazama I, Muramatsu Y, Suzuki M, kimura T, Sasaki Ss, Imai Y, Matsubara M. Reverse pharmacological effect of loop diuretics and altered rBSC1 expression in rats with lithium nephropathy. Kidney Int 2003;63(1):165-171.
55. Agre P, Preston GM, Smith BL, Neilsen S. Aquaporin CHIP: the archetypal molecular water channel. Am J Physiol 1993; 265: F463-F476.
56. Nielsen S, Marples D, Knepper M, Agre P. The Aquaporin family of water channels in the kidney: An update on physiology and pathophysiology of Aquaporin-2. Kidney Int 1996; 49: 1718-1723.
57. Fushimik Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. Nature 1993; 361(6412): 549-552.
58. Marples D, Christensen S, Oltosen P, Nielsen S. Lithium-induced Down-regulation of Aquaporin -2 water channel experession in the rat kidney medulla. J Clin Invest 1995; 95 (4): 1838-1845.
59. Walker RG, Bennett WM, Davies BM. Structural and functional aspects of long-term lithium therapy. Kidney Int 1982; 21: 513-519.
60. Albrecht J, Kampf D, Muller-Oerlinghausen B. Renal function and biopsy in patients on lithium therapy. Pharmako-psychiatne 1980; 13: 228-234.
61. Bendz H, Andersch S, Aurell M. Kidney function in an unselected lithium population. A cross-sectional study. Acta Psychiatry Scand 1983; 68: 325-334.
62. Lokkegaard H, Anderson HF, Henriksen E, Bartels PD, Brahm M, Baastrup PC, Jorgensen HE, Larsen M. Munck O, Rasmussen K. Renal function in 153 manic depressive patients treated with lithium for more than five years. Acta Psychiatry Scand 1985; 71: 347-355.
63. Baylis PH, Heath DA. Water disturbances in patients treated with oral lithium carbonate. Ann Intern Med 1978; 88: 607-609.
64. Uldall PR, Awad AG, McCormick WO, Caner LB, Gonsick T, Grass CL, Kugelmass ML. Renal function in patients receiving long-term lithium therapy. Can Med Assoc J 1981; 124(11): 1471-1474.
65. Wahlin A, Bucht G, Von Knorring L, Smigan L. Kidney function in patients with affective disorders with and without lithium therapy. Pharmacopsych 1980; 15: 253-259.
66. Wallin L, Aling C, Aurell M. Impairment of renal function in patients on long-term lithium treatment. Clin Nephrol 1982; 18: 23-28.
67. Donker AJM, Prins E, Meijer S, Sluiter W, Van Berkestijn JW, Dols LC. A renal function study in 30 patients on long-term lithium therapy. Clin Nephrol 1979; 12: 254-262.
68. Vestergaard R, Amdisen A, Hansen AE, Schou M. Lithium treatment and kidney function. Acta Psychiatry Scan 1979; 60: 504-520.
69. Johnson GF, Hunt G, Duggin GG, Horvath JJ, Tiller DJ. Renal function and lithium treatment: initial and follow up tests in manic-depressive patients. J Affective Disord 1984; 6: 249-263.
70. Coppen A, Bishop ME, Bailey JE, Cattell WR, Price RG. Renal function in lithium and non-lithium treated patients with affective disorders. Acta Psychiatry Scand 1980; 62: 343-355.
71. Grof P, MacCrimmon DJ, Smith EKM, Daigle L, Saxena B, Varma R, Grof E, Keitner G, Kenny J. Long-term lithium treatment and the kidney. Can J Psychiatry 1980; 25: 535-544.

72. Jorgensen F, Larsen S, Spanager E, Clausen E, Tango M, Brinch E, Brun C. Kidney function and quantitative histological changes in patients on long-term lithium therapy. Acta Psychiatry Scand 1984; 70: 455-462.
73. Rabin EZ, Garston RG, Weir RV, Posen GA. Persistent nephrogenic diabetes insipidus associated with long term lithium carbonate treatment. Can Med Assoc J 1979; 121(2): 194-198.
74. Lee R, Jampol L, Brown W. Nephrogenic diabetes insipidus and lithium intoxication: complications of lithium carbonate therapy. N Engl J Med 1971; 284: 93-94.
75. Simon NM, Garber E, Arieff AJ. Persistent nephrogenic diabetes insipidus after lithium carbonate. Ann Intern Med 1977; 86: 446-447.
76. Hansen HE, Hestbech J, Olsen S, Amdisen A. Renal function and renal pathology in patients with lithium induced impairment of renal concentrating ability. Proc EDTA 1977; 14: 518-527.
77. Price TRP, Beisswenger PJ. Lithium and diabetes insipidus. Ann Int Med 1989; 88: 576-577.
78. Forrest J Jr. Lithium-induced polyuria: cellular mechanisms and response to diuretics. In: Lithium - controversies and unresolved issues. Cooper TB, Gershon S, Kline NS (editors). Excerpta Medica, Amsterdam 1979; p. 632-641.
79. Bucht G, Wahlin A. Renal concentration capacity in long-term lithium treatment and after withdrawal of lithium. Acta Med Scand 1980; 207: 309-314.
80. Nascimento L, Rademacher D, Hamburger R, Arruda JA, Kurtzman A. On the mechanism of lithium-induced renal tubular acidosis. J Lab Clin Med 1977; 89: 455-462.
81. Mehta PK. Sodhi B, Arruda JAL, Kunzman NA. Interaction of amiloride and lithium on distal urinary acidificalion. J Lab Clin Med 1979; 93: 93-94.
82. Batlle DC, Gaviria M, Grupp M. Distal nephron function in patients receiving chronic lithium therapy. Kidney Int 1982; 21: 477-485.
83 Arruda JAL, Dytko G, Mola R. Kurtzman NA. On the mechanism of lithium-induced renal tubular acidosis; studies in the turtle bladder. Kidney Int 1980; 17: 196-204.
84. Laski ME, Kurtzman NA. Characterization of acidification in the cortical and medullary collecting tubule of the rabbit. J Clin Invest 1983; 72: 2050-2059.
85. Eiam-Ong B, Dafnis E, Spohn M, Kurtzman NA, Sabatini S. H/K/ATpase in distal renal tubular acidosis: urinary tract obstraction, lithium, and amiloride. Am J Physiol 1993; 265 (6Pt2): F675-F680.
86. Galla JN, Forrest JN, Hecht B, Kashgarian M, Hayslett JP. Effect of lithium on water and electrolyte metabolism. Yale J Biol Med 1975; 48: 305-314.
87. Batlle DC, Tarka J, Kurtzman NA. Renal handling of potassium after chronic lithium administration. Clin Res 1983; 31: 424A.
88. Jefferson JW. Potassium supplementation in lithium patients: a timely intervention or premature speculation ? J Clin Psychiatry 1992; 53: 370.
89. Hestbech J, Hansen H, Amdisen A, Olsen S. Chronic renal lesions following long-term treatment with lithium. Kidney Int 1977; 12: 205-213.
90. Hansen HE, Hestbech J, Sorenson JL, Norgaard K, Heilskov J, Amdisen A. Chronic interstitial nephropathy in patients with long term treatment. Q J Med 1979; 192: 577-591.
91. Aurell M, Svalander C, Wallin L, Alling G. Renal function and biopsy finding in patients on long-term lithium treatment. Kidney Int. 1981; 20: 663-670.
92. Markowitz GS, Radhakrishnan J, Kambhaam N, Valer AM, Hines WH, D'Agati VD. J Am Soc Nephrol 2000; 11: 1439-1448.
93. Walker RG, Escott M, Birchall J, Dowling JP, Kincaid-Smith P. Chronic progressive renal lesions induced by lithium. Kidney Int 1986; 29: 875-881.
94. Walker RG, Davies BM, Holwill BJ. A chronic pathological study of lithium nephrotoxicity. J Chro Dis 1982; 35: 655-695.
95. Rafaelson OJ, Bolwig TG, Ladefoged J. Kidney function and morphology in long-term lithium treatment. In: Lithium. Cooper TB, Gershon S, Kline NS (editors). pta Medica 1979; p. 578-583.
96. Davies B, Kincaid-Smith P. Renal biopsy studies of lithium and pre-lithium patients and comparison with cadaver transplant kidneys. Neuropharmacol 1979; 18: 1001-1002.
97. Walker RG, Dowling JF, Alcorn D, Ryan GB, Kincaid-Smith P. Renal pathology associated with lithium therapy. Pathology 1983; 15: 403-11.
98. Kincaid-Smith P, Burrows GD, Davies BM. Renal biopsy findings in lithium and pre-lithium patients. Lancet 1979; 2: 700-701.
99. Burrows GD, David B, Kincaid-Smith P. Unique tubular lesion after lithium. Lancet 1978; 1: 1210.
100. McAuliffe WG, Olesen OV. Effects of lithium on the structure of the rat kidney. Nephron 1983; 34: 114-124.
100a. Min G, Christensen S, Marcussen N, Osterby R. Glomerular structure in lithium-induced chronic renal failure in rats. APMIS 2000; 108(10): 652-62.
101. Ottosen PD, Sign B, Kristensen J, Olsen S, Christensen S. Lithium-induced interstitial nephropathy associated with chronic renal failure. Reversibility and correlation between functional and structural changes. Acta Pathol Microbiol Immunol Scand 1984; 92: 447-454.

102. Gerner RH, Psarras J, Kirschenbaum MA. Results of the clinical renal function tests in lithium patients. Am J Psychiatry 1980; 137: 834-837.
103. Hullin RP, Coley VP, Birch NJ, Thomas TH, Morgan DB. Renal function after long-term treatment with lithium. Brit Med J 1979; 1: 1457-1459.
104. Thysell H, Brante G, Sjostedt L, Lindergard B, Lindholm T, Franzen G, Rorsman B. Glomerular filtration rate and calcium metabolism in long-term lithium treatment. Neuropsychobiol 1981; 7: 105-111.
105. Depaulo JR Jr, Correa EJ, Sapir DG. Renal glomerular function and long-term lithium therapy. Am J Psychiatry 1981; 138: 324-327.
106. Halgren R, Alm PO, Hellsing K. Renal function in patients on lithium treatment. Brit J Psychiatry 1979; 135: 22-27.
107. Hestbech J, Aurell M. Lithium-induced uremia. Lancet 1979; 1: 212-213.
108. Nue C, Manschreck TC, Flockx JM. Renal damage associated with long-term use of lithium carbonate. J Clin Invest 1979; 40: 460-463.
109. Vestergaard P. Lithium-induced uremia ? Lancet 1979; 1: 491.
110. Chugh S, Yager H. End stage renal diseases after treatment with lithium. J Clin. Psychopharmacol 1997; 17 (6): 495-497.
111. Von Knorring K, Wahlin A, Nystrom K, Bohman SO. Urema induced by long term lithium treatment. Lithium 1990; 1: 251-253.
112. Lazarus JH. Endocrine and metabolic effects of lithium. New York: Plenum Medical, 1986.
113. Mitchell JE, Mackenzie TB. Cardiac effects of lithium therapy in man: a review. J Clin Psychiatry 1982; 43: 47-51.
114. Timmer RT, Sands JM. Disease of the month: lithium intoxication. J Am Soc Nephrol 1999; 10: 666-674.
115. Okusa MD, Crystal LJT. Clinical manifestations and management of acute lithium intoxication. Am J Med 1994; 97: 383-389.
116. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J. Lithium. In: Medical toxicology - diagnosis and treatment of human poisoning. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J (editors). Williams and Wilkins, Baltimore 1997; p. 1579.
117. Henry GC. Lithium. In: Toxicologic emergencies. Goldfrank LR, Flomenbaum NE, Lewin NA, Weisman RS, Howland MA, Hoffman RS (editors). Appleton and Lange, Stampford CT 1998; p. 969.
118. Dupuis RE, Cooper AA, Rosamund LJ, Campbell-Bright S. Multiple delayed peak lithium concentrations following acute intoxication with an extended release product. Ann Pharmacother 1996; 30: 356-360.
119. Leblanc M, Raymond M, Bonnardeaux A, Isenring P, Pichette V, Geadah D, Quimet D, Ethier J, Cardinal J. Lithium poisoning treated by high-performance continuous arteriovenous and venovenous hemodiafiltration. Am J Kidney Dis 1996; 27: 365-372.

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Aristolochic acid nephropathy after Chinese herbal remedies

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Introduction

Numerous myths have grown around medicinal herbs and their healing powers [1]. The myth of beneficent nature is resistant to the accumulated evidence of health problems resulting from unknown or underestimated toxicity as well as from adulteration and misidentification of medicinal herbs [2]. Many plants contain substances toxic to humans and therefore, not surprisingly, to the human kidney. In early 1993, a rapidly progressive kidney failure leading to end-stage renal disease was reported in a number of women who had ingested slimming pills that contained powdered Chinese herbs [3]. Further investigations showed that this so called "Chinese herbs nephropathy" was, in fact, secondary to the replacement of one of the prescribed Chinese herb, *Stephania tetrandra*, by other mixtures of Chinese herbs containing *Aristolochia Fang chi* [4]. The term "Aristolochia nephropathy" should thus preferably replaced the previously used term of "Chinese herb nephropathy (CHN)" [5].

History

In early 1992, two women presented to our nephrology department in Brussels (Belgium) with an extensive interstitial fibrosis of the kidney that rapidly progressed to terminal renal failure [3]. These two women who had no previous history of renal disease had followed, just before the onset of the renal disease, the same weight loss regimen in the same medical clinic in Brussels. This clinic specialized in weight loss regimens for more than 15 years and no renal problems had been previously encountered. Interestingly, the diet regimen was changed in mid 1990 by introducing powdered extracts of Chinese herbs, nominally *Stephania tetrandra* and *Magnolia officinalis* in the slimming pills [3]. A 1992 – epidemiological survey of the nephrology centers of Brussels showed that seven other women with "interstitial nephritis of unknown origin" were admitted for dialysis in 1991 and 1992. They had all followed the same slimming regimen including the Chinese herbs in the same medical clinic [3]. *Stephania*

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tetrandra and *Magnolia officinalis* were withdrawn from the Belgian market at the end of 1992. However, the outbreak of renal failure after absorption of these Chinese herbs eventually resulted in about 100 cases in 1998, 70 % of them being in end stage renal disease [6].

Phytochemical analyses of 12 different samples of *Stephania tetrandra* delivered in Belgium from 1990 to 1992 showed that only one sample corresponded to uncontaminated *Stephania* while the others were most probably *Aristolochia* sp [4], confirming the results of the analyses performed in Hong Kong on a sample sent by the Belgian importers [7].

After the publication of the index cases [3], similar cases were reported all around the world: four cases in France secondary to the intake of Arkomedika n°28 slimming pills containing *Stephania tetrandra* which was, in fact, *Aristolochia Fangchi* [8,9], one case in Spain after the chronic intake of an infusion made with a mixture of herbs containing *Aristolochia pistolochia* [10], two cases in United Kingdom after the treatment of eczema with aristolochic acids containing Mu-tong [11] and another case following a 5-year period of ingesting a Chinese herbal preparation to treat hepatitis B [12]. Two series of respectively 12 [13] and 20 cases [14] were also reported in Taiwan related to the use of various unidentified herbal medications. In Japan, four cases presenting with a Fanconi syndrome were related to the use of different Chinese herb remedies (Boui and Mokutsu) containing aristolochic acids [15,16]. A reversible Fanconi syndrome after the intake of a Chinese herbal remedy (*Akebia*) containing aristolochic acids was also reported in Germany [17]. Finally, the case of a woman with interstitial renal fibrosis progressing rapidly to end-stage renal disease after Chinese herbal medicine was reported in USA [18].

Clinical features and functional aspects

Renal failure was usually not suspected and was, in most of the cases, discovered by routine blood testing. Dipstick analysis for proteinuria was negative and urinary sediment was unremarkable. Blood pressure was initially normal in half of the patients. Anemia was present and usually more severe that might be anticipated from the degree of renal failure [19]. Further investigations of renal functions indicated that proximal tubular cells were a primary target in Aristolochia nephropathy. First, some cases presented with a Fanconi syndrome [15,16,17]. Second, urinary excretion of five low molecular weight proteins (β2-microglobulin, cystatin C, Clara cell protein, retinol binding protein and α1 microglobulin) was markedly increased in five patients with CHN and the urinary low molecular weight protein/albumin ratio was higher than in control patient with glomerular diseases [20]. Third, levels of urinary neutral endopeptidase, an ectoenzyme of the proximal tubule brush border were significantly decreased in patients with renal failure secondary to CHN as compared to patients with glomerular diseases. Moreover, neutral endopeptidase enzymuria correlated positively with creatinine clearance and negatively with low molecular weight protein urinary levels [21]. Finally, the pattern of aminoaciduria in four cases of CHN with Fanconi syndrome (increased excretion of proline, hydroxyproline and citrulline with an almost normal excretion of glycine) suggested that aristolochic acids would predominantly affect the low affinity transport system of proline in the brush border membrane of proximal tubule [16].

Despite the interruption of the exposure to Chinese herbs, progression of renal failure is usually relentless over a period of few months to several years. Six year after the withdrawal of the incriminated herbs from the Belgian market, more than 100 patients with CHN were recorded in Belgium, 30 % of them having a moderate renal failure and 70 % being treated by maintenance dialysis or renal grafting [6].

A pilot study involving 35 CHN patients showed that a steroid therapy was able to slow the progression of the renal failure: after one year, only two of the 12 CHN patients treated with steroids required dialysis as compared with 16 of the 23 CHN control patients [22]. The beneficial effect of steroid therapy was confirmed 8 years later in a larger group of patients [23]. Curiously, an asymptomatic aortic insufficiency was observed in one third of the patients with CHN [19, 24]. This cardiac complication was supposed to be the result of an extrarenal toxicity of Chinese herbs [25]. However, the attention was drawn to the role of appetite suppressants in the development of valvular heart diseases [26]. Since most of the CHN patients we have seen have been given appetite suppressants (fenfluramine, dexfenfluramine, phentermine alone or in combination) besides the Chinese herbs, the puzzling association of aortic insufficiency with CHN may more likely be linked to the concomitant use of (dex)-fenflu-

ramine than to an extrarenal effect of the Chinese herbs [24]. In fact, the presence of aortic regurgitation was detected in 21 out of 40 CHN patients and was significantly correlated in a dose-response relationship with the cumulative dose of fenfluramine [27].

Pathology

Macroscopically, the kidneys were shrunk, asymmetric in about half of the cases with irregular outlines in one third [25].

Microscopically, the description of the pathologic aspects were derived from the analysis of 4 pieces of native nephroureterectomies obtained at the time of transplantation [28] and of 33 renal biopsies performed at different levels of renal failure [29].

As shown on Figure 1, extensive interstitial fibrosis with atrophy and loss of the tubules was the major lesion. It was predominantly located in superficial cortex. The glomeruli were relatively spared. They nevertheless showed a mild collapse of the capillaries and a wrinkling of the basement membrane. Thickening of Bowman's capsule was the rule.

Interlobular and afferent arterioles showed thickening of their walls due to a swelling of the endothelial cells. These aspects suggest that the primary lesions could be located in the vessel walls leading to ischemia and interstitial fibrosis [29]. In one case, an extension of the fibrotic process to the pelvis and the ureter was observed [28], what may explain the unusual presentation of this case with a bilateral hydronephrosis [30].

Association with urinary tract carcinomas

Moderate atypia and atypical hyperplasia of the urothelium were first described in 4 pieces of nephroureterectomies performed in 3 CHN patients prior or at time of transplantation [28]. Then, three cases of cancers of the urinary tract were reported: the first case, a 28 year old woman with CHN, developed two papillary transitional cell carcinomas in the posterior bladder wall 12 months after a renal transplantation [31]; the second case, a 42 year old woman with CHN, presented with hematuria secondary to a papillary transitional cell carcinoma of the right pelvis [32]. The third case was a 49 year old woman previously published as a CHN case in UK [11]. She developed a hydronephrosis of the left native kidney after a successful renal transplantation. The piece of nephroureterectomy showed a multifocal transitional cell carcinoma of the ureter [33]. Patients with CHN in end-stage renal diseases treated by dialysis or renal grafting were therefore systematically offered bilateral removal of their native kidneys and ureters. Doing that, multifocal high grade transitional cell carcinomas, mainly in the upper urinary tract, were observed in four patients among 10 in one series [34] and in 18 among 39 in an other series [35]. The cumulative dose intake of *Stephania* (in fact, *Aristolochia*) was shown to be a significant risk factor for the development of urothelial carcinomas [35].

Urothelial cancer seemed to be a late complication of CHN since all the cases had been detected in patients with ESRD. However, the observation of a generalized urinary tract cancer in a 69 year old woman after intake of Chinese herbal medicine containing aristolochic acids but without a significant renal failure suggests that a dissociation between carcinogenicity and nephrotoxicity of aristolochic acids is possible [36].

Pathogenesis: The role of aristolochic acids

The time between the introduction of Chinese herbs in weight loss regimens and the outbreak of renal diseases in Brussels (Belgium) circumscribed the search for the culprit to the Chinese herbs [3]. Further epidemiological survey demonstrated that only the so-called *Stephania* was associated with all the cases of renal interstitial fibrosis [29].

Replacement of *Stephania* by *Aristolochia* was suspected [3] because : 1) *Stephania tetrandra* (Han Fang-ji) belongs to the family of Fang-ji besides *Aristolochia Fang chi* (Guang Fang ji); 2) pathological aspect of CHN is very similar to that of Balkan endemic nephropathy [3,28,29], the cause of which is still under controversies but some suggested causes included fungal and plant toxins such as ochratoxin A from *Penicillium* and aristolochic acids from *Aristolochia clematis* [37].

Actually, the replacement of *Stephania* by *Aristolochia* species was confirmed on different batches of powders delivered in Belgium under the name of *Stephania tetrandra.* Most of these batches did not contain tetrandrine but aristolochic acids (0.65 ± 0.56 mg/g) [4].

Aristolochic acids (AA) were thus considered as the offending substance because AA induced nephrotoxic effects in experimental animals [38] as well as in human beings [39]. They also induced carcinomas in rodents [40].

However, some controversies were raised against the AA hypothesis. First, promoters of Chinese herbs claimed that the renal disease originated, in fact, for the injection of a "hidden" serotonin-like substance, with the mesotherapy which was a part of the slimming regimen [41, 42]. Serotonin was indeed shown to induce ischemic renal lesions progressing in a short time to renal fibrosis [43]. Moreover the Belgian patients were also given (dex)fenfluramine which is a serotonin agonist [44]. Second, Chinese herbs originated from batches imported in Belgium at the same time were used without apparent untoward effects [44]. Third, similarities with Balkan endemic nephropathy suggested that ochratoxin A could be an alternative hypothesis.

However, further evidences support the AA hypothesis. The presence of 7(desoxyadenosin-N^6-yl) aristolactam I DNA adducts (dA-AAI) was demonstrated in renal tissue samples obtained from five patients with CHN while dA-AAI was absent in the renal tissue of six patients with other renal diseases [45]. That was also the case for 7(deoxyguanosine-N^2-yl) aristolactam I DNA adducts (dG-AAI) and 7(deoxyadenosin-N^6-yl) aristolactam II DNA adducts (dA-AAII) [46]. A larger series of kidney samples from 38 patients with CHN confirmed the presence of DNA adducts six year after the exposure to Chinese herbs; the levels ranging from 1.2 to 165 per 10^9 normal nucleotides for dA-AAI, from 0.6 to 6.8 per 10^9 normal nucleotides for dA AAII and from 0.4 to 8.2 per 10^9 normal nucleotides for dG AAI. These adducts were absent in kidney samples obtained in eight patients with renal diseases of other origin [35]. The renal tissue sample of 25 among these 38 patients with CHN were also analyzed for ochratoxin A related adducts. Levels of these adducts were low and close to the background level of the assay [35]. On the other hand, for 71 patients with CHN followed in our department, a comprehensive analysis of the medical charts and of the prescriptions filled between 1990 and 1992 directly obtained from the pharmacists was conducted. This survey showed that eleven patients with Chinese herbs - related end stage renal disease did not receive mesotherapy. Moreover, with a multiple regression analysis, the cumulative dose of *Stephania* (in fact, *Aristolochia*) appeared as the only significant factor predicting the slope of the time course of the inverse of plasma creatinine levels [47]. Although these observations can not rule out a possible potentiating effect of anorexigens [44], the description of similar renal diseases after the intake of *Stephania* without slimming pills [48, 49] as well as in different clinical settings all around the world [8-18] indicates that (dex)fenfluramine should not be necessary to induce renal disease. Moreover, dexfenfluramine did not enhance the nephrotoxicity of AA in a rat model of CHN [50].

On the other hand, AA are activated by nitroreduction in aristolactams which form DNA adducts with adenosine and guanosine. The formation of AA-DNA adducts was studied *in vitro*: cytochrome P450 1A1 and 1A2 [51] as well as prostaglandin H synthetase [52] were shown to be involved in the metabolic activation of AA. These observations could explain variations between individuals in the susceptibility to aristolochic acid toxicity as well as the preferential localization in the kidney and the urinary tract. Carcinogenicity of AA DNA adducts has been related to the mutation in the codon 61 of the protooncogen Ha-ras [53] as well as in a mutation of p53 [34]. Finally, the effects of AA on proximal tubules were investigated on the opossum kidney (OK) cell line. Aristolochic acids impaired the process of receptor-mediated endocytosis of albumin and β2-microglobulin, decreased megalin expression and formed specific DNA adducts in OK cells. These data support the involvement of AA in the proximal tubule dysfunction found in CHN patients [54].

Experimental aristolochic acid nephropathy

First attempts to experimentally reproduce CHN failed: two groups of seven Wistar rats were orally given either pure aristolochic acids (10 mg/kg for 5 days a week during 3 months) or herbs powders (containing aristolochic acids) mixed with fenfluramine. At sacrifice animals in both groups had developed the expected tumors but not fibrosis of the renal interstitium [55]. On the contrary, when 12 female New Zeeland white rabbits were injected intraperitoneally with 0.1 mg aristolochic acids per kg, 5 days a week, for 17 to 21 months, they developed a severe hypocellular in-

terstitial fibrosis, urothelial atypias and, in 3 of them, tumors of the urinary tract [56].

In the salt depleted Wistar rat model, the daily administration of 10 mg per kg body weight of aristolochic acids induce after 35 days, renal failure with interstitial fibrosis (Figure 2) as well as a papillary urothelial carcinoma of the pelvis in some of them [57].

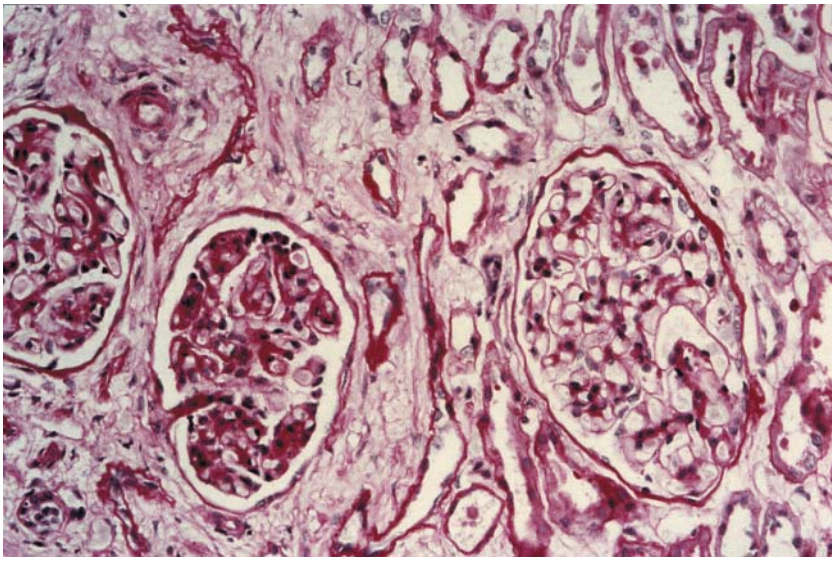

Figure 1. *Pathological aspect of Chinese herb nephropathy. Paucicellular interstitial fibrosis around atrophic tubules (✻). Fibrous thickening of the arteriolar walls (→). No glomerular lesion. H&E staining, orig. magn. x300.*
By courtesy of Dr. M. Depierreux.

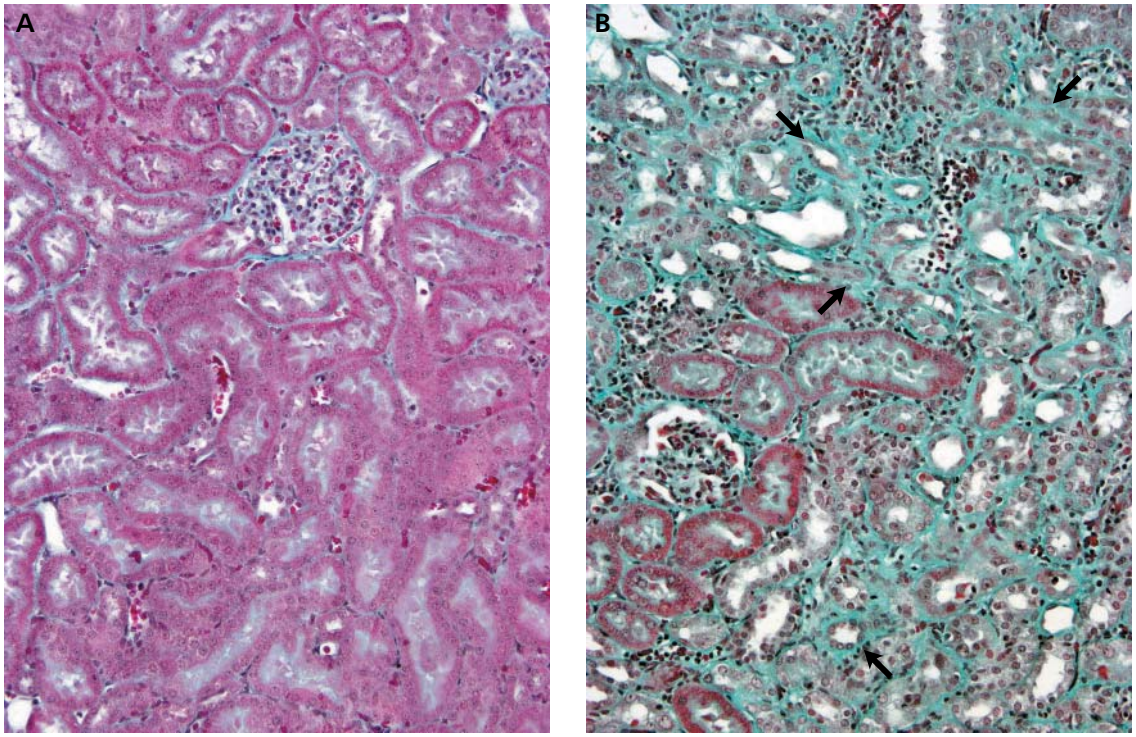

Figure 2. *Photomicrographs of renal cortex.* ***A****. For a control rat on day 35, no abnormalities were noted.* ***B****. For an aristolochic acid-treated (10 mg/kg bw) rat on day 35, severe tubular atrophy and interstitial fibrosis (→) were observed. Goldner's trichrome staining, orig. magn. x100.*

Conclusion

From an outbreak of end stage renal disease occurring around a slimming clinic in Brussels [3] a new cause of renal interstitial fibrosis [3,28,29] complicated by urinary tract carcinomas [34, 35] was identified. Finally, the disease was related to the intake of Chinese herbs containing aristolochic acids (see above). After the publication of the Belgian cases, other similar cases were reported all around the world [8-18]. The existence of more cases should be suspected. Indeed, Fang ji, a commonly used traditional Chinese medicine, purchased from herbs shops in Hong Kong contained aristolochic acids [58] as well as *Akebia* used in traditional Sino Japanese prescriptions ("Kampo") [59]. Indian traditional medicine used more than 7500 plant species which include *Aristolochia bracteata*, *Aristolochia tagala* and *Aristolochia indica* and chronic interstitial nephritis of unknown origin is a frequent cause of terminal renal disease in Indians [60].

Nephrotoxins are usually easy to identify when deriving from well known therapeutic agents. However, their identification requires a detective work when causes involve the uncontrolled use of herbs ingredients in home remedies or folk medicine. The difficulty is compounded by the fact that patients do not mention their regular use of herbal powders or infusions because they consider these natural products to be harmless [1].

Faced with a case of interstitial renal nephritis of unknown origin, all nephrologist should be encouraged to examine with the utmost care whether herbal remedies containing aristolochic acids as depicted by the US Food and Drug Administration [61] (Table 1) can genuinely be ruled out. Moreover, taking into account that herbal remedies containing plant species of the genus *Aristolochia* are carcinogenic to humans [62], this attitude should be extended to the diagnosis of urinary tract carcinoma.

Table 1. Botanicals known or suspected to contain or to be adultered with aristolochic acids.

Botanical names
Aristolochia sp (n=30), *Asarum sp* (n=6), *Akebia sp* (n=3), *Bragantia sp* (n=1), *Clematis sp* (n=6), *Cocculus sp* (n=17), *Sinomenium sp* (n=1), *Stephania sp* (n=1).
Common names
• Aristolochia, Akebia, Clematis, Clematidis, Cocculus, Serpentaria, Stephania
• Dutchman's pipe, Birthwort, Snakeroot, wild (Indian) Ginger, False Coltsfoot, Colic root, Chocolate vine, Virgin bower, Indian cockle, Colombo, Columba, Ukulwe, Orient vine,
• Fang-ji [1], Mu-tong [1], Boui, Mokutsu, Saishin, Mokku, Ma dou ling, Tian Xian teng, Mokuboi, Kwang banggi, Moktong, Yu Zhi zi, Bei Xi Xin, Xin Xin, Ireisen, Wojoksum, Weiling Xian, Fengteng, Kanboi.

[1] Fang-ji and Mu-tong are ingredients in the following products : Ba Zheng Wan, Chan Yang Zheng Ji Wan, Da Huang Quing Wei Wan, Dang Gui Si Ni Wan, Dao Chi Wan, Dieda Wan, Fu Ke Fen Quing Wan, Guan Xin Su He Wan, Ji Sheng Ju He Wan, Kat Kit Wan, Long Dan Xie Gan Wan, Quell Fire, Shi Xiang Fan Shen Wan, Xin Yi Wan (From ref. 61 in which more details can be found).

References

1. Vanherweghem JL. Nephropathy and Herbal Medicine. Am J Kidney Dis 2000; 35:330-332
2. Huxtable RJ. The myth of beneficient nature: the risks of herbal preparations. Ann Intern Med 1992; 117: 165-166
3. Vanherweghem JL, Depierreux M, Tielemans C, Abramowicz D, Dratwa M, Jadoul M, Richard C, Vandervelde D, Verbeelen D, Vanhaelen-Fastre R, Vanhaelen M. Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet 1993; 341:387-391
4. Vanhaelen M, Vanhaelen-Fastre R, But P, Vanherweghem JL. Indentification of aristolochic acid in Chinese herbs. Lancet 1994; 343: 174 (letter).
5. Vanherweghem JL. Aristolochia nephropathy in humans: an outbreak in Belgium. J Am Soc Nephrol 1993; 4: 327 (abstract).
6. Vanherweghem JL. Misuse of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). J Altern Complement Med 1998; 4: 9-13.
7. But PPH. Need for correct identification of herbs in herbal poisoning. Lancet 1993; 341: 637 (letter).

8. Pourrat J, Montastruc JL, Lacombe JL, Cisterne JM, Rascol O, Dumazer P. Nephropathie associée à des herbes chinoises – 2cas. Presse Med 1994; 23: 1669 (letter).
9. Stengel B, Jones E. Insuffisance rénale terminale associée à la consommation d'herbes chinoises en France. Néphrologie 1998; 19: 15-20.
10. Pêna JM, Borras M, Ramos J, Montoliu J. Rapidly progressive interstitial renal fibrosis due to a chronic intake of a herb (*Aristolochia pistolochia*) infusion. Nephrol Dial Transplant 1996; 11: 1359-1360.
11. Lord GM, Tagore R, Cook T, Gower P, Pusey CD. Nephropathy caused by Chinese herbs in the UK. Lancet 1999; 354: 481-482.
12. Cronin AJ, Maidment G, Cook T, Kite GC, Simmonds MS, Pusey CD, Lord GM. Aristolochic acid as a causative factor in a case of Chinese herbal nephropathy. Nephrol Dial Transplant 2002 ; 17: 524-525.
13. Yang CS, Lin CH, Chang SH, Hsu HC. Rapidly progressive fibrosing interstitial nephritis associated with Chinese Herbal drugs. Am J Kidney Dis 2000; 35:313-318.
14. Chang CH, Wang YM, Yang AH, Chiang SS. Rapidly progressive interstitial renal fibrosis associated with Chinese herbal medications. Am J Nephrol 2001; 21 :441-448
15. Tanaka A, Nishida R, Maeda K, Sugawara A, Kuwahara T. Chinese herb nephropathy in Japan presents adult onset Fanconi syndrome: could different components of aristolochic acids cause a different type of Chinese herb nephropathy ? Clin Nephrol 200; 53: 301-306.
16. Tanaka A, Nishida R, Yokoi H, Kuwahara T. The characteristic pattern of aminoaciduria in patients with aristolochic acid – induced Fanconi syndrome: could iminoaciduria be the hallmark of this syndrome ? Clin Nephrol 2000; 54: 198-202.
17. Krumme B, Endmeir R, Vanhaelen M, Walb D. Reversible Fanconi Syndrome after ingestion of a Chinese herbal "remedy" containing aristolochic acid. Nephrol Dial Transplant 2001; 16:400-402.
18. Meyer MM, Chen TP, Bennett WM. Chinese herb nephropathy. BUMC proceedings 2000; 13: 334-337.
19. Van Ypersele De Strihou C and Vanherweghem JL. The tragic paradigm of Chinese herbs nephropathy. Nephol Dial Transplant 1995; 10:157-160.
20. Kabanda A, Jadoul M, Lauwerys R, Bernard A, Van Ypersele De Strihou C. Low molecular weight proteinuria in Chinese herbs nephropathy. Kidney Int 1995; 48:1571-1576.
21. Nortier JL, Deschodt-Lanckman MM, Simon S, Thielemans NO, De Prez EG, Depierreux MF, Tielemans CL, Richar C, Lauwerijs RR, Bernard AM, Vanherweghem JL. Proximal tubular injury in Chinese herb nephropathy: monitoring by neutral endopeptidase enzymuria. Kidney Int 1997; 51: 288-293.
22. Vanherweghem JL, Abramowicz D, Tielemans C, Depierreux M. Effects of steroids on the Progression of Renal Failure in Chronic Interstitial renal fibrosis: a pilot study in Chinese herb nephropathy. Am J Kidney Dis 1996; 27: 209-215.
23. Muniz Martinez MC, Nortier J, Vereerstraeten P, Vanherweghem JL. Steroid therapy in chronic interstitial renal fibrosis: the case of Chinese-herb nephropathy. Nephrol Dial Transplant 2002; 17: 2033-2034.
24. Vanherweghem JL. Association of valvular heart disease with Chinese herb nephropathy. Lancet 1997; 350:1858 (letter).
25. Reginster F, Jadoul M, Van Ypersele De Strihou C. Chinese herbs nephropathy: presentation, natural history and fate after transplantation. Nephrol Dial Transplant 1997; 12:81-86.
26. Connoly HM, Crary JL, Mc Goon MD, Hensrud DD, Edwards BS, Edwards WD, Schaff HV. Valvular heart disease associated with fenfluramine - phentermine. N Engl J Med 1997; 337:581-588.
27. Unger P, Nortier J, Muniz Martinez MC, Plein D, Vandenbossche JL, Vereerstraeten P, Vanherweghem JL. High prevalence of fenfluramine-related aortic regurgitation in women with end-stage renal disease secondary to Chinese herb nephropathy. Nephrol Dial Transplant 2003; (in press).
28. Cosyns JP, Jadoul M, Squifflet JP, De Plaen JF, Ferluga D, Van Ypersele De Strihou C. Chinese herbs nepropathy: A clue to Balkan endemic nephropathy ? Kidney Int 1994; 45: 1680-1688.
29. Depierreux M, Van Damme B, Vanden Houte K, Vanherweghem JL. Pathologic aspects of a newly described nephropathy related to the prolonged use of Chinese herbs. Am J Kidney Dis 1994; 24: 172-180.
30. Jadoul M, De Plaen JF, Cosyns JP, Van Ypersele De Strihou C. Adverse effects from traditional Chinese medicine. Lancet 1993; 341:892-893.
31. Cosyns JP, Jadoul M, Squifflet JP, Van Cangh PJ, Van Ypersele De Strihou C. Urothelial malignancy in nephropathy due to Chinese herbs. Lancet 1994; 344:188 (letter).
32. Vanherweghem JL, Tielemans C, Simon J, Depierreux M. Chinese herbs nephropathy and renal pelvic carcinoma. Nephrol Dial Transplant 1995; 10: 270-273.
33. Lord GM, Cook T, Arlt VM, Schmeiser HH, Williams G, Pusey CD. Urothelial malignant disease and Chinese herbal nephropathy. Lancet 2001; 358:1515-1516.
34. Cosyns JP, Jadoul M, Squifflet JP, Wese FX, Van Ypersele De Strihou C. Urothelial lesions in Chinese herb nephropathy. Am J Kidney Dis 1999; 33: 1011-1017.

35. Nortier J, Muniz Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, Depierreux MF, De Pauw L, Abramowicz D, Vereerstraeten P, Vanherweghem JL. Urothelial carcinoma associated with the use of a Chinese herb (*Aristolochia Fangchi*). N Engl J Med 2000; 342: 1686-1692.
36. Nortier JL, Schmeiser HH, Muniz Martinez MC, Arlt VM, Vervaet C, Garbar Ch, Daelemans P, Vanherweghem JL. Invasive urothelial carcinoma after exposure to Chinese herbal medicine containing aristolochic acid may occur without severe renal failure. Nephrol Dial Transplant 2003; 18: 426-428.
37. Stephanovic V, Polenakovic MH. Balkan nephropathy: kidney disease beyond the Balkans. Am J Nephrol 1991; 11: 1-11.
38. Mengs U. Acute toxicity of aristolochic acid in rodents. Arch Toxicol 1987; 59: 328-331.
39. Jackson L, Kofman S, Weiss A, Brodovsky H. Aristolochic Acid (NSC-50413) : Phase I clinical study. Cancer Chemother 1964; 42 : 35-37.
40. Mengs U, Lang W, Poch JA. The carcinogeric action of aristolochic acid in rats. Arch Toxicol 1982; 51: 107-119.
41. Mc Intyre M. Chinese herbs: risk, side effects, and poisoning: the case for objective reporting and analysis reveal serious misrepresentation. J Altern Complement Med 1998; 4: 15-16.
42. Malak J. Chinese herb nephropathy is not (dex)fenfluramine nephropathy but serotonin nephropathy. J Altern Complement Med 1998; 4: 131-132 (letter).
43. Colson CR, De Greef KE, Duymelinck C, Simoens P, Verpoorten GA, De Broe ME. Role of serotonin in the development of Chinese herbs nephropathy. J Am Soc Nephrol 1998; 9: 593 A (Abstract).
44. De Broe ME. On a nephrotoxic and carcinogenic slimming regimen. Am J Kidney Dis 1999; 33: 1171-1173.
45. Schmeiser HH, Bieler CA, Wiessler M, Van Ypersele De Srihou C, Cosyns JP. Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy. Cancer Res 1996; 56: 2025-2028.
46. Bieler CA, Stiborova M, Wiessler M, Cosyns JP, Van Ypersele De Strihou C, Schmeiser HH. 32 P post labelling analysis of DNA adducts formed by aristolochic acid in tissues from patients with Chinese herbs nephropathy. Carcinogenesis 1997; 18: 1063-1067.
47. Muniz Martinez MC, Nortier J, Vereerstraeten P, Vanherweghem JL. Progression rate of Chinese herb nephropathy: impact of *Aristolochia Fangchi* ingested dose. Nephrol Dial Transplant 2002; 17: 408-412.
48. Vanherweghem JL, Cuykens JJ, Vandenbergh P, Bouman KP, Hagers Y. Valvular heart disease and Chinese herb nephropathy. Lancet 1998; 351: 991 (letter).
49. Gillerot G, Jadoul M, Arlt VM, van Ypersele De Strihou C, Schmeiser HH, But PP, Bieler CA, Cosyns JP. Aristolochic acid nephropathy in a Chinese patient: time to abandon the term "Chinese herbs nephropathy"? Am J Kidney Dis 2001; 38: E26
50. Debelle FD, Nortier JL, Arlt VM, De Prez EG, Vienne AR, Salmon IJ, Deschodt-Lanckman MM, Vanherweghem JL. Effect of dexfenfluramine on aristolochic acids nephrotoxicity in a rat model of Chinese herb nephropathy. Arch Toxicol 2003; (in press).
51. Stiborova M, Frei E, Wiessler M, Schmeiser HH. Human enzymes involved in the metabolic activation of carcinogenic aristolochic acids: evidence for reductive activation by cytochromes P450 1A1 and 1A2. Chem Res Toxicol 2001; 14: 1128-1137
52. Stiborova M, Frei E, Breuer A, Wiessler M, Schmeiser HH. Evidence for reductive activation of carcinogenic aristolochic acids by prostaglandin H synthase - (32)P-postlabeling analysis of DNA adduct formation. Mutat Res 2001; 493: 149-160.
53. Schmeiser HH, Scherf HR, Wiessler M. Activating mutations at codon 61 of the c-Ha-ras gene in thin-tissue sections of tumors induced by aristolochic acid in rats and mice. Cancer Lett 1991; 59: 139-143.
54. Lebeau C, Arlt VM, Schmeiser HH, Boom A, Verroust PJ, Devuyst O, Beauwens R. Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells. Kidney Int 2001; 60: 1332-1342
55. Cosyns JP, Goebbels RM, Liberton V, Schmeiser HH, Bieler CA, Bernard AM. Chinese herbs nephropathy associated slimming regimen induces tumours in the forestomach but not interstitial nephropathy in rats. Arch Toxicol 1998; 72: 738-743.
56. Cosyns JP, Dehoux JP, Guiot Y, Goebbels RM, Robert A, Bernard AM, Van Ypersele De Strihou C. Chronic aristolochic acid toxicity in rabbits: a model of Chinese herb nephropathy ? Kidney Int 2001; 59: 2164-2173.
57. Debelle FD, Nortier J, Deprez E, Garbar CH, Vienne AR, Salmon IJ, Deschodt-Lanckman M, Vanherweghem JL. aristolochic acids induce chronic renal failure with interstitial fibrosis in salt depleted rats. J Am Soc Nephrol 2002; 13: 431-436.
58. Zhu M, Phillipson JD. Hong Kong samples of the traditional Chinese Medicine "Fang ji" contain aristolochic acid toxins. Int J Pharmacognosy 1996; 34: 283-289.
59. Hashimoto K, Higuchi M, Makino B, Sakakibara I, Kubo M, Komatsu Y, Maruno M, Okada M. Quantitative analysis of aristolochic acids, toxic compounds, contained in some medicinal plants. J Ethnopharmacology 1999; 64: 185-189.
60. Vanherweghem JL. Aristolochia sp and chronic interstitial nephropathies in Indians. Lancet 1997; 349: 1399 (letter).
61. Lewis CJ, Alpert S. Letter to health Care Professionals on FDA concerned about botanical products, including dietary supplements containing aristolochic acid. Us Food and Drug Administration – Center for Food Safety and Applied Nutrition – Office of Natritional Products. Labeling and Dietary supplements. May 31, 2000.

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Balkan endemic nephropathy

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Introduction

Balkan (or endemic) nephropathy is a chronic tubulo-interstitial disease of unknown, presumably exotoxic etiology. It has been shown to exist only in some parts of the southeastern Europe.

There were about 30 scientific meetings on Balkan nephropathy. Over 500 papers on the disease were published by the year 1970 [1]. By late 1980's, this number rose to 3000 [2]. Sociopolitical turmoil, including wars, and economical hardship prevented any serious research on the problem during the 1990's. In spite of numerous proceedings and a large number of publications on the subject, many features of Balkan nephropathy, its etiology and natural history in particular, remained nearly as mysterious as when described in the mid-fifties.

Meetings organized by international organizations [3-7] had a key role in informing the international scientific community on the disease. An updated source of information is a bilingual (in English and Serbian) monograph published in 2000 [8].

Epidemiological features

Distribution and frequency

Though exclusive geographical restriction of the agent(s) of Balkan nephropathy is not very likely, the disease has been diagnosed only among people living (or those who used to live) in more or less well defined areas of the Balkans. Along with Bulgaria and Romania, three republics of the former Yugoslavia have been affected: Bosnia, Croatia and Serbia (Figure 1).

As recently summarized [9], the affected territory has a shape of a rhomboid. Its longer diameter spreads over 500 km (from the Vratza municipality in Bulgaria to villages west of Slavonski Brod in Croatia), while its transversal diameter has about 300 km (from endemic foci in eastern Romania to Vitina municipality in Kosovo). The disease affects individuals who live (or used to live) in rural environment. There are some spared households even in the most affected areas, leading to frequently cited remarks on mosaic distribution of the disease.

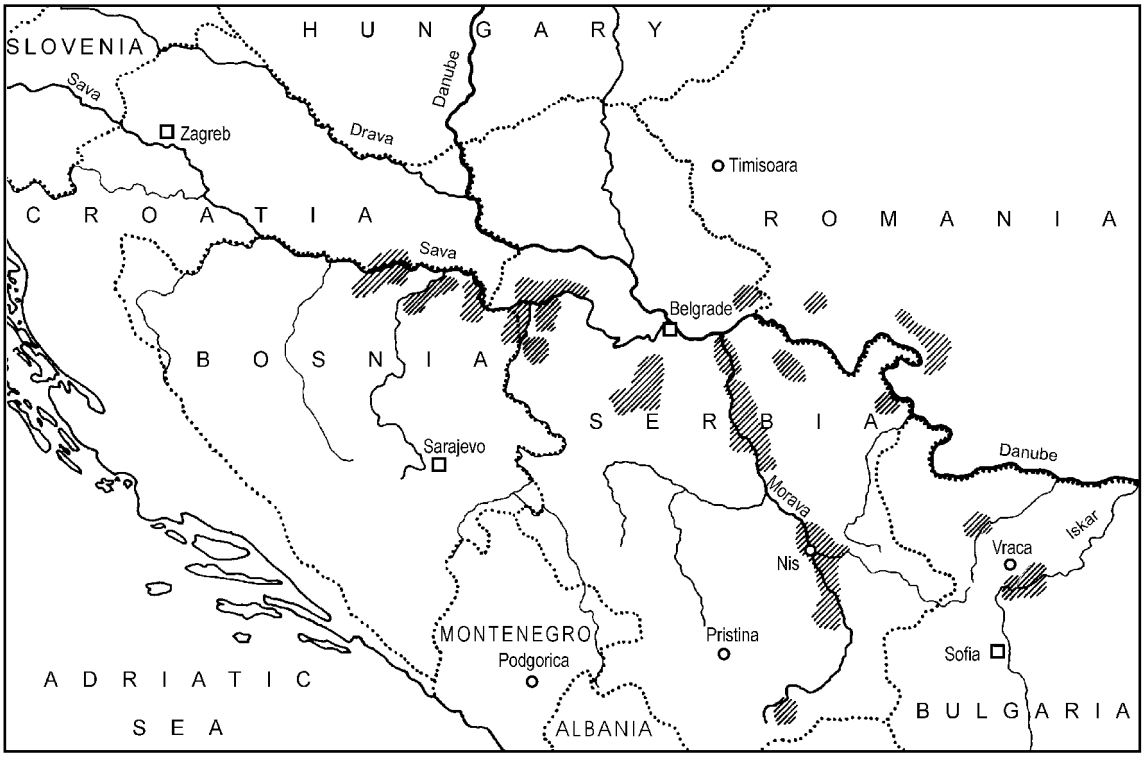

Figure 1. *Medical geography of Balkan nephropathy.*

Topography of the terrain differs between endemic regions. All 14 endemic villages in Croatia are located in a single lowland municipality, at an altitude of about 100 m, while Bosnian foci are found up to 130 m. About 90% of all endemic settlements in Serbia are also situated at a low altitude, below 200 m [10], either in large plains, river valleys or, much more seldom, in hilly regions. There have been no studies of medical geography of Balkan nephropathy in Romania for the last 30-35 years and endemic localities are yet to be determined [11], but the original findings pointed to hilly areas, with endemic villages laying at the bottom of valleys eroded by flooding, at an altitude of 200-300 m. The endemic regions in Bulgaria are described as mountainous or semi-mountainous, without any relationship between endemicity and altitude. Hydrogeological features [12] and lack of floods differ (at least some) Bulgarian foci from other typical endemic regions.

Controversial data on the frequency of Balkan nephropathy are mainly the result of methodological shortcomings [9]. A main obstacle is the operational definition of the disease, leading to huge differences in estimated prevalence rates. The highest reported average annual incidence rate is 16.6 per 1000 in Cakonica, Bulgaria. The average cause-specific mortality rate over 15 years in one of the most affected Serbian foci was 3.3 per 1000 [13].

Demographic data

During initial Balkan nephropathy research, patients were frequently in their thirties [14], and it was widely accepted that azotemia usually affects the age group 30-50 [3]. Later an apparent shift towards the older ages occurred, with most identified patients being above the age of 60 [9]. The diagnosis of clinical forms before the age of 20 was rare and never independently confirmed. Despite occasional statements on laboratory and bioptic abnormalities in the first decade of life among clinically healthy children from endemic areas, no follow up study ever confirmed that these children developed subsequent kidney disorder.

Both genders are similarly affected, especially considering mortality. As explained in details elsewhere [9], higher prevalence rates among women reported by some authors appears to be a consequence of unreliable diagnostic criteria.

A vast majority of experts believe that a link exist between agricultural activity and exposure to the agent(s) of Balkan nephropathy. There is also near consensus on the absence of ethnic and/or religious differences as a risk factor for developing the disease. The most convincing data come from Croatia, where the large group of Ukrainians who settled a century ago had the same odds of being affected as the indigenous population. In addition, the first generation immigrants also develop Balkan nephropathy, usually a couple of decades after moving into an endemic region.

Initial studies of affected households show a low standard of living, poor hygienic level, and insufficient nutrition. However, socio-economic factors, including living conditions and well water quality, did not differ between contiguous affected and non-affected households, or between endemic and neighboring non-endemic villages.

Chronological characteristics

The initial description of Balkan nephropathy eminated from Bulgaria [15, 16] and Serbia [17-19]. By 1957, the disease was recognized in Bosnia and Croatia, and by 1958 in Romania [20].

On retrospect, it was not a newly emerging condition but rather recognition of an already existing endemic process, a previous epidemic wave having occurred in the early forties. Unfortunately, attempts to trace the disease prior to World War 2 are speculative, due to the absence of reliable data and a high frequency of the competing causes of morbidity, notably tuberculosis and malaria.

Data on the secular trend of the disease are contradictory. The earliest affected Romanian endemic village, Ergevitza, now appears disease-free; however, the village was almost deserted and later repopulated by relatively young people (C. Tatu – personal communication). The duration of exposure is too short to make any judgement. The other extreme is the population of two best-known Serbian foci, Sopic and Petka, where the intensity of endemic process is unchanged (D. Bukvic – personal communication). In spite of such controversies, two facts, common to all endemic areas, are crucial in assessing any future trends. These are, an apparent shift of the age distribution of the incidence towards the older age groups, and a much longer natural history of the condition compared to previous data [9].

Other epidemiological characteristics

Clustering of cases within a household is one of the most conspicuous features of the disease. It is generally agreed that the disease affects both blood related and non-blood related family members. The "phenomenon of simultaneous deaths" (dying of parents and their children within a short interval) has also been observed.

Between 1/3 and 1/2 of patients with Balkan nephropathy develop urothelial tumors [21]. An exceptionally high frequency of these tumors was also observed in the general population of endemic regions [22]. The attributive risk of developing upper urothelial tumors in inhabitants of endemic foci amounts to several dozen [23] or even to as much as 100-200.

There is no evidence that domestic and/or wild animals in endemic regions develop a similar condition.

Overview of the descriptive epidemiological research

There is general agreement on the following descriptive-epidemiological characteristics of Balkan nephropathy [9]: The disease is known to exist only in some parts of the southeastern Europe, with Central Serbia as the most affected region. Balkan nephropathy has not spread beyond its already defined foci; the disease is distributed mosaically: non-endemic villages exist in the most affected regions, and there are spared families and households in the most affected settlements. There are clustering of cases in families and households. Children and adolescents are spared the clinical disease. Incidence is proportional to age, except for the oldest age groups. There are no clear differences in the cause-specific mortality rates. The excess risk of developing transitional cell urothelial tumors is expressed by two- or even three-digit numbers.

The large majority of researchers support the following statements [9]: autochthonous urban population is spared; rural way of life, i.e., agricultural activity is needed for exposure to the agent(s). Separation from an endemic focus early may prevent the disease, while immigration to an endemic area provides risk of disease development, providing that the exposure was sufficient. Prevalence of the disease has been stable over many years, but now appears to decline in most affected settlements. Incidence rates are shifting towards the older age groups, and the clinical course is much more protracted, suggesting a less intensive contact with the agent(s) and, consequently, possible future spontaneous disappearance of Balkan nephropathy (providing, of course, that standard of living does not deteriorate too much).

Etiology

Genetic factors

The most elaborate and, seemingly consistent, hypotheses regarding etiology come from proponents of heredity as an explanation of the disease occurrence. These authors assume that the risk of developing the disease is restricted only to specific, ethnically distinct, population groups, irrespectively of their place of birth and residence history. Wider acceptance of these hypotheses has been hampered by the different perception of descriptive epidemiology of Balkan nephropathy by a majority of researchers on the topic.

Currently one research group argues in favor of a crucial role of genetic factors. They identified a specific chromosome marker (3q25) in Balkan nephropathy patients [24-26]. Another research team saw some aberrations of the X chromosome, but they resembled changes occurring after exposure to ocharatoxin A [27].

Some authors described major anomalies of urinary organs in a high percentage of otherwise healthy children from affected households. "Foetal" and abnormal glomeruli were also observed among these children. Unfortunately no explanation exists for how severe anomalies, notably renal agenesis or double ureters, might disappear in adult life.

Genetic epidemiological approach suggest two possibilities, either polygenic type of inheritance with an insufficient expression of the main gene [28], or monofactorial model with a crucial role of a single gene of incomplete penetrance [29]. In both cases, a contributing environmental factor is needed.

There is no evidence supporting an immunological mechanism in Balkan nephropathy.

Biological agents and their products

Unspecified viral particles [30], an unidentified cytopathogenic agent, serially propagated slow viruses [31], and an unknown virus associated with foci of

natural infection [32] have been mentioned in the context of Balkan nephropathy etiology. Several specific viruses, notably West Nile [33], coronavirus [34], and papova virus [35], were also suggested as a causative agent. A common feature of all these hypotheses was the unimpressive supporting evidence and ignorance of basic epidemiological features of the disease, in particular its absence of spreading [36].

Bacteria received particular attention in initial stages of the Balkan nephropathy research but, for the last 20 years, their possible etiological importance has been unanimously ruled out [2]. Protozoa have never attracted any attention.

Toxic fungal products are the principle and prime potential culprits. Most efforts have concentrated on ochratoxin A, a mycotoxin responsible for porcine (swine) nephropathy [37]. The substance is found in endemic foci but is also present in neighboring non-endemic areas, and the differences are not statistically significant [38, 39]. Still, the consistent isolating of ochratoxin A in greater frequency and higher concentrations from food and sera samples obtained from endemic, compared to control villages, provides strong support for this hypothesis.

Association of ochratoxin A with chronic interstitial nephropathy in Tunisia [40] and its relation to renal tumors [41] provides additional support for the idea of the etiological role of this mycotoxin. Other fungal toxins such as zerealenone, citrinin [42] and aflatoxin were also isolated in endemic foci. Experimental models suggested that a combination of mycotoxins, rather than a single one, might be involved in the etiology of Balkan nephropathy [43].

Aristolochic acid and its salts, originated from a weed, *Aristolochia clematitis*, have toxic and carcinogenic effect to the kidneys and urothelium [44], respectively. Ivic [45] postulated that this plant may be a cause of Balkan nephropathy, but failed to provide convincing evidence from field surveys. Considerable evidence that *A. clematitis* plays a central role in the etiology of Chinese herb nephropathy [46-48], a condition similar to Balkan nephropathy, requires a second look at this previously abandoned hypothesis.

No local practice, in terms of the use of teas or folk medicine, could be implicated. No one has ever studied flora of the local wells.

Agents from the inanimate environment

Chronologically, lead poisoning was first offered as an explanation for the occurrence of Balkan nephropathy [17-19]. The idea on lead-contaminated flour led to abandonment of water mills in a part of Central Serbia. This energetic public health action had no impact on the disease frequency.

Effects of non-occupational exposure to cadmium [49], itai-itai disease in particular [50, 51], were occasionally compared with kidney damage seen in Balkan nephropathy patients. In spite of some resembling features, the idea of a common etiology between cadmium nephropathy (including itai-itai disease) and Balkan nephropathy was refuted [50, 52].

Many other metals, including radioactive ones such as uranium [53], were also suggested as possible causative agents of the disease. Results were both non-convincing and non-reproducible.

Inability to identify a single toxic effect of any metal or metalloid as a cause of Balkan nephropathy led researchers to two alternative approaches. First, deficiency, rather than abundance of such a chemical element was proposed [54], with selenium as the most likely candidate [55]. Second, attention was paid to a combined adverse effect of several elements. Synergism of uranium and some other elements, none of which exceeding maximal allowed levels, was proposed [56]. It was also noted that criteria used in occupational medicine (exposure only during working hours) have been applied to an ecological problem (constant exposure) and that concentrations of lead or cadmium within formally acceptable levels, combined with other factors, such as selenium deficiency, might lead to the disease [56]. All these suggestions remained speculative.

As for non-metals, there were attempts to relate Balkan nephropathy to silicon [57-60]. However, when affected and non-affected households were compared, there was even an inverse relationship between the silica content and endemicity. On one occasion, small differences in silica content happened to reach the level of statistical significance but the association was explained as a result of confounding variables [61].

A common hydrogeological characteristic of endemic foci [12] and an inverse relationship to altitude of wells and disease frequency in a longitudinal (cohort) study [61], point to potable water as a vehicle of

the agent(s). However, none of the previously mentioned or several dozen other non-organic substances were associated with the disease [62].

Organics in water have been investigated and provided some interesting data [63]. Except for nitrites [64], chemically unstable substances have not been studied. Wells associated with the disease are situated on alkali soil [65], but the finding was restricted to a single endemic area and has not been reproduced.

Based on chronological data, it is clear that no pesticides, fertilizers or chemicals introduced during the last few decades can be blamed for the occurrence of Balkan nephropathy. Except for exposure to agricultural activities, no occupation, habit (e.g., smoking, alcohol consumption), or hobby (e.g., hunting, fishing) has been shown to precede the disease onset.

Overview of the etiological research

Genetic factors may play a role, given the same exposure, in developing Balkan nephropathy, upper urothelial tumors, both diseases, or none of them [66]. However, epidemiological data indicate that one or more external, environmental factors are crucial for the occurrence of both Balkan nephropathy and excessive frequency of these tumors in endemic areas.

Among biological agents and their products, probable candidates for etiological agents are mycotoxins and possibly viruses. Toxic role of a plant, notably *Aristolochia clematitis*, is less likely, but cannot be excluded.

As for inanimate environment, there is no chemical element that could be singled out but two facts appear likely. First, insufficient rather than abundant concentrations should be looked at, and second, a combination of factors rather than a single one should be searched for. Organics in water and/or soil are an interesting track to be followed. The same applies to chemically unstable substances.

Pathomorphological changes

Balkan nephropathy is non-destructive and non-inflammatory tubulointerstitial renal disease [67]. The changes are non-specific and in the chronic, sclerotic phase they are similar to changes observed in other chronic interstitial diseases such as analgesic nephropathy [68], vascular nephrosclerosis [67] cyclosporine-induced nephropathy [69], radiation nephritis [70, 71] and aging [70], intoxication with silicate, cadmium, lead, uranium [72], Aristolochia clematitis [45], mycotoxin ochratoxin A [73], and Chinese herbs [46, 48].

Macroscopic features

Before introduction of hemodialysis in the treatment of chronic renal patients, the kidneys of patients who died of Balkan nephropathy used to be the smallest seen at post mortem examinations, weighing 14.8-80 g each (Figure 2A), the differences between the left and right kidneys being small (5-20 g) [72, 74-76]. Surface of the kidneys is smooth, occasionally wavy, but never granulated or roughly nodular. The section shows markedly narrowed cortex, pyramid and Bertin's columns are fairly well preserved, and corticomedullary border is well differentiated. Papillary necrosis of the pyramids has not been found.

Small, papillary, usually multiple tumors of the renal pelvis and ureters are also one of the characteristic findings (Figure 2B). In post-mortem studies tumors were reported in 8-50% of cases [72, 77].

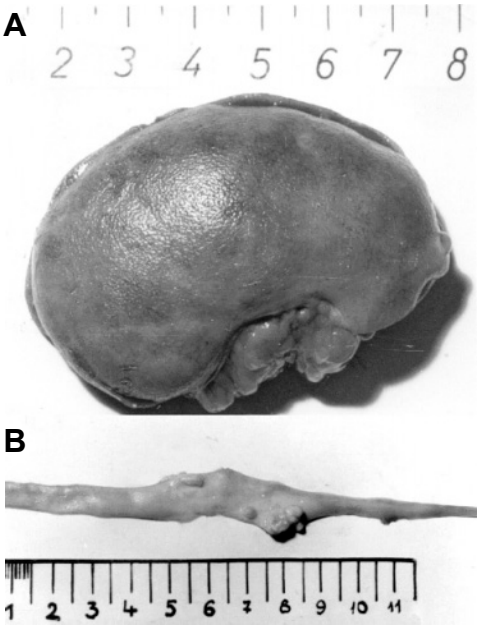

Figure 2. A. *Macroscopic appearance of the right kidney weighing 35 g in a case of BN, surface is smooth, occasionally mildly wrinkled (autopsy, a man aged 48).* ***B.*** *Multifocal papillary tumor of the right ureter (autopsy).*

Morphological studies of renal changes in post mortem material

Diffuse fibrosis of cortical interstitium and tubular atrophy may be observed in the absence of significant cellular interstitial infiltration. In contrast to the cortex, Bertin's columns are less markedly affected. Even in severe tubulointerstitial cortical changes, glomeruli are well preserved, partially collapsed, and subsequently subjected to focal or generalized sclerosis causing collapse. Glomeruli in Bertin's column are occasionally compensatorily enlarged. Pyramids are preserved or less markedly affected [72, 74, 77]

Blood vessels, arcuate or interlobular arteries, and arterioles are affected in the form of intimal sclerosis and thickening of lamina elastica interna. In addition, the blood vessels are compressed and torsioned [77].

In cases of tumors of the renal pelvis and ureters, morphological signs of pyelonephritis are recognized [72].

Optic microscopic, immunofluorescent and electron microscopic studies of renal biopsies

In oligosymptomatic clinical cases, rare disseminated foci of interstitial fibrosis and tubular atrophy with preserved glomeruli are seen. These changes have no special predilection of distribution and are not inflammatory. They tend to be triangular, with the base oriented toward the renal surface [67, 77]. In cases with initial renal failure, the fields of acellular interstitial fibrosis are larger and diffuse.

The striking atrophic process observed in Balkan nephropathy suggests that apoptosis may play a role in this disease. In this context it is of interest that Savin et al. observed an increased apoptosis to proliferation ratio at the level of the tubuli [78].

The glomeruli are usually affected by generalized [80%] or segmental sclerosis [10%] and only in 8% is hyalinosis recorded. Double contour glomerular basement membrane occurs in 22% of the cases. In 2.7-6% of cases fetal-like glomeruli can be seen in the kidneys, while glomerular hypercellularity was recorded in 4% [70, 77, 79].

The most interesting changes are present in pre- and postglomerular blood vessels. In about 50% of cases PAS positive proteins are deposited in vasa afferens walls in a focal segmental or circumferential manner in the form of droplets, bands or granules [70, 77, 79]. No tissue reaction is seen around these arterioles.

Interlobular capillaries are filled with thick, proteinaceous substances that are also deposited below the capillary endothelium and may even be found free in the interstitium. These changes are described as capillary sclerosis [68]. Although renal vascular changes in Balkan nephropathy have been pointed out as very important, they are not specific and can be encountered in other renal diseases. Ferluga et al. [70] and Sindjic [77] point out to similarities in early changes encountered in Balkan nephropathy with cyclosporine-induced nephrotoxic changes.

Immunofluorescence revealed irregular and scarce deposits of C3, fibrin and IgM, and occasionally IgA, C1q and C4, mainly on the vascular walls, Bowman's capsule and some sclerotic glomeruli [67, 80, 81].

Electron microscopic findings are either normal or correspond to degenerative and sclerotic changes. While some authors describe virus-like particles [34, 82, 83], others point out that such particles were not found [70, 71].

Despite these findings, some authors described Balkan nephropathy as a form of glomerulonephritis [79, 84]. However, the lack of reliable evidence supporting glomerulonephritis has lead to it being discarded [71, 75] and abandoned even by its advocates [85].

Optic microscopic, immunofluorescent and electron microscopic studies of renal biopsies in children aged 5-15 from affected families in endemic regions failed to detect any Balkan nephropathy related changes [77].

Overview of morphological studies

It is generally agreed that the morphologic changes of Balkan nephropathy are not specific and correspond to non-destructive, non-inflammatory kidney disease accompanied by marked changes on the blood vessels in both early and late stages of the disease, interstitial, multifocal fibrous expansion and severe tubular atrophy mainly in the upper cortex [67, 70, 71, 77, 79].

Changes on kidney arterioles have been described suggesting that the changes in early stage of the disease may be responsible for the development of multifocal, ischemic, vascular nephrosclerosis encountered in chronic stages of the disease [67, 70]. On the other hand, close similarity of Balkan nephropathy with an-

algesic and cyclosporine-induced nephropathy has been recognized [69, 70, 77]. All this leads to a suggestion that Balkan nephropathy develops following a model of toxic nephropathy, targeting primarily the vascular endothelium where the tubular epithelium is affected either directly or indirectly due to accompanying ischemia.

Clinical features, diagnostics and treatment

Clinical picture and course

Balkan nephropathy is a chronic tubulointerstitial disease with occult, insidious onset, usually progressing slowly without apparent signs and symptoms. After a long asymptomatic period, the disease is manifested as chronic renal failure. Less commonly blunt lumbar pain or renal colic may develop or, occasionally, dysuric symptoms induced by urinary tract infection. If hematuria exists, urothelial tumor should be suspected. In an advanced case polyuria and nocturia are present due to impaired concentrating ability of the kidneys. The disease is tolerated well and the patients preserve their working ability until advanced stages of renal failure [18, 74, 86, 87].

Objective examination reveals a characteristic skin tan of Balkan nephropathy patients: a pale yellow with copperish glow on the cheeks has been recognized since the augural reports on the disease [18, 86]. Besides, xantochromia of the palms and soles is also frequently observed.

Patients with Balkan nephropathy do not suffer from edema, and their blood pressure is usually normal [18, 86-88]. In the advanced phase of the disease physical examination detects signs of dysfunction of other organs or systems as a consequence of chronic renal failure [19].

As Balkan nephropathy is characterized by a slow asymptomatic course, most authors identify two main stages of the disease: the first, asymptomatic (latent, subclinical) and second, manifest (symptomatic). The latter is usually subdivided into the stage without renal failure (early, compensated Balkan nephropathy, with no azotemia) and chronic renal failure (decompensated Balkan nephropathy, uremia) [19, 86, 87].

An important feature of Balkan nephropathy is its association with a high incidence of tumors of the renal pelvis and ureters [22, 89]. Controversially, urinary bladder tumors are not more frequent in the regions of Balkan nephropathy compared to non-endemic ones [90]. Upper urothelial tumors of patients originating from the region with Balkan nephropathy differ from the same tumors identified in patients from other regions because of their similar incidence in both sexes, bilateral occurrence, slower evolution and more common association with chronic renal failure [90].

Laboratory findings

Appearance and urine color are unchanged in most patients with Balkan nephropathy. Urine sediment is usually scarce; when microhematuria or leukocyturia are encountered they are usually associated with the occurrence of tumors or urinary tract infection [86, 87].

Bacteriological studies usually reveal sterile urine, but in 8.3-31.8% significant bacteriuria was confirmed and considered due to superimposed urinary tract infection [86, 87].

Proteinuria is a common finding in patients with Balkan nephropathy [18, 86]. It is usually intermittent, less than 1 g per day but becomes permanent in advanced renal failure [91]. Although proteinuria is one of the criteria for diagnosis of Balkan nephropathy, it has been reported in healthy members of endemic families [29, 91, 92]. Tubular proteinuria is most common and increased excretion of low-molecular weight proteins such as beta2 -microglobulins, lysozyme, ribonuclease, light chains of immunoglobulin, retinol-binding protein has been reported [93-98]. Beside tubular proteinuria, smaller numbers of patients manifest mixed proteinuria, while in patients with renal failure, glomerular proteinuria may be encountered [96, 98].

Anemia has been noted in patients with Balkan nephropathy in early studies [18] and described as normocytic and normochromic or mildly hypochromic [86]. It has been suggested that anemia occurs earlier in the course of the disease progression than is the case in other renal diseases and that it precedes azotemia [86, 99], however, recent studies have failed to substantiate this claim [91, 100]. Also, there is no evidence that the anemia in Balkan nephropathy differs from anemia accompanying other renal diseases in either features [100] or rate of deterioration in the progression of renal failure [101]. Nevertheless, anemia in Balkan nephropathy patients treated with hemodialysis is

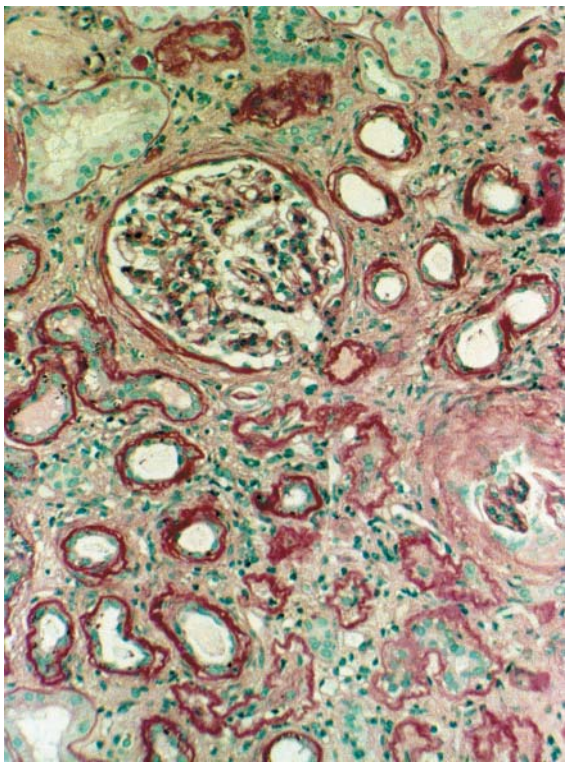

Figure 3. *Interstitial fibrosis and tubular atrophy; glomerulus with mild mesangial hypercellularity and another with incomplete hyalinosis. PAS, x120.*

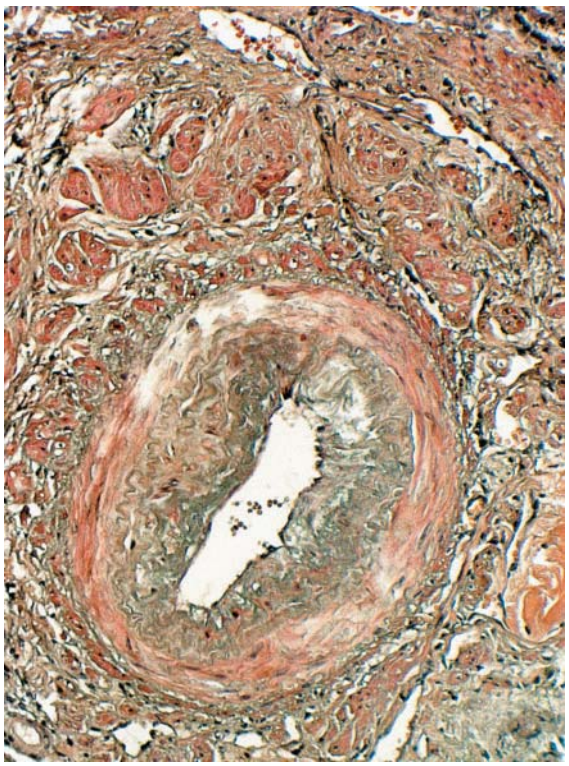

Figure 4. *Interlobar artery showing intimal fibrosis. PAS, x240.*

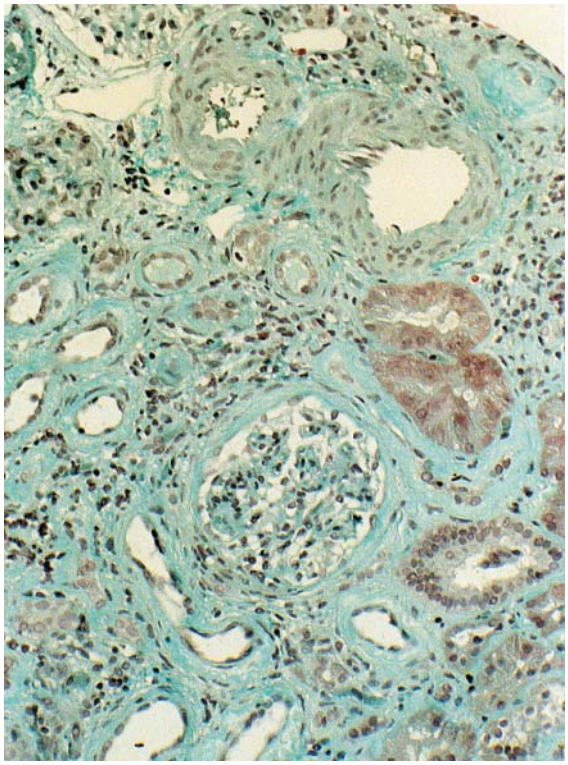

Figure 5. *Reduced number of tubules; fibrotic interstitium; few infiltrating cells. Masson's trichrome, x120.*

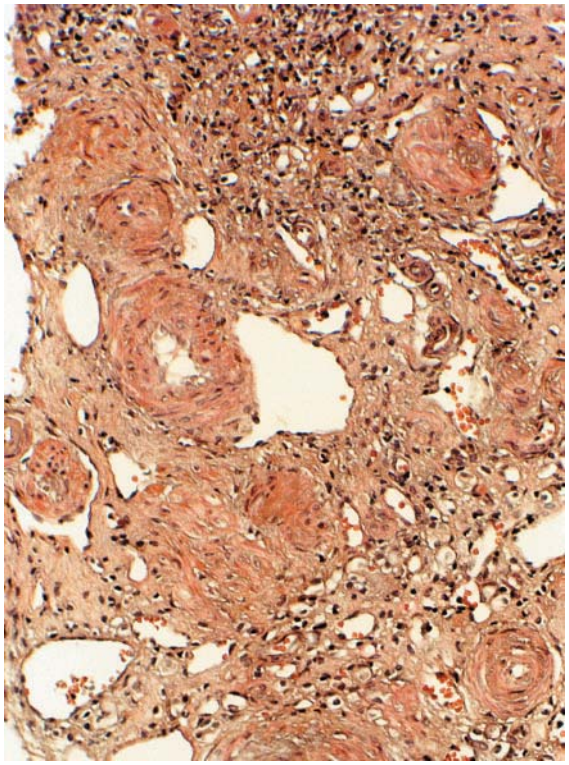

Figure 6. *Extensive interstitial scarring associated with severe arterio and arteriolosclerosis. PAS, x120.*

more severe than in patients with other renal diseases [101].

The leukocyte count in the peripheral blood of patients with Balkan nephropathy is normal and without pathological changes in the differential count and bone marrow [19, 86].

Investigation of renal function in patients with endemic nephropathy has revealed tubular dysfunction in the earliest stage of the disease: renal glycosuria, increased uric acid and amino acid excretion [94], as well as increased excretion of low molecular weight proteins [97]. Significantly higher activity of cellular enzymes in the urine and increased urinary excretion of Tamm-Horsfall protein have been described in patients with Balkan nephropathy, as well as in healthy members of endemic families [102]. Findings of a distal tubular disorder (impaired urinary acidification, impaired urine concentrating ability) were described in earlier studies [86, 87] but could not be confirmed in studies conducted in larger groups of patients with normal or mildly impaired glomerular filtration rate [91, 103]. The occurrence of certain disorders of the tubular function recorded in the course of chronic renal failure (increased natriuria, phosphaturia) can be considered as the result of kidney adaptation to the lost nephron mass, instead of Balkan nephropathy properties [103].

The immunological studies have failed to indicate that immune disorders participate in the pathogenesis of Balkan nephropathy, with some of the detected changes having been attributed to advanced renal failure [104].

Imaging methods

Different methods of kidney imaging have shown that Balkan nephropathy patients with chronic renal failure have symmetrically shrunken kidneys with smooth surface and no calcifications [88]. The time at which the shrinking occurs remains to be determined. While some authors suggest that the size of the kidneys remains normal in patients in the latent phase of the disease and with normal renal function, others report cases of shrunken kidneys in patients in an early phase with normal glomerular filtration rate, and it was even proposed that the disease was characterized with primarily small kidneys [91, 103, 105].

Excretory urography does not reveal changes in the pyelocaliceal system, except in cases with secondary infection or urothelial tumors.

Radionuclide methods have shown that renal plasma flow impairment is the first sign of the early phase of renal failure. Glomerular and tubular functions correspond to the severity of the disease.

Diagnosis

The most commonly used criteria for the diagnosis of Balkan nephropathy are those proposed by Danilovic et al [99]. They include: (1) farmers in the endemic villages, (2) familial history positive for endemic nephropathy, (3) mild proteinuria, (3) low specific gravity of the urine, (4) anemia, early occurrence, normochromic or hypochromic, (5) retention of nitrous compounds in the blood (urea > 50 mg/dl, creatinine > 1.5 mg/dl), (6) symmetrically shrunken kidneys. Using these criteria, Danilovic classified patients into the following groups:

1. *potential*, a group with intermittent proteinuria and positive familial history, including those that fulfill at least the first three criteria;
2. *suspected*, a group that in addition to the first three fulfill at least one of the remaining three criteria;
3. *affected*, a group that fulfill at least 5 out of 6 criteria;
4. *decompensated* patients that fulfill at least 5 out of 6 criteria and have urea values > 150 mg% and manifested signs of uremia.

Analysis of these criteria leads to the conclusion that they enable detection only of patients with overt disease, and the criteria are not sufficiently specific to enable a reliable diagnosis. Therefore, numerous studies have focused on developing sufficiently sensitive and specific criteria to allow diagnosis in the early phase. Although indications of tubular disorders, particularly tubular proteinuria, may be used as sufficiently specific diagnostic criteria, so far no single clinical or laboratory finding is considered pathognomonic for Balkan nephropathy when differentiate it from other tubulointerstitial diseases.

The diagnosis of Balkan nephropathy is now established according to epidemiological criteria suggested by Danilovic (criteria 1-3) [99], presence of tubular proteinuria and ruling out other renal diseases.

Histopathological analysis makes the diagnosis of

Balkan nephropathy much easier [70, 77], and it is considered indispensable in classifying the following groups of patients with urinary abnormalities suggestive of endemic nephropathy:

1. Patients from families that were not previously been affected with endemic nephropathy, but live in an endemic village;
2. In cases of nephropathy of unknown etiology in villages close to endemic foci;
3. In immigrants to endemic regions and in emigrants from these regions [104].

Differential diagnosis of Balkan nephropathy should include all chronic, slowly progressive renal diseases, primarily chronic tubulointerstitial diseases. Although no specific indicators of Balkan nephropathy have been recognized, epidemiological data, familial history as well as clinical characteristics of the disease enable differential diagnosis. Thus, shrunken kidneys with smooth surface are characteristic of Balkan nephropathy and they differentiate it from analgesic nephropathy, pyelonephritis or reflux nephropathy that are characterized by shrunken kidneys with uneven surface. Pyelocaliceal system of the kidneys remains unaffected in patients with Balkan nephropathy, unlike the characteristic changes observed in pyelonephritis or obstructive nephropathy. Absence of papillary calcifications also enables differentiation of Balkan nephropathy from analgesic, obstructive, reflux nephropathy [103, 106].

Recently similarity of Balkan nephropathy and nephropathy induced by Chinese herbs used in slimming diets have been suggested [47]. Nevertheless, Chinese herb nephropathy is a rapid progressive tubulointerstitial disease with pronounced fibrosis and progression towards end-stage renal disease within few years, clearly different from the protracted clinical course of Balkan nephropathy.

Prevention and treatment

Balkan nephropathy is a disease of unknown etiopathogenesis, so that recommendations regarding effective prevention are not possible. Efforts have been made to improve the living conditions, bring high quality drinking water to endemic villages and undertake other hygienic measures. Treatment is planned according to the stage of renal impairment. In principle, the treatment involves treating any reversible factors, which deteriorate the renal function and measures applied in chronic renal failure [107].

End-stage renal disease is treated with dialysis and kidney transplantation. Hypertension and cardiovascular diseases affect the Balkan nephropathy patients less frequently, so they tolerate hemodialysis rather well compared to patients with other renal diseases. The Balkan nephropathy patients on long-term hemodialysis frequently develop upper urothelial or urinary bladder carcinoma.

Although the number of reported cases with kidney transplant is small, neither specific post-transplantation problems nor disease recurrence on the transplanted kidney have been described.

Overview of clinical and laboratory studies

Balkan nephropathy is a chronic tubulointerstitial disease with insidious occult onset which progresses without symptoms. Agreement as to how to define the early asymptomatic phase of the disease is lacking, since no specific indicators for the diagnosis have been recognized. The diagnosis is established according to epidemiological criteria (farmers in endemic villages, familial history positive for endemic nephropathy), presence of tubular proteinuria, findings of symmetrically shrunk kidneys with smooth surface, without calcifications and ruling out of other renal disease. Renal biopsy may make the diagnosis easier, although the changes are non-specific. One important feature of Balkan nephropathy is the associated high incidence of tumors of the renal pelvic and ureters.

So far, laboratory studies have failed to detect any disorder as a specific marker for early detection of the disease or a reliable indicator for differential diagnosis. Laboratory studies have confirmed that Balkan nephropathy is a tubulointerstitial disease so that tubular disorders precede impairment of glomerular filtration. Although anemia is one of the criteria for the diagnosis of the disease, it has not been evidenced that pathogenesis and features of this anemia differ from that observed in other chronic renal diseases. It is only more severe in end-stage Balkan nephropathy patients than in patients with other kidney diseases.

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References

1. Astrug A. (editor). Endemic Nephropathy – Bibliography 1956-1970, Sofia: Ministry of Public Health; 1971.
2. Radovanovic Z. Aetiology of Balkan nephropathy: a reappraisal after 30 years. Eur J Epidemiol 1989; 5: 372-377.
3. World Health Organization. The "Endemic Nephropathy" of South-Eastern Europe. Bull Wld Hlth Org 1965; 32(3): 431-448.
4. Wolstenholme GEW, Knight J (editors). The Balkan Nephropathy. Ciba Foundation Study Group No. 30. London: Churchill; 1967.
5. World Health Organization. WHO Meeting of Investigators on Endemic Nephropathy, Belgrade and Lazarevac, November 1974, NCD/WP/74.
6. Castegnaro M, Plestina R, Dirheimer G, Chernozemsky IN, Bartsch H (editors). Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours. IARC Scientific Publications No. 115. Lyon: International Agency for Research on Cancer; 1991.
7. Hall P, Batuman V (guest editors). Kidney Int 1991; 40: Suppl 34 (Proc IUPAB meetings, Belgrade, 1989 and 1990).
8. Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic Lj, Petronic V. Endemic Nephropathy. Belgrade: Office for Textbooks and Teaching Aids, 2000.
9. Radovanovic Z. Epidemiology and aetiology of endemic nephropathy. In: Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic Lj, Petronic V. Endemic Nephropathy. Belgrade: Office for Textbooks and Teaching Aids, 2000.
10. Radovanovic Z. Topographical distribution of the Balkan endemic nephropathy in Serbia (Yugoslavia). Trop geogr Med 1979; 31: 185-189.
11. Gluhovschi G, Stefanovic V, Dimitrov Tz, et al. Endemic Balkan Nephropathy. Helicon, Timisoara, 1994.
12. Radovanovic Z, Peric J. Hydrogeological characteristics of endemic nephropathy foci. Public Health 1979; 93: 76-81.
13. Radovanovic Z, Danilovic V, Velimirovic D et al. Beta$_2$-microglobulinuria as a predictor of death in a population exposed to Balkan endemic nephropathy. Kidney Int 1991; 40(S34): 32-34.
14. Danilovic V. Effet des recherches des néphrites endemiques en Yougoslavie. Arch. Union Méd Balkan 1964; 2(1): 31-40.
15. Tancev I, Evstatijev P, Dorosiev D, Penceva Z, Cvetkov G. Proucvanija na nefritite v'v Vracanska okolija. S'vr Med 1956; 7(9): 14-29.
16. Ivanov L. Proucvanija v'rhu etiologijata na b'brecnite zaboljavanija v'v Vracanska okolija. S'vr Med 1956; 7(9): 30-34.
17. Danilovic V, Djurisic M, Mokranjac M, Stojimirovic B, Zivojinovic J, Stojakovic P. Porodicna oboljenja bubrega u selu Sopic izazvana hronicnom intoksikacijom olovom. Srp arh celok lek 1957; 85(10): 1115-1125.
18. Danilovic V, Djurisic M, Mokranjac M, Stojimirovic B, Zivojinovic J, Stojakovic P. Néphrites chroniques provoquées par l'intoxication au plomb par voie digestive (farine). Presse Méd 1957; 65(90): 2039-2040.
19. Danilovic V. Chronic nephritis due to ingestion of lead-contaminated flour. Brit Med J 1958; 1: 27-28.
20. Milojcic B. Epidemic of chronic nephritis of unknown aetiology. Brit Med J 1960; i: 244-245.
21. Radovanovic Z, Jankovic S, Jevremovic I. Incidence of tumors of urinary organs in a focus of Balkan endemic nephropathy. Kidney Int 1991; 40(S34): 75-76.
22. Petkovic S, Mutavdzic M, Petronic V, Markovic V. Les tumeurs du bassinet et de l'uretere. Recherches cliniques et étiologique. J Urol Néphrol 1971; 77(6): 429-439.
23. Radovanovic Z, Krajinovic S, Petkovic S, Hall PW. Papillary transitional cell tumours, Balkan nephropathy, and ß2-microglobulin. Lancet 1981; 2: 689.
24. Toncheva D, Dimitrov Ts, Tzoneva M. Cytogenetic studies in Balkan endemic nephropathy. Nephron 1988; 48: 18-21.
25. Toncheva D, Gergov T, Tzoneva M, Bouchakliev Z. Spontaneous and induced chromosome aberrations in Balkan endemic nephropathy. Kidney Int 1991; 40 (Suppl 34): 97-101.
26. Toncheva D, Dimitrov T. Genetic predisposition to Balkan endemic nephropathy. Nephron 1996; 72(4): 564-569.
27. Manolova Y, Manolov G, Parvanova L, Petkova-Bocharova T, Castegnaro M, Chernozemsky IN. Induction of characteristic chromosomal aberrations, particularly X-trisomy, in cultured human lymphocytes treated by ochratoxin A, a mycotoxin implicated in Balkan endemic nephropathy. Mutat Res 1990; 231(2): 143-149.
28. Tucic N, Marinkovic D. The inheritance of liability of Balkan nephropathy. Genetika 1979; 11(3): 213-220.
29. Banjevic D, Tucic N. The monofactorial model for inheritance of liability to disease and its implications for relatives at risk. Genetika 1979; 11(3): 221-230.
30. Georgescu L, Litvac B, Manescu N, Petrovici A, Schwartzkopf A, Zosin C. Particules virales dans le rein de la nephropathie endemique Balcanique. Sem Hôp Paris 1970; 46: 3526.
31. Draganescu N, Antipa C, Girjabu E, Melencu M. Endemic Balkan nephropathy, a slow virus disease? Rev Roum Med Virol. 1983; 34(1): 3-6.
32. Birtasevic B, Vukovic B, Drndarevic D, Seguljev Z, Obradovic M, Pokorni D, Cobeljic M, Stojic P, Stefanovic S, Stankovic A, et al. Endemska (Balkanska) nefropatija kao prirodno-zarisna infekcija? Vojno-sanitetski pregled 1983; 40: 319-324.
33. Kraus N. Studien über die endemische Nephrozirrhose der Balkanhalbinsel. Zeitschrift für ärztliche Fortbildung 1966; 60: 829-834.

34. Apostolov K, Spasic P, Bojanic N. Evidence of a viral etiology in endemic (Balkan) nephropathy. Lancet 1975; 2: 1271-1273.
35. Nastac E, Stoian M, Hozoc M, Iosipenco M, Melencu M. Further data on the prevalence of serum antibodies to papova viruses (BK and SV40) in subjects from the Romanian area with Balkan endemic nephropathy. Rev Roum Med-Virol 1984; 35(1): 65-67.
36. Radovanovic Z. Epidemiological evidence on Balkan nephropathy as a viral disease. Medical Hypotheses 1987; 22: 171-175.
37. Krogh P. Causal association of mycotoxic nephropathy. Acta Path Microbiol Scand Sect A (Suppl) 1978; 269: 1-28.
38. Petkova-Bocharova T, Castegnaro M. Ochratoxin A contamination of cereals in an area of high incidence of Balkan endemic nephropathy in Bulgaria. Food Addit Contam 1985; 2: 267-270.
39. Cvetnic Z, Pepeljnjak S. Ochratoxinogenicity of Aspergillus ochraceus strains from nephropathic and non-nephropathic areas in Yugoslavia. Mycopathologia 1990; 110(2): 93-99.
40. Maaroufi K, Achour A, Betbeder AM, Hammami M, Ellouz F, Creppy EE, Bacha H. Foodstuffs and human blood contamination by the mycotoxin ochratoxin A: correlation with chronic interstitial nephropathy in Tunisia. Arch Toxicol 1995; 69: 552-558.
41. Maaroufi K, Pfohl-Leszkowicz A, Achour A, el May M, Grosse Y, Hammami M, Ellouz F, Creppy EE, Bacha H. Ochratoxin A genotoxicity, relation to renal tumors. Arch. Inst. Pasteur Tunis 1994; 71(1-2): 21-31.
42. Yeulet SE, Mantle PG, Rudge MS, Greig JB. Nephrotoxicity of *Penicillium aurantiogriseum*, a possible factor in the aetiology of Balkan endemic nephropathy. Mycopathologia 1988; 102: 21-30.
43. Bach PH, Gregg NJ, Delacruz L. Relevance of a rat model of papillary necrosis and upper urothelial carcinoma in understanding the role of ochratoxin A in Balkan endemic nephropathy and its associated carcinoma. Food Chem. Toxicol 1992; 30(3): 205-211.
44. Mengs U, Lang W, Poch JA. The carcinogenic action of aristolochic acid in rats. Arch Toxicol 1982; 51: 107-119.
45. Ivic M. Problem etiologije endemske nefropatije. Acta Fac Med Naiss 1970; 1: 29-38.
46. Vanherweghem JL, Depierreux M, Tielemans C, Abramowicz D, Dratwa M, Jadoul M, Richard C, Vandervelde D, Verbeelen D, Vanhaelen-Fastre R, et al. Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet 1993; 341:387-391.
47. Cosyns JP, Jadoul M, Squifflet JP, De Plaen JF, Ferluga D, van Ypersele de Strihou C. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy ? Kidney Int 1994; 45(6): 1680-1688.
48. Cosyns JP, Dehoux JP, Guiot Y, Goebbels RM, Robert A, Bernard AM, van Ypersele de Strihou C. Chronic aristolochic acid toxicity in rabbits: a model of Chinese herbs nephropathy ? Kidney Int. 2001; 59(6): 2164-2173
49. Nogawa K, Kobayashi E, Honda R, Ishizaki A, Kawano S, Matsuda H. Renal dysfunctions of inhabitants in a cadmium-polluted area. Environ Res 1980; 23(1): 13-23.
50. Danilovic V (editor). Bulletin, T. LXIX, Classe des sciences medicales No. 12. Beograd: Academie Serbe des Sciences et des Arts, 1980.
51. Frieberg L, Elinder C-G, Kjellstrom T, Nordberg GF (editors). Cadmium and health: a toxicological and epidemiological appraisal. CRC Press, Boca Raton 1986.
52. Piscator M, Kostial K, Plestina R. Urinary excretion of cadmium and zink among women with Balkan endemic nephropathy. Trace Elements Med 1984; 1: 134-138.
53. Ignjatovic B. Prilog proucavanju etiologije hronicnog nefrita. Narodno zdravlje 1962; 18: 329-334.
54. Maksimovic Z, Radovanovic Z. Balkan endemic nephropathy in Yugoslavia and geochemical studies. In: Trace Substances in Environmental Health (18). Hemphill DD (editor). Univ Missouri 1984; p 230-236.
55. Maksimovic Z. Selenium deficiency and Balkan endemic nephropathy. Kidney Int 1991; 40 (suppl 34): S12-S14.
56. Wedeen RP. Environmental renal disease. Lead, cadmium and Balkan endemic nephropathy. Kidney Int 1991; 40 (suppl 34): S4-S8.
57. Duancic V. Essai d'explication de la manifestation de l'affection rénale de masse å Slavonski Kobas. Biol glasnik 1960; 3: 43-50.
58. Markovic B, Lebedev S. Role étiopathogénique des silicates érosifs dans la néphrite endémique. Presse méd. 1965; 73(8): 401-404.
59. Markovic B, Arambasic M. Experimental chronic interstitial nephritis compared with endemic human nephropathy. J Pathol 1971; 103: 35-40.
60. Goldsmith JR, Goldsmith DF. Fiberglass or silica exposure and increased nephritis or ESRD (end-stage renal disease). Am J Ind Med 1993; 23: 873-881.
61. Radovanovic Z, Markovic-Denic Lj, Marinkovic J, Jevremovic I, Jankovic S. Well water characteristics and the Balkan nephropathy. Nephron 1991; 57: 52-54.
62. Radovanovic Z, Edmunds WM. Groundwater chemistry and the incidence of Balkan endemic nephropathy. Environ Geochem Health 1991; 13(2): 43-49.
63. Finkelman RB, Feder GL, Orem WH, Radovanovic Z. Relationship between low-rank coal deposits and Balkan endemic nephropathy. AGID News 1991; 65: 23.

64. Radovanovic Z, Stevanovic D. Different forms of mineral nitrogen in drinking water and the Balkan nephropathy. Arch Environm Contam Toxicol 1988; 17: 813-815.
65. Filipovic A, Abinun A, Nijemcevic S, Lekovic G, Popovic N. Ispitivanje ovisnosti pojave endemske nefropatije i sastava zemljista iz kojeg stanovnistvo uzima vodu za pice u opstini Modrica. Medicniski arhiv 1977; 31(5): 277-284.
66. Radovanovic Z, Velimirovic D, Naumovic T. Upper urothelial tumours and the Balkan nephropathy - Inference from the study of a family pedigree. Eur J Cancer 1990; 26: 391-392.
67. Sindjic M. Pathomorphology of the kidneys in endemic nephropathy. Academie Serbe des Scientes et des Arts Bulletin 1980; 69: 127-140.
68. Zollinger HU, Mihatsch MJ, Torhost J. Nephropathy due to analgesics. Morphological aspects. XIII Inter. Congr Inter Acad Pathology, Paris, 1980; 17 (Abstract).
69. Mihatsch MJ, Thuel G, Ryffel N. Morphology of cyclosporin nephropathy. Prog Allergy 1986; 38: 447-465.
70. Ferluga D, Hvala A, Vizjak A, Trnavcevic S, Halibasic A. Renal function, protein excretion and pathology of Balkan endemic nephropathy. III Light and electron micorscopy studies. Kidney Int 1991; 40 (Suppl 34): S54-S67.
71. Hall PW III, Dammin GJ Balkan nephropathy. In: Renal pathology with clinical and functional correlations. Fisher G, Brenner BM (editors). JB Lippincott Co, Philadelphia, 1989; p. 913-924.
72. Sindjic M. Resultats des recheches patho-anatomiques, faites jusqu'a present, concernant la nephropathie endemique. In: I Symposium sur la nephropathie endemique. 1973 Beograd. Danilovic V (redacteur). Academie Serbe des Scientces et des Arts, Beograd 1973; 187-202.
73. Krogh P, Hald B, Pleština R, Ceovic S. Balkan (endemic) nephropathy: evidence for support of a causal relationship. In: Endemic (Balkan) Nephropathy. Proceedings of the 4th Symposium on Endemic (Balkan) Nephropathy. Strahinjic S, Stefanovic V (editors). University Press, Niš, 1979; 35-43.
74. Radonic M, Radoševic Z, •upanic V. Endemic nephropathy in Yugoslavia. In: The Kidney. Mostby S, David E (editors). Wiliams & Wilkins Co, Baltimore 1966; p. 503-522.
75. Zosin L et al. Aspects anatomo-cliniques de la néphropathie endémique balkanique. Sém Hôp, 1966; 42(3/1): 194-201.
76. Hall PW et al. Investigation of chronic endemic nephropathy in Yugoslavia. II Renal Pathology. Am J Med 1965; 39(2): 210-217.
77. Sindjic M. Morphological changes in kidneys affected by endemic nephropathy. In: Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic LJ, Petronic V. Endemic nephropathy. Belgrade: Office for Textbooks and Teaching Aids, 2000.
78. Savin M, Bumbaširevic V, Djukanovic LJ, Petronic V. The significance of apoptosis for early diagnosis of Balkan nephropathy. Nephrol Dial Transplant 2001; 16 (Suppl 6): 30-32.
79. Doichinov D, Todorov D, Dinev I, Milev B. Biopsy specimen examination during early stage of endemic nephropathy. In: II Simposium sur la nephropathie endemique. 1977 Beograd-Lazarevac. Danilovic V (redacteur). Academie Serbe des Sciences et des Arts, Beograd 1979; p. 189-194
80. Polenakovic M, Hrizoho D. Endemska nefropatija – imunofluorescentna mikroskopska ispitivanja. In: II Simpozijum o endemskoj nefropatiji, Beograd-Lazarevac 1977; Danilovic V (editor) SANU, Beograd 1979; p. 137-150.
81. Vizjak A, Trnacevic S, Ferluga D, Halilbašic A. Renal function, protein excretion and pathology of Balkan endemic nephropathy. IV-Immunohistology. Kidney Int 1991; 40(34): 68-74.
82. Georgescu L, Litvac B, Diosi P, Plavosin L, Herzog G. Viruses in endemic (Balkan) nephropathy. Lancet 1976; i: 1086.
83. Uzelac-Keserovic B, Vasic D, Ikonomovski J, Bojanic N, Apostolov K. Isolation of a coronavirus from urinary tract tumours of endemic Balkan nephropathy patients. Nephron 2000; 86: 93-94
84. Pantic VR, Suša S. Ultrastructure of nephrons in kidney of endemic nephropathy patients. In: Endemic (Balkan) Nephropathy. Proc 4th Symp, Nis 1979. Strahinjic S, Stefanovic V (editors). Inst Nephr Haemod, Nis 1981; p. 123-132.
85. Doitchinov D. Immunohistochemical studies in Balkan endemic nephropathy. In: Endemic (Balkan) Nephropathy. Proc 4th Symp, Nis 1979. Strahinjic S, Stefanovic V (editors). Inst Nephr Haemod, Nis 1981; p. 265.
86. Puchlev A, Popov N, Astrug A, Dotchev D. Clinical studies on endemic nephropathy in Bulgaria. In: Int Symp on End Nephr, Sofia, 1963, Bulg Acad Sc Press, Sofia 1965; p. 17-24.
87. Bruckner I, Zosin C, Lazarescu R, et al. A clinical study of nephropathy of an endemic character in the People's Republic of Rumania. In: Int Symp on Endemic Nephropathy, Sofia 1963. Bulg Sofia: Acad Sc Press; 1965; p. 25-35.
88. Djukanovic LJ, Velimirovic D, Sindjic M. Balkan Nephropathy. In: Clinical nephrotoxins - renal injury from drugs and chemicals. De Broe ME, Porter GA, Bennett WM, Verpooten GA (editors). Kluwer Academic Publishers, Dordrecht 1988; p. 426-436.
89. Petkovic S. Epidemiology and treatment of renal pelvic and ureteral tumours. J Urol 1971; 114: 858.
90. Petronic V. Tumors of the upper urothelium and endemic nephropathy. In: Endemic nephropathy. Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic LJ, Petronic V (editors). Office for Textbooks and Teaching Aids, Beograd, 2000.
91. Trnacevic S, Halilbašic A, Ferluga D, Plavljanic D, Vizjak A, Durakovic H, Habul V, Mesic E, Imamovic G, Hranisavljevic J, et al. Renal function, protein excretion and pathology of Balkan endemic nephropathy. I. Renal function. Kidney Int (Suppl) 1991; 34: S49-S51.

92. Cvorišcec D, Radonic M, Ceovic S, Aleraj B. Characteristics of proteinuria in endemic nephropathy. J Clin Chem Clin Biochem 1983; 21: 569-571.
93. Bruckner I, Stoica G, Serban M. Studies on urinary proteins. In: The Balkan Nephropathy. Wolstenholme GEW and Knight J (editors). Ciba Foundation Study Group No. 30. Churchill, London 1967; p. 15-20.
94. Hall P W, III, Piscator M, Vasiljevic M, Popovic N. Renal function studies in individuals with the tubular proteinuria of endemic Balkan nephropathy. Q J Med 1972; 41: 385-393.
95. Radoševic Z, Traeger J, Radonic M, Manuel Y, Revillard J P. Etude elctrophoretique de la proteinurie de 31 sujet crates vivant en pays de nephropathie endemique. J Urol Nephrol 1968; 74: 703-710.
96. Bruckner I, Stoica Gh. Urinary proteins in renal diseases. II. Alpha$_2$-microglobulins in tubular protienuria with special reference to endemic nephropathy. Rev Roum Med Med Int 1978; 16: 2-8.
97. Hall PW, Vasiljevic M. Beta2-microglobulin excretion as an index of renal tubular disorders with special reference to endemic Balkan nephropathy. J Lab Clin Med 1973; 81: 897-904.
98. Raicevic S, Trnacevic S, Hranisavljevic J, Vucelic D. Renal function, protein excretion, and pathology of Balkan endemic nephropathy. II. Protein excretion. Kidney Int 1991; 40 (Suppl 34): S52-S56.
99. Danilovic V. Endemic nephropathy in Yugoslavia. In: Endemic (Balkan) Nephropathy. Proc 4th Symp, Niš 1979. Strahinjic S, Stefanovic V (editors). Inst Nephr Haemod, Niš 1981; p. 1-5.
100. Pavlovic-Kentera V, Djukanovic LJ, Clemons GK, Trbojevic S, Dimkovic N, Slavkovic A. Anaemia in Balkan endemic nephropathy. Kidney Int 1991; 40 (Suppl 34): S46-S48.
101. Pavlovic-Kentera V, Clemons GK, Trbojevic S, Dimkovic N, Djukanovic LJ. Erythropoietin and anemia in the progression of Balkan endemic nephropathy and other renal diseases. Nephron 1990; 54: 139-143.
102. Cvorišcec D, Stavljenic A, Radonic M. Relationship between tubular and Tamm-Horsfall proteinuria in Balkan endemic nephropathy. Nephron 1998; 42: 152-155.
103. Djukanovic LJ. Laboratory studies. In: Endemic nephropathy. Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic LJ, Petronic V (editors). Office for Textbooks and Teaching Aids, Beograd 2000.
104. Polenakovic M, Stefanovic V. Balkan Nephropathy. In: Oxford textbook of clinical nephrology, Oxford medical Publications, Sec. Davison A, Cameron JS, Grunfeld JP, Kerr DNS, Ritz E, Winearls Ch (editors). Oxford University Press, Oxford 1998; p. 1202-1210.
105. Hrisoho D T. Balkan Endemic Nephropathy. Study of pathogenesis based on genetic factors. In: Endemic (Balkan) Nephropathy. Proc 4th Symp, Niš 1979. Strahinjic S, Stefanovic V (editors). Inst Nephr Haemod, Niš 1979; 1981; p. 147-154.
106. Elseviers MM, De Schepper A, Corthouts R, Bosmans JL, Cosyn L, Lins RL, Lornoy W, Matthys E, Roose R, Van Caesbroeck D, et al. High diagnostic performance of CT scan for analgesic nephropathy in patients with incipient to severe renal failure. Kidney Int 1995; 48(4): 1316-1323
107. Polenakovic M, Djukanovic LJ. Clinical picture, diagnostics and treatment of endemic nephropathy. In: Endemic nephropathy. Radovanovic Z, Sindjic M, Polenakovic M, Djukanovic LJ, Petronic V (editors). Belgrade: Office for Textbooks and Teaching Aids, 2000.

31

Nephrotoxins in Africa

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Introduction

Toxin-induced acute renal failure (ARF) is a common cause of morbidity and mortality in Africa. However reports in the medical literature are limited because in the majority of cases, identification of toxin is missing [1].

A majority of the toxin induced acute renal failure in South Africa follows a visit to the traditional diviner (the "sangoma"). This often results in a conspiracy of silence; the patients are reluctant to admit such a visit and in most instances deny both the consultation, and subsequent ingestion of prescribed herbal therapy [2]. This handicaps the planning of management, particularly as some toxins have multi-system effects, e.g. acute renal failure accompanied by hepatitis and colitis, as occurs in dichromate poisoning [3]. While the majority of patients admitted with poisoning have been prescribed by traditional healers, approximately 12% of the patients have obtained their medications from "African" shops (equivalent of a western–style chemist) [1]. It is not always the diviner who is responsible

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 603-611

for the prescribing these toxins, but rather the patients who buy medicines without completely understanding their content.

De Smet [4] and others [5] have advocated the need to disseminate knowledge about the risks and benefits of herbal and alternative medicines. Such information would allow 'ingestors' of such medicines the knowledge to decide whether or not to consume herbal concoctions.

The ingestion of alternative medicines for the improvement of well-being is a global problem. This probably reflects, in part, the dissatisfactions many patients express concerning western style medical practice. Larrey [6] points out that the trend in the use of herbal medicines is growing due to a belief that natural products are both good and innocuous when compared with western style medicines. De Smet [4] summarized this global problem using a series of selected case reports. In the summary he described the intake of herbal tea (contained the toxic pyrolizidine alkaloids) leading to hepatotoxicity and death. Another example was the use of azarcon in Mexico (lead tetroxide) causing severe lead poisoning with resultant seizures, encephalopathy and death in a three-year old child. Furthermore, he presented another tragic case of a woman, whom, despite repeated warnings, had continued to eat raw dried rattlesnake meat, contaminated with Salmonella Arizona. She succumbed from sepsis. He concludes that there is a strong placebo effect derived from the ritual of taking herbal medicines and this entices many to try alternative treatments.

Psychosomatic complaints may in fact benefit from this ritual and where health resources are restricted – as in South Africa - may save the State millions of rands in health costs! Chan [7] supports this view and mentions that in many developing countries, traditional methods of treatment (as opposed to the conventional western style prescription methods) are the only affordable and available forms of health care for the majority of the population.

We are all aware of the substantial benefits patients have derived from the use of botanical derivatives to treat medical conditions (digitalis comes to mind immediately). The clinical results with feverfew, which has benefits as an anti-migraine agent is but one example [4]. However, the acceptability of these plant extracts arose only after safety and efficacy was assured. An example is research conducted by the Chinese on the leaves of Artemisia annua and the discovery of a new and exciting anti-malarial artemisinin. While the anti-febrile effects of Artermisia annua herb have been recognized in China since the 4th century AD [8], it was only in 1972 that the research into the anti-malarial properties began.

A starting point would be to assemble a catalogue of safe herbal remedies, which the traditional healers could use for their patients. Watt and Breyer-Brandwijk [9] published such a catalogue and listed the local names of numerous medicinal plants. The poisonous ones were also identified in their publication. However, insuring that this information is easily accessible to the traditional healers is challenging. In addition to the need for a revised, updated and expanded version, the document must be written in the language of the traditional healer and be user friendly.

Bye and Dutton [2] have researched the culture of the Zulu people (concentrated mainly in the KwaZulu/Natal region) and the use of traditional remedies. The Zulu believe that disease is a reflection of disharmony between an afflicted person and his/her ancestors. The sangoma (diviner) diagnoses the problem by consulting with the spirits and thus identifies the source of the disharmony. The inyanga prepares and dispenses the herbal treatment required to dispel the disharmony and in so doing hopes to cure the affliction. Although this work concentrated on the Zulu population [2], there exists this common thread of belief, in the need for ancestral placation, during times of illness amongst the blacks in Africa. Therefore, the administration of toxic herbal substances (or chemical substances e.g. mixtures of solutions with battery acid and others with dichromates) is intrinsic to the whole African continent.

The lack of good toxicological services in a large part of the African continent is a major contributor to the inability to identify most of the culprit toxins. Another major problem is that the registration of herbalists has not been uniform, which can lead to a situation where ignorant persons are dispensing substances of which they, and anyone else for that matter, know little. When herbal remedies are recommended, there are no checks and balances in the treatment protocols. Unhappily, fatalities expected to be the result of herbal use are a major problem in infants including the death of healthy babies. Similar to western-type medical practice, charlatans are encountered amongst the sangomas and inyangas. These quacks are usually ignorant of the

safer, tried and tested traditional remedies. They may prescribe the hard-core, more toxic substances. An example of such an occurrence is a case of cresol poisoning discussed below.

This chapter will attempt to outline the extent of the problem in the major provinces in South Africa. We have confined our comments to toxins that have a major impact on renal dysfunction – although some (if not most!) toxins have secondary effects with consequent kidney failure.

We have also incorporated a description of specific problems, which do not involve diseases as such (and therefore the sangoma and inyanga is definitely innocent in this instance). These specific problems fall into the category of cosmetics used as hair-dyes and skin-lightening creams (in the Sudan and from Kenya).

Toxins in the different regions

Western Cape

This region has two major referral hospitals for treating those people in renal failure. It is our opinion that in the majority of toxin induced acute renal failure 'unknown' toxin is by far the commonest etiological agent! In a minority of cases the definite agent is know. One of these is potassium dichromate. The excellent work done by Wood et al [3] in describing the extent of the toxicity of potassium dichromate has been of great educational value.

Potassium dichromate

Potassium dichromate is the principal active ingredient in purgative solutions; the indications for its use are broad and may encompass any complaint. The substance is toxic in the hexavalent state but metallic chromium is inert. It is used in the leather industry for tanning, as an industrial cleaning agent and in electroplating. It is a bright yellow crystalline substance in its natural state.

When taken orally or rectally it is irritant to the mucosa and can cause acute tubular necrosis, hepatitis and colitis. The toxic hexavalent chromium becomes rapidly bound to tissue (in the trivalent form). Therefore clinical measures to reduce absorption must be administered immediately in order to have an effect. When inhaled, it causes chronic bronchitis, interstitial pneumonitis and fibrosis. The indications for which dichromates may be prescribed are numerous. Figure 1 is an example of a purgative, the contents of which, on analysis was found to have a mixture of dichromates and faeces. This mixture was given to a patient to relieve mild constipation. The patient initially denied that she had consulted a sangoma but later, after she had undergone dialysis for two weeks followed by complete recovered from her acute tubular necrosis (ATN), brought in the medicine bottle and admitted that she had taken the substance orally on prescription of a healer. This had produced severe diarrhea and dehydration with subsequent ATN. Note (Figure 1) the healer has used western-style prescription methods including standard abbreviations such as t.d.s. and p.o. Furthermore note that the indications for use of the medicine range from "blood" disorders to diarrhea to libido problems!

Wood has presented several cases of known dichromate poisoning [3]. In six of the seven cases, the patients were able to produce the ingested compound for analysis and dichromate proved to be the principal active ingredient. All his patients had blood levels of chromium well in the toxic range. They all required dialy-

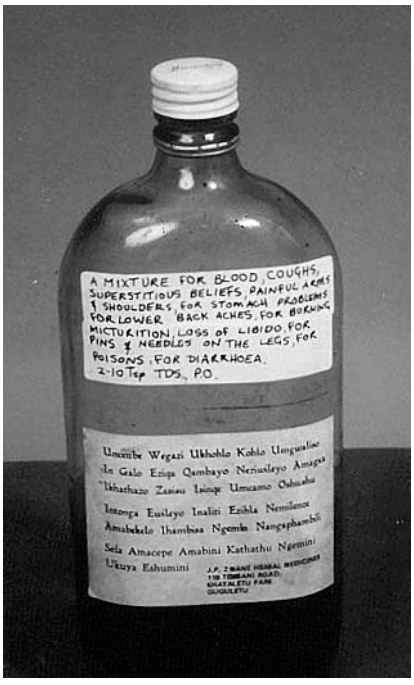

***Figure 1**. Medicine bottle containing dichromates. Note indications for use.* *By courtesy of Prof Wood.*

sis support and in one tissue was obtained at post-mortem examination. This patient died from sepsis and massive gastrointestinal hemorrhage. It is noteworthy that the liver had the highest chromium content followed by the kidney and this was found 26 days after the initial presentation!

The clinical presentation of these 7 cases was that of a spectrum involving the kidneys with GIT manifestations in all, as well as hepatic failure in two. Sepsis and hemorrhage were secondary manifestation of mucosal damage and in one case, rectal perforation (probably traumatic enema) was an additional finding.

Treatment consists of early hemodialysis to remove a larger amount of chromium (due to the rapid tissue binding). There is no role for chelating agents. Otherwise, treatment for dichromate poisoning is entirely supportive. Fortunately, the renal failure is reversible. It is interesting to point out that the chromium is taken up by the kidneys and produces a good nephrogram on straight abdominal X-ray!

Cresols

Transcutaneously absorbed substances may lead to severe toxic systemic effects. Substances, which fall into this category, include the phenols and the closely related compounds, cresols. The commercially available Jeyes fluid is a cresol. Jeyes fluid ingestion and administration by enema are well-documented forms of poisoning in South Africa. Berg et al. (personal communication) are the first in South Africa to describe the development of ATN following the transcutaneous absorption of a cresol. The case was a woman who may have presented to a healer (Berg cannot be sure that it was not a charlatan that was consulted) complaining of nausea. She was "painted" with a solution of Jeyes fluid. Whilst being painted she immediately lost consciousness and was taken to a nearby hospital. In addition to being deeply comatose, superficial chemical burns where noted on admission. Her vital signs were normal including a normal blood pressure. She regained consciousness after approximately 3 hours; her urine was bloody and she became oliguric. It was thought that she had aspirated and she was subsequently treated for pneumonia (confirmed on X-ray and located to the right upper lobe).

Examination of the urine revealed macroscopic blood and granular casts. Her serum biochemistry revealed hyperkalemia and elevated urea and creatinine levels. She ultimately required dialysis and recovered sufficient renal function within 3 days to allow cessation of dialysis therapy. In view of the close association between the exposure to the Jeyes fluid and the development of ATN, a renal biopsy was not performed and a causal role was assumed.

It appears that cresols are absorbed across intact skin. Once absorbed, phenols are widely distributed throughout the body and are toxic to various cell types. Green reports on a one-year old child who died 4 hours following the accidental application of a phenol solution to his head. At post-mortem examination, the presence of the phenols in the internal organs was detectable by the typical odor of phenol [10]. ATN was also documented histologically. Bruce et al. document 2 cases of cresol poisoning and their resultant deaths [11].

Tygerberg Hospital, the other reference hospital in the Western Cape, provides an additional toxin, which has caused ATN. This toxin is cantharidin.

Cantharidin

Is commonly known as Spanish fly and is derived from blister beetles. Karras speculates that it may be a more common cause of morbidity than is recognized [12]. It is used as a sexual stimulant and is an ingredient in some wart removal remedies [13]. Poisoning is noteworthy for its dramatic effect on the gastrointestinal and urinary tracts, as well as occasionally inducing cardiac abnormalities and seizures [12, 13]. The patient may present with massive hematemesis and hematuria. The kidney is often involved with ATN and glomerular damage. Treatment is supportive and includes dialysis when indicated.

Kwazulu-Natal

Seedat, in 1978 [14], concluded that the commonest medical causes of acute renal failure in Natal were toxins. The toxins were mainly herbal in nature and the composition of the majority was unknown. However the best studied toxin from this area is the ox-eye daisy or impila (Callilepsis Laureola) [15].

The **impila** (which means health in Zulu) bears a root similar to a sweet potato. It is harvested in winter and stored after drying and crushing. The solution is

administered after 30 min in boiling water either rectally or orally [2]. It is a multi-purpose muthi (medicine) and is given for general health, impotence and HIV symptoms. It is also believed that if the root is buried near a person's home, then it will intercept any evil directed towards that household [2].

Impila causes massive centrilobular liver necrosis with hypoglycemia and liver failure. It also causes acute tubular necrosis [16, 17]. Watson described 50 black children who had died at the King Edward VIII Hospital [16]. Post-mortem examination was conducted in all cases confirming the diagnosis of impila poisoning. No common trend was noted in the clinical presentation of these children. They all thus concluded that hypoglycemia and evidence of hepatic and renal dysfunction, were strong indicators of impila poisoning.

Atractyloside is a component in the root of the impila and it is this substance, which has been demonstrated to cause acute tubular necrosis in rats [17].

Gauteng

A substantial experience of toxin induced renal failure has been gained at the Chris Hani Baragwanath Hospital. This 3000 – bed teaching hospital serves approximately 4 million people from Soweto. Once again patients often visit traditional healers, usually prior to, or instead of consultation with a medical doctor [1, 18]. A recent study done at the hospital [Katz - personal communication] revealed that 13% of cases of ARF were caused by herbal toxins. Segal and others reported on ritual–enema-induced colitis [19]. Their report incorporates 11 patients where the clinical hallmarks of the injury were peritonitis and rectal bleeding. The injury in some cases extended to involve the whole colon. Of the enema ingredients, potassium dichromate was prominent. Therefore this is a ubiquitous toxic substance used widely throughout South Africa. Other ingredients were vinegar, caustic soda and dettol. The severe cases were complicated by renal failure. Obviously – with the exception of the dichromates, which we know are directly nephrotoxic - the cause of the renal impairment was multifactorial. Sepsis probably played a major role in the pathogenesis of the acute renal failure.

In the experience of Katz (personal communication), males predominate in the group with herbal induced ARF. This observation is supported by a report from Zimbabwe describing the pattern of poisoning from traditional medicines in that country [20]. Apparently men resort to taking muthi because it is perceived as being manly and also because they have easier access to the sangomas than do women. Interestingly, from the Katz group again, comes a recent report (personal communication), which described a male who presented in ARF after drinking one spoonful of a sangoma prescription. He had visited the sangoma with the complaint of vomiting and abdominal cramps. He required 2 months of dialysis and – although no longer dialysis dependent - the latest biochemistry shows incomplete recovery. A sample of the ingested compound was found to contain Cape Aloe. This was a surprise finding, since the aloe is considered to be safe. Therefore one must be cautious in interpreting this as the agent responsible for the ARF, since there may have been other substances present in the concoction, which could not be detected. An unanswered question here is what was the cause of the vomiting and cramps, which led the man to consult the sangoma in the first place? Did that have any deleterious, causal effect on renal function?

Aloes occur throughout the world. The genus Aloe includes herbs, shrubs and trees. The leaves are used for the preparation of medicine or cosmetics [9, 21, 22].

Cape aloe

This is a common species of aloe and is derived from Aloe ferox. It is reported to be the most extensively used plant substance as an herbal remedy in South Africa [9]. The aloe is also identified as one of the most commonly used herbal propriety products [21]. It is not considered toxic. Therefore the case from the Chris Hani Baragwanath Hospital discussed above, must have had other additives that were not measurable or the "dose" may have been too high. Or, as must always be considered, did the original disease for which help was sought from the sangoma, not play a role in causing the ARF [31]?

To support its safety, Van Wyk [22] mentions the medicinal uses of the Cape aloe. The yellow juice from the leaves is dried and a small crystal (the size twice that of a match head) of the dried substance is taken orally as a laxative. Its use as a laxative has also been important from a commercial point of view. The export market has been a valuable source of revenue for

SA. It may also be used for arthritis, but at a much smaller dose than that required for a catharsis. Van Wyk mentions that eczema, hypertension and stress have also been included in the list of indications for this product. One is uncertain as to how these indications were arrived at and whether there is any substantive evidence of efficacy, with the use of aloes, in the treatment of these conditions.

The active purgative ingredient in the aloe is called barbaloin. The barbaloin is a prodrug and once in the colon is it converted to the active substance, aloe-emodin anthrone [21, 22]. The conversion to active drug is facilitated by the colonic flora. The laxative action results from the inhibition of colonic Na–K-ATPase with the resultant increase in the water content of the colon.

Eastern Cape

Rose described the toxicity of the senecio plant in 1972 [23]. It is the most common plant species to contain the pyrolizidine alkaloids. Toxicity includes hepatic necrosis and later intrahepatic veno-occlusion. A major secondary component is ATN. There are over 50 species of senecio plants in the Eastern Cape. The plants are used extensively as enemas and purgatives. Rose mentions that, despite the deaths resulting from the use of these plants, the local inhabitants are not aware of the danger these plants pose to their well-being.

Figure 2 is a photograph of a cow horn and segment of hollowed out reed. These objects in the photograph have actually been used in sangoma/inyanga treatment procedures. They were obtained (after much negotiation!) from a sangoma practicing in Cape Town. They are the standard instruments used by the sangomas to administer the various herbal remedies, via the rectum. The Higginson's syringe has also been used [3]. The funnel-shape of the cow horn makes it easy to use this form of treatment – however we must remember the report from Segal [19] in which the complications of rectal perforation and colitis are ascribed to the instruments and methods used to administer treatments rectally. The hollowed out reed is only used in children. Here the prescribed solution is first aspirated into the hollow reed and the blackened tip is inserted into the rectum. The sangoma will then blow through the hollow reed forcing the herbal medicine into the rectum of the child.

The following discussion relates to renal disease occurring as a consequence of substances used for **cosmetic** reasons.

Kenya

Barr in 1972, discussed the nephrotic syndrome in adult Africans in Nairobi [24]. In this report he reported that young, English-speaking women, with the nephrotic syndrome, were in the majority. They were able to separate these patients from the rest by the cosmetics that they used. In fact, more specifically, by the habit of applying skin lightning creams. On further analysis, it was found that they had used creams containing amino-mercuric chloride. Analysis of the urine revealed high levels of mercury. After cessation of the mercury containing creams, the urinary mercury levels rapidly fell to normal. This study was of interest in that only 12% of the biopsies obtained were diagnosed as membranous nephropathy. The majority (50%) had minimal change disease.

The mean duration of use of the creams before presentation with leg edema, was 13 months. The remission rate was 50% in those with minimal change disease after withdrawal of the creams.

Mercury

Human exposure is either to mercury vapor or methyl mercury compounds [25]. See also chapter 26.

Both of these forms of mercury can lead to kidney involvement with nephrotic range proteinuria. The ef-

***Figure 2**. Cow horn and hollow reid used by the traditional healers for the administration of herbal enemas.*

fect on the kidney is suggested to be on the basis of mercury-stimulated T lymphocytes [25]. These T lymphocytes produce damaging antibodies to the basement membrane with consequent heavy proteinuria. The damage may manifest as membranous nephropathy with the nephrotic syndrome [26, 27] or as minimal change disease [24]. Of importance, there are no case reports of nephrotoxicity resulting from exposure to mercury from amalgam tooth fillings [28].

There is no specific treatment for mercury poisoning of the kidneys but removal of the source of the metal is important. This maneuver may result in spontaneous improvement in 50% of cases [24]. Brown, in a study from Malawi, described the failure to improve in 2 out of 6 patients with membranous nephropathy who were known to have used skin creams [27]. This occurred despite removal from exposure to the mercury as well as the administration of steroids.

The Sudan

In a personal communication, Sulieman from the Sudan described renal toxicity of a particular hair dye [29]. This dye contains paraphenylenediamine and when mixed with henna, blackens the hair in a very short time. The substance is a common cause of ATN in the Sudan. It is also toxic to the heart and liver. It is absorbed through the skin but people have ingested the dye in suicide attempts. Within 3-4 hours after ingestion they develop angioneurotic edema soon followed by renal failure. Renal biopsy shows the typical features of acute tubular necrosis. See chapter 32.

Discussion

The study and identification of all the herbal medicines in South Africa will be an important contribution to the well-being of the majority of our population. In this regard, the University of Cape Town's department of Pharmacology has set up a unit to study herbal medicines. They are collaborating with the sangomas in an exciting exchange of information. The benefits from such collaboration are not limited to defining a therapeutic option point of view, but also will provide an understanding of the culture behind the sangoma/inyanga influence. Suspicion and distrust between the western-style doctors and traditional healers needs to be eliminated.

Savage and Hutchings, in their thought provoking article in 1987, pointed out the failings of the western-style doctors [30]. They made the valid point that much of the adverse publicity given herbal remedies was as a result of guesswork. Doctors often ascribed the various disease manifestations to herbal treatment without confirmation. The finding that the original problem for which the patient consulted the sangoma was not pursued, was a cause for concern for these researchers.

Their statement of an "aloof attitude of mild contempt" on the part of the medical staff says it all [30]. The sangoma/inyanga influence must be understood; solutions to end this poisoning of, in many instances, healthy patients must be found. The problem will only be solved once trust between the two (traditional and western-style) healthcare givers is established.

References

1. Joubert PH. Poisoning admissions of Black South Africans. Clin Toxicology 1990; 28(1): 85-94.
2. Bye SN, Dutton MF. The inappropriate use of traditional medicines in South Africa. J Ethnopharmacol 1991; 34: 253-259.
3. Wood R, Mills PB, Knobel GJ, Hurlow WE, Stokol JM. Acute dichromate poisoning after use of traditional purgatives. A report of seven cases. S Afr Med J 1990; 77: 640-642.
4. De Smet PAGM. Is there any danger in using traditional remedies ? J Ethnopharmacol 1991; 32: 43-50.
5. Perharic L, Shaw D, Colbridge M, House I, Leon C, Murray V. Toxicological problems resulting from exposure to traditional remedies and food supplements. Drugs Safety 1994; 11(4): 284-294.
6. Larrey D. Hepatotoxicity of herbal remedies. J of Hepatol 1997; S26: 47-51.
7. Chan TYK. Monitoring the safety of herbal medicines. Drug Safety 1997; 17(4): 209-214.
8. De Smet PAGM. The role of plant-derived drugs and herbal medicines in healthcare. Drugs 1997; 54(6): 801-840.
9. Watt JM, Breyer-Brandwijk MG. The medicinal and poisonous plants of Southern and Eastern Africa. Livingstone, Edinburgh, 1962.
10. Green MA. A household remedy misused – fatal cresol poisoning following cutaneous absorption (a case report). Med Sci Law 1975; 15(1): 65-66.

11. Bruce AM, Smith H, Watson AA. Cresol poisoning. Med Sci Law 1976; 16(3): 171-176.
12. Karras DJ, Farrell SE, Harrigan RA, Henretig FM, Gealt L. poisoning from "Spanish fly" (cantharidin) 1996; 14(5): 478-483.
13. Till JS, Majmudar BN. Canthanridin poisoning. South Med J. 1981; 74(4): 444-447.
14. Seedat YK. Acute renal failure among blacks and Indians in South Africa. S Afr Med J 1978; 54: 427-431.
15. Bhoola KDN. A clinicopathological and biochemical study of the toxicity of Callilepsis laureola (impila). MD thesis. University of Natal 1983.
16. Watson AR, Coovadia HM, Bhoola KD. The clinical syndrome of Impila (Callilepis laureola) poisoning in children. S Afr Med J 1979; 55: 290-293.
17. Wainwright J, Schonland MM, Candy HA. Toxicity of Callilepsis laureola. S Afr Med J 1977; 52: 313-315.
18. Buchanan N, Cane RD. Poisoning associated with witchdoctor attendance. S Afr Med J 1976; 50: 1138-1140.
19. Segal I, Tim LO, Hamilton DG, Lawson HH, Solomon A, Kalk F, Cooke SAR. Ritual –enema-induced colitis. Dis Col Rect 1979; 22(5): 195-199.
20. Kasilo OMJ and Nhachi CFB. The pattern of poisoning from traditional medicines in urban Zimbabwe. S Afr Med J 1992; 82: 187-188.
21. Iwu MM. Handbook of African Medicinal Plants. CRC press 1993.
22. Van Wyk B-E, Van Oudtshoorn B, Gericke N. Medicinal plans of South Africa, 1st edition. Briza publications 1997.
23. Rose EF. Senecio species: toxic plants used as food and medicines in the Transkei. S Afr Med J 1972;1039-1043.
24. Barr RD, Rees PH, Cordy PE, Kungu A, Woodger BA, Cameron HM. Nephrotic syndrome in adult Africans in Nairobi. B Med J 1972; 2: 131-134.
25. Clarkson TW. Mercury-an element of mystery. N Engl Med J 1990; 323(16): 1137.
26. Oliviera DBG, Foster G, Savill J, Syme PD, Taylor A. Membranous nephropathy caused by mercury-containing skin lightening cream. Postgr Med J 1987; 63: 303-304.
27. Brown KGE, Abrahams C, Meyers AM. The nephrotic syndrome in Malawian Blacks. S Afr Med J 1977; 55: 275-278.
28. Sandborgh-Englund G, Nygren AT, Ekstrand L, Elinder CG. No evidence of renal toxicity from amalgam fillings. Am J Physiol 1996; 271(4 pt 2): R941-R945.
29. Suleiman SM, Homeida M, Aboud OI. Paraphenylenediamine induced acute tubular necrosis following hair-dye ingestion. Hum Toxicol 1983; 2(4): 633-635.
30. Savage A, Hutchings A. Poisoned by herbs. Br Med J 1987; 295; 1650-1651.
31. Luyckx VA, Ballantine R, Claeys M, Cuyckens F, Van den Heuvel H, Cimanga RK, Vlietinck AJ, De Broe ME, Katz IJ. Herbal remedy - associated acute renal failure secondary to Cape aloes (Case report). Am J Kidney Dis 2002; 39(3): E13 (www.ajkd.org).

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Paraphenylene diamine hair dye poisoning

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Introduction

Since 1883, paraphenylene diamine (PPD) has traditionally been used for dyeing (dark color) hair in Europe [1- 2] as a fresh preparation mixed with hydrogen peroxide (H_2O_2) [3]. In Sudan PPD is used by women to color their hair and as a body dye when added to henna (*Lawasonia alba*). Henna on it's own need to be applied two or three times for several hours to give the desired color (dark red or black). In contrast this can be achieved with one single application in less than one hour by adding PPD to the henna. The toxicity of PPD, when added to henna occurs through skin absorption. This toxicity can not be attributed to henna, (*L. Alba*) which is a herb used for cosmetic purposes and also used in folk medicine for the treatment of some skin diseases, as an antiinflammatory, antipyretic and analgesic agent [4-5]. In Morocco, Takaout beldia indicates a non-toxic vegetable product extracted from the gallnut of Tamaris Orientalis (Figure 1). This non-toxic substance is highly appreciated by woman for its hair dyeing properties. Its rarefaction resulted in the use of paraphenylene diamine as substitute under the name of Takaout Erroumia. If nowadays accidental ingestion is exceptional, its use for suicidal attempt in young woman in Morocco and Tunisia become highly prevalent [6].

For the last seventy-five years the dermatological effects of PPD are well studied and as a result of these studies the European Union restricted the concentration of PPD in hair dye formulations to a maximum of 6% [3]. Systemic intoxication is not well studied although there are many studies of its mutagenicity and carcinogenicity [7-8]. Its ingestion is responsible for a

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respiratory (cervico-facial edema), muscular (rhabdomyolysis) and a renal syndrome (acute renal failure due to hypovolemia and myoglobulinuria). The respiratory syndrome mainly determines its prognosis.

Paraphenylene diamine (PPD) characteristics

PPD [$C_6H_4 (NH_2)_2$] is an aromatic amine not found in nature. It is a derivative of paranitroanaline and it is available in the form of white crystals when pure and rapidly turns to brown when exposed to air [9]. Paraphenylene diamine has a molecular weight of 108 Dalton; its boiling point is 267°C and melting point 140°C. It is soluble in ethanol, ether, benzene, chloroform, and acetone and with agitation in water [10].

Rinne and Zinke first prepared PPD in 1874 by reducing 1, 4 dinitrobenzene with tin and hydrochloric acid. Now it is produced commercially by reducing 1-amino-4-nitrobenzene by (1) iron and hydrochloric acid or (2) iron, ammonium polysulphide and hydrogen or (3) iron and ferrous chloride [2].

PPD is used in a variety of industrial products. Along with its derivatives, it has important antioxidant actions - used in the manufacturing of synthetic and natural rubbers, petroleum products, cellulose ethers and alfalfa meals [2]. PPD also has commercial application as photographic developers and in a variety of antioxidants and is also used in dyeing furs and for printing of cellulosic textile materials. PPD hydrochloride has been used as an analytical reagent in the testing of blood, hydrogen sulphide, amyl alcohol and milk [11].

PPD is used in hair dye formulations and can produce a variety of shades depending on the formulation. The concentration of PPD in hair dye formulation range from 0.20% in golden blond dyes to 3.75% in black hair dyes. Exact concentrations of PPD in different formulations are not known because most hair dye formulations are proprietary.

For safety reasons, different occupational health authorities, in the countries where PPD is produced, have developed standards regarding the degree of air contamination. It has been stated that employees' exposure to PPD should not exceed 0.1 mg/m^2 in the working atmosphere in any eight-hours work shift of forty-hour week. This is the maximum allowable concentration in Germany, Japan and UK [9].

Pharmaco-toxicology

PPD has two modes of reactions by which it has a biological effect:

Oxidation**:** PPD gives benzoquinone imines as a result of oxidation. The imines react rapidly with the couplers (another chemical material in the formulation) and/or an oxidized PPD to produce indo dyes. The most frequent couplers are 2, 4-diaminoanisole (blue forming coupler), resorcinol (green brown), metaminophenol (magenta/brown) and 1- naphtol (purple blue color). The most commonly used oxidant is hydrogen peroxide. Free ammonia is present to promote the oxidation reaction and the pH of the mixture on the dyed area is about 9.5 [3].

Deamination**:** Deamination has been suggested as a mode of action of PPD, which results in the production of aniline, which may contribute in part to the toxic effects of the compound [12].

PPD induces one of the most striking experimental edema. The edema appears to be grossly specific and selectively localized in the head and neck. It was suggested that the toxic effect of the PPD might be produced by the conversion of the PPD on mucus surfaces to its oxidation product quinondimine, which is responsible for intense local irritation [13]. Some authors believed that PPD toxicity is due to some effect either on the blood colloids or on vascular permeability [15]. Also it was believed that the PPD toxicity is due to altered vascular permeability and involvement of the parasympathetic nervous system [13]. Deamination and formation of analine is claimed to be responsible in part for the toxic symptoms [12]. These different views as to the cause of PPD edema appear to be due to the fact that the exact number and nature of the oxidation products is not known [14].

At high concentrations and after a long period of exposure PPD produces cell death. This effect together with lipid peroxidation can be the cause of the production of superoxide and hydrogen peroxide by the autooxidation of PPD [15].

It was proved that at non-toxic doses, PPD induces intercellular adhesion molecule-1 (ICAM-1) expression on the keratinocytes [16]. These results were consistent with the view that oxidative stress may be an essential part of the pre-immunological phase in the induction of the allergic contact dermatitis by PPD [16].

PPD can cause methemoglobinemia by oxidation

of the ferrous form (Fe^{2+}) of haemoglobin to the ferric (Fe^{3+}) form. Aniline, nitrobenzene, phenacetin and other nitro and amino organic compounds are powerful methemoglobin formers.

From the studies of the intracutaneous sensitization of guinea pigs using PPD, hydroquinone, quinhydrone and benzoquinone it has been suggested that benzoquinone formation plays an important role in the allergic action of PPD [15].

Studies in rats demonstrate that subcutaneous administration of 3 mg of the PPD hydrochloride induces skeletal muscle lesions in the form of rhabdomyolysis with infiltration of inflammatory cells, necrosis, accumulation of neutral lipids and dilatation of sacroplasmic reticulum [17].

In rats, teratogenicity was studied by testing four commercially available hair dye formulations containing 1, 2, 3 and 4% PPD and several aromatic amine derivatives among their constituents [18]. No abnormal foetal effects were noted, except with the formulation containing 2% PPD, which induced skeletal deformities [18].

Experimental studies in guinea pigs revealed that there is an increase in malondialdehyde, which indicates lipid peroxidation, suggesting that increased free radical formation is responsible for the histopathologically demonstrated tissue damage [15]. The increase in histamine level in the blood as a sign of hypersensitive reaction results in increased permeability and the increased activities of the cytoplasmic enzymes AST and ALT and that of tyrosinase, observed in skin following repeated exposure to PPD. This indicates a metabolic disturbance in amino acid metabolism, which may be responsible for the epidermal thickening and erythematous changes [15].

There are many reports about the dark coloration of urine after topical application of commercial hair dye formulations containing PPD. It was shown that PPD is excreted in urine after topical application [19]. It is believed that the darkening of urine was caused by oxidizing agents and was taken as evidence of the excretion of unchanged PPD [19].

It was found that the LD50 of PPD was 250 mg/kg BW in rabbits and 100 mg/kg BW in cats. The subcutaneous LD50 was found to be 170 mg/kg BW in rats, 200 mg/kg bw in rabbits and 100 mg/kg BW in dogs. The intraperitoneal LD50 was found to be 37 mg/kg BW in rats [3]. The lethal dose for humans was estimated to be 10 grams of pure PPD [6].

Clinical presentation

Epidemiology

In Sudan, PPD in its pure form (90-99%) is available in the local markets and there are no restrictions for its use or trade (Figure 2).

The major problem of PPD toxicity results from the ingestion of the compound accidentally, in suicidal or homicidal attempts. However, there are some reported cases of severe intoxication after topical application of the pure PPD mixed with henna or for dyeing hair [20] (Figure 3). Samples of the PPD collected from the local market were found to have a purity of 97% when analyzed [6]. A survey of suicidal attempts in Khartoum, the capital of Sudan, in the period 1987-1990 revealed a number of 264 cases, with an age range between 10 to 30 years. In 35% of these cases PPD was used [21].

In reported series of 24 patients who presented with PPD intoxication and were admitted to Om Durman hospital in Sudan within a period of 12 months, twelve patients took the PPD intentionally and eight of them died [22].

Over a period of 2 years a series of 18 cases were reported in Khartoum North Hospital and there were two babies among them aged eighteen months, 70% were suicidal attempts. The mortality rate in this series was 22% [6]. A number of 150 cases with PPD intoxication had been admitted to the renal unit in Khartoum Teaching Hospital from 1985 to 1995. Sixty percent of them developed ARF requiring dialysis [23].

Recent statistics from the ENT teaching hospital in Khartoum showed that the number of patients admitted with PPD intoxication has risen from 45 cases in 1995 to 289 (87% in the context of a suicidal attempt) in 1999 giving a rise of around six folds over 5 years with an average of about 110% per year. The majority of the cases were in the age group of 15-24 years and more than 90% of them were females. Mortality rate was 12.5% over the 5 years peaking up to 27% in 1995 and falling to 9% in 1999. Patients requiring tracheostomy averaged 36%.

In Morocco, intoxication with PPD is a major health problem. A reported series of 171 cases of PPD poisoning admitted to the medical resuscitation service in Ibn Roshd hospital between January 1994 and October

1997. In this series, there were 5 men and 166 women, with a mean age around 26 years. Twenty four percent of the patients developed severe ARF and 55 deaths (38.7%) were observed in this study [24]. In 90% of the cases PPD was ingested in the context of a suicidal attempt. The amount ingested varied between 3 and 15 grams.

Cases with PPD poisoning were reported in the UK, France, Israel, Japan and other countries [25-29].

Clinical features and systemic toxicity

Acute systemic toxicity: Cases reported with systemic toxicity of PPD had shown various clinical manifestations as well as biochemical and histological changes.

The intoxication represents 30% of the intensive care admittance during the last 4 years. On admission the clinical presentation consist mainly in edema, important and/or sudden onset of the cervico-facial region. Facial dermatitis is observed in the case of application of the toxin (Table 1) [30].

Chronic systemic toxicity: Repeated and prolonged exposure to PPD is believed to increase the risk of non-Hodgkin's lymphomas and multiple myeloma and cancer of the bladder [31–32]. Hair dye formulations containing PPD was incriminated in the increased risk of systemic lupus erythematosus (SLE) and breast cancer, however other studies, showed that there is no significant relationship [33-34]. Aplastic anemia due to PPD exposure also has been reported [35].

Determination of PPD has a great value in diagnosis, follow up of the treatment and also for medico-legal purposes.

Table 1. Frequency of clinical symptoms observed in 171 patients with PPD intoxication in Morocco between 1991 and 2000.

Edema	94%
Acute respiratory insufficiency - tracheal intubation (72%) - tracheotomy (21%)	56%
Signs of rhabdomyolysis	88%
Gastrointestinal symptoms (abdominal pain)	53%
Oliguric acute renal failure	32%

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PPD was detected first in the urine of experimental animals [19] and in urine of humans by Yagi and colleagues [6], using thin layer chromatography. Determination of PPD in the serum is not mentioned in the literature.

Nephrotoxicity

The kidneys are particularly vulnerable to effects of noxious agents because of their high perfusion rate. Renal damage induced by chemicals is well known. Renal lesions associated with PPD intoxication received much attention because most of the clinical investigators reported renal failure [6, 31-32].

Experimental studies in mice exposed to PPD showed no histological changes in the kidneys [34]. However, evidence of severe nephrotoxicity has been reported in humans [22-25]. Histological changes typical of acute tubular necrosis have been also reported [36].

A case report of systemic vasculitis and crescentic glomerulonephritis has been published in patients chronically exposed to henna containing PPD [37].

In a prospective study performed in Khartoum Kidney Dialysis Centre and Sheffield Kidney Institute 19 renal biopsies out of a series 23 patients with severe (39%), moderate (35%) and mild intoxication (26%) were studied under light microscopy. Glomerular injury observed in 94% of the biopsies in the form of hypercellularity, membranous proliferation, glomerular swelling, capsular drop and accentuated lobular architecture [38].

Tubular lesions were found in 78.9% of the studied samples. Different epithelial necrosis is the most common lesion observed (78.9%) while tubular atrophy had been found in (15.8%) of the studied samples.

Interstitial lesions were observed in 16 samples from the studied biopsies (84.2%). Focal inflammation (neutrophils and eosinophils) was the most common injury (47.3%).

No vascular injury was observed in all of the studied biopsies [38].

Other systems involvement

Dermatological manifestations

PPD is a top listed allergen [39-40]. Erythematous urticarial papules, plaques and target lesions (erythema

Figure 1. *Natural Takaout beldia (Morocco).*

Figure 2. *PPD from the local market.*

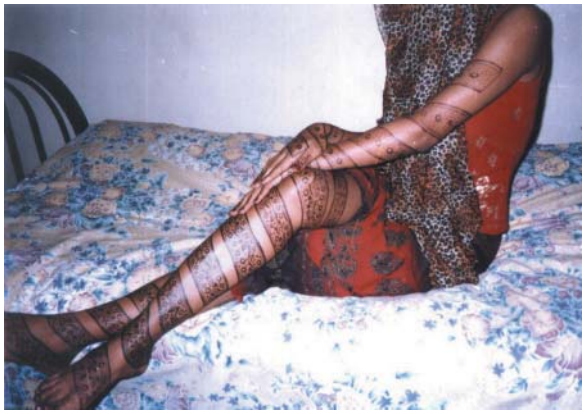

Figure 3. *Intoxication due to massive topical use (Sudan).*

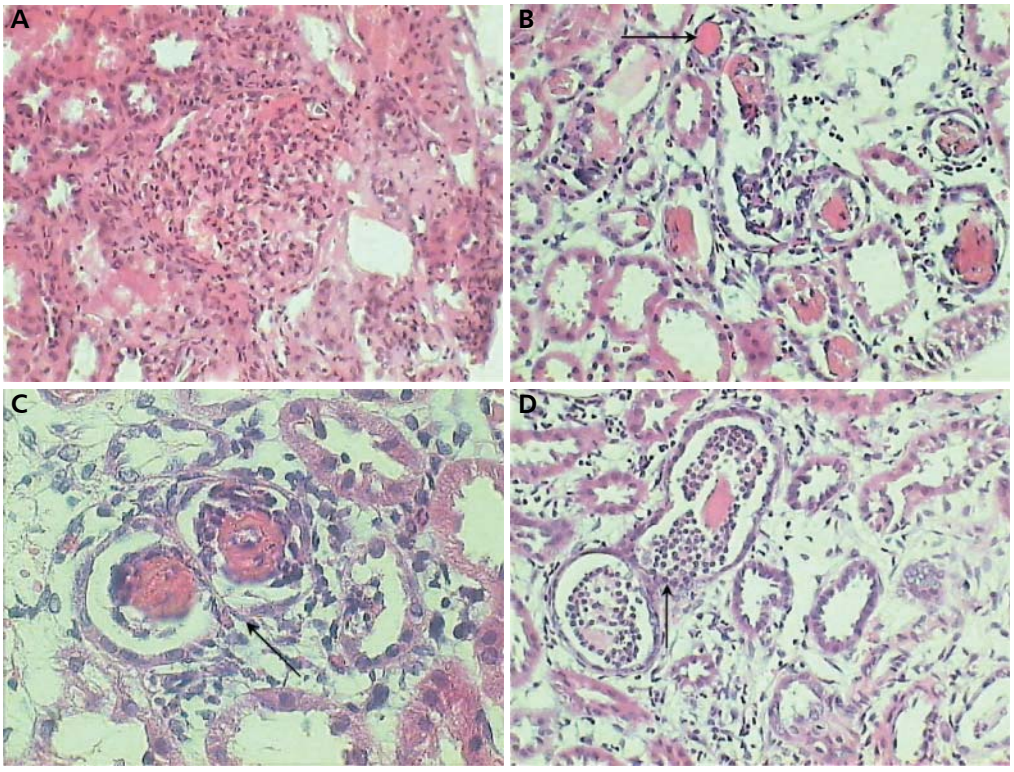

Figure 4. *Glomerular (**A**) and tubular (**B-D**) lesions in PPD intoxication. H&E staining. **A**. Glomerular swelling and hypercellularity. **B**. Inflammatory cells are present around eosinophilic intraluminal material (arrow). Some tubular epithelial cytoplasm is attenuated. **C**. Tubular basement membrane is distinct in some areas. **D**. Intraluminal inflammatory cells are seen with a small proteinaceous casts. The tubular basement membrane is indistinct.*

multiform like eruptions) were described. These skin manifestations occur as a result of an allergic contact dermatitis, which generally manifest as an eczematous rash [41].

Rhabdomyolysis

Cases of rhabdomyolysis following intoxication with PPD have been reported [25] [27].

Skeletal muscle biopsy of patients showed scattered coagulation necrosis with associated muscular inflammatory cellular infiltration [27]

Cardiovascular system

In many reports of PPD toxicity cardiac arrest was the main cause of death. In these cases cardiac arrest is attributed to arrhythmia. Most notably ventricular tachyarrhythmia including ventricular fibrillation has been the major feature of PPD cardiac toxicity [28-29]. Cases have been also reported of myocardial infarction associated with cardiac rhabdomyolysis [42].

Respiratory system

Dyspnoea, tachypnoea and asphyxia with chest pain following acute PPD poisoning have been reported in a number of studies [6] [12] [25]. PPD was proved to be the cause of asthmatic attacks in the sensitive individuals [43]. A case of Goodpasture's syndrome was reported to be induced by exposure to PPD [44]. Extrinsic allergic alveolitis also has been reported [45].

Ophthalmic effects

In animal study it was reported that 89% of the mice fed PPD developed lenticular changes indicating that PPD has cataractogenous effects, which are related to the duration, amount and individual sensitivity [46]. It was concluded that PPD is potentially toxic to human lens. Exophthalmia and permanent blindness due to optic nerve atrophy following PPD poisoning were reported [6]. Using a patch test to determine PPD phototoxicity, it was proved that PPD could cause a phototoxic reaction and photoallergy [47- 48].

Hepatotoxicity

Experimental studies in guinea pigs showed that the skin absorbs PPD [17]. The histopathological findings of the livers of the sacrificed animals showed signs of focal and early degenerative changes in hepatocytes, along with mild fatty changes. There was a moderate congestion of sinusoids and a focal granulomatous reaction with occasional Langerhans type giant cells. Subacute toxic hepatitis due to PPD poisoning was early reported [30]. In contrast others reported that there were no hepatic changes seen in their patients [6].

Neurotoxicity

In animal studies it was proved that PPD has a toxic effect on the parasympathetic nerves [14]. In humans, neurotoxicity consisted in reported cases of mental status alterations ranging from drowsiness to coma. Also, reports of paraplegia and paraparesis have been published [6] [23].

Treatment

There is no specific antidote for the PPD. The early challenge threatening the patient's life is asphyxia due to edema of the upper respiratory tract and the airways. Tracheostomy is a life saving measurement in this condition [6]. Nasotracheal intubation was proven also to be effective [26].

Vascular refilling is installed promptly in order to prevent as much as possible the development of acute renal failure.

Acute renal failure (ARF) was found to be the second life threatening effect. Hemodialysis had been used as a method of treatment with variable success [24-26]. On the other hand, peritoneal dialysis was used in the treatment of the ARF due to PPD toxicity in other reports [36].

Symptoms related to PPD poisoning seem to be due to histamine release; the use of antihistamines was suggested [49]. Intensive medical treatment by steroids and chlorpheneramine maleate was given to all patients together with prophylactic penicillin in one report [6]. Pethidine was given for relief of muscle pain in another report [27].

Numerous questions concerning PPD remain: physiopathological mechanisms of neurological myocardial and renal damage induced by the toxin, availability of an antidote and the extraction by hemodialysis/hemoperfusion.

References

1. Proger B, Jacobson P, Shmidt P and Stern D. Beilsteins handbouch der organischen chemie. In: IARC monograph on evaluation of the carcinogenic risk of chemical to man. 1977; 16: 127.
2. Thirtle JR. Phenylene diamines and toluen diamines. In: Encyclopedia of chemical technology (2nd edition, volume 15). Kirk RE, Othmer OE (editors). John Wiley and Sons, New York 1968, p. 216-224.
3. Burnett C, Goldenthal EI, Harris SB, Wazeter FX, Strausburg J, Kapp R, Voelker R. Teratology and percutaneous toxicity studies on hair dyes. J Toxicol Environ Health 1976; 1(6):1027-1040.
4. Gupta S, Gupta SS. Anti-inflammatory and antihyaluronidase activity of (Lwasonia alba). Indian Pharmacol 1985; 10: 90-91.
5. Singh S, Modi NT, Saifi AGQ. Anti-inflammatory activity of (Lwasonia inermis). Curr Sci1982; 51: 470-471.
6. Yagi H, El hindi AM, Khalil SI. Acute poisoning from hair dye. East African J 1991; 68: 404-411.
7. Searl CE, Havenden DG, Venih S, Gyde OHB. Carcinogenicity and mutagenicity test of some hair colourents and consituents. Nature 1975; 225: 506-509.
8. Nishioka H. The examination of cancer causing property of pigmented cosmetics through microbiol test. Fourth meeting of Environmental Mutagen Society of Japan, Kyoto 1975, September. 27.
9. IARC Working Group 1987. IARC Monographs on the evaluation of the carcinogenic risk of chemical to man. IARC publication.
10. Grasseli JG (editor). CRC Atlas of Spectral Data and Physical Constants for Organic Compounds. Chemcial Rubger Co., Cleveland, Ohio 1973, p. B-223.
11. Windholz M, Budavaria S. Merck index 1983. Published by Merck and Co. Inc. Rathway J. USA, p. 214-227.
12. Nott HW. Systemic poisoning by hair dye Brit Med J 1924; 1: 421-422.
13. Tainter ML, Hanzlik PJ. The mechanism of edema productionby paraphenylene diamine. J Pharmacol Exp Ther 1924; 24: 179-211.
14. Tainter ML, James M, Vandeventer W. Comparative edemic actions of ortho meta and para-phenylene diamines in different species. Arch int Pharmacodyn 1929; 36: 152-162.
15. Mathur AK, Gupta BN, Muther N, Singh A, Shukla IJ, Ravi Shanker. Biochemical and histopathological changes following dermal exposure to paraphenylene diamine in guinea-pigs. J Applied Toxicology 1990; 10: 383-386.
16. Picardo M, Zompella C, Marchese C, De-Luca C, Faggioni A, Schmidt RJ, Santucci B. Paraphenylene diamine a control allergen, induces oxidative stress and ICAM-1 expression in human keratinocytes. Brit J Dermatol 1992; 126: 450-455.
17. Mascres C, Jasmin G. Alterations de la fibre musculaire induites chez la rat par la p-phenylene diamine. Path Biol (Paris) 1975; 23: 193-199.
18. Burnett C, Loehr R, Corbett J. Dominant lethal mutagenicity study on hair dyes. J Toxicol Envirom Hlth 1977; 2: 657-662.
19. Kiese M, Raucher E. The absorption of P-toluene diamine through human skin hair dye. Toxicol Appl Pharmacol1968; 13: 325-327.
20. D'Arcy PF. Fatalities with the use of a henna dye. Pharmacy international 1982; 3: 217-218.
21. Abdelkarim EE, Ali HM, Harron DWG and Ali KhM. Suicide attempt with Paraphenylene diamine (PPDA) dye in Sudan. Health Services Journal of the Eastern Mediterranean Region. WHO 1992; 6, 2: 44-48.
22. El-Ansary EH, Ahmed MEK, Adam SE. Systemic toxicity of paraphenylene diamine. Lancet 1983; 1: 1341-1342.
23. Salma MS, Mirghani F, Mohamed EN, et al. Poisoning with hair dye containing Paraphenylene diamine: ten years experience. Saudi J Kidney Dis Transplant 1995; 6: 286-289.
24. Charra B, Menebhil L, Bensalama A, Mottaoukkil S. Systemic toxicity of paraphenylene diamine. Works of the Sixth Congress of the Arab Society of Nephrology and Renal Transplantation. February 21-24, 2000- Marrakech, Morocco.
25. Averbukh Z, Modai D, Leonov Y, Weissgarten J, Lewinsohn G, Fucs L, Golik A, Rosenmann E. Rhabdomyolysis and acute renal failure induced by paraphenylenediamine. Hum Toxicol 1989; 8(5): 345-348.
26. Bourquia A, Jabrane AJ, Ramdani B, Zaid D. Systemic toxicity of paraphenylene diamine 4 cases. Press Med 1988; 17: 1798-1800.
27. Saito K, Murai T, Yabe K, Hara M, Watanabe H, Hurkawa T. Rhabdomyolisis due to paraphenylene diamine (hair dye). Report of an autopsy case. Nippon-Hoigaku-Zasshi 1990; 44: 469-474.
28. Lifshits M, Yagoubsky P, Sofer S. Fatal paraphenylene diamine (hair dye) intoxication in child resembling Ludwig's angina. J Toxicol Clin Toxicol 1993; 31: 653-656.
29. Ashraf W, Dawling S, Farrow LJ. Systemic paraphenylene diamine (PPD) poisoning a case report and review. Hum Exp Toxicol 1994; 13: 167-170.
30. Israels MGG, Manch MB, William S. Systemic poisoning by paraphenylene diamine with report of fatal case. Lancet 1934; 1: 505-510.

31. Pearce N, Bethwaite P. Increasing incidence of non-Hodgkin's lymphoma: occupational and environmental factors. Cancer Res 1992; 52: 5496-5500.
32. Brown LM, Evert GD, Burmeister LF, Blair A. Hair dye use and multiple myeloma in white men. Am J Public Health 1992; 82: 1673-1674.
33. Petri M, Allbritton J. Hair product use in systemic lupus erythematosus. A case control study. Arthritis Rheum 1992; 35: 625-629.
34. Koening KL, Pasternack BS, Shore RE, Strax P. Hair dye use and breast cancer: a case control study among screening participants. Am J Epidemiol 1991; 133: 985-995.
35. Hopkins JE, Manoharan A. Severe aplastic anaemia following the use of hair dye: report of two cases and review of literature. Postgrad Med J 1985; 61: 1003-1005.
36. Suliman SM, Homeida M, Aboud OI. Paraphenylene diamine induced acute tubular necrosis following hair dye ingestion. Human Toxicol 1983; 2: 633-635.
37. Brown JH, Conway B, Hill CM. Chronic renal failure associated with topical application of paraphenylene diamine. Brit Med J 1987; 294: 155-156.
38. Hamdouk M. PPD nephrotoxicity. In: Paraphenylene diamine (Hair Dye) Acute Systemic toxicity. Thesis for MMed Sci in Nephrology, Sheffield Kidney Institute, UK. 2001, p. 34-47.
39. Burnett C, Corbett JF. The Chemistry and Toxicology of Hair Dyes. Academic press, New York 1977, p. 203-224.
40. Seidnari S, Di-Nardo A, Motolese A, Pincelli C. Erythema multiforme associated with contact sensitization description of six clinical cases. J Ital Dermato Venerol 1990; 125: 35-40.
41. Nethercott JR, Macpherson M, Choi BC, Nixon B. Contact dermatitis in hairdressers. Contact Dermatitis 1986; 14: 73-79.
42. Ababou A, Ababou K, Mosadik A, Lazreq C, Sbihi A. Myocardial rhabdomyolysis following paraphenylene diamine poisoning. Ann Fr Anesth Reanim 2000; 19: 105-107.
43. Bardana EJ, Andrach RH. Occupotional asthma secondary to low molecular weight agents used in the plastic and resin industries. Eur J Respr Dis 1983; 64: 241-251.
44. Bernis P, Homles J, Quoidbach A, Mahier PH, Bouvy P. Remission of Goodpasture's Syndrome after withdrawal of an unusual toxic. Clinical Nephrol 1985; 23: 312-317.
45. Wang GH, Jiang XF, Luo WC. Report of two cases of extrinsic allergic alveolitis and review of the relevant literature. Chung Hua Chich Chih 1994; 1: 376-8, 384.
46. Jain IS, Jain GC, Kaul RL, Dhir SP. Catractogenous effect of hair dye. A clinical and experimental study. Ann Ophtalmol 1979; 11: 686-687.
47. Kroons S. Standard photopatch testing with waxter, para-aminobenzonic acid, potassium chromate and balsam of Peru. Contact Dermatitis 1983; 9: 5-9.
48. Horio T. Allergic and photoalergic dermatitis from diphenhydramine. Arch Dermatol 1976; 112: 1124-1126.
49. Gleason MN, Glosselin RE, Hodge HC. Clinical Toxicology of commercial Products. Williams and Wilkins Co., 1963, p. 93.

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Urinary biomarkers and nephrotoxicity

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Introduction

In the broadest sense, biologic markers are substances present in biologic specimens that, when measured, help to determine the relationship between xenobiotic exposures and human diseases. In sum, they are biological indicators that signal a changed physiological state, indicate the presence of cell, tissue or organ stress or are indicative of overt injury. The purposes served by biomarkers are twofold: firstly, to achieve the earliest identification of health impairment resulting from a xenobiotic exposure; and secondly, to gain insight into the mechanism(s) responsible for any adverse impact of such exposure on the health of individuals or specific populations at risk.

Categories of biomarkers

In 1987, the National Research Council of the National Academy of Sciences through its Committee on Biological Markers developed a model for conducting environmental health research that defines the progression from xenobiotic exposure to clinical disease and identifies four stages during that process [1]. These stages mark the course by considering the magnitude of the internal dose, its relationship to the biologically effective dose, the presence of early biologic effects and eventually on alterations in the structure and/or function of the target organ. At each point along this line, individual susceptibility - which is also subject to various external factors - determines whether or not the process progresses to the development of clinical disease (Figure 1).

In this schema, biomarkers are considered to fall in the three general designations. These include biomarkers of exposures, biomarkers of effect, and biomarkers of susceptibility. Each of these types of biomarkers has specific and relevant applications.

Biomarkers of exposure

A biomarker of exposure is defined as "an exogenous substance or its metabolite(s) or the product of the interaction between a xenobiotic agent and target molecule or cell that is measured within a compartment of an organism" [1]. A marker of external exposure is simply the amount of a xenobiotic substance to which a person is subjected, whereas a marker of internal exposure is the amount of a substance absorbed into the body. Markers of internal exposure are a more accurate means of estimating exposure than are markers of external exposure and require the analysis of biological samples.

Biomarkers of exposure to xenobiotics causing nephrotoxicity may take one of several forms. The measurement of *blood or tissue levels* of drugs known to have adverse effects on the kidney, such as cyclosporine, aminoglycoside antibiotics, or lithium, is a standard practice. The awareness of the *total amount of drug administered* is frequently important when considering amphotericin and cisplatin nephrotoxicity. More difficulty is encountered with the determination of the *body burden of a toxicant,* although under certain circumstances such a value is necessary to determine the health effects of exposure to heavy metals such as cadmium and lead, and some analgesics [2].

Ideally, biomarkers of exposure should have a direct and quantitative relationship to the xenobiotics' *biologically effective dose*. This term refers to the internal dose of xenobiotic that produces a predictable biologic

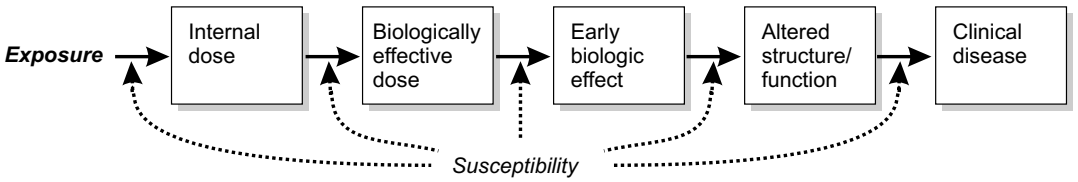

Figure 1. *Simplified flow chart of classes of biologic markers (indicated by boxes). Solid lines indicate progression, if it occurs to the next class of marker. Dashed lines indicate that individual susceptibility influences the rates of progression, as do other variables. Biologic markers represent a continuum of changes, and the classification of change might not always be distinct (Adapted from Committee on Biological Markers of the National Research Council, USA), 1987.*

effect. To gain an understanding of the biologically effective dose, several facts are required (Table 1). These include the knowledge of the amount of xenobiotic which is present in the external environment, its route of entry and the extent of absorption, distribution and accumulation within the body, the target cell or receptor site of the xenobiotic, the route and extent of its metabolism, the modification of the effective dose by associated metabolic, physiologic and pathologic conditions, and finally the pathways of elimination.

Biomarkers of effect

A biomarker of effect is defined as "a measurable alteration of an endogenous component within an organism that, depending on magnitude, can be recognized as a potential or established health impairment or disease" [1]. Markers of effect represent points on a continuum of health impairment and may be measured qualitatively or quantitatively. Early responses to exposure may include changes in the function of target tissues or responses in organs or tissues such as chromosomal damage, mutations of critical target genes, or altered hormone status. Biomarkers of effect are classified according to their impact on health status. The utility of a biomarker of effect may range from enabling prediction of future health impairment to confirming the presence of clinical disease. The biomarker may either be an indirect manifestation of a disease process or may be a direct result of impaired organ function.

An example of an indirect marker of xenobiotic-induced renal disease is the elevated level of red cell content of either delta amino-levulinic acid dehydrase or free erythrocyte protoporphyrin in patients with lead nephrotoxicity [3, 4]. Direct examples of biomarkers of effect are dependent upon the nature of the disease process itself. To mention a few, the presence of small amounts of albumin in the urine of patients with diabetes mellitus is an early warning sign of diabetic nephropathy [5] and may also be found in individuals chronically exposed to cadmium [6]. The appearance in the urine of abnormal amounts of low molecular weight proteins such as β-2 microglobulin (β_2-m) and/or retinol binding protein have been useful in the detection and stratification of workers with industrial exposure to various heavy metals [7-9]. Abnormal patterns of urinary electrolyte excretion and impaired acidification have long been recognized in patients with amphotericin-induced renal injury [10]. Structural lesions within the kidneys may be found in certain electrolyte depletion syndromes such as in the case of the prolonged use of potassium-depleting diuretics [11].

Patients with either acute or chronic renal failure may present with many and varied manifestations of uremia [12]. In these patients, the application of biomarkers of effect to detect clinical disease in its earliest stages is of great importance. Table 2 contains a list of various groups of xenobiotics associated with acute or chronic renal disease.

Table 1. Determinants of the biologically effective dose of a xenobiotic.

Amount in external environment
Route of entry
Extent of absorption, distribution and accumulation
Target cell or receptor site
Modification by associated conditions
Route and extent of metabolism
Pathways of elimination

Table 2. Xenobiotics associated with renal disease.

Occupational and environmental xenobiotics
Organic solvents
Heavy metals
Pesticides
Recreational drugs
Heroin
Cocaine
Diagnostic and therapeutic agents
Antibacterial agents
Antiviral agents
Antifungal agents
Antineoplastic agents
Immunosuppressive agents
Non-steroidal anti-inflammatory drugs
Osmotic agents
Radiographic contrast material
Natural toxic compounds
Aflotoxins
Hemolytic agents and myotoxins

Early effects

Among the occupational and environmental xenobiotics associated with acute renal injury are specific substances such as toluene (organic solvents), lead (heavy metals) and chlordane (pesticides). Of the diagnostic and therapeutic agents, the aminoglycosides (antibacterials), acyclovir (antivirals), amphotericin (antifungals), cisplatin (chemotherapeutic agents), and cyclosporine (immunosuppressives) stand out. Non-steroidal anti-inflammatory drugs (analgesic agents) continue to be associated with acute renal dysfunction [13]. Notably, people who often take non-steroidal anti-inflammatory drugs or acetaminophen have an increased risk of developing chronic renal failure [14]. The parenteral administration of high doses of certain polyols (mannitol, sorbitol), sugars (glucose, fructose, sucrose, lactose), polysaccharides (inulin), and other products (e.g. radiocontrast agents) may be associated with renal injury marked by vacuolation and subsequent swelling of renal tubular epithelial cells - the so-called "resorptive vacuolation". An increasing concern is the renal dysfunction associated with the use of heroin and cocaine (recreational drugs).

Some agents such as arsine may trigger a severe hemolytic reaction, causing hemoglobinuria and subsequent acute renal failure. Others may lead to the destruction of striated muscle, and myoglobinuria will result. In both cases, the consequent "pigment nephropathy" is not an uncommon cause of acute renal failure.

Late effects

Chronic renal failure as a result of toxic or environmental exposures usually involve progressive chronic interstitial nephropathy, which, in addition to prolonged analgesic abuse, may result from chronic lithium ingestion, heavy metal exposure or treatment with cyclosporine [2]. Exposure to hydrocarbons may accentuate the renal insufficiency in patients with pre-existing renal disease or result in the appearance of the nephrotic syndrome or a form a rapidly progressive glomerulonephritis.

Highly specific and sensitive chemical and immunologic techniques for identifying biological substances in urine provide a variety of tests of "tubular proteinuria" for the early detection of kidney damage. Patterns of biomarkers excretion characterize both specific toxins and the anatomical sites of damage within the nephron. Urine biomarkers (high- and low-molecular weight proteins, enzymes, prostaglandins, and growth factors) have proven of particular value following industrial exposure to metals and hydrocarbon solvents (e.g. perchloroethylene, PCE). Renal injury from heavy exposure to known toxins in the workplace indicates the potential for environmental renal damage in large populations subjected to long-term, low-level exposure. Lead causes increased N-acetyl-β-D-glucosaminidase (NAG), thromboxane B_2 (TXB_2) and 6-ketoprostaglandin F_{1a} (PGF_{1a}) in the urine. Urinary NAG correlates directly with blood but not with bone lead concentration, i.e., current not cumulative lead exposure. Occupational exposure to mercury results in increased intestinal alkaline phosphatase (IAP), NAG, and Tamm-Horsfall protein excretion. Cadmium leads to diffuse increases in circulating low-molecular weight proteins and lysosomal enzymes in urine. PCE results in increased urinary 6-keto-PGF_{1a}. Absorption of solvents regularly induces tubular proteinuria for a variety of circulating low molecular weight proteins and lysosomal enzymes. However, this tubular dysfunction is not clearly related to the solvent nephropathy that presents clinically as immunologically mediated glomerulonephritis. In contrast to cadmium, tubular proteinuria associated with lead, mercury, chromium, and PCE has not been shown to predict the later development of kidney failure.

Biomarkers of susceptibility

A biomarker of susceptibility can be defined as "an indicator of an inherent or acquired limitation of an organism to respond to the challenge of exposure to a specific xenobiotic substance" [1]. These markers indicate differences in individuals or populations that affect the body's response to xenobiotic exposure. They may include genetic characteristics, preexisting disease that results in an increase in the amount of agent absorbed or the target tissue responses, differences in metabolism, variations in immunoglobulin levels, or the capacity of an organ to recover from environmental insult.

The first type of susceptibility marker is based on the fact that enzymes alter most chemicals, and these alterations may increase or decrease the ability of a chemical to interact with DNA, RNA, or proteins. The balance between enzymes that detoxify or enhance the

toxicity of chemicals differs among individuals and ethnic groups. These differences often are inherited and can lead to pronounced differences in a person's sensitivity to the effects of chemical exposure.

A second type of susceptibility marker reflects genetic differences in the capacity of cells to repair DNA damage caused by environmental insult. People deficient in DNA repair genes may exhibit more DNA damage manifest as DNA adducts; alterations in chromosome number; structural modifications such as chromosome breaks, rearrangements, and exchanges; micronuclei, which are fragments of nuclear material left in the cytoplasm after replication; activated oncogenes and their protein products; and much higher incidences of cancer.

A third type of susceptibility marker is preexisting inherited genetic defects that increase the risk of cancer. Cancer is generally understood to be a multistage process, requiring several genetic alterations or mutations to produce a clinically detectable tumor. If a person has inherited one or more of the necessary genetic alterations, fewer steps are needed for a chemical to cause cancer, putting this person at a greater risk.

Enzyme metabolism

There is considerable variation among humans in the production of enzymes that either activate formation of electrophilic metabolites that covalently bind to DNA or catalyze detoxification of chemical carcinogens. A large number of enzymes involved in regulation of metabolic pathways, many of which originally evolved in humans to handle naturally occurring toxicants, play an important role in detoxifying compounds humans are exposed to today as a result of environmental pollution. Within each group of enzymes, there are many forms that have evolved to metabolize different chemicals. For example, there are approximately 40 cytochrome P450 enzymes that evolved from a common ancestral cytochrome gene, estimated to be 2000 million years old. Each of the 40 forms is composed of variants that have different capacities to metabolize drugs and chemicals.

DNA repair

In addition to signaling exposure or susceptibility to a mutagenic agent, biomarkers also indicate inability to repair damage. DNA has a network of repair mechanisms to prevent injury and maintain the integrity of the genetic material that drives normal cellular and biological processes. Under normal circumstances, during replication of DNA, repair enzymes travel along the molecule and excise mismatches of nucleotide base pairs or aberrations in molecular structure, such as adducts. When there is a deficiency in the system of repair genes of an individual, that person is predisposed to developing cancer. Excision repair enzymes also provide backup protection for correcting errors in the genetic code and architectural distortions during replication. Just as metabolic responses to chemical exposure vary from one person to another, DNA excision repair is highly variable among individuals of any population.

It should be appreciated that a major research goal is to link markers of exposure with markers of effect. Unfortunately, for the vast majority of patients with suspected toxic renal injury the precise knowledge of the offending agent is speculative and not measurable by current techniques. As a result, more is known about the risk factors associated with an adverse health effects than is known about the parameters of exposure (Table 3).

Definitions

The usefulness of a biomarker requires that its measurement be standardized, reproducible and convenient. In addition, a correlation must exist between the assay values and the extent of injury. In strict terms, the *usefulness* of a test is related to its ability to deter-

Table 3. Some factors influencing nephrotoxicity.

Urine flow rate
Urine pH
Renal blood flow
Sodium balance
Pre-existing disease
Other drug therapy
Tolerance
Pharmacokinetic factors
Microsomal enzyme activity
Dosage and route of administration
Duration of exposure

mine whether or not a particular disease is present or absent. The term *true positive* (TP) refers to a person with the disease or condition under investigation who has a "positive" test result. If the test is "negative" despite the presence of the disease, the result is a termed a *false negative* (FN). In the absence the disease or condition under investigation, a "negative" result is termed a *true negative* (TN) while a "positive" result is termed a *false positive* (FP) (Figure 2). The *true positive fraction* (TPF) is expressed as TPF = TP/(TP + FN). The *true negative fraction* (TNF) is expressed as TNF = TN/(FP + TN). The *false positive fraction* (FPF) is expressed as FPF = FP/(FP + TN). The *false negative fraction* (FNF) is expressed as FNF = FN/(TP + FN). The false negative fraction plus the true positive fraction equals one. Similarly, the false positive fraction plus the true negative fraction equals one.

Test *sensitivity* indicates the percentage of individuals in whom a test is positive for the disease in question. It is the same as the TPF. The better the sensitivity of the test, the fewer are the false negatives. Tests with a very high sensitivity can, if negative, be used to exclude the relevant disease. Test *specificity* refers to the percentage of individuals without the disease in question in whom a test is negative. It is the same as the TNF. The better the specificity of the test, the fewer are the false positives. Expressed somewhat differently, the false-positive rate = 1 – (specificity). Also, the false-negative rate = 1 – (sensitivity).

There are at least two major problems that can cause errors in the interpretation of the "sensitivity" and "specificity" indexes of individual tests [15]. Since the sensitivity and specificity calculated for any diagnostic test are derived from data obtained in a selected group, it is necessary to choose a large enough sample of both diseased and nondiseased subjects so that the test in question does not receive falsely high values for its sensitivity and specificity. Notably, test performance in a population changes as the prevalence of the disease changes. That is, how well a test with a given sensitivity and specificity identifies affected and unaffected persons is itself affected by the prevalence of disease in the population being studied. There is often an inverse relationship between the sensitivity and specificity of a given test whereby an increase in one is accompanied by a decrease in the other. Thus, for each *'cut-off'* point or *decision threshold,* a different sensitivity/specificity pair may be recorded. That is, a high sensitivity may be associated with a low specificity.

It is also necessary to establish the true diagnosis independently. If not, *bias* may falsely elevate the test's efficacy. The diagnostic performance of a test may be subject to bias when the test has different sensitivity and specificity in subjects with different manifestations of the disease, and the study population is not truly representative. A more accurate approach is to define a series of sensitivities and specificities which are based on the clinical features of each subject, with the sensitivity and specificity reported in the evaluation of a diagnostic test actually representing an "average" of these values [16].

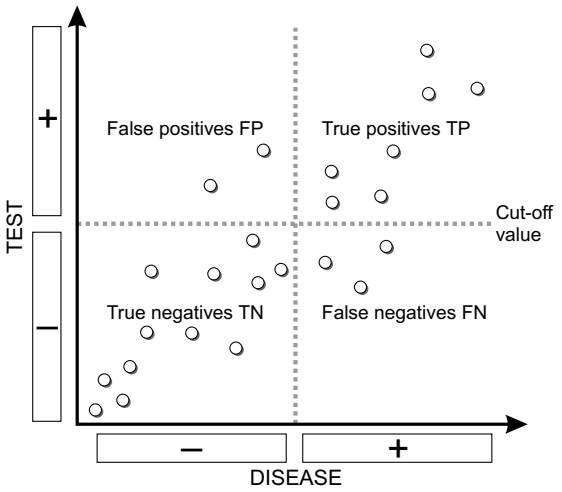

Figure 2. *Illustration of the test/disease concept.*

The clinical performance of a laboratory test can be described in terms of *diagnostic accuracy,* or the ability to correctly classify subjects into clinically relevant subgroups. The overall accuracy of a test is the measure of "true" findings divided by the test results from the total population (TP + TN)/(TP + FP + FN + TN). This is also termed the *efficiency* of the test. Since the diagnostic accuracy refers to the quality of the information provided by the classification device, it should be distinguished from the usefulness, or actual practical value, of the information - although it is true that the usefulness of a diagnostic test is largely determined by its accuracy.

One measure of accuracy is the *likelihood ratio* (LR) [17]. The likelihood ratio is based on the relationship between the true positive fraction and the false positive fraction. Thus, LR = TPF/FPF. Substituting terms, the LR = sensitivity/(1-specificity). *Receiver-operating characteristic* curves are graphical summaries of likeli-

hood ratios. The Receiver Operating Curve (ROC) plots the sensitivity versus 1-specificity over the complete range of decision thresholds [18]. The Receiver Operating Curve (ROC) originated during World War II with the use of radar in signal detection. This was extended to the use of diagnostic tests for identifying disease states, using plots of sensitivity versus specificity for each test result in the diagnosis of the disorder in question. The area under a ROC curve serves as a measure of the diagnostic accuracy (discrimination performance) for a test. Thus, the likelihood ratio is the slope of the receiver-operating characteristic curve at that point.

The *odds ratio* is a ratio of two odds. Odds are the ratio of the number of people in a group with an event to the number without an event. Thus, if a group of 100 people had an event rate of 0.20, 20 people had the event and 80 did not, and the odds would be 20/80 or 0.25. In other terms, it is equal to the probability of disease divided by 1- the probability of disease. An odds ratio of one indicates no difference between comparison groups. For an undesirable outcome, OR that is less than one indicates that the intervention was effective in reducing the risk of that outcome. When the event rate is small, odds ratios are very similar to relative risks.

The relative risk (RR) is the ratio of risk in the intervention group to the risk in the control group. The risk is the ratio of people with an event in a group to the total in the group. A relative risk of one indicates no difference between comparison groups. For an undesirable outcome, an RR that is less than one indicates that the intervention was effective in reducing the risk of that outcome.

The *predictive value* of a test relates positive or negative results to the prevalence of the disease in the population. The positive predictive value is true-positive test results divided by all positive test results (that is, the sum of true positives and false positives = all positive test results). This is also referred to as the predictive value of a positive test. The negative predictive value is the true-negative test results divided by all patients with negative results (that is, the sum of false negatives and true negatives = all negative test results). This is also referred to as the predictive value of a negative test. A high predictive value for a positive test indicates a strong likelihood that a person with a positive test result actually has the disease. A high predictive value for a negative test means that a negative result virtually rules out the disease.

Prevalence indicates the number of patients per 100,000 population who have the disease at a given time. That is, prevalence is the sum of the true positives and the false negatives for the test condition as proportion of population. *Incidence* is derived from a stated period of time. The incidence rate for a disease is the number of patients per 100,000 population who develop the disease in a given year.

Translating this concept of progressive appearance of biomarkers from exposure to disease into actual practice remains a challenge. The dual aspects of renal function, i.e., filtration/elimination and reabsorption/secretion, assure that no single test or measure can define global renal function. Furthermore, the substantial metabolic and endocrine functions of the kidney are not considered in the classical techniques used to analyze renal function. This has led to the use of a separate category of tests designed to serve as markers of renal dysfunction or injury (Table 4). Also, considerable attention has been directed to the immunological responses that follow xenobiotic exposure. Finally, as the mechanisms responsible for cell injury, death and regeneration become more apparent, a new and promising set of biomarkers is emerging.

Table 4. New parameters and techniques applicable to monitor nephrotoxicity.

Parameters	Techniques
Clearance of lithium, H_2O and N-methylnicotinamide metabolites	High pressure liquid chromatography
Enzymes and antigens	Fluorimetric and luminometric immunoassays
Microproteins	2-Dimensional electrophoresis Immunoblotting techniques Nephelometry, turbidimetry
DNA, mRNA	Southern blotting Pulse field electrophoresis Northern blotting Restricted fragment length analysis
In vivo imaging	Nuclear magnetic resonance spectroscopy [219, 220]
In vitro imaging	Electron probe analysis
Surface markers	Cell sorting

Considerations in the selection of biomarkers

Performance specifications

There are several performance specifications that need to be considered in the use of a particular biomarker. Each depends on the question being addressed. Both diagnostic and prognostic biomarkers are used for patient selection. Diagnostic biomarkers either identify a disease process, specific patient characteristics, or establish the severity and extent of involvement, or disease burden. Prognostic biomarkers help predict which patients will progress most rapidly, respond to a particular therapy, or develop complications.

To be useful for monitoring progression or treatment response, markers must change with disease and therapy. Key metrics of markers for monitoring therapy include: responsiveness (rate of change) to disease and therapy, measurement precision, and link to clinical outcomes (surrogate validity). Complications caused by therapy must be distinguished from those associated with the disease itself.

Performance criteria

A fundamental set of performance criteria can be defined by which the utility of different biomarkers can be compared. The most important quality of a surrogate endpoint is its link to the "true" clinical outcome of interest. In fact, very few biomarkers have been widely accepted as true surrogate endpoints. The use of a surrogate endpoint assumes that the morphological, compositional or physiological feature it represents lies directly along the disease pathway to the clinical outcome of interest. To be useful, the surrogate must also lie on the intervention pathway. Alternative mechanisms of disease or therapy that bypass this biomarker undermine its validity as a surrogate endpoint.

The so-called dynamic range refers to the proportion of change in the "true" outcome that is captured by changes in the surrogate endpoint. Inability of the surrogate endpoint to detect early, mild changes in the true outcome is sometimes referred to as a "floor effect". Failure to register late, severe changes is called a "ceiling effect". Ideally, any such floors or ceilings lie outside of the range of changes in the true outcome that is relevant to the question under study.

The rate of change of the chosen molecular endpoint determines the minimum study duration possible. Regardless of whether the aim is internal decision-making about which compounds and doses to test or to confirm therapeutic efficacy for regulatory approval, faster readouts accelerate clinical testing and allow earlier entry into the market and increased revenues over the patent life of a drug. Measurement precision defines the statistical power with which changes in the biomarker can be resolved. Three sources of variation must be considered: biological variability, variability associated with sample collection, and variability associated with the sample analysis.

Urinary biomarkers

Urinalysis

Test strip screening: The examination of the urine using qualitative test strip provides an estimate of glucose, pH, hemoglobin, protein, specific gravity and a number of other substances including ketones, bilirubin, urobilinogen, leukocytes and nitrate. The degree of sophistication has progressively increased to the extent that reading of test strips with reflectometers is possible. There is a good probability that urines negative by dipstick for protein, blood, leukocytes, nitrates, glucose and ketones will be negative on microscopic examination, with only 5.3% having any abnormality. However, urines positive for one or more of these findings may not correlate well with the microscopic findings due to a number of false positive and false negative by dipsticks for red cells and leukocytes. Sensitivities for dipsticks have been reported to be 75.3% and 81.0% and specificities were 88.6% and 64.3% for red cells and leukocytes, respectively [19]. It is recommended that microscopic analysis be done on dipstick abnormal urines. Some other limitations occur. For example, patients with microalbuminuria or tubular proteinuria are not detected by current test strip methods. Newer immunological techniques, which enable the determination of specific protein molecules, may make this possible [20].

Urine microscopy: The microscopic examination of the urine sediment provides enhanced diagnostic efficiency. *Hematuria:* The normal number of erythrocytes in resuspended urine sediment is no more than 1 to 2 per high-powered field. When an abnormal number

of erythrocytes are present it is necessary to distinguish between a renal or non-renal origin. The simultaneous presence in the urine of casts and protein favor a renal origin. With phase-contrast microscopy, a high percentage of dysmorphic erythrocytes support a renal source of hematuria [21]. The urine should be examined immediately after voiding. Since erythrocytes may be lysed in low specific gravity urine, a concentrated sample should be used for analysis. *Pyuria:* The normal number of white blood cells in the concentrated, resuspended urine sample does not exceed 1 to 2 per high-powered field. In patients with pyelonephritis or nephrotoxic interstitial nephritis, neutrophils may be found whereas with allergic interstitial nephritis, eosinophils may appear. Macrophages and lymphocytes can be found in the urine of some patients with glomerulonephritis and be useful in monitoring the activity of the disease [22]. *Tubular epithelial cells:* The appearance in the urine of epithelial cells is most likely a result of tubular injury. These cells may be present alone or in casts and be indicative of either acute or chronic tubulointerstitial nephritis. Since casts may dissolve in alkaline urine, an acid urine sample is preferred for analysis.

Eosinophiluria: The finding of eosinophils in the urine has been suggested to be useful in establishing the diagnosis of acute interstitial nephritis. However, the positive predictive value in screening samples may be too low, and the number of false positives and negatives in selected groups may be too high for eosinophiluria to stand alone in making the diagnosis of acute interstitial nephritis [23].

Blood urea nitrogen concentration (BUN) and urea clearance (C_{urea})

The BUN is not a satisfactory measurement of the glomerular filtration rate because the plasma concentration of urea is affected by nitrogen metabolism. In addition, the C_{urea} is proportional to the urine flow rate. For example, at low and high rates of urine flow, the minimal and maximal values of the C_{urea} may vary from 30% to 60% of the glomerular filtration rate. This occurs because various tubular segments are permeable to urea and allow passive reabsorption to occur under conditions of antidiuresis. The fractional excretion of urea (FE_{urea}) is calculated as [(urine urea/plasma urea)/(urine creatinine/plasma creatinine) x 100]. A low FE_{urea} may be used as an index of decreased renal perfusion [24].

Serum creatinine concentration (Scr) and creatinine clearance (Ccr)

The Scr is a more commonly used marker for the estimation of glomerular filtration rate. In addition to the level of renal function, its absolute value is related to muscle mass and varies from person to person as a consequence of differences in age, gender and body weight. All commonly used methods of creatinine measurement suffer from a lack of precision within the normal range. For individuals with glomerular filtration rate greater than 30 ml/min, the 95% confidence interval for Scr is ± 22%, whereas it is ± 13% in patients with glomerular filtration rate less than 30 ml/min [25]. The actual Scr may be increased by blocking renal tubular secretion. For example, trimethoprim and/or trimethoprim/sulfamethoxazole have been demonstrated to cause a 15 to 35% increase in Scr due to an inhibition of tubular secretion [26]. Also, various drugs {i.e. cephalosporins) cross react with the Jaffe method for determining creatinine to cause false elevations of the Scr.

Using either nomograms or formulae, Ccr can be estimated and the glomerular filtration rate approximated [27]. It is possible to estimate the Ccr from a stable Scr if the age and gender of the patient are taken into consideration. The most widely used equations [28-30] are as follows:

Cockroft and Gault, 1976 [27]:

Males

$$\text{Ccr \{ml/min)} = \frac{(140 - \text{age in years}) \times (\text{weight in kg})}{72 \times \text{serum creatinine (mg/100 ml)}}$$

Females = males x 0.85

Jelliffe, 1973 [28]:

Males

$$\text{Ccr (ml/min)} = \frac{98 - 16\,[(\text{age} - 20) \div 20]}{\text{serum creatinine (mg/l00 ml)}}$$

Females = males x 0.90

Kampmann et al., 1974 [29]:

$$\text{Ccr (ml/min)} = \frac{[\text{Ucr (mg/kg/min)}]\ (\text{weight in kg}) \times 100}{\text{serum creatinine (mg/100 ml)}}$$

Levey et al, 1999 [30]:

Estimated GFR =

$186.3 \times (sCr)^{-1.154} \times age^{-0.203} \times (0.742 \text{ if female}) \times (1.21 \text{ if black})$

In general, there is a wide degree of scatter when values of glomerular filtration rate are predicted by these equations, although the equation derived from the MDRD study provides more accurate estimates of GFR than the other formulae or measured clearances [30] and is comparable to values obtained using iothalamate clearance. The variation in calculated Ccr is particularly true in the elderly or others with large decreases in muscle mass, in patients with liver disease, and individuals ingesting a high-protein diet or those receiving parenteral nutrition containing amino acid solutions. Absolute variation is also more evident at higher estimated GFR.

The endogenous Ccr gives an acceptable estimate of the glomerular filtration rate and is the most widely used method in clinical practice for routine purposes. However, in normal individuals, the majority of measurements tend to yield values of Ccr that exceed the actual glomerular filtration rate by a substantial amount, owing to the fact that there is a small but significant amount of creatinine which appears in the urine as a result of tubular secretion. This problem is accentuated when the glomerular filtration rate declines. Ccr measurements may be twofold higher than the actual glomerular filtration rate because of continued tubular secretion of creatinine at a time when the rate of filtration is severely curtailed. Indeed, the amount of secreted creatinine varies inversely with the glomerular filtration rate [31, 32]. Recently, Coresh et al [33], using the NHANES III data base identified laboratory calibration of SCr as another factor which significantly distorted calculated GFR at values > 30 ml/min.

Glomerular filtration rate

Any substance used to measure glomerular filtration rate should be metabolically intact, freely filtered through the glomerular capillary wall, and be neither secreted nor reabsorbed by the tubules. Accurate plasma and urine quantitation also should be easily achievable. In addition to inulin, several compounds are useful for the measurement of glomerular filtration rate. These include the urologic contrast media diatrizoate, iohexol, ^{57}cocyanocobalamin, ^{51}Cr-ethylenediaminetetraacetic acid (EDTA) or sodium [^{125}I] iodothalamate and ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) provide reliable measurement of glomerular filtration rate [*34*]. Inulin is a polymer of fructose and is an ideal glomerular filtration rate marker because it is freely filtered and neither reabsorbed or secreted by the tubules. It is widely used as a research tool but because of a number of technical difficulties, it is not widely used in clinical settings. Isotopic methods offer a high level of reliability but the impracticality of using these methods in a clinical setting makes them unsuitable for routine use. On the other hand, Iohexol is a convenient, reliable technique for measuring GFR and has the same precision as ^{125}I-Iodothalamate [35].

As an alternative to the standard clearance techniques which involve the collection of urine over a known period of time plus maintaining a constant plasma level of an appropriate marker, the glomerular filtration rate can be calculated from the rate of disappearance from the plasma of any tracer, where:

$$\text{clearance} = \frac{\text{injected dose}}{\text{area under plasma concentration curve}}$$

Additional techniques to obtain more reliable estimates of glomerular filtration rate without resorting to steady-state infusions involve the plotting of the declining plasma level of radio isotopic agents [36] or non-radioactive iodinated contrast agents [37] if they are cleared by glomerular filtration. The glomerular filtration rate as measured with iohexol shows excellent agreement with the values obtained using inulin and chelates throughout a wide range of kidney function. As a result, the method is gaining favor [34, 38-39].

The "renal reserve" is determined by measuring the percentage increase in glomerular filtration rate following ingestion of a high protein meal [40]. The failure of the glomerular filtration rate to increase in response to such a challenge suggests that underlying chronic disease and nephron atrophy has been masked by hypertrophy of other nephrons so that overall renal function seems to be well maintained.

Renal blood flow

If a marker is extracted from the blood exclusively by the kidney resulting in a renal venous concentration of 0% (i.e. the arterio-venous extraction fraction is 100%), then the calculated value of the clearance of the marker (Cx) is equal to renal plasma flow. In practice, a compound, such as *para*-amino hippurate (PAH) with an extraction fraction of about 87%, is used. To acknowledge the fact that there is discrepancy between the PAH clearance and renal plasma flow, the term effective renal plasma flow is used when the extraction factor is not measured. In sum, renal plasma flow = effective renal plasma flow + extraction factor and renal blood flow = effective renal plasma flow + the hematocrit.

A decrease in the PAH clearance might be due to either an actual decline in renal plasma flow or a decrease in the extraction factor of *PAH*. The latter occurs when the tubular secretion of *PAH* in proximal tubules is impaired due to tubular disease or the presence of substances, which compete with transcellular *PAH* transport. Thus, the PAH clearance cannot be considered a reliable measure of renal plasma flow, unless the extraction factor of *PAH* is measured simultaneously. This requires that a sample of renal venous blood be obtained.

Tubular function

The identification of a reliable and convenient method for the estimation of the reabsorptive and secretory capacity of the kidney has proven to be a considerable challenge to Nephrology. This is not unexpected when one considers the complex and integrated functions contributed by the various tubular segments to insure proper composition of bladder urine. General estimates of integrated tubular function include the capacity of the kidneys to concentrate or dilute the urine in response to water deprivation or administration; the ability to excrete an administered acid load; and the precision with which sodium balance is maintained. But lacking is a technique for assessing tubular function, which rivals the measurement of glomerular filtration rate.

Specific gravity and osmolality: The urinary specific gravity and osmolality are indicators of the ability of the kidney to concentrate and dilute the urine. The urinary specific gravity depends upon the size and weight of urinary solutes. The normal range is 1.003 to 1.025 whereas the possible range is 1.001 to 1.040. Osmolarity indicates the total number of solute particles per kilogram of urine water. The normal range is from 150 to 900 mosm/kg with a possible range from 50 to 1200 mosm/kg.

pH: A hydrogen ion concentration gradient of 1 to 1000 may be established across tubular cell membranes of the kidney. Since the pH is the negative logarithm of the hydrogen ion concentration, this translated into a decrease from the normal plasma pH value of 7.4 to the minimal urine pH of 4.4.

Lithium clearance: The lithium clearance is a used to estimate the amount of sodium and water delivery from the pars recta of the proximal tubule into the descending limb of the loop of Henle [41]. This information may be helpful in the assessment of the state of hydration. The method is based on several assumptions the most important of which are that lithium reabsorption parallels sodium and water along the entire proximal tubule; that lithium is neither reabsorbed 'in measurable amounts beyond the pars recta of the proximal tubule; nor is it secreted by the tubular cells [42]. Recently, Anastasio et al [43] have used LiCl to evaluate salt and water handling in cirrhotic patients and found increased sodium reabsorption in the distal tubule accounts for the salt retention that characterizes this clinical condition.

Proteinuria

Under normal circumstances, the glomerular filtration barrier restricts the transfer of high molecular weight proteins from plasma to the nephron lumen. In certain pathologic states, the permselectivity of the filtration barrier changes allowing high molecular weight proteins to appear in the urine. These proteins undergo pinocytotic reabsorption creating cytoplasmic vesicles that then fuse with primary lysosomes to form secondary lysosomes. In this final form the proteins are hydrolyzed to amino acids, which are delivered into the blood stream. In contrast, under normal conditions a finite amount of low molecular weight proteins are filtered which then undergo reabsorption by proximal tubular cells. Exopeptidases situated on the brush border membrane are responsible for splitting peptides up to a molecular weight of 10,000 daltons. Following

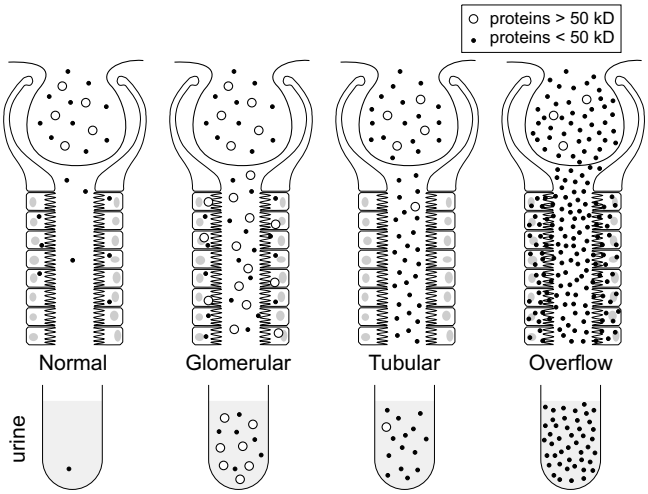

Figure 3. *Three kinds of proteinuria.*

Table 5. Classification of proteinuria according to site of origin.

Plasma proteins
Kidney-derived proteins
Proteins from the urogenital tract
Proteins released from tissue outside the urogenital tract
Pregnancy associated proteins
Tumor-derived proteins

metabolic conversion, reabsorption of the amino acids or dipeptides occurs by specific sodium-dependent carriers [44]. When the reabsorptive capacity of the proximal tubular epithelium is disrupted, various low molecular weight proteins escape reabsorption and can be measured in the urine. Thus, the distinction between so-called "glomerular" proteinuria and "tubular" proteinuria is based on both the quantity and quality of the proteins measured in the urine [45] (Figure 3). A recent refinement of this differentiation in protein selectivity has been the "so-called" urine protein expert system [46, 47]. This expert system, which includes total protein, albumin, α_1-microglobulin, IgG, α_2-macroglobulin, NAG and creatinine, has proven to be more discriminatory in providing correct clinical diagnoses which are histologically confirmed, as compared to human expert diagnosis. Another approach to differentiating glomerular from tubular disease involves analyzing urinary proteins with the SDS-PAGE system that separates various urinary protein species. In a recent report, Lau and Woo [48] found an excellent correlation between SDS-PAGE prediction and findings on renal biopsy. In general, proteins in the urine may be classified into six main categories according to their origin (Table 5).

Measuring urinary protein excretion has been simplified by the introduction of the urine protein to creatinine ratio [UP:Ucr] [49, 50]. The advantages to using the P:Cr ratio are: 1) compliance issues in collecting a 24 hour sample are eliminated, 2) managing large volumes of urine are eliminated, 3) eliminates the need for accurate timing of the sample collection [51]. To minimize the effect of the diurnal excretion of protein, the sample for P:Cr ratio should be collected following the first morning voiding or before midday [51].

In addition to serving as markers of renal dysfunction, it is now evident that the filtration of abnormal amounts and/or types of proteins influences the progression of renal disease by promoting secondary injury to tubular epithelial cells and interstitial structures. For example, the upregulation of various cytokines in tubular epithelial cells may contribute to the development of interstitial fibrosis and cell cycle activation leading to tubular cell proliferation and/or apoptosis [52-54].

High-molecular weight proteinuria

The appearance in the urine of serum proteins with a molecular weight (Mr) in excess of 40,000 to 50,000 daltons is an early marker of glomerular damage. The commonly measured high molecular weight proteinuria includes: albumin (Mr 69,000), transferrin (Mr 77,000) and IgG (Mr 146,000) (Table 6).

A. Albumin is quantitatively the major urinary protein derived from plasma. Its average concentration in normal urine is at least 50 times higher than most other low molecular weight proteins. Albumin's molecular size (molecular radius: 3.6 nm) and strong negative charge, effectively retarded filtration at the glomerular barrier since the vast majority of pores perforating the glomerular filtration barrier have a radius of 2.9–3.1 nm. The loss of negative charges causes the effective small pore radius to increase to approximately 4.5 nm, which allows the passage of albumin. The small amounts of albumin that ordinarily escape into the glomerular filtrate are reabsorbed by the proximal tubule with a presumed efficiency of 99%. Transient and totally reversible increases in the albumin excretion may be observed in various "physiologic" situations

that induce increases in the glomerular filtration rate such as heavy exercise, fever, or assuming an orthostatic position.

Albuminuria in excess of 3.5 g/day can undoubtedly be ascribed to increase in glomerular permeability. Glomerular proteinuria can be suspected when the amount of protein in the urine exceeds 2.5 grams in a 24-hour period [55]. At either level, the proteinuria may be nonselective in that it contains the spectrum of molecular sizes. Albuminuria greater than 0.5 g/day is also likely to be a consequence of enhanced glomerular filtration since the relative increase in urinary excretion exceeds that which could be expected from a complete failure of tubular reabsorption. Recently, technical advances have allowed for the measurement in the urine of milligram quantities of albumin i.e. 20 to 250 mg/24 hours [5]. Detection of these small quantities of albumin - referred to as microalbuminuria - has become an early biomarker of altered glomerular permeability and is considered to be the earliest marker of developing diabetic nephropathy [5]. It has also been used as an endpoint in therapeutic intervention studies [56, 57]. Thus, the definition of "glomerular" proteinuria has shifted from quantity to quality with assays of microalbuminuria being conducted in a variety of glomerular diseases to provide earlier detection [58]. When microalbuminuria (20 to 200 mg/min) is observed in the absence of low molecular weight proteinuria, it may be ascribed to enhanced glomerular permeability. When accompanied by an increased urinary excretion of low molecular weight proteins, microalbuminuria results wholly or partly from impaired albumin reabsorption. The significance of microalbuminuria in the assessment of nephrotoxicity is presently unknown. In persons exposed to nephrotoxic agents, the presence of microalbuminuria may be used as an indicator of risk for late nephropathy. Capillary zone electrophoresis, a recent improvement in measuring small quantities of protein, has been used to monitor renal injury during the administration of nephrotoxic drugs such as carboplatin, etoposide, and ifosfamide [59].

B. Transferrin, the iron-transporting protein, occurs in urine at concentrations that are about 15 times lower than that of albumin. The protein has a slightly larger effective molecular radius (around 4.0 nm) than albumin (3.6 nm). Its detection in the urine allows a more sensitive indicator of early glomerular involvement in some nephropathies such as cadmium nephropathy. A strong association has been found between the presence of albumin and transferrin in the urine of patients with the nephrotic syndrome. In these patients, increased transferrin synthesis is insufficient to compensate for urinary losses and plasma levels are reduced

Table 6. Characteristic of urinary proteins [281].

Protein	Abbv.	Molecular Weight kD	GSC*	Normal Urinary Excretion mg/mmol creatinine	Normal Plasma Levels Mg/l
β_2-microglobulin	β_2-m	11.6	18.3 (4.4-32.2)	< 0.05	1.3
Retinol-binding protein (free)	RBP	21	13.6 (5.1-22.1)	<0.017	5.8
Thyroid-stimulating hormone	TSH	28	0.99 (0.3-1.68)	<0.05	2
α_1-microglobulin	α_1-m	31	21.1 (9.5-32.9)	<2	32
α_1-acid glycoprotein	α_1-AG	40	—	<0.02	770
Zinc-α_2-globulin	ZAG	41	—	<0.02	140
β_2-Glycoprotein I	β_2-GI	50	12.3 (6.1-18.5)	<0.03	150
Vitamin D binding protein	DBP	51.3	—	<0.01	400
Transthryetin	TTR	55	—	<0.01	300
Albumin	ALB	65.5	13.8 (6.6-21)	<0.025	45000
Transferrin	TRF	78	1.2 (0.6-1.8)	<0.19	2700
Immunoglobulin G	IgG	160	58 (34-82)	<0.2	120000

**GSC= glomerular sieving coefficient*

[60].

C. Gamma globulins excreted in the urine include IgG, IgM, IgA, and immunoglobulin light chains. IgG and IgM are large proteins with molecular radii of 5.5 and 12 nm, respectively. The appearance in the urine of IgG indicates an increased density of large pores in the glomerular filtration barrier with a radius of 8 to 9 nm whereas the presence of IgM in the urine indicates an increased density of shunts in the glomerular capillary wall [61, 62]. Coupled with the measurement of urinary albumin concentrations, the determination of urinary levels of IgG and IgM are useful for assessing the selectivity of the glomerular-type proteinuria [63]. The urinary excretion of IgG is regarded as a reliable index of a non-selective pathway shunt through the glomerular capillary wall. In this regard, it has been reported that proteinuria in patients with type 1 diabetes mellitus is mainly due to impaired charge-selective properties of the glomerular capillary wall, while proteinuria in type 2 diabetes mellitus is predominantly caused by a decreases size selectivity of the glomerular capillary wall [64]. Moreover, proteinuric patients with a high urinary content of IgG and IgM have poor renal survival [65]. IgG, transferrin, albumin, and α_1 microglobulin were used to predict progression of renal failure and extent of tubulointerstitial disease in patients with idiopathic membranous nephropathy [66]. As a result it was found that IgG excretion has a direct, positive correlation with the extent of tubulointerstitial disease and α_1-microglobulin excretion rates. An increased excretion of monoclonal light chains, i.e. Bence-Jones proteins, is usually the sign of an overproduction from a neoplastic origin such as multiple myeloma or Waldenstrom's macroglobulinemia. A variety of new techniques for improving the detection and diagnosis of monoclonal gammopathies have been recently reported [67-71]. When multiple proteins are analyzed in patients with multiple myeloma, it is possible to identify patients with minimal renal impairment and thus initiate early treatment [72].

Low-molecular weight proteinuria

In contrast, "tubular" proteinuria is often less than 1.0 g/24 hours and composed of LMW proteins [55]. Several LMW proteins normally appear in the urine and have been evaluated as potential biomarkers of effect in renal tubular damage [9]. Included are β_2-microglobulin (β_2-m), retinol binding protein and α-l microglobulin (α_1-m) [73]. Other LMW proteins of interest include protein 1, amylase, lysozyme, ribonuclease [74] and cystatin C [75]. Combining LMW proteins with prostanoids, growth factors and enzymes of renal and non-renal origin, excretory patterns have been identified which provide insight as to the site and mechanism of nephrotoxic injury [76].

A. β2-microglobulin *(*β_2-m*)* is a low molecular weight (Mr: 11,800) globular protein located on the surface of virtually all nucleated cells. It was the first low molecular weight protein isolated from the urine of patients with tubular disease [77]. It is closely related to the class I histocompatibility antigens which consist of a heavy, variable chain and a light chain that binds to the heavy chain domain nearest to the cell membrane. The light chain consists of the β_2-m molecule. Due to its molecular weight and small radius, β_2-m is readily filtered at the glomerulus. Under normal circumstances, approximately 99.9% of the filtered β_2-m is reabsorbed by the proximal tubular epithelial cells and ultimately catabolized. A very small amount, around 70 to 80 mg/24 hours, appears in the urine. The urinary excretion of β_2-m is considerably increased in cases of renal tubular impairment. As a result, the, determination of urinary β_2-m has been widely used for the screening of proximal tubular damage. For example, in surveys of occupationally exposed individuals, a dose-dependent relationship between the body burden of cadmium and β_2-m excretion has been established [12]. However, since β_2-m undergoes degradation at urinary pH of 5.5 or less, proteins such as retinol binding protein or α_1-m have been proposed as preferred biomarkers better suited for epidemiologic surveying due to their stability and resistance to proteolytic degradation over a wide range of urinary pHs [9, 78]. The simultaneous measurement of serum and urinary levels of β_2-m permits a determination of its clearance that provides greater precision regarding tubular function, but also demands alkalinization of the urine to insure complete recovery of urinary β_2-m [79]. Despite the technical problems associated with its measurement, the experience with β_2-m far exceeds that of all other low molecular weight proteinuria assays [79].

B. Retinol-binding protein, also called α_2-microglobulin, is a low molecular weight protein (Mr: 21,400). It is synthesized in the endoplasmic reticulum of the liver where it binds to retinol (vitamin A). It appears

in the plasma bound to transthyretin (or prealbumin). Once the retinol is given up at the appropriate target tissue, retinol-binding protein undergoes a conformational change and looses its affinity for transthyretin. In its new configuration, it is rapidly eliminated from plasma by glomerular filtration, then reabsorbed and catabolized by proximal tubular cells. Retinol-binding protein reabsorption involves megalin [80], a large glycoprotein and member of the low-density lipoprotein receptor family. Because of its stability in acid urine and since the serum level of free retinol binding protein is influenced only by renal function, the assay of urinary retinol binding protein is preferred over that of β_2-m. It has been considered a good marker of renal injury in clinical settings evaluating the potential nephrotoxic agents [81].

C. α1-microglobulin (protein HC; α_1-m) is a glycosylated protein with a Mr of 27 kD. It has been shown to be associated with a novel family of small secretory proteins, the so-called lipocalin superfamily that includes retinol-binding protein. Protein HC is mainly synthesized in the liver and occurs in the serum in both a free form (free protein HC) and bound to several high molecular weight proteins such as immunoglobulin A (HC-IgA) and albumin (HC-albumin). The renal handling of protein HC is less well characterized than that of β_2-m or retinol binding protein. It has a glomerular sieving coefficient close to the benchmark separating LMW and HMW proteins. While half the amount in the plasma is complexed with immunoglobulin A, the free form is readily filtered through the glomerular basement membrane. The free form of this protein has been used as an indicator of proximal tubular dysfunction [82-84]. It is stable in native urine and its normal urine concentration is sufficiently high to be determined with rapid and cheap immunochemical techniques [85]. Notably, the excretion rate decreases with recovery of the damaged tubular cells [86, 87]. In proteinuric glomerular disease, urinary protein HC concentration correlates to the degree of IgG present in the urine [88].

Other proteins

Protein 1 is an α-microprotein. It is unique in that it is a sex-dependent protein excreted in greater amounts by males than females after puberty. It is excreted in increased amounts in the urine of patients with proximal tubule dysfunction. The Mr of 18,700 and isoelectric points between 4.6 to 5.2 of rat α_{2m}-globulin are very close to that estimated for protein 1.

Amylases are excreted in greater amounts in tubular proteinuria. However, since the fractional uptake by the proximal tubule is substantially lower than that of β_2-m or retinol-binding protein, they are less sensitive indicators of tubular injury. Serum amylases are synthesized mainly in salivary glands and the pancreas. Both isoenzymes have the same molecular size (29A) but different net charges in plasma. Salivary amylase is more anionic (pI 5.9 to 6.4) than pancreatic amylase (pI 7.0). A decrease in the number of anionic proteoglycans of the glomerular basement membrane and subsequent diminished rejection of anionic plasma proteins would facilitate the excretion of greater quantities of the more anionic isoamylase, salivary amylase. Thus, the use of the urinary ratio of salivary to pancreatic amylase could be useful in exploring changes in negative charges of the glomerular basement membrane [89].

Lysozyme or muramidase, is an enzyme that catalyses the hydrolysis of the peptidoglycan layer of bacterial cell walls. The urinary excretion of lysozyme increases during urinary tract infections, proximal tubular damage, and excessive endogenous lysozyme synthesis that overwhelm the absorption capacity of the proximal tubule.

Cystatin C is a non-glycosylated basic protein with practically the same Mr (13,300) as β_2-m. It is a member of the cystatin superfamily and the major inhibitor of the cysteine proteinases. The cysteine proteinases are one of four major classes of endoproteinases that possess the ability to degrade intact glomerular basement membranes [90]. In the rat it has been isolated from urine after sodium chromate induced tubular dysfunction. All nucleated cells produce cystatin at a stable rate that is not influenced by inflammation. Serum cystatin C concentrations change with age in parallel with changes in glomerular filtration rate. It has been suggested that serum concentrations correlate inversely with the glomerular filtration rate [76]. Automated techniques for the measurement of cystatin C are now available [91]. In some circumstances, high concentrations of potentially bioactive hormones such as PTH, insulin, IGF-1 and the chemokine monocyte chemoattractant protein-1 (*vida infra*) are present and may themselves contribute to progressive renal failure [92].

Tamm-Horsfall glycoprotein

Tamm-Horsfall glycoprotein (THP) is a 616 amino acid, 80 kD protein with a carbohydrate component that accounts for nearly 30% of the molecular weight. It is the most abundant protein of renal origin in normal urine and is the major constituent of urinary casts. Synthesis of THP occurs in cells of the thick ascending limb of the loop of Henle where it is localized on the epithelial cell membrane. It is excreted in the urine at a relatively constant rate (20 to 60 mg/24 hours). The carbohydrate side chains are responsible for binding certain cytokines while an arginine-glycine-asparagine sequence is involved in the binding of integrins [93]. The isoelectric point (3.2) is very low, so that the protein has a net negative charge at physiologic pH. Viscosity of solutions containing this protein increase markedly when the [NaCl] is > 60 mM. Increasing the concentration of Tamm-Horsfall glycoprotein, $[H^+]$, and $[Ca^{2+}]$ also increases viscosity [94]. These factors are determinants of cast formation. The urinary excretion can increase following injury to the distal part of the tubule, but it can be abnormal when the renal mass is reduced.

Its unique site of origin provides potential as a biomarker of renal tubular dysfunction [95]. The quest has been aided by the recent development of a simple yet sensitive radioimmunoassay of human THP [95]. An inverse relationship between the extent of tubular damage and diminished THP excretion has been described in a study of biopsy-proven cases of chronic glomerulonephritis [96]. It has been suggested that the lessened excretion of THP in renal disease may be a result of a reduction in the number of functional distal tubular cells [97].

Enzymuria

The acceptance by nephrologists of urinary enzyme activity as a measure of renal tubular dysfunction has been limited for several reasons. Paramount among these has been the difficulty to establish correlations have been made between specific disease states and the presence or absence of enzymuria. In addition, a relationship between the severity of cellular injury and the magnitude of enzymuria has been difficult to establish. This has been due in part to the fact that various factors alter urinary enzyme activity that are independent of cellular integrity, i.e., urinary pH, osmolarity, and the presences of various enzyme inhibitors or activators [98]. Nonetheless, the ease with which urine can be sampled, the impressive improvements in the technical aspects of assaying urinary enzyme activity [99-101] and the more complete understanding of the significance of enzymuria, has rekindled interest in their application as monitors of both acute and chronic renal injury.

The interpretation of urinary enzyme titers is founded on the premise that the sole source of high-molecular weight enzymes is damaged tubular cells [102]. In addition to normal cell shedding [103-105] enzymes also gain urinary access because of altered cell membrane permeability, increased rate of enzyme synthesis, and cell necrosis. Obviously, extraneous sources of urinary enzyme activity must be excluded including filtered plasma enzymes, cells and secretions from genitourinary tract, non-renal cells escaping into the urine, and the effect of drugs such as salicylates which can cause the desquamation of renal cells [106].

The ideal criteria for interpretation of enzymuria [107] include the following: (i) to evaluate glomerular function the enzyme should be present in blood, absent in renal tissue and have a molecular size that precludes its filtration; (ii) to evaluate tubular reabsorption the enzyme should be present in blood, absent from renal tissue, have a molecular weight that allows it to be freely filtered and be reabsorbed by the tubule; and (iii) to evaluate anatomical and functional condition of the tubular epithelium the enzyme should be restricted to the renal tissue. Other criteria for the diagnostic use of enzymuria [108] including various technical and biological considerations are summarized in Table 7.

The unique distribution of various enzymes along the length of the nephron provides the potential for identifying the specific injury site. Enzymes may not be uniformly distributed along or between nephrons thus the site selectivity of single enzymes is questionable, however it should be possible to localize the area of kidney damage on the basis of the pattern of enzymuria. While over one hundred urinary enzymes have been evaluated [109], only a small number of enzymes, that are well defined and widely reported, are valuable as biomarkers (Table 8).

When considering the application of urinary enzymes to monitor subtle renal dysfunction and/or to clarify mechanisms of nephrotoxicity, only a limited number of enzymes have been generally accepted as valuable urinary biomarkers. These include: lactic de-

Table 7. Criteria for diagnostic use of urinary enzymes.

Technical considerations
Precision
Standardization (accuracy)
Interference
Technical performance (automation)
Cost
Biological considerations
Nephron origin
Intracellular site
Mechanisms of release into urine
Stability in urine at 37°C
Factors known to influence sampling conditions

Table 8. Some enzymes used as index of nephrotoxicity.

Enzyme	Cellular location
Alanine aminopeptidase (AAP)	brush border
Alkaline phosphatase	
γ-glutamyltransferase (GGT)	
Maltase	
Trehalase	
Glutamic oxaloacetic transaminase	cytosol
Lactate dehydrogenase	
Malate dehydrogenase	
N-acetyl-β-D-glucosaminidase	lysosome
Acid phosphatase	
β-glactosidase	
β-glucosidase	
β-glucuronidase	
Glutamate dehydrogenase	mitochondria

hydrogenase, N-acetyl-β-D-glucosaminidase (NAG), and alanine aminopeptidase (AAP), while others, such as intestinal alkaline phosphatase and glutathione-S-transferase, are emerging as useful markers.

Urinary enzymes have the potential of determining the primary site of renal damage because different sections of the nephron have a characteristic complement of enzymes. In dogs, increases in brush border enzymes, including γ-glutamyl transferase and alkaline phosphatase, have been associated with renal proximal tubular damage, while increases in NAG have been observed in the early stage of renal papillary necrosis. Urinary enzymes have been particularly useful in detection of acute renal damage in dogs, specifically tubular damage: however, it is important to note that their corresponding value in providing information about chronic renal damage remains to be established. Although elevation of certain enzymes appears to be a relatively sensitive measure of nephrotoxicity in the dog, there is no current agreement regarding which enzyme assays are the most appropriate for routine use in human safety assessment studies. In addition, elevation of a single enzyme is of limited diagnostic value in detection of renal damage because spurious increases in urinary enzymes sometimes occur in normal dogs. Therefore, if one wishes to conduct special assessment of nephrotoxicity in dogs, evaluation of several enzymes at multiple time points is needed to compensate for normal enzyme variation and to identify potential anatomic site selectivity of the toxin [110].

In addition to albumin and retinol binding protein, the Center for Disease Control's renal battery includes NAG and AAP, which are used for different reasons. For example, NAG is a lysosomal bound enzyme that may indicate an increase in lysosomal activity, while AAP may indicate turnover of brush-border tissue. They behave differently in response to the effects on the kidney of different toxicants and chronic diseases, each providing potentially valuable pieces of information [111].

A. Lactate dehydrogenase. The use of urinary enzymes in the investigation and diagnosis of renal injury or disease was initiated by Rosalki and Wilkinson [112], who reported increased activity in the urine of patients with renal disease. However, lactate dehydrogenase soon gave way to more site-specific, easier to determine urinary enzymes.

B. N-acetyl-β-D-glucosaminidase (NAG) is found in both the S3 segment of proximal tubular cells and the distal nephron as a lysosomal enzyme. It has its highest activity in the straight (S3) location of the proximal tubule of man, with less activity in the collecting duct portion of the distal nephron. With a molecular weight of approximately 150,000 daltons, it is normally retarded from passage through the glomerulus, and elevated urinary levels are indicative of tubular cell injury. The presence of NAG, an intracellular lysosomal enzyme, in the urine indicates organelle damage within the proximal tubule. In addition to occurring in the urine of individuals with tubular injury, it has also been found in the urine of patients with various forms of glomerular disease, obstructive uropathy and nephrosclerosis. Other non-specific increases in urinary NAG activity have been described. With the refinement

of the colorimetric assay technique, it is one of the most useful and best studied of the diagnostic urinary enzymes. The enzyme activity is apparently not influenced by variations in urinary pH. Urinary NAG activities vary little throughout 24 hours if the urine creatinine concentration of the sample is used to correct the varying rates of urine flow. Thus, random samples of urine may be used for enzyme assay. Increased urinary NAG appears to be dependent both upon the activity of the disease process and the functioning renal cell mass. Since the renal cell mass decreases in older individuals and there is lower excretion of creatinine, an increased excretion of NAG occurs in individuals over 70 years of age.

NAG, along with other urinary enzymes, has been used to evaluate drug induced tubular damage as in the case of acetaminophen [113], 5-aminosalicyate/sulfasalazine in patients being treated for inflammatory bowel disease [114], and the relative nephrotoxicity of differing aminoglycoside dose schedules in neonates [115]. Assess of the urinary excretion of NAG have also been reported in hypertensive patients [116] and in patients with chronic renal failure due to various causes [117]. However, to date, it is considered to be an ancillary but non-definitive marker of renal disease.

C. Alanine aminopeptidase is restricted to the proximal tubule [118-120]. It shares with NAG great popularity as a measure of tubular injury. Increased excretion of NAG and alanine aminopeptidase has been reported in a variety of renal diseases including: pyelonephritis, glomerulonephritis, urologic cancers and renal transplant rejection. In addition, increased excretion has been reported in association with many well-defined nephrotoxins, i.e., exposure to cadmium, mercury, lead, cisplatin, aminoglycosides, cyclosporine, tacrolimus (FK-506), non-steroidal anti- inflammatory drugs, radiocontrast media in both clinical [40, 79, 121-129] and experimental situations [130-136]. Thus, the experience with N-acetyl-β-D-glucosaminidase and alanine aminopeptidase indicates that while neither is specific with regard to discriminating between glomerular and tubular disease, they are very sensitive to acute tubular injury in which either the offending agent is known or the exposure incident is well characterized [137].

D. Intestinal alkaline phosphatase and human tissue non-specific **tissue alkaline phosphatase** are two urinary isoenzymes that have elicited interest as potential segment specific markers of the human nephron [138]. Both are members of the closely related group of alkaline phosphatases. Intestinal alkaline phosphatase is the intestinal isoenzyme that is localized on the brush border of human intestinal epithelial cells. It is also present in normal human kidney, where it is exclusively expressed on the brush border of tubuloepithelial cells of the S3-segment of the proximal tubule. The intestinal alkaline phosphatase, which is released in urine, has its origin in the kidney. As a result, intestinal alkaline phosphatase is considered to be a specific and sensitive marker for alterations of the S3-segment of the human proximal tubule. Tissue alkaline phosphatase, in contrast, is localized on the membrane of liver cells, osteoblasts, and fibroblasts, and on the brush border all along the different segments of the proximal tubule. By measuring both enzymes, judgments as to the involvement of S1-S2 versus S3 segments can be achieved during either occupational screening [139] or when conducting clinical pharmacology studies [140]. Their usefulness as markers has been enhanced because specific monoclonal antibodies have been developed against each and because spot urine collections using appropriate preservative will remain stable for up to five months [138]. The two alkaline phosphatase isoenzymes have been validated as independent markers of proximal tubular cell alterations in over twenty occupationally exposed cohorts and clinical groups [141].

E. Glutathione-S-transferases (GST) are cytosolic enzymes. Four GST isoenzyme classes have been identified – alpha, mu, pi and theta [142]. Alpha GST isoform is localized to the human proximal tubular cells. The pi GST isoform is localized in the human distal convoluted tubules, the thin limb of the loop of Henle, and the collecting ducts. An increased urinary excretion of alpha GST correlates with brush border damage and decreased staining of proximal tubules for that isoenzyme [143]. It appears that radioimmunologic or immunochemical quantitation of alpha and pi forms of the enzyme can be used as sensitive and relatively simple markers for early detection of toxic effects with respect to the renal tubule [144]. The measurement of urinary glutathione S-tranferases has been used as a parameter to predict early graft function after transplantation [145], to raise suspicion of graft rejection in patients with delayed graft function [146], and to dis-

tinguish between cyclosporine A-induced nephrotoxicity and acute rejection [147].

Emerging biomarkers

With the rapid increase in understanding of the mechanisms of cell injury and repair, a number of new substances have been identified that may prove to be useful markers of acute injury or disease activity. These include various cytokines and growth factors, several lipid mediators, a complex array of extracellular matrix components and cell adhesion molecules, plus a variety of miscellaneous compounds. At the present time, the clinical utility of their measurement in biologic samples is unknown, although in selected instances, clinical correlates have emerged. Unfortunately, not all of these markers are present in urine or blood samples. For some, detection involves histologic or histochemical techniques applied to renal tissue samples. Nonetheless, the substances discussed below are intimately involved in the control and modification of cell function, the response to stress and/or the processes of repair. It is anticipated that with proper amplification, one or more may be useful as a marker of susceptibility, exposure or effect.

Cytokines

Cytokines are polypeptides that regulate a number of important biologic functions. They act as systemic mediators of inflammatory and immune responses, are closely involved in tissue repair, and under certain circumstances promote tissue destruction and fibrosis. The cytokines include, among others, the interleukins and interferons, tumor necrosis factor, colony-stimulating growth factors and various other growth factors (Table 9). It is now appreciated that among the mechanisms responsible for glomerular and tubulointerstitial disease, cytokines play a prominent role.

A. Interferons are a group of cytokines that include interferon-α (IFN-α), interferon-β (IFN-β) and interferon-γ (IFN-γ). The interferons are naturally protective substances. For example, IFN-α and IFN-β are produced in response to viral infection and inhibit viral replication plus assist the induction of viral resistance. Not only do they possess antiviral activity but they also mediate the response to other infectious agents, demonstrate antitumor activity, and play a role in the regulation of growth, differentiation and development [148]. In contrast, IFN-γ has more potent immunoregulatory effects than either IFN-α or -β. Among its properties, IFN-γ is capable of activating human macrophage oxidative metabolism and microbicidal activity.

B. Interleukins are produced by a variety of cells including lymphocytes and monocytes. They modulate inflammatory and immune responses by regulating the growth, differentiation and mobility of effector cells. **Interleukin-l** (IL-l), a pro-inflammatory cytokine, is associated with the systemic acute phase response producing both fever and neutrophilia. Locally, it mediates tissue injury and remodeling. In the kidney, the synthesis and release of IL-l may contribute to progressive glomerular injury due to its action on mesangial cells. IL-l has the capacity to stimulate matrix production by glomerular epithelial cells and induce proliferation by glomerular mesangial and endothelial cells. **Interleukin-6** (IL-6) is a glycoprotein

Table 9. Various cytokines.

Interleukins
Interleukin-1
Interleukin-2
Interleukin-4
Interleukin-5
Interleukin-6
Interleukin-8
Colony-stimulating factors
Interleukin-3
Granulocyte-Macrophage-CSF
Macrophage-CSF
Granulocyte-CSF
Interferons
Interferon-α
Interferon-β
Interferon-γ
Growth factors
Epidermal Growth factor
Insulin-like growth factor
Transforming growth factor β
Platelet-derived growth factor
Tumor necrosis factor
TNF-α
TNF-β

[149] that also is involved in regulating mesangial cell proliferation [150]. IL-6 can be measured in the urine where its presence is a reflection of local production by either glomerular mesangial cells or by cells that have infiltrated the glomeruli. In patients with mesangial proliferative glomerulonephritis [151, 152] IL-6 can be detected in the urine, presumably as a result of its production by mesangial cells. Curiously, it is not found in the urine from patients with other types of glomerulonephritis. In the patients with mesangial proliferative glomerulonephritis a relationship exists between the urinary levels of IL-6 and the level of disease activity and it has been suggested that the urinary IL-6 levels may be a useful marker for detecting progressive injury [152]. Recently, IL-6 has been reported to be elevated in the serum of with reflux nephropathy [153] for which a pathogenic role was proposed. **Interleukin-8** (IL-8) is a potent neutrophil and lymphocyte chemotactic cytokine and is the most widely studied member of the so-called intercrine superfamily of cytokines [154]. Urinary levels of immunoreactive IL-8 may be elevated with various glomerular diseases. The glomerular production of IL-8 promotes the infiltration of leukocytes - particularly neutrophils - into glomeruli where they contribute to progressive renal injury [155]. As can be appreciated, assays for interleukins are expected to become useful for evaluating renal damage and monitoring disease activity. In addition, elevated levels of IL-8 have been reported in patients undergoing cardiac bypass surgery [156]. These same authors found elevated excrete of several anti-inflammatory cytokines, IL-10, IL-1ra, and TNFsf2, and raised the possibility that they were derived from the kidney as a auto-protective mechanism to limit the extent of renal damage secondary to the filtration of the inflammatory cytokines.

C. Tumor necrosis factor α is not detected under normal physiological conditions. Although first identified for its anticancer activity, in addition to neoplastic cells., TNF_{α} is produced in response to tissue invasion by bacteria, viruses, fungi or parasitic agents. Various cells, including glomerular mesangial cells, synthesize Tumor Necrosis Factor [157]. In the mesangial cells, tumor necrosis factor may stimulate the synthesis of various prostaglandins along with platelet activating factor. It also induces cyclic AMP and cyclic GMP accumulation, promotes the generation of reactive oxygen metabolites, upregulates the expression of intercellular adhesion molecule-l (ICAM-l) and may have either a stimulatory or inhibitory effect on mesangial cell proliferation [158]. Some of these products, including oxygen radicals [159] and various cytokines [160] may be injurious to the mesangial cells themselves.

The stimulation of mesangial cells to release and respond to tumor necrosis factor may accelerate the glomerular infiltration of polymorphonuclear leukocytes and monocytes. Indeed, the injection of tumor necrosis factor enhances glomerular damage in some forms of experimental glomerulonephritis [161]. Another important target is the vascular endothelium where an increase in the local production of tumor necrosis factor-α may result in the formation of capillary thrombi. An increase in plasma and urinary levels of two soluble tumor necrosis factor receptors has be found in patients with chronic renal failure [162].

D. Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that plays an important role in the recruitment of monocytes/macrophages into renal tubulointerstitium [163, 164]. It is known to be produced mainly by tubular epithelial cells in kidney [165], and to contribute to renal interstitial inflammation and fibrosis [166]. Furthermore, protein overload in renal tubular cells is shown to up-regulate MCP-1 gene and its protein [167, 168]. These lines of evidence collectively suggest that increased urinary protein excretion probably aggravates renal tubular damage by enhancing MCP-1 expression in tubular cells. Protein overload in renal tubular cells has been found to up-regulate MCP-1 gene and its protein [13, 14]. It has been suggested that MCP-1 expression in renal tubuli is enhanced in proteinuric states, irrespective of the types of renal disease, and that increased MCP-1 expression probably contributes to renal tubular damage in proteinuric states [169].

Growth factors

Several growth factors have been isolated from kidney tissue. They include, among others, epidermal growth factor, insulin-like growth factors, transforming growth factors, and platelet-derived growth factor.

A. Epidermal growth factor is a polypeptide that stimulates the proliferation and differentiation of epidermal and epithelial cells and is a potent mitogen. In

the kidney, cells of the distal convoluted tubules and the thick ascending loop of Henle produce a precursor molecule, pre-pro-epidermal growth factor. Binding sites for epidermal growth factor are present in various locations of the renal tubules and the glomeruli [170]. Epidermal growth factor is thought to be important both for the maintenance of renal tubule integrity and for the regenerative response of tubular epithelial cells to injury [171]. There is little evidence to suggest that epidermal growth factor plays a role in compensatory hypertrophy. In experimental models, changes in the processing of pre-pro-epidermal growth factor, and in epidermal growth factor receptor density have been found along with altered expression and distribution of epidermal growth factor [172, 173]. In humans, urinary epidermal growth factor concentrations range from 50 to 70 ng/mg creatinine [174] and are higher in females than in males [175]. Epidermal growth factor levels may be reduced in the urine of patients with chronic renal failure [176].

B. Insulin-like growth factors (insulin-like growth factor-I and insulin-like growth factor-II) were originally called somatomedins because of their role in mediating the action of growth hormone. Growth hormone stimulates the synthesis and release of insulin-like growth factors that then exert negative feedback on growth hormone secretion. Insulin-like growth factors are also produced in a growth hormone-independent fashion, acting as local growth factors. In the kidney, insulin-like growth factors-I is synthesized in the glomerular mesangial cells and in the cortical and medullary collecting duct cells. They are bound to high-affinity carrier proteins and interact with distinct cell surface receptors in glomeruli and in all nephron segments. Insulin-like growth factor-I is a proinsulin-like peptide that exerts a variety of actions in the kidney whereas the physiologic role of insulin-like growth factor-II remains poorly understood. Although the infusion of insulin-like growth factor-I increases the glomerular filtration rate and renal plasma flow in humans with normal renal function, the primary physiologic role of the insulin-like growth factors appears to be the modulation of cell division and growth. In the fetal kidney, locally produced insulin-like growth factors-I may be important in organogenesis [177]. Insulin-like growth factor-I may also influence the regeneration of tubular epithelial cells after ischemic injury [178]. However, it may also be involved in mesangial proliferation and the expansion of the extracellular collagenous matrix, forerunners of glomerular sclerosis. Finally, insulin-like growth factors-1 may play an important role in the late but not the initiating phase of compensatory hypertrophy and the accelerated renal growth in early diabetes mellitus.

C. Transforming growth factor β is a family of growth peptides that are intimately involved in extracellular matrix formation, cellular proliferation and differentiation. It is present in both the renal cortex and medulla. It is a prototypical cytokine that plays a central role in regulating tissue repair and remodeling after cell injury. Transforming growth factor-β is unique among the cytokines in that it has a direct effect on the synthesis of extracellular matrix components such as collagen and fibronectin, is capable of inhibiting matrix protein degradation by proteases, and modulates matrix receptors to increase adhesion of cells to matrix. Indeed, the regulation of extracellular matrix homeostasis is thought to represent a major part of its action [179]. Transforming growth factor-β also controls the interaction of cells with the extracellular matrix by regulating the expression of the integrin family of cell adhesion receptors. Transforming growth factor-β stimulates cell proliferation and differentiation and is involved in the regenerative activity that follows ischemic injury. It may be a mediator of the renal fibrosis that occurs in response to the administration of antiglomerular basement membrane IgG; has been implicated in the pathogenesis of glomerulonephritis; and it may contribute to the development of progressive kidney fibrosis.

D. Platelet-derived growth factor: platelet-derived growth factor is disulphide-bonded dimer composed of an A and B chain. It exists in three forms (platelet-derived growth factor-AB, platelet-derived growth factor-AA or platelet-derived growth factor-BB), each with different functional properties. The platelet-derived growth factor family plays a role in cell growth and differentiation, is involved in tissue repair processes and is one of the growth factors whose unregulated activity may be involved in the progression of renal damage toward sclerosis. It stimulates chemotaxis, influences the production of extracellular matrix and regulates its subsequent metabolism. Platelet-derived growth factor produced by mesangial cells or inflammatory cells may contribute to the development of glomerulonephritis through autocrine or paracrine

mechanisms [180]. Platelet-derived growth factor expression has been studied in renal biopsy samples from patients with mesangial proliferative glomerulonephritis and found to be elevated when compared to normals or those with nonproliferative forms of glomerulonephritis [181]. Platelet-derived growth factor stimulates the production of transforming growth factors-β by mesangial cells [182]. Also, an increase expression of platelet-derived growth factor receptors has been found at the mesangial level in patients with various forms of mesangial proliferative glomerulonephritis [183].

CCN gene family

The CCN gene family encodes multifunctional, extracellular matrix-associated signaling proteins that regulate cell adhesion, migration, proliferation, survival, and differentiation [184]. Six members of this gene family are known and include *Cyr61* (cysteine-rich angiogenic protein 61). *Cyr61* supports the adhesion of fibroblasts, endothelial cells, epithelial cells, blood platelets, and other cell types and is able to synergize with other mitogenic growth factors to enhance growth factor-induced DNA synthesis [185]. The rapid induced *Cyr61* has been reported in proximal straight tubules following renal ischemia in both rats and mice. Because it is excreted in the urine, it has been suggested that it might serve as an early biomarker of renal injury [186].

Lipid mediators

The eicosanoids are locally active hormones (autocoids) that are derived from precursor polyunsaturated fatty acids. The rate-limiting step in the synthesis of eicosanoids is the phospholipase-regulated release of arachidonic acid from membranes. Arachidonic acid metabolism may follow one of three possible pathways. In the first, the cyclooxygenase- peroxidase pathway leads to the formation of the prostenoids - prostaglandins and thromboxanes. In the second, the lipoxygenase pathway yields the leukotrienes and lipoxins. A third pathway, the cytochrome P-450 mono oxygenase pathway is also involved in the metabolism of arachidonic acid.

Cyclooxygenase pathway - prostanoids

Prostaglandins and thromboxanes have a number of important functions that can be of considerable importance in determining the acute or chronic response to injury. Among their actions, prostaglandins and thromboxanes either enhance or inhibit inflammation depending on the specific mediator and its local concentration. A ratio of the local synthesis of thromboxane A2 (TXA2), a potent vasoconstrictor, and prostacyclin (PGI2), a potent vasodilator, have been shown to have a major impact on the hemodynamic changes observed with certain models of acute renal failure. In addition, chronically diseased kidneys produce increased quantities of TXA2 and often reduced quantities of PGI2.

Arachidonic acid metabolites can induce the production of tumor necrosis factor-α and may directly participate in the stimulation of gene expression. On the one hand, TXA2 stimulates mRNA for the genes encoding type IV collagen, laminin and fibronectin; on the other hand, PGI2 appears to suppress expression of type IV collagen, laminin and fibronectin [187]. As can be appreciated, stimulation of extracellular matrix proteins by TXA2 may contribute to the progression of renal disease. Other potentially deleterious effects include constriction of glomerular afferent and efferent arterioles, intraglomerular platelet aggregation, and contraction of glomerular mesangial cells [188].

Glomerular production of TXA2 is increased in a number of models of renal injury. In addition to its vascular effects, TXA2 is a potent inducer of platelet aggregation, with the consequent release of vasoactive and growth-promoting factors. The vasodilatory prostaglandins (such as PGE2 and PGI2 serve as homeostatic mechanisms to oppose the effects of TXA2. They maintain blood flow and glomerular filtration in the setting of decreased renal perfusion or chronic kidney disease.

Lipoxygenase pathway

A. Leukotrienes: The oxygenation of arachidonic acid by 5-lipoxygenase enzymes, produce leukotriene A4 (LTA4) with subsequent production of leukotrienes B4, C4, D4, and E4, all of which have important biological effects on the kidney. They are mediators of both acute inflammation and the slow reacting substances important in hypersensitivity. The effects of LTB4 tend to be pro-inflammatory and immunomodulatory even

though the peptidoleukotrienes, formerly known as the slow reacting substances of anaphylaxis, have effects that are primarily vascular and hemodynamic. Lipoxygenase products appear to be involved in the recruitment, as well as the attachment, of inflammatory cells to the glomerulus, thereby providing a potentially major stimulus for the perpetuation of injury. For example, LTB4 is a potent chemotactic agent that promotes adhesion of leukocytes to endothelial cells. LTB4 also induces aggregation of polymorphonuclear cells, stimulates the generation and release of reactive oxygen intermediates, and enhances the production of cytokines such as IL-l, IL-2, and IFN-γ. LTB4 is devoid of constrictor action in the normal rat kidney. However, the leukotrienes, LTC4 and LTD4, are potent renal vasoconstrictors and can increase the permeability of postcapillary venules. LTD4 exerts preferential constrictor effects on postglomerular arteriolar resistance. In addition, LTC4 and LTD4 leukotrienes stimulate contraction of glomerular mesangial cells. They may be important mediators of inflammation in glomerulonephritis. LTB4 and other products of the lipoxygenase pathway have been shown to modulate glomerular filtration, arteriolar resistance, and mesangial contractility.

B. Lipoxins: A combination of actions by 15- 12- and 5-lipoxygenase enzymes on arachidonic acid leads to the generation of the lipoxins, LXA4 and LXB4. LXA4 dilates afferent arterioles without affecting efferent arteriolar tone. This response may be mediated by the secondary release of cyclooxygenase products such as PGE_2 and PGI_2. LXB4 possess vasoconstrictor actions in the rat kidney.

P-450 Mono oxygenase pathway

P-450 arachidonic acid-derived metabolites have been identified in human urine [189]. The renal cytochrome P-450 system is involved in catalyzing the enzymatic transformation of arachidonic acid. This reaction can involve either an epoxygenase system yielding various epoxides or a mono oxygenase system yielding other oxidation products. Cells of the proximal tubule, thick ascending limb of Henle and the collecting duct contain the major amount of cytochrome P-450 enzyme in the kidney.

Platelet activating factor

Platelet activating factor is a family of phosphocholines produced by inflammatory cells, platelets, endothelial cells, isolated glomeruli, mesangial cells, and renal medullary cells. Platelet activating factor's biologic effects include platelet aggregation and activation, chemotaxis and chemokinesis, polymorphonuclear leukocyte aggregation and degranulation, stimulation of oxygen-free radical formation, smooth muscle contraction, and increased vascular permeability. Due to the concomitant cytosolic release of arachidonic acid that accompanies platelet activating factor biosynthesis, its formation has been linked to the generation of the biologically active products resulting from the activation of the cyclooxygenase and lipoxygenase pathways.

Extracellular matrix components and cell adhesion molecules

Extracellular matrix components

In glomeruli, the extracellular matrix is of two types. In the glomerular basement membrane the major components are type IV collagen, heparin sulphate proteoglycans, laminin, nidogen, entactin, and fibronectin. In mesangial cells, type V collagen and chondroitin sulphate proteoglycans predominant.

A. Collagens are classified as fibril-forming collagens (types I, II, III, V and XI), non-fibril-forming collagens that form networks (type IV and X), and microfibrils (type VI collagen). Type IV collagen is the major component of the glomerular basement membrane where it contributes structural support, provides a matrix for cell adhesion and is integral to the permselectivity properties of the glomerulus. Type IV collagen has the ability to bind to other matrix proteins, such as laminin, proteoglycan, and nidogen. It has been measured in human urine [190] where it is released as part of its turnover. It has been found to be significantly elevated in cases of diabetic nephropathy, membranous nephropathy and rapidly progressive glomerulonephritis. Presumably, the increase in urinary type IV collagen excretion reflects increased glomerular basement membrane degradation by inflammatory cells. It appears that the assay of collagen IV is a way of investigating fibrotic changes to the glomerulus.

Type V collagen along with Type VI collagen has been found in the mesangium, glomerular basement membrane, and renal interstitium. Type VI collagen is a ubiquitous protein present in many types of extra-

cellular matrices. It is a major component of microfibrils and possesses distinct cell binding properties. Type VI collagen may be a requirement to provide an appropriate extracellular environment for cultured fibroblasts to arrest proliferation. An increased deposition of type VI collagen has been found in the late stages of nodular glomerulosclerosis [191].

B. Glycoproteins are basic constituents of normal renal tissue and are structurally and functionally active components of basement membranes.

Laminins are the major glycoprotein, with distinct cell binding properties that account for the ability to bind to type IV collagen. Indeed, laminins are capable of many interactions with cell surface receptors. The major functions of laminin take advantage of the ability to interact with cells and affect cell behavior. This may involve increasing cell adhesion, adjusting cell polarization, or allowing cell spreading, migration, and differentiation. Breakdown products of laminin are present in serum as a result of both the normal an abnormal turnover of basement membranes. It has been suggested that these fragments have the potential for serving as a marker for the progression of renal disease [192]. It is also the case that changes in the glycoconjugates of renal tubules accompany tubular damage.

Fibronectin is a glycoprotein found on the surface of many cells where it plays an important role in organizing the extracellular matrix. It is present in many basement membranes and is especially abundant in the mesangium and Bowman's capsule matrices. It is capable of interacting with proteoglycans, collagens of extracellular matrices, and various cell surface receptors. Fibronectin may mediate cell adhesion to collagen. In rats with either immune or toxic glomerular diseases, increased plasma fibronectin levels may occur in the early phases prior to the onset of proteinuria and before the development of widespread glomerular lesions [193].

A ubiquitous laminin-associated glycoprotein is *entactin* (also known as nidogen), a glycoprotein now thought to provide linkage between laminin and type IV collagen in basement membranes.

C. Proteoglycans can be found in all basement membranes. Proteoglycans with three-heparin sulphate attached to the core protein perlecan predominate. A decrease in the glomerular content of heparin sulphate proteoglycans occurs in a number of renal diseases. This results in a loss of the negative charge of the basement membrane and is viewed as one of the key factors responsible for high-molecular weight proteinuria. The chondroitin/dermatan-sulphate proteoglycans, such as biglycan and decorin, are quite distinct from heparin sulphate proteoglycans. Proteoglycans are able to bind to several growth factors such as transforming growth factors-β. Binding of transforming growth factors-β to the extracellular matrix proteoglycans decorin and biglycan serves to neutralize the growth-stimulatory effects of transforming growth factors-β. In this way, these proteoglycans act as negative feedback regulators in the process of extracellular matrix formation [194].

Cell adhesion molecules

Cell adhesion molecules (CAMs) are transmembrane glycoproteins that act at the cell surface to mediate specific binding interactions with other cell adhesion molecules on adjacent cells or with proteins in the extracellular matrix. They are responsible for the adhesion of various leukocytes with each other, with extracellular matrix and with other cell types. There are four families of CAMs that facilitate these interactions. The classification is based on differences in structure and includes selectins, cadherins, integrins, and immunoglobulin superfamily (Table 9). CAMs play a role in renal morphogenesis and are expressed in the adult human kidney. These molecules can influence growth factor expression, and conversely growth factors can modulate production of cell surface adhesion molecules and the expression of extracellular matrix proteins.

A. Selectins are a family of so-called homing receptors that include P-selectin (platelet selectin), E-selectin (endothelial cell selectin), and L-selectin (leukocyte selectin). They are involved in the homing of lymphocytes to lymph nodes. Selectins support leukocyte-endothelial cell and leukocyte-platelet adhesion and mediate the migration of neutrophil granulocytes in developing inflammatory reactions. Selectins bind at least three broad categories of natural or synthetic carbohydrates [195].

B. Cadherins constitute a family of cell-surface glycoproteins that mediate cell adhesion in a Ca^{2+}-dependent manner. They tend to concentrate at cell junctions and require interaction with cytoplasmic-based protein to function. Three subclasses have been described: E-cadherins, N-cadherins, and P-cadherins. Maintenance of cell polarity, a critical function that allows for

vectorial tubular transport in the kidney, appears to be inducible by E-cadherins.

C. Integrins are a family of large integral transmembrane glycoproteins, involved in the adhesive interactions of cells [196]. They consist of two subunits, an α and β chain. Each subunit is a transmembrane protein with a large extracellular domain and a small cytoplasmic domain. The variability in available α and β chains allows for a large family of integrins and provides cells with the ability to recognize a variety of adhesive substrates. They appear to be the primary mediators of cell adhesion to extracellular matrix adhesion and basement membranes and contribute to cell-cell adhesion [197]. They are thought to link the cytoskeleton of one cell with that of another or with the extracellular matrix. Integrins associate with cytoskeletal proteins viatalin, vinculin, and probably other cytoskeletal proteins [198]. β1 integrins are found on mesangial cells where they appear to be the principle mediators of cell-extracellular matrix adhesion, with fibronectin, laminin and collagens as their major ligands. They are known as the very late activation antigen proteins. β2 integrins are involved in leukocyte cell-cell adhesion. The very late activation antigen-4 β1 integrin (very late activation antigen-4) and the CD11-CD18 β-2 integrins are important in leukocyte-endothelial adhesion. Adhesion molecules may play an important role in reperfusion injury of the kidney [199, 200].

D. Immunoglobulin superfamily of cell adhesion molecules are large plasma-membrane glycoproteins, which function primarily in cell-cell adhesion in a Ca^{2+}-independent manner. They include among others, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These two members of the immunoglobulin supergene family play an important role in a variety of inflammatory and immune- mediated mechanisms, mediating both cell migration and activation. ICAM-1 is a glycoprotein expressed on endothelial cells of larger vessels, glomeruli and peritubular capillaries, epithelial, fibroblast and leukocyte cells. ICAM-2 is a glycoprotein expressed by endothelial cells, lymphocytes and some other leukocytes. VCAM-1 is a glycoprotein widely distributed on endothelial, epithelial, macrophages and dendritic cells. It supports the adhesion of eosinophils, basophiles, monocytes and lymphocytes. ICAM-1 and VCAM-1 appear to be particularly important for the firm attachment and transendothelial migration of leukocytes through their interactions with lymphocytes via the leukocyte integrins CD11a/CD18 1-2 (LFA-1) and very late activation antigen-4, respectively.

Recent reports have shown the presence of soluble forms of ICAM-1 and VCAM-1 in human sera and have demonstrated increased levels of these soluble markers in patients with inflammatory diseases as well as with other immunologic mediated disorders. Changes in ICAM-1 expression have been reported in glomerulonephritis, tubulointerstitial inflammation, and renal allograft rejection. Circulating levels of ICAM-1 are elevated in some forms of glomerulonephritis [201]. Expression of VCAM-1 has been observed on proximal tubule cells in patients with vasculitis and crescentic nephritis, lupus nephritis, IgA nephropathy, and acute interstitial nephritis induced by non-steroidal anti-inflammatory drugs [202]. The neural cell adhesion molecules (N-CAM) and the leukocyte-function-associated antigens also belong to this family.

E. Kidney injury molecule 1 (KIM-1) is a member of the immunoglobulin gene superfamily. KIM-1

Table 9. Cell adhesion molecules.

Selectins
P-selectin
E-selectin
L-selectin
Cadherins
E-cadherins
N-cadherins
P-cadherins
Integrins
β1-integrins
α4-β1 (VLA-4)
β2-integrins
CD11a/CD18
CD11b/CD18
CD11c/CD18
Immunoglobulin-like molecules
Intercellular adhesion molecule-1 (ICAM-1)
Intercellular adhesion molecule-2 (ICAM-2)
Intercellular adhesion molecule-3 (ICAM-3)
Vascular cell adhesion molecule-1 (VCAM-1)
CD2, CD3, CD4, CD8
Major histocompatibility complex classes 1 & 2
Neural cell adhesion molecule-1 (NCAM-1)
Carcinoembryonic antigen
Kidney injury molecule 1 (KIM-1

mRNA and protein are expressed at a low level in normal kidney but are increased dramatically in the kidney following ischemic injury. It is expressed in proliferating and dedifferentiated epithelial cells in regenerating proximal tubules. KIM-1 is an epithelial cell adhesion molecule up regulated in the cells, which are dedifferentiated and undergoing replication. It has been suggested that KIM-1 may play an important role in the restoration of the morphological integrity and function following renal ischemic injury [203]. In patients with acute tubular necrosis, the amount of soluble released form of KIM-1 is markedly increased. Patients with milder degrees of tubular injury related to contrast nephropathy and interstitial nephritis, also had elevated urinary levels of immunoreactive KIM-1 [204].

Miscellaneous biomarkers

Tubule antigens

Tissue constituents (including enzymes) of the kidney and urogenital tract are physiologically shed into the urine as a consequence of cell turnover and metabolism. When they are detected by immunochemical methods, they are referred to as antigens. It has been demonstrated that urinary excretion of specific proximal tubular antigens is increased in a variety of experimentally induced renal diseases. By raising monoclonal antibodies to membrane and other cellular derived antigens a new sensitive, specific, and readily available biomarker of renal cell injury is possible. For example, increased excretion of tubular antigens occurs in a variety of nephropathies including those due to exposure to cadmium, hydrocarbons, cisplatin, and radiographic contrast- media [102]. Monoclonal antibodies to human brush-border antigens (BBA, BB-50) have been produced [205]. The advantage of tubular antigen determination is the possibility of detecting site-specific renal damage. As described above, monoclonal antibodies have been produced that react with *intestinal alkaline phosphatase* which is specific for the S3 segment of the proximal tubule. A monoclonal antibody-based assay for *adenosine deaminase binding protein,* a proximal tubular antigen present on the brush border of proximal tubular epithelial cells, has been described. Adenosine deaminase-binding protein release appears to result from acute tubular injury, with the level rising before an increase occurs in the Scr. Conversely, adenosine deaminase-binding protein levels return towards normal with cessation of proximal tubular injury, often before the rise in the Scr is reversed. *Villin* is a cytoskeletal protein of brush borders. It is linked to actin and stabilizes the actin filaments that anchor the individual microvilli. In conditions of high Ca^{2+} concentrations, which are present during cellular damage such as hypoxia or direct toxic agents, villin acts as an F-actin severing protein. This causes the release of brush-border bundles that are shed into the lumen of the tubule and excreted with urine. The appearance of villin in the urine is therefore an indicator of renal proximal tubular damage [206].

Table 10. Human conditions with elevated urinary endothelin levels.

IgA nephropathy
Membranous proliferative glomerulonephritis
Focal glomerulosclerosis
Lupus nephritis
End stage renal disease
Acute hemolytic uremic syndrome
Subarachnoid hemorrhage
Normal pregnancy
Cisplatin nephropathy
Cyclosporine nephrotoxicity

Endothelins

are a family of locally generated peptides that possess a number of biological functions. They are potent, if not the most potent renal vasoconstrictors [207] and stimulate vascular smooth muscle cell and mesangial cell proliferation [208]. Four endothelins (ET-1, ET-2, ET-3 and ET-β) have been identified along with at least two ET receptor subtypes: the ETA and ETB receptors. The predominant isotype in humans is ET-1 ("classical" endothelin). Endothelial cells appear to be the primary source of ET-1 found circulating in plasma while glomerular ET is thought to arise mostly from the glomerular endothelium and from mesangial cells themselves. Endothelin release may be initiated by thrombin or endothelial cell damage. Endothelin induces mesangial cells to contract thus contributing to glomerular hypertension and cause expression of genes in mesangial cells so that matrix proteins are produced. Endothelins often act via the intermediary of thromboxane biosynthesis, and they release platelet-derived

growth factors. In rats following subtotal nephrectomy, a significant correlation exists between urinary ET-1 levels and the percent of the remaining glomeruli affected by sclerosis.

It has been shown that subjects with renal diseases such as IgA nephropathy, membranous proliferative glomerulonephritis, focal sclerosis, and lupus nephritis have levels of endothelin that are significantly higher than those in healthy subjects [209]. Increased circulating ET-1 concentrations and urinary excretion of ET-1 have been observed in patients treated with the nephrotoxic immunosuppressive agents cyclosporine A and tacrolimus (FK-506) [210]. Other nephrotoxic agents, such as cisplatin, also increase urinary excretion of ET [211]. In patients with chronic renal disease, urinary excretion of ET-1 is significantly elevated when compared to normal values (Table 10).

Clusterin

Clusterin is a dimeric glycoprotein that has been isolated from several tissues including the kidney and is reported to be induced during renal and other tissue damage [212]. Clusterin production by the kidney has been associated with a number of physiologic functions, including regulation of complement, reproduction, cell aggregation and programmed cell death or apoptosis. In the kidney, clusterin is a component of immune deposits and its expression is increased after ischemia or obstruction. In gentamicin-treated rats, an increase in urinary clusterin protein may provide an early sign of nephrotoxicity [213]. An increase in urinary excretion may persist after other markers of nephrotoxicity – such as urinary NAG – have fallen [214].

Heat shock proteins

Exposure of cells to a variety of stresses induces a modification of cell metabolism called the heat shock or stress response [215], which is accompanied by the rapid synthesis of the so-called heat-shock proteins (HSPs). HSPs are considered to have essential protective function in cells. They are classified according to their function or apparent molecular weight into four families: the HSP90 family, the HSP70 family, the HSP60 family and other low-molecular weight HSPs. Events such as progression through the cell cycle and differentiation or environmental stresses such as heat, oxidative injury, heavy metals, inhibitors of energy metabolism, or pathological conditions such as inflammation, all result in the expression of HSPs which are considered to have essential protective functions in cells [216].

Antibody detection

A. Antiglomerular basement membrane antibodies: Some xenobiotics may induce an immune-type glomerulonephritis associated with the occurrence of antibodies against some constituents of the glomerular basement membrane. These antibodies may be directly responsible for the nephropathy or they may be produced as a consequence of non- specific alterations of the normal glomerular basement membrane lattice.

B. Antineutrophil cytoplasmic antibodies (ANCA) are a class of autoantibodies with varied specificities against myeloid-specific lysosomal enzymes. Indirect immunofluorescence microscopy using alcohol-fixed neutrophils demonstrates one of two types of ANCA patterns: one causing cytoplasmic staining (C-ANCA) frequently reacting with proteinase 3 (PR-3) of the α granules, and a second causing perinuclear staining (P-ANCA) that frequently has specificity for myeloperoxidase. ANCA are associated with necrotizing granulomatosis and with pauci-immune necrotizing vasculitis involving many tissues and are useful for the diagnosis of Wegener's granulomatosis, microscopic polyarteritis, Churg-Strauss syndrome, systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis.

Uronic acid

The measurement of urinary uronic acid has been used as an early indicator in the development of renal papillary necrosis in rats given multiple doses of N-phenylanthranilic acid or mefenamic acid [217]. A significant elevation of uronic acid in urine occurred was well ahead of the development of histological evidence of renal papillary necrosis. The biochemical basis of these changes appears to be related to acid mucopolysaccharides accumulation [218].

References

1. National Research Council. Commission on Life Sciences Board on Environmental Studies and Toxicology. Committee on Biological Markers, Subcommittee on Biologic Markers in Urinary Toxicology. In: Biologic Markers in Urinary Toxicology. National Academy Press, Washington DC 1995; p. 16-21.
2. Bennett WM, Elzinga LW, Porter GA. Tubulointerstitial disease and toxic nephropathy. In: The Kidney. Brenner BM, Rector FC, Jr, (editors). WB Saunders, Philadelphia. London. Toronto, Montreal, Sydney, Tokyo 1991; p. 1430-1496.
3. Deistan HP. Renovascular hypertension and azotemia. N Engl J Med 1984; 311: 1114-1116.
4. Goering PL, Fowler BA. Regulation of lead inhibition of delta aminolevulinic acid dehydratase by high affinity lead-binding protein. J Pharmacol Exp Ther 1984; 231: 66-71.
5. Morgensen CE. Urinary albumin excretion in early and long term juvenile diabetes. Scand J Clin Lab Invest 1971: 281: 101-109.
6. Mueller PW, Paschal DC, Hammel RR, Klincewicz SL, McNeal ML, Spierto B, Steinberg K. Chronic renal effects in three studies of men and women occupationally exposed to cadmium. Arch Environ Contam Toxicol 1992; 23: 125-136.
7. Goyer R. Cadium Nephrotoxicity. In: Nephrotoxic mechanisms of drugs and environmental toxins. Porter GA (editor). Plenum Med Bk Co, New York, London 1982; p. 305.
8. Buchet JP, Roels H, Bernard A, Lauwerys R. Assessment of renal function of workers exposed to inorganic lead, cadmium, and mercury vapors. J Occup Med 1980; 22: 741-750.
9. Bernard AM, Moreau D, Lauwerys R. Comparison of retinal binding protein and β-2-microglobin in urine for the early detection of tubular proteinuria. Clin Chim Acta 1982; 116: 1-7.
10. Douglas JB, Healy JK. Nephrotoxicity effect of amphotericin B, including renal tubular acidosis. Am J Med 1969; 46: 154-162.
11. Bennett WM, Plamp C, Porter GA. Drug related syndromes in clinical nephrology. Ann Int Med 1977; 87: 582-590.
12. McMurray SD, Luft FC, Mazwell DR, Hamburger DH, Lleit S. Prevailing patterns and predictor variables in patients with acute tubular necrosis. Arch Intern Med 1978; 138: 950-955.
13. Cooper K, Bennett WM. Nephrotoxicity of common drugs used in clinical practice. Arch Intern Med 1987; 147: 1213-1218.
14. Perneger TV, Whelton PK, Klag MJ. Risk of kidney failure associated with the use of acetaminophen, aspirin, and nonsteroidal antiinflammatory drugs. N Engl J Med 1994; 331: 1675-1679.
15. Ransohoff DF, Feinstein AR. Problems of spectrum and bias in evaluating the efficiency of diagnostic tests. N Engl J Med 1978; 299: 926-930.
16. Lachs MS, Nchamkin I, Edelstein PH, Goldman J, Feinstein AR, Schwartz JS. Spectrum bias in the evaluation of diagnostic tests: lessons from the rapid dipstick test for urinary tract infection. Ann Intern Med 1992; 117: 135-140.
17. Jaeschke R, Guyatt GH, Sackett DL for the Evidence-Based Working Group. Users' guides to the medical literature, III: how to use an article about a diagnostic test. B: what are the results and will they help me in caring for my patients? JAMA 1994; 271: 703-707.
18. Zweig MH, Campbell G. Receiver-operating characteristic plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993; 39: 561-577.
19. Bonnardeaux A, Somerville P, Kaye M. A study on the reliability of dipstick urinalysis. Clin Nephrol 1994; 41: 167-172.
20. Voswinckel P. A marvel of colors and ingredients. The story of urine test strips. Kidney Int 1994; 46 (Suppl 47): S3-S7.
21. Janssens PMW. New markers for analyzing the cause of hematuria. Kidney Int 1994; 46 (Suppl 47): S111-S114.
22. Hotta O, Taguma Y, Yusa N and Ooyama M. Analysis of mononuclear cells in urine using flow cytometry in glomerular diseases. Kidney Int 1994; 46 (Suppl 47): S117-S121.
23. Ruffing KA, Hoppes P, Blend D, Cugino A, Jarjoura D, Whittier FC. Eosinophils in urine revisited. Clin Nephrol 1994; 41: 163-166.
24. Kaplan AA, Kohn OF. Fractional excretion of urea as a guide to renal dysfunction. Am J Nephrol 1992; 12: 49-54.
25. Brochner-Mortensen J. Routine methods and their reliability for assessment of glomerular filtration rate in adults. Danish Med Bull 1978; 25: 181-202.
26. Kastrup J, Petersen P, Bartram R, Hansen JM. The effect of trimethoprim on serum creatinine. Brit J Urol 1985; 57: 265-268.
27. Cockroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31-41.
28. Jelliffee RW. Creatinine clearance: a bedside estimate. Ann Intern Med 1973; 79: 604-605.
29. Kampmann J, Siersbaek-Nielsen K, Kristensen M, Molholm-Hansen J. Rapid evaluation of creatinine clearance. Acta Med Scand 1974; 196: 517-520.
30. Levey, AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999; 130: 461-470.
31. Shemish O, Golbetz H, Kriss JP, Myers B. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int 1985; 28: 830-838.

32. Bauer JH, Brooks CS, Burch RN. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. Am J Kidney Dis 1982; 2: 337-347.
33. Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, Levey AS. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. Am J Kidney Dis 2002; 39: 920-929.
34. Gaspari F, Perico N, Remuzzi G. Measurement of glomerular filtration rate. Kidney Int 1997; 63: S151-S154.
35. Rocco MV, Buckalew VMJr, Moore LC, Shihabi ZK: Measurement of glomerular filtration rate using nonradioactive Iohexol: comparison of two one-compartment models. Am J Nephrol 1996; 16: 138-143.
36. Maher FT, Nolan NG, Elverback LR. Comparison of simultaneous clearances of 125I-labeled sodium iothalamate and of inulin. Mayo Clin Proc 1971; 46: 690-691.
37. Gaspan F, Amuchastegui CS, Guerini E, Perico N, Mosconi L, Ruggenenti P, Remuzzi G. Plasma clearance of nonradioactive iohexol as an alternative to renal clearance of inulin for measurement of glomerular filtration rate in humans. J Am Soc Nephrol 1993; 4: 315.
38. Aurell M. Accurate and feasible measurements of glomerular filtration rate - is the iohexol clearance the answer. Nephrol Dial Transplant 1994; 9: 1222-1224.
39. Brown SCW, O'Reilly PH. Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard. J Urol 1991; 148: 675-679.
40. Bennett WM, Porter GA. Overview of clinical nephrotoxicity. In: Toxicology of the kidney. Hook JB, Goldstein RS (editors). Raven Press, New York 1993; p. 61-97.
41. Thomsen K, Schou M. Renal lithium excretion in man. Am J Physiol 1968; 215: 823-827.
42. Whiting PH. The use of lithium clearance measurements as an estimate of glomerulo-tubular function. Renal Failure 1999; 21:421-426.
43. Anastasio P, Frangiosa A, Papalia T, DeNapoli N, Capodicasa L, Loguercio C, DelVecchio BC, DeSanto NG. Renal tubular function by lithium clearance in liver cirrhosis. Sem Nephrol 2001; 21: 323-326.
44. Guder WG, Hofman W. Markers for the diagnosis and monitoring of renal tubular lesions. Clin Nephrol 1992; 38 (Suppl 1): S3-S7.
45. Peterson PA, Evrin PE, Berggard I. Differentiation of glomerular, tubular, and normal proteinuria: determinations of urinary excretion of β-2-microglobin, albumin and total protein. J Clin Invest 1969; 48:1189-1198.
46. Ivandic M, Hofmann W, Guder WG. Development and evaluation of a urine protein expert system. Clin Chem 1996, 42: 1214-1222.
47. Lun A, Ivandic M, Priem F, Filler G, Kirschstein M, Ehrich JH, Guder WG. Evaluation of pediatric nephropathies by a computerized Urine Protein Expert System (UPES). Ped Nephrol 1999; 13: 900-906.
48. Lau YK, Woo KT. SDS-PAGE is underutilized as a tool for investigating renal patients. Nephron 2002; 90: 227-229.
49. Ginsberg JM, Chang BS, Matarese RA, Garella S. Use of single voided urine samples to estimate quantitative proteinuria. N Engl J Med 1983; 309:1543-1548.
50. Schwab SJ, Christensen RL, Dougherty K, Klahr S. Quantitation of proteinuria by the use of protein to creatinine ratio in single urine sample. Arch Intern Med 1987; 147: 943-949.
51. Torng S, Rigatto C, Rush DN, Nickerson P, Jeffery JR. The urine protein to creatinine ratio (P/C) as a predictor of 24-hour urine protein excretion in renal transplant patients. Transplantation 2001; 72:1453-1456.
52. Bohle A, Mackensen-Haen S, von Gise H, Grund K-E, Wehrmann M, Batz Ch, Bogenschütz O, Schmitt H, Nagy J, Müller C, Müller G. The Consequences of Tubulo-Interstitial Changes for Renal Function in Glomerulopathies. A Morphometric and Cytological Analysis. Path Res Pract 1990; 186: 135-144.
53. Nath KA. Tubulointerstitial changes as a major determinant in the progression of renal damage. Am J Kidney Dis 1992; 1: 1-17.
54. Bernard A, Lauwerys RR. Proteinuria: changes and mechanisms in toxic nephropathies. Crit Rev Toxicol 1991; 21: 373-405.
55. Dennis V, Robinson RR. Proteinuria. In: The Kidney: physiology and pathophysiology. Seldin DW, Giebisch G (editors). River Press, New York 1985; p. 1805.
56. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The eftect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. N Engl J Med 1993; 329: 1456-1462.
57. Parving HH, Hommel E, Kamkjaer M, Gieses NJ. Effect of captopril on blood pressure and kidney function in normotensive insulin-dependent diabetics with nephropathy. Brit Med J 1989; 299: 533-536.
58. Parving HH, Mogensen CE, Jensen HA, Evrin PE. Increased urinary albumin-excretion rate in benign essential hypertension. Lancet 1974; 1: 1190-1192.
59. Gysler J, Schunack W, Jaehde U. Monitoring of chemotherapy-induced proteinuria using capillary zone electrophoresis. J Chromatography 1999; 721: 207-216.

60. Prinsen BHCMT, De Sain-Van der Velden MGM, Kaysen GA, Straver HWHC, Van Rijn HJM, Stellaard F, Berger R, Rabelink TJ. Transferrin synthesis is increased in nephrotic patients insufficiently to replace urinary losses. J Am Soc Nephrol 2001; 12: 1017-1025.
61. Tencer J, Frick IM, Oquist BW, Alm P, Rippe B. Size-selectivity of the glomerular barrier to high molecular weight proteins: upper size limitations of shunt pathways. Kidney Int 1998; 53(3): 709-715.
62. Schurek HJ, Neuman KH, Flohr, et al: the physiological and pathophysiological basis of glomerular capillarry permeability for plasma proteins and erythrocytes. Eur J Clin Chem Clin Biochem 1992; 30: 627-633.
63. Schurek HJ, Neumann KH, Flohr H, Zeh M, Stolte H. Diagnostic and prognostic significance of proteinuria selectivity index in glomerular diseases. Clin Chim Acta 2000; 297:73-83.
64. Bakoush O, Tencer J, Tapia J, Rippe B, Torffvit O. Higher urnary IgM excretion in type 2 diabetic nephropathy compared to type 1 diabetic nephropathy. Kidney Int 2002; 61: 203-208.
65. Tencer J, Bakoush O, Torffvit O. Diagnostic and prognostic significance of proteinuria selectivity index in glomerular diseases. Clin Chim Acta 2000; 297: 73-83.
66. Bazzi C, Petrini C, Rizza V, Arrigo G. Beltrame A, Pisano L, D'Amico G. Urinary excretion of IgG and α (1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. Am J Kidney Dis 2001; 38: 240-248.
67. Levinson SS. An algorithmic approach using kappa/lambda ratios to improve the diagnostic accuracy of urine protein electrophoresis and to reduce the volume required for immunoelectrophoresis. Clin Chim Acta 1997; 262: 121-130.
68. Wong WK, Wieringa GE, Stec Z, Russell J, Cooke S, Keevil BG, Lockhart S. A comparison of three procedures for the detection of Bence-Jones proteinuria. Ann Clin Biochem 1997; 34: 371-374.
69. Marshall T, Williams KM. Electophoretic analysis of Bence Jones proteinuria. Electrophoresis1999; 20: 1307-1324.
70. Chew ST, Fitzwilliam J, Indridason OS, Kovalik EC. Role of urine and serum protein electrophoresis in evaluation of nephritic-range proteinuria. Am J Kidney Dis 1999; 34:135-139.
71. Levinson SS. Urine protein electrophoresis and immunofixation electrophoresis supplement one another in characterizing proteinuria. Ann Clin Lab Sci 2000; 30: 79-84.
72. Corso A, Serricchio G, Zappasodi P, Klersy C, Bosoni T, Moratti R, Castagnola C, Lazzarino M, Pagnucco G, Bernasconi C. Assessment of renal function in patients with multiple myeloma, the role of urinary proteins. Ann Hematol 1999; 78: 371-375.
73. Bernard AC, Buchet JP, Roels H, Lauwerys R. Renal excretion of proteins and enzymes in workers exposed to cadmium. Eur J Clin Invest 1979: 9: 11-22.
74. Friberg L, Procator M, Norberg GF, Kjellstrom T (editors). Cadmium in the environment. CRC Press, Cleveland 1974.
75. Simonsen O, Grubb A. Thysell H. The blood serum concentration of cystatin C (g-trace) as a measure of the glomerular filtration rate. Scand J Clin Lab Invest 1985: 45: 97-101.
76. Wedeen RP, Udasin I, Fiedler N, D'Haese P, De Broe M, Gelpi E, Jones KW, Gochfeld M. Urinary biomarkers as indicators of renal disease. Renal Failure 1999; 21: 241-249.
77. Berggard I, Beam AG. Isolation and properties of a low molecular weight β2-globulin occurring in human biological fluids. J Biol Chem 1968; 243: 4095-4103.
78. Beetham R, Dawanay A, Landon J, Cattell R. A radioimmunoassay for retinal-binding protein in serum and urine. Clin Chem 1985; 31: 1364-1367.
79. Price RG. The role of NAG (N-acetyl-β-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol 1992; 38 (suppl 1): S14-S19.
80. Marino M, Andrews D, Brown D, McLuskey RT. Trancytosis of retinol-binding protein across renal proximal tubule cells after megalin (gp 330)-mediated endocytosis. J Am Soc Nephrol 2001; 12: 637-648.
81. Camara NO, Matos AC, Rodrigues DA, Pereira AB, Pacheco-Silva A. Urinary retinol binding protein is a good marker of progressive cyclosporine nephrotoxicity after heart transplant. Transplant Proc 2001; 33(3): 2129-2131.
82. Ekstrom B, Peterson PA, Berggard I. A urinary and plasma α-l-glycoprotein of low molecular weight: isolation and some properties. Biochem Biophys Res Commun 1975; 65: 1472-1535.
83. Weber MH, Verwiebe R. α1-Microglobulin (Protein HC): features of a promising indicator of proximal tubular dysfunction. Eur J Clin Chem Clin Biochem 1992; 30: 683-691.
84. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 1992; 38(Suppl 1): S20-S27.
85. Tencer J, Thysell H, Grubb A: Analysis of proteinuria: Reference limits for urine excretion of albumin, protein HC, immunoglobin G, k- and l-chain immunoreactivity, orosomucoid and α1-antitrypsin. Scand J Clin Lab Invest 1996; 56: 691-700.
86. Teppo AM, Honkanen E, Ahonen J, Gronhagen-Riska C. Changes of urinary α1-microglobullin in the assessment of prognosis in renal transplant recipients. Transplantation 2000; 70:1154-1159.
87. Tsukahara H, Hiraoka M, Kuriyama M, Saito M, Morikawa K, Kuroda M, Tominaga T, Sudo M. Urinary α1-microglobulin as index of proximal tubule function in early infancy. Pediatr Nephrol 1993; 7: 199-201.

88. Bakoush O, Grubb A, Rippe B, Tencer J. Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria. Kidney Int 2001; 60: 1904-1909.
89. Recio F, Villamil F. Charge selectivity and urine amylase isoenzymes. Kidney Int 1994; 46 (Suppl 47); S89-S92.
90. Baricos WH, Cortez SL, Le QC, Zhou Y, Dicarlo RM. O'Connor SE, Shah SV. Glomerular basement membrane degradation by endogenous cysteine proteinases in isolated glomeruli. Kidney Int 1990; 38: 395-401.
91. Newman DJ, Thakkar H, Edwards RG, Wilke M, White T, Grubb AO, Price CP. Serum cystatin C: a replacement marker for creatinine as a biochemical marker of glomerular filtration rate. Kidney Int 1994: 46 (Suppl 47): S20-S21.
92. Norden AGW, Lapsley M, Lee PJ, Pusey CD, Scheinman SJ, Tam FWK, Thakker RV, Unwin RJ, Worng O. Glomerular protein sieving and implications for renal failure in Fanconi syndrome. Kidney Int 2001; 60: 1885-1892.
93. Kumar S, Muchmore A. Tamm-Horsfall protein-urimodulin (1950-1990). Kidney Int 1990; 37: 1395-1401.
94. Hoyer JR, Seiler MW. Pathophysiology of Tamm-Horsfall protein. Kidney Int 1979; 16: 279-289.
95. Goodall AA, Marshall RD. Effects of freezing on the estimated amounts of Tamm-Horsfall glycoprotein in urine, as determined by radioimmunoassay. Biochem J 1980; 189: 533-539.
96. Thornley C, Dawnay A, Cattell WR. Human Tamm-Horsfall glycoprotein: urine and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay. Clin Sci 1985; 68: 529-535.
97. Lynn KL, Marshall RO. Excretion of Tamm-Horsfall glycoprotein in renal disease. Clin Sci 1985; 68: 253-257.
98. Plummer DR. Noorazar S, Obatomi DK, Haslan JD. Assessment of renal injury by urinary enzymes. Uremia Invest 1985; 9: 97-102.
99. Lockwood TD, Bosmann HB. The use of urinary N-acetyl-B-D-glucosaminidase in human renal toxicology I. Partial biochemical characterization and excretion in humans and release from the isolated perfused rat kidney. Toxicol Appl Pharmacol 1979; 49: 323-336.
100. Jung K, Scholz D. An optimized assay of alanine aminopeptidase activity in the urine. Clin Chem 1980; 26: 1251-1254.
101. Price RG. Urinary enzymes. Nephrotoxicity and renal disease. Toxicol 1982; 23: 99-134.
102. Dubach UC, LeHir M. Conical evaluation of the diagnostic use of urinary enzymes. Contrib Nephrol 1984; 42: 74-80.
103. Mutti A. Detection of renal disease in humans. Developing markers and methods. Toxicol Lett 1989; 46: 177-191.
104. Vanderlinde RE. Urinary enzymes measurements of renal disorders. Ann Clin Lab Sci 1981; 11: 189-201.
105. Maruhn D, Fuchs I, Mues G, Bock KD. Normal limits of excretion of eleven enzymes. Clin Chem 1976; 22: 1567-1574.
106. Koenig H, Goldstone A, Hughes C. Lysosomal enzymuria in the testosterone treated mouse. Lab Invest 1978; 39: 329-341.
107. Ceriotti G. A new look at the measurement and interpretation of enzyme assays. Ann Clin Biochem 1976; 13: 345-353.
108. Guder WG. Hofmann W. Future markers for the diagnosis of renal lesions. In: Nephrotoxicity: mechanism, early diagnosis and therapeutic management. Bach PH, Gregg NJ, Wilks MF, Delacruz L. editors. Marcel Dekker, New York 1991; p. 575-580.
109. Dubach UC, LeHir M, Gandhi R. Use of urinary enzymes as markers of nephrotoxicity. Toxicol Lett 1988; 46: 193-196.
110. Clemo FA. Urinary enzyme evaluation of nephrotoxicity in the dog. Toxicol Pathol 1998; 26: 29-32.
111. Price RG, Berndt WD, Finn WF, Aresini G, Manley SE, Fels LM, Shaikh ZA, Mutti A. Urinary biomarkers to detect significant effects of environmental and occupational exposure to nephrotoxins. III. Minimal battery of tests to assess subclinical nephrotoxicity for epidemiological studies based on current knowledge. Renal Failure 1997; 19(4): 535-552.
112. Rosalki SB, Wilkinson JH. Urinary lactic dehydrogenase in renal disease. Lancet 1959; 2: 327-328.
113. Mitic-Zlatkovic M, Stefanovic V. Acute effects of acetaminophen on renal function and urinary excretion of some proteins and enzymes in patients with kidney disease. Renal Failure 1999; 21: 525-532.
114. Kreisel W, Wolf LM, Grotz W, Grieshaber M. Renal tubular damage: an extraintestinal manifestation of chronic inflammatory bowel disease. Eur J Gastroent Hepatol 1996; 8: 461-468.
115. Langhendries JP, Battisti O, Bertrand JM, Francois A, Darimont J, Ibrahim S, Tulkens PM, Bernard A, Buchet JP, Scalais E. Once-a-day adminstration of amikacin in neonates: assessment of nephrotocicity and ototoxicity. Dev Pharm Ther 1993; 20: 220-230.
116. Ruilope LM. Renal damage in hypertension. J Cardiovas Risk 1995; 2: 40-44.
117. Rustom R, Costigan M, Shenkin A, Bone JM. Proteinuria and renal tubular damage: urinary N-acetyl-β-D-glucosaminidase and isoenzymes in dissimilar renal disease. Am J Nephrol 1998; 18: 179-185.
118. Mondorf AW. Urinary enzymatic markers of renal damage. In: The aminoglycosides: microbiology, clinical use and toxicology. Whelton A, Neu HC (editors). Marcel Dekker Inc., New York, Basel 1982; p. 283.
119. Meyer BR, Fischhein A, Rosenman K. Increased urinary enzyme excretion in workers exposed to nephrotoxic chemicals. Am J Med J 1984; 76: 989-998.
120. Mueller PW. MasNeil ML, Steinberg KK. Stabilization of alanine aminopeptidase. γ-glutamyltransferase and N-acetyl-β-D-glucosaminidase in normal urine. Arch Environ Contam Toxicol 1986; 15: 343-347.
121. Mattenheimer H. Enzymes in renal disease. Ann Clin Lab Sci 1977; 7: 422-432.
122. Davey PG, Cowley DM, Geddes AM, Terry J. Clinical evaluation of β-2-microglobulin, murmamidase, and alanine aminopeptidase as markers of gentamicin nephroxicity. Contrib Nephrol 1984; 42: 100-106.

123. Scherberich JE, Mondorf W, Falkenberg FW, Pierard D, Scoeppe W. Monitoring drug nephrotoxicity. Contrib Nephrol 1984; 42: 81-92.
124. Mueller PW, Smith SJ, Steimberg KK, Thun MJ. Chronic renal tubular effects in relation to urine cadmium levels. Nephron 1989; 52: 45-54.
125. Porter GA. Contrast associated nephropathy. Am J Cardiol 1989; 64: E22-E26.
126. Mueller PW, Delaney V, MacNeil ML, Caudill SP, Steimberg KK. Indicators of acute renal transplant rejection in patients treated with cyclosporine. Clin Chem 1990; 36: 759-764.
127. Tataranni G, Zavagli G, Farinelli R, Malacarne F, Fiocchi O, Nunzi L, Scaramuzzo P, Scorrano R. Usefulness of the assessment of urinary enzymes and microproteins in monitoring cyclosporine nephrotoxicity. Nephron 1992; 60: 314-318.
128. Zafirovska KG, Bogdanovska SV, Marina N, Gruev T, Lozance L. Urinary excretion of three specific renal tubular enzymes in patients treated with nonsteroidal anti-inflammatory drugs (NSAID). Renal Failure 1993; 15: 51-54.
129. Donadio C, Tramont G, Giordani R, Lucchetti A, Calderazzi A, Ferrari P, Bioanchi C. Renal effects and nephrotoxicity of contrast media in renal patients. Contrib Nephrol 1993; 101: 241-250.
130. Hofmeister R, Bhargaya AS, Gunzel P. Value of enzyme determinations in urine for the diagnosis of nephrotoxicity in rats. Clin Chem Acta 1986; 160: 163-167.
131. Whiting PH, Thomson AW, Simpson JG. Cyclosporine and renal enzyme excretion. Clin Nephrol 1986; 25 (suppl 1): S100-S104.
132. Obatomi DK. Plummer DT, Haslam JD. Enzymuria as an index of nephrotoxicity over long term exposure of rats to gentamicin. In: Nephrotoxicity: mechanism, early diagnosis and therapeutic management. Bach PH, Gregg NJ, Wilks MF, Oelacruz L (editors). Marcel Dekker Inc, New York 1991; p. 555-561.
133. Burdmann EA, Andoh TF, Franceschini N,Lindsley J, Vora l, Bennett WM. Renal and metabolic effects of short-term rapamycin (RAPA) treatment in salt de- pleted rats. J Am Soc Nephrol 1993: 4: 910.
134. Burdmann EA, Rosen S, Lindsley J, Elzinga L, Andoh T, Bennett WM. Production of less chronic nephrotoxicity by cyclosporine G than cyclosporine A in a low salt rat model. Transplantation 1993: 55: 969-966.
135. Andoh TF, Burdmann EA, Lindsley J, Houghton DC, Bennett WM. Enhancement of FK 506 nephrotoxicity by sodium depletion in an experimental rat model. Transplantation 1994; 57: 483-489.
136. Burdmann EA, Andoh TF, Lindsley J, Russell J, Bennett WM, Porter GA. Urinary enzymes as biomarkers of renal injury in experimental nephrotoxicity of immunosuppressive drugs. Renal Failure 1994; 16: 161-168.
137. Lauwerys RR, Bernard A. Early detection of the nephrotoxic effects of industrial chemicals: state of the art and future prospects. Am J Ind Med 1987; 11: 275-285.
138. Verpooten GF, Nuyts GD, Hoylaerts MF, Nouwen EJ, Vssanyiova Z, Dlhopolcek P, De Broe ME. Immunoassay in urine of a specific marker for proximal tubular S3 segment. Clin Chem 1992; 38: 642-647.
139. Nuyts GD, Roels HA, Verpooten GF, Bernard AM, Lauwerys RR, De Broe ME. Intestinal type alkaline phosphatase in urine as an indicator of mercury induced effects on the S3 segment of the proximal tubule. Nephrol Dial Transplant 1992; 7: 225-229.
140. Verpooten GF, Nouwen EJ, Hoylaerts MF, Hendrix PG, De Broe ME. Segment specific localization of intestinal type alkaline phosphatase in human kidney. Kidney Int 1989; 36: 617-625.
141. Nouwen EJ, De Broe ME. Human intestinal versus tissue-nonspecific alkaline phosphatase as complementary urinary markers for the proximal tubule. Kidney Int 1994; 46 (Suppl 47): S43-51.
142. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995; 30: 445-600.
143. Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. Urinary excretion of glutathione S transferase α and pi in patients with proteinuria: reflection of t site of tubular injury. Nephron 2000; 85: 120-126.
114. Sundberg AG, Appelkvist EL, Backman L, Dallner G. Urinary pi-class glutathione transferase as an indicator of tubular damage in the human kidney. Nephron 1994; 67: 308-316.
145. Sundberg A, Appelkvist EL, Dallner G, Nilson R. Glutathione transferases in the urine: sensitive methods for detection of kidney damage induced by environmental agents in humans. Environm Health Persp 1994; 102 (Suppl 3): 293-296.
146. Savary E, Nemes B, Jaray J, Dinya E, Borka P, Varga M, Sulyok B, Remport A, Toth A, Perner F. Prediction of early renal graft function by the measurement of donor urinary glutathione S-transferase. Transplantation 2000; 69: 1398-1402.
147. Polak WP, Kosieradzki M, Kwiatkowski A, Danielewicz R, Lisik W, Michalak G, Paczek L, Lao M, Walaszewski J, Rowinski WA: Activity of glutathione S-transferases in the urine of kidney transplant recipients during the first week after transplantation. Annals Transplant 1999; 4: 42-45.
148. Baron S, Tyring SK, Fleischmann WR Jr, Coppenhaver DH, Niesel DW, Klimpel GR, Stanton GJ, Hughes TK. The interferons. Mechanisms of action and clinical applications. JAMA 1991; 266(10): 1375-1383.
149. Hirano T. The biology of interleukin-6. Chem Immunol 1992; 51: 153-180.
150. Ruef C, Budde K, Lacy J, Northemann W, Baumann M, Sterzel RB, Coleman DL. Interleukin-6 is an autocrine growth factor for mesangial cells. Kidney Int 1990; 38: 249-257.

151. Horii Y, Muraguchi A, Iwano M, Matsuda T, Hira-yama T, Yamada H, Fujii Y, Dohi K, Ishikawa H, Ohmoto Y, Yoshizaki K, Hirano T, Kishimoto T. Involvement of interleukin-6 in mesangial proliferative glomerulonephritis. J Immunol 1989; 143: 3949-3955.
152. Horii Y, Iwano M, Hirata E, Shiiki H, Fujii Y, Dohi K, Ishikawa H. Role of interleukin-6 in the progression of mesangial proliferative glomerulonephritis. Kidney Int 1993; 43 (Suppl 39): S71-S75.
153. Jutley RS, Youngson GG, Eremin O, Ninan GK. Serum cytokine profile in reflux nephropathy. Ped Surg Interna 2000; 16: 64-68.
154. Gomez-Chiarri M, Ortiz A, Seron D, Gonzalez E, Egido J. The intercrine superfamily and renal disease. Kidney Int 1993; 43 (Suppl 39): S81-S85.
155. Wada T, Yokoyama H, Tomosugi N, Hisada Y, Ohta S, Naito T, Kobayashi K-I, Mukaida N, Matsushima K. Detection of urinary interleukin-8 in glomerular disease. Kidney Int 1994; 46: 455-460.
156. Gormley SM, McBride WT, Armstron MA, Young IS, McClean E, MacGowna SW, Campalani G, McMurry TJ: Plasma and Urinary cytokine homeostasis and renal dysfunction during cardiac surgery. Anesthesiology 2000; 93: 1210-1216.
157. Baud L, Oudinet JP, Bens M, Noe L, Peraldi MN, Etienne J, Ardaillou R. Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide. Kidney Int 1989; 35: 1111-1118.
158. Baud L, Fouqueray B, Philippe C, Amrani A. Tumor necrosis factor α and mesangial cells. Kidney Int 1992; 41: 600-603.
159. Radeke HH, Meier B, Topley N, Floge J, Habermehl GG, Resch K. Interleukin l-α and tumor necrosis factor-α induce oxygen radical formation in mesangial cells. Kidney Int 1990; 37: 767-775.
160. Zoja C, Wang JM, Bettoni S, Sironi M, Renzi D, Chiaffarino F, Abboud HE, Van Damme J, Mantovani A, Remuzzi G, Rambaldi A. Interleukin-1b and tumor necrosis factor-α induce gene expression and production of leukocyte chemotactic factors, colony-stimulating factors, and interleukin-6 in human mesangial cells. Am J Pathol 1991; 138: 991-1003.
161. Tomosugi N, Cashman S, Hay H, Pusey C, Evans D, Shaw A, Rees A. Modulation of antibodiy-mediated glomerular injury *in vivo* by bacterial lipopolysaccharide, tumor necrosis factor, and IL-l. J Immunol 1989; 142: 3083-3090.
162. Brockhaus M, Bar-Khayim Y, Gurwicz S, Frensdorff A, Haran N. Plasma tumor necrosis factor soluble receptors in chronic renal failure. Kidney Int 1992; 42: 663-667.
163. Boucher A, Droz, D, Adafer E, Noel L. Characterization of Mononuclear Cell Subsets in Renal Cellular Interstitial Infiltrates. Kidney Int 1986; 29: 1043–1049.
164. Danoff TM. Chemokines in Interstitial Injury. Kidney Int 1998; 53: 1807–1808.
165. Prodjosudjadi,W, Gerritsma JSJ, Klar-Mohamad N, Gerritsen AF, Bruijn J.A., Daha MR, Van Es LA. Production and Cytokine-mediated Regulation of Monocyte Chemoattractant Protein-1 by Human Proximal Tubular Epithelial Cells. Kidney Int 1995; 48: 1477–1486.
166. Lloyd CM, Minto AW, Dorf ME, Proudfoot A, Wells TNC, Salant DJ, Gutierrez-Ramos JC. RANTES and Monocyte Chemoattractant Protein-1 (MCP-1) Play an Important Role in the Inflammatory Phase of Crescentic Nephritis, but only MCP-1 is involved in Crescent Formation and Interstitial Fibrosis. J Exp Med 1997; 185: 1371–1380.
167. Wang Y, Chen J, Chen L, Tay Y, Rangan GK, Harris DCH. Induction of Monocyte Chemoattractant Protein-1 in Proximal Tubule Cells by Urinary Protein. J Am Soc Nephrol 1997; 8: 1537–1545.
168. Eddy, A.A.; Giachelli, C.M. Renal Expression of Genes that Promote Interstitial Inflammation and Fibrosis in rats with Protein-overload Proteinuria. Kidney Int 1995; 47: 1546–1557.
169. Tsukasa Morii, Hiroki Fujita, Takuma Narita, Jun Koshimura, Takashi Shimotomai, Hiromi Fujishima, Naomi Yoshioka, Hirokazu Imai, Masafumi Kakei, and Seiki Ito: Increased Urinary Excretion of Monocyte Chemoattractant Protein-1 in Proteinuric Renal Diseases (Renal Failure in press).
170. Nouwen EJ, Dauwe S, De Broe ME. EGF and TGF - are differentially localized along distal nephron segments in the adult and fetal human monkey. J Am Soc Nephrol 1993; 4(3): 472.
171. Kennedy WA II, Buttyan R, Sawczuk IS. Epidermal growth factor (EGF) suppresses renal tubular apoptosis following ureteral obstruction. J Am Soc Nephrol 1993; 4(3): 738.
172. Safirstein R, Zelent AZ, Price PM. Reduced renal prepro-epidermal growth factor mRNA and decreased EGF excrtetion in ARF. Kidney Int 1989; 36: 810-815.
173. Verstrepen WA, Nouwen El, Yue XS, De Broe ME. Altered growth factor expression during toxic proximal tubular necrosis and regeneration. Kidney Int 1993; 43: 1267-1279.
174. Joh T, Itoh M, Katsumi K, Yokayama Y, Takeuchi T, Kato T, Wada Y, Ranaka R. Physiological concentrations of human epidermal growth factor in biological fluids: use of a sensitive enzyme immunoassay. Clin Chim Acta 1986; 158: 81-90.
175. Moel DI, Safirstein RL, Sachs HK, Agrawal R. Epidermal growth factor (EGF) excretion 17-23 years after chelation therapy for childhood plumbism. J Am Soc Nephrol 1993; 4(3): 321.
176. Mattila A-L, Pasternack A, Viinikka L, Perheentupa J. Subnormal concentration of urinary epidermal growth factor in patients with kidney disease. J Clin Endocrinol Metab 1986; 62: 1180-1183.
177. Han YKM, D'Ercole AJ, Lund PK. Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 1987; 236: 193-197.
178. El Nahas AM, Sayed-Ahmed N. Insulin-like growth factor I and the kidney: friend or foe ? Exp Nephrol 1993; 1: 205-17.

179. Bruijn JA, Roos A, de Geus B, de Heer E. Transforming growth factor-β and the glomerular extracellular matrix in renal pathology. J Lab Clin Med 1994; 123: 34-47.
180. Johnson RJ, Floege J, Causer WG, Alpers CE. Role of platelet-derived growth factors in glomerular disease. J Am Soc Nephrol 1993; 4: 119-128.
181. Gesualdo L, Raniere E, Pannarale G, DiPaolo S, Schena P. Patelet-derived growth factor and proliferative glomerulonephritis. Kidney Int 1993; 43 (Suppl 39): S86-S89.
182. Abboud HE, Woodruff KA, Snyder SP, Bonewald LF. Polypeptide growth factors regulate the production of latent transforming growth factor B in human mesangial cells. J Am Soc Nephrol 1991; 2: 434.
183. Fellstrom B, Klareskog L, Heldin CH, Larsson E, Ronnstrand L, Terracio L, Tufveson G, Wahlberg J, Rubin Kelley YE. Platelet-derived growth factor receptors in the kidney. Up-regulated expression in inflammation. Kidney Int 1989; 36: 1099-1102.
184. Brigstock DR. The connective tissue growth factor/cystein-rich 61/nephroblastoma overexpression (CCN) family. Endocr Rev 1999; 20: 189-206.
185. Jedsadayanmata A, Chen CC, Kireeva ML, Lau LF, Lam SC. Activation-dependent adhesion of human platelets to Cyr61 and Fisp12/mouse connective tissue growth factor is mediated through integrin apha(Iib)β(3). J Biol Chem 1999; 274: 24321-24327.
186. Muramatsu Y, Tsujie M, Kohda Y, Pham B, Perantoni AO, Zhao Hong, Sang-Kyung J, Yuen PST, Craig L, Hu X, Star RA: Early detection of cysteine rich protein 61 (CYR61, CCN1) in urine following renal ischemic reperfusion injury. Kidney Int 2002; 62: 1601-1610.
187. Bruggeman LA, Pellicoro JA, Horigan EA, Klotman PA. Thromboxane and prostacyclin differentialy regulate murine extracellular matrix gene expression. Kidney Int 1993; 43: 1219-1225.
188. Remuzzi G, FitzGerald GA, Patrono G. Thromboxane synthesis and action within the kidney. Kidney Int 1992; 41: 1483-1493.
189. Toto R, Siddhanta A, Manna S, Pramanik B, Falck JR, Capdevila J. Arachidonic acid epoxygenase: detection of epoxyeicosatrienoic acids (EETs) in human urine. Biochim Biophys Acta 1987; 919: 132-139.
190. Donovan KL, Coles GA, Williams JD. An ELISA for the detection of type IV collagen in human urine - Application to patients with glomerulonephritis. Kidney Int 1994; 46: 1431-1437.
191. Nerlich AG, Schleicher ED, Wiest I, Specks U, Timpl R. Immunohistochemical localization of collagen VI in diabetic glomeruli. Kidney Int 1994; 45: 1648-1656.
192. Price RG. Taylor SA, Crutcher E. Assay of laminin fragments in the assessment of renal disease. Kidney Int 1994; 46 (Suppl 47): S25-S28.
193. Quiros J. Gonzalez-Cabrero J, Herrero-Beaumont G, Egido J. Elevated plasma fibronectin levels in rats with immune and toxic glomerular disease. Renal Failure 1990; 12: 227-232.
194. Yamaguchi Y. Mann DM. Ruoslahti E. Negative regulation of transforming growth factor-β by the proteoglycan decorin. Nature 1990; 346: 281-284.
195. Rabb HAA. Cell adhesion molecules and the kidney. Am J Kidney Dis 1994; 23: 155-166.
196. Brady HR. Leukocyte adhesion molecules and kidney disease. Kidney Int 1994: 45: 1285-1300.
197. Bosman FT. Integrins: cell adhesives and modulators of cell function. Histochem J 1993; 25: 469-477.
198. Ruoslahti E, Noble NA, Kagami S, Border WA. Integrins. Kidney Int 1994; 45 (Suppl 44): S17-S22.
199. Burridge K, Fath K, Kelly T, Nuckolis B, Turner C. Foca adhesion: transmembrane junctions between the extracellular matrix and the cytoskeleton. Ann Rev Cell BioI 1988; 4: 487-525.
200. Kelly KJ, Williams WW Jr, Colvin RB, Bonventre JV. Antibody to intercellular adhesion molecule I protects the kidney against ischemic injury. Proc Natl Acad Sci 1994; 91: 812-816.
201. Simon EE. Potential role of integrins in acute renal failure. Nephrol Dial Transplant 1994; 9 (Suppl 4): 26-33.
202. Rothlein R, Mainolfi EA. Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991; 147: 3788-3793.
203. Seron D, Cameron JS, Haskard DO. Expression of VCAM-1 in the normal and diseased kidney. Nephrol Dial Transplant 1991; 6: 917-922.
204. Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, Sanicola M. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998; 273: 4135-4142.
205. Han WK, Bailey V, Abichandami R, Thadhani RI, Ichimura T, Bonventre JV: Kidney injury molecule-1 (KIM-1) is a new biomarker for human renal proximal tubule injury. J Am Soc Nephrol 2000; 11: 129A.
206. Mutti A. Lucertini S, Valcavi P. Urinary excretion of brush-border antigen revealed by monoclonal antibody: early indicator of toxic nephropathy. Lancet 1985; 2: 914-917.
207. Coudrier E, Kerjaschki D, Louvard D. Cytosheletal organization and sub membranous interactions in intestinal and renal brush borders. Kidney Int 1988; 34: 309-320.

208. Gandhi CR, Berkowitz DE. Watkis D. Endothelins: biochemistry and pathophysiologic actions. Anesthesiology 1994; 80: 892-905.
209. Simonson MS, Wann S, Mene P, Dubyak M, Kester Y, Nakazato Y, Sedor JR, Dunn MJ. Endothelin stimulates phospholipase C, Na/H exchange, c-fos expression and mitogenesis in rat mesangial cells. J Clin Invest 1989; 83: 708-712.
210. Ohta K, Hirata Y, Shichiri M, Kanno K, Emori T, Tomita K, Marumo F. Urinary excretion of endothelin-1 in normal subjects and patients with renal disease. Kidney Int 1991; 39: 307-311.
211. Kon V, Sugiura M, Inagami T, Harvie BR, Ichikawa I, Hoover RL. Role of endothelin in cyclosporine-induced glomerular dysfunction. Kidney Int 1990; 37: 1487-1491.
212. Ohta K, Hirata Y, Shichiri M, Ichioka M, Kubota T, Marumo F. Cisplatin-induced urinary endothelin excretion. JAMA 1991; 265: 1391-1392.
213. Abassi ZA, Klein H, Golomb E, Keiser HR. Urinary endothelin: a possible biological marker of renal damage. Am J Hypertens 1993; 6(12): 1046-1054.
214. Dvergsten J, Manivel JC, Correa-Rotter R, Rosenberg ME. Expression of clusterin in human renal diseases. Kidney Int 1994; 45(3): 828-835.
215. Rulitzky WK, Schlegel PN. Wu D, Cheng CY, Chen C-LC, Li PS, Goldstein M, Reidenberg M, Bardin CW. Measurement of urinary clusterin as an index of nephrotoxicity. Proc Soc Exp Biol Med 1992; 199: 93-96.
216. Lovis C, Mach F, Donati YRA, Bonventure JV, Polla BS. Heat shock proteins and the kidney. Renal Failure 1994; 16: 179-192.
217. Nguyen TK, Obatomi DK, Bach PH. Increased urinary uronic acid excretion in experimentally-induced renal papillary necrosis in rats. Renal Failure 2001; 23: 31-42.
218. Eti S, Cheng CY, Marshall A. Reidenberg MM. Urinary clusterin in chronic nephrotoxicity in the rat. Proc Soc Exp Biol Med 1993; 202: 487-490.
219. Videen JS, Ross BD. Proton nuclear magnetic resonance urinalysis: coming of age. Kidney Int 1994; 46 (suppl 47): S122-S128.
220. Anthony ML, Sweatman BC, Beddell CR, Lindoon JC, Nicholson JK. Pattern recognition classification of the site of nephrotoxicity based on metabolic data from proton nuclear magnetic resonance spectra of urine. Mol Pharmacol 1994; 46: 199-211.
221. Bueler MR, Wiederkehr F, Vonderschmitt DJ. Electrophoretic, chromotographic and immunological studies of human urinary proteins. Electrophoresis 1995; 16: 124-134.

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Pharmacological aspects of nephrotoxicity

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Clinically overt nephrotoxicity is the result of the intrinsic capacity of a toxin to damage renal cells or tissue, the susceptibility of the patient, and changes in disposition of the toxin that result in increased delivery to the target organ or tissue. In this chapter we will examine the latter aspect of nephrotoxicity and will focus on (i) drug interactions that potentially lead to nephrotoxicity and (ii) changes in drug disposition induced by renal failure.

Drug interactions

Usually a distinction is made between *pharmacodynamic drug interactions*, which describes changes in drug activity or toxicity, and *pharmacokinetic drug interactions*, which include changes in drug disposition. Examples of pharmacodynamic drug-drug interactions that lead to an increased risk for nephrotoxicity are covered in the chapters dealing with the individual drugs.

It is now recognized that the majority of drug-drug interactions are mediated by two intimately related mediators, the cytochrome P-450 enzyme system and the drug transporter P-glycoprotein. A considerable overlap has been found in drugs that act as inducers, inhibitors or substrates for both systems [1-3].

Cytochrome P-450 (CYP) enzymes are membrane-bound, heme-containing terminal cellular oxidases that are part of a multienzyme system that also includes a FAD/FMN-containing NADPH-cytochrome P-450 reductase and cytochrome b5 [4]. CYP enzymes oxidize, peroxidize, and/or reduce cholesterol, vitamins, steroids, xenobiotics and numerous pharmacological substances in an oxygen-and NADPH-dependent manner; therefore, they play a key role in the metabolism of a wide variety of endogenous substrates and exogenously administered drugs and chemicals. Metabolism of foreign chemicals usually results in successful detoxication of the irritant. However, the actions of CYP-450 enzymes can also generate toxic metabolites that contribute to increased risks of birth defects, cancer and other toxic effects. Moreover, expression of many P-450 enzymes is often induced by accumulation of a substrate.

Currently, CYP proteins are conveniently arranged

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into families and subfamilies on the basis of amino acid sequence identity: proteins with approximately 40% homology identity or greater are included in the same family designated by an Arabic number. Proteins with greater than 55% homology are grouped together in the same subclass and designated by a capital letter; the last number identifies specific gene products. To date, several hundred CYP genes have been identified in nature, but only 18 gene families play a role in mammalian metabolism [5]. Xenobiotics or foreign chemicals including thousands of environmental pollutants are metabolized almost exclusively in CYP 1-4 families. Expression of the CYP1 gene family is induced by the arylhydrocarbon receptor, a transcription factor that is activated by binding of polycyclic aromatic hydrocarbons [5]; CYP2 is the largest P-450 family in mammals. The CYP3 family has four members. CYP3A4 and CYP3A5 are most abundantly expressed P-450 enzymes in the human liver and gastrointestinal tract and metabolize more than 120 frequently prescribed drugs and endogenous substrates such as steroids and bile acids.

Most CYPs are primarily expressed in the liver, with significantly lower levels of expression in extrahepatic tissues. However, some CYPs are predominantly detected in the heart, gastrointestinal tract, lung and even kidney. The renal cytochrome CYP system catalyzes the metabolism of a variety of chemicals and drugs with an activity that may equal that of the liver. Recent data have suggested that specific CYPs localized in the vascular smooth muscle and endothelium contribute to the regulation of vascular tone and homeostasis [4]. There is evidence that CYP expression is increased in the kidney in hypertension [6] and during salt loading [7]. During infection and inflammation, the expression of CYP450 and its dependent biotransformation are also modified at the pre-translational steps in protein synthesis [9].

In addition to an efficient-metabolism phenotype, variant alleles encode a poor-metabolism phenotype, with low or no enzyme activity towards a particular drug. On occasion, because of one or more gene duplications, a variant genotype might involve a very high ultra-metabolism phenotype resulting in treatment failure and toxic effects. Similarly, rates of detoxification and sometimes of metabolic activation of environmental chemicals can be strikingly different between individuals with different CYP haplotypes. Of particular clinical importance, metabolism of certain antifungal and immunosuppressive drugs by CYP3A4 and CYP3A5 could lead to insufficient amounts in extensive-metabolized patients [9], and excessive concentrations in those with the poor metabolism phenotype, when either type of patient is given the recommended prescribed dose.

Essentially all families of drug-metabolizing enzymes in humans have genetic variants, many of which translate into functional changes in the proteins encoded. Pharmacogenetic monogenic traits of several CYP isoenzymes are not uncommon and may be present in a variable percentage of the population (1-18%) [10]. For instance, subjects who have inherited two copies of a gene or genes that encode either an enzyme with decreased CYP2D6 activity or one with no activity may present with decreased metabolization of drugs as diverse as codeine, metoprolol or the antihypertensive drug debrisoquin [11]. Many other CYP enzymes display genetic variation that can influence a person's response to drugs including 2C9, metabolizing the antihypertensive drug losartan, 2C19, which metabolizes omeprazole and 3A5, which metabolizes a very large number of drugs.

P-glycoprotein, a 170-kDa glycoprotein encoded by the human multi-drug resistance-1 (MDR-1) gene, belongs to the ATP-binding cassette (ABC) proteins. These proteins bind ATP and use its energy to drive the transport of various molecules across plasma as well as intracellular membranes [12-15]. Initially, P-glycoprotein was identified by its ability to confer multidrug resistance on tumor cells by acting as a cellular efflux pump for a variety of anticancer drugs. It was quickly realized that P-glycoprotein was also present at high levels in normal tissues including the luminal membrane of the renal proximal tubule, the biliary canicular membrane of the hepatocyte, the apical surface of the mucosal cells in the small and large intestine and the capillary endothelial cells of the brain. This tissue distribution suggests that P-glycoprotein plays a role in the excretion of xenobiotics and metabolites into the urine and bile and into the intestinal lumen and in preventing their accumulation in the brain. Several members of the ABC protein family are involved in bile formation. In the liver, P-glycoprotein is located at the canicular hepatocyte membrane and is critical for the removal of cytotoxic cations [16]. In the gut, P-glycoprotein is directly involved in drug absorption.

Its activity results in expulsion of absorbed drug from the enterocyte back into the lumen. Studies in MDR-1-knockout mice using the P-glycoprotein probe drug digoxin have demonstrated *in vivo* that elimination of P-glycoprotein activity resulted in increased absorption and altered pharmacokinetics of P-glycoprotein substrates [17]. Recent clinical studies have emphasized the role of human MDR-1 gene polymorphism in the variable bioavailability of several drugs including potential nephrotoxins like tacrolimus [9, 18-21].

In the kidney P-glycoprotein is expressed at the brush border of the proximal convoluted tubule. The physiological role of P-glycoprotein in the kidney is currently being explored, but it is already clear that it plays a major role in the tubular secretion of endogenous and exogenous substances. There is some experimental evidence that xenobiotic transport is under the hormonal control of endothelin [22-24]. Digoxin is a commonly used probe drug to study proximal tubular P-glycoprotein-mediated transport *in vivo*. Studies in humans using concomitant administration of P-glycoprotein inhibitors like clarithromycin and itraconazole have shown that P-glycoprotein activity is crucial in tubular secretion of digoxin [25, 26]. Potential nephrotoxins like cyclosporine [27], rapamycin [28] and the HIV protease inhibitors ritonavir and saquinavir [29] have also been shown to be transported by the renal P-glycoprotein-mediated transport. Cyclosporine as well as other P-glycoprotein substrates seem to act as both substrate and inhibitor, with inhibition leading to up-regulation of (inactive) P-glycoprotein [27, 30] (Figure 1). One could speculate about the role of the inhibition by cyclosporine of this detoxifying system in the kidney in the development of cyclosporine nephrotoxicity as well in the additive toxicity observed when cyclosporine is used concomitantly with other nephrotoxins like vancomycin and aminoglycosides (see Chapter 8).

Drug-drug interactions mediated by the CYP and P-glycoprotein system arise from the induction of the proteins or from inhibition of their function. While induction may lead to decreased bioavailability and therapeutic failure, inhibition can give rise to increased plasma levels, altered tissue distribution and in the case of nephrotoxic drugs overt clinical toxicity. As previously noted, there is considerable overlap in the substrate specificity of CYP3A4 and P-glycoprotein. Certainly at the level of the intestine, both proteins fulfill

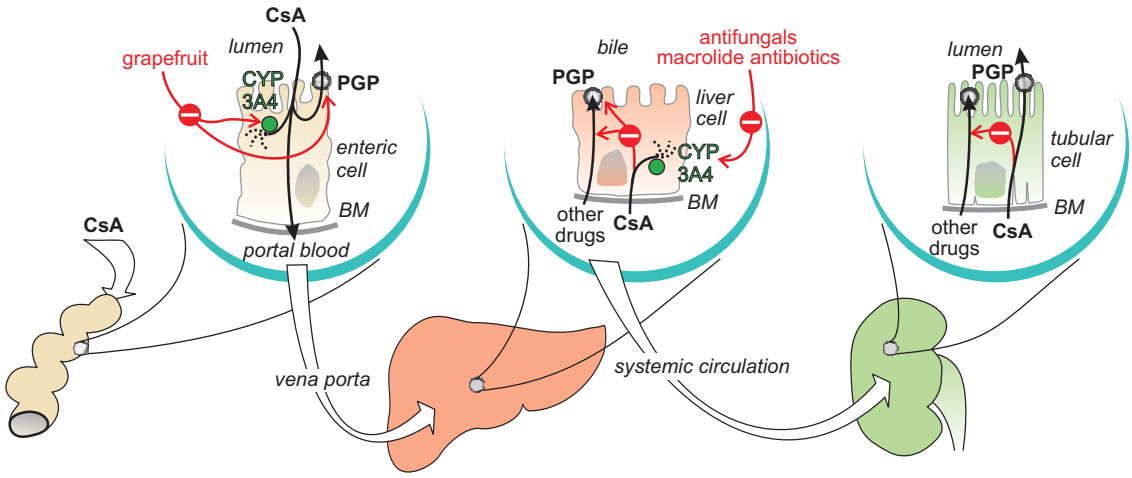

Figure 1: *Cyclosporine (CsA) as an example of a substrate and inhibitor of cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (PGP).* CYP3A4 and P-glycoprotein share their location in the mucosal cell of the small intestine. Through their coordinate action they limit the oral bioavailability of cyclosporine. The expression of P-glycoprotein is highly variable and influenced by genetic factors and induction by other drugs [57, 58]. This may explain the interindividual range from 5% to 89% of the bioavailability of cyclosporine. In the liver cyclosporine undergoes extensive biotransformation again mediated by CYP3A4. Inhibition of hepatic CYP3A4 by imidazole antifungals or macrolide antibiotics may entail toxic cyclosporine levels. By inhibiting the hepatocyte P-glycoprotein, cyclosporine may modulate the efflux into the bile of other drugs or metabolites (e.g. mycophenolate) [59]. In the kidney cyclosporine is transported by glycoprotein into the lumen of the proximal tubule. Although there is upregulation of the expression of P-glycoprotein at the level of the brush border [30], transport activity of other xenobiotics may be inhibited.

a complementary role in the so-called first-pass effect [31, 32].

Enzyme induction, and subsequent increased enzyme activity, may be caused by the administration of approximately 400 drugs and/or chemicals. Even folk remedies and herbs, previously regarded safe and well tolerated, are often overlooked as real drugs and may have detrimental effects on the levels and the therapeutic effect of concomitantly administered drugs. The intake of St. John's Wort (Hypericum perforatum), contains naphtodiantrons, potent inducers of CYP3A, may cause decreased cyclosporine levels and hence organ rejection in patients who have undergone kidney or other solid organ transplantation [33].

Inversely, inhibition of drug-metabolizing enzymes and efflux transporters generally decrease the rate of metabolism of the object drug. This, in turn, can result in increased serum concentrations and potential drug toxicity if the drug has a narrow therapeutic index. For instance, a major adverse effects of both fibrates and statins cause myopathy, which is dose related with statin monotherapy and more frequently observed with the lipophilic than the hydrophilic statins. If not recognized, rhabdomyolysis and acute renal insufficiency may result. Although combination therapy in serious hyperlipidemia can provide better control of lipid levels, cases of severe rhabdomyolysis, renal failure and rarely death have been described as a result of the interference with the CYP3A4 system, due to a marked increase in reductase inhibitory activity [34]. Moreover, the risk for acute renal failure is also significantly increased when statins are combined with other drugs such as cyclosporine, macrolides or itraconazole, known to influence CYP [35].

Grapefruit juice, a beverage consumed in large quantities by the general population, is a potent inhibitor of the intestinal CYP3A4 and P-glycoprotein system, leading to inhibition of the first-pass metabolism of many medications (Figure 1) and therefore increasing serum concentrations. Long term ingestion of large amounts may result in enhanced blood pressure reduction when co-ingested with certain 1, 4 dihydropyridine calcium antagonists, unpredictable and hazardous interactions with immunosuppressants such as cyclosporine in solid organ transplant patients and theoretically increase the risk for HMG-CoA reductase inhibitors myopathy and rhabdomyolysis [36]. Many calcium channel blockers inhibit the metabolism of cyclosporine. In particular, and possibly because of the metabolite intermediate complex that forms between diltiazem and CYP3A4, diltiazem in doses as low as 10 mg increased the bioavailability of cyclosporine thus mandating a lower dose to maintain efficacy or avoid toxic effects, including renal insufficiency. Similarly, the change in cyclosporine pharmacokinetics induced by the P-glycoprotein inhibitor verapamil allows the dose of cyclosporine to be reduced by one-third to one-half [37] (Table 1).

Drug disposition in renal failure

The principles of drug dosing in renal failure relate to the concept of individualization of drug therapy. The goal of this concept is to achieve the desired intensity of drug effect in the patient being treated. Dosage adjustment to produce the "therapeutic drug level" has become the major means to achieve this goal. The reason that this is usually effective is that most of the individual variation in dose-response is due to variation between individuals in their rates of elimination of drugs, not in their receptor or tissue sensitivity to drugs. Thus, individualizing the dose to produce the desired drug level leaves only the variation in tissue sensitivity to affect the intensity of a drug's effect.

In renal failure, there are changes in some aspects of drug absorption, distribution, and metabolism as well as excretion that influence the drug level achieved

Table 1. Some cytochrome P450 3A4 enzyme and P-glycoprotein efflux transporter: inhibitors, inducers, and shared substrates with nephrotoxic potential.

Inhibitors	Inducers	Substrates
Erythromycin	Amiodarine	Cyclosporine
Clarithromycin	Phenytoin	Tacrolimus
Itraconazole	Phenobarbital	Rapamycin
Ketoconazole	Rifampicine	Colchicine
Fluconazole	St John's Wort	Indinavir
Cimetidine	Glucocorticoids	Ritonavir
Diltiazem	Carbamazepine	Saquinavir
Verapamil		
Cyclosporine		
Grape fruit		

at normal drug doses. There are also changes in sensitivity to some drugs that should be considered in addition to pharmacokinetic changes produced by renal failure. An understanding of the changes in drug handling and in drug sensitivity in renal failure enables one to individualize therapy for such patients.

Absorption

Most orally administered drugs are lipid soluble molecules that are absorbed in the small intestine by diffusion. This appears to be normal in renal failure. If the renal failure is associated with delayed gastric emptying, then drug absorption may be slowed, the maximum drug concentration achieved in the plasma decreased, and the length of time for complete absorption to occur may be prolonged. The total amount of drug absorbed, the fraction bioavailable, is usually unchanged.

A study of D-xylose absorption in renal failure found that the absorption rate of the xylose was slowed and the amount absorbed was decreased [38]. This is a water-soluble rather than a lipid-soluble substance. What relationship this impaired intestinal absorption of xylose has to usual drug absorption remains to be determined.

Recent experimental studies in rats with acute glycerol-induced renal failure and with chronic renal failure showed a decreased expression of intestinal, renal and brain P-glycoprotein [39-41]. Other authors could not confirm this finding [42]. The clinical relevance of these experimental observations remains unclear.

Distribution

Drugs distribute throughout the body in a heterogeneous manner. Their concentrations differ in various tissues and organs. But the ratio of the concentration of a drug in one tissue relative to its concentration in another tissue is rather constant in one individual compared to another individual. Furthermore, it is the concentration of drug in plasma water that creates the diffusion gradient for the drug to diffuse in all tissues of the body including its site of action. Thus, under "steady state" conditions, the ratio of drug concentration in plasma water to that anywhere in the body is a constant. If one knows the amount of drug in the body at any time plus its plasma concentration, then the ratio of these two values, amount/concentration, produces a value that has units of volume. This ratio of amount/concentration is known as "volume of distribution". It is a characteristic of a drug and is the same from person to person if the people are metabolically normal and of the same size. The volume of distribution of a drug depends on the size of the patient, the smaller the patient, the smaller the volume of distribution of a drug. Since patients with chronic renal failure are often smaller than healthy people, their volumes of distribution of drugs will be smaller. Because of this, the dose of any drug would produce a higher drug concentration in plasma water in these patients than in patients with other illnesses who are of ordinary size.

For drugs that are highly bound to plasma proteins, anything that lowers the protein binding of the drug will lower the drug concentration in plasma and thus the amount of drug in the body. The reason for this is that the analytical methods used to measure drug concentrations usually measure both free drug in plasma water and drug bound to plasma proteins. If the drug bound to proteins is less, then the total amount of drug in the plasma is less, and since this is the denominator in the volume of distribution equation, the value for the volume of distribution is increased.

An important assumption in medical practice is that normal protein binding exists when interpreting drug level measurements. Patients with azotemia retain acidic metabolites that bind to the anion binding sites on serum albumin. They may also have abnormal albumin [43]. For these reasons, azotemic patients have decreased binding of anionic drugs to serum albumin and desirable "therapeutic levels" for these drugs in plasma or uremic patients will be lower than the "therapeutic levels" established for metabolically normal people. Since it is the drug concentration in plasma water that sets the diffusion gradient for the drug to its site of action, this adjustment is an important consideration. These kinetics has been worked out in detail for phenytoin, which is the most common drug for which protein-binding displacement occurs, and drug level monitoring is important.

It is also possible that drug binding to tissues rather than to plasma proteins is decreased in renal failure. This apparently occurs with digoxin, which has extensive tissue binding but little plasma protein binding. Under these circumstances, the decreased tissue binding of digoxin leads to a decreased body content of the

drug for any drug level in plasma and therefore a low volume of distribution. The significance of this is that a standard loading dose of digoxin will produce a higher drug level and greater intensity of effect in a uremic patient compared to a metabolically normal patient.

Metabolism

Investigations of the effect of chronic renal failure on hepatic enzyme activity in animals have demonstrated reductions in some, but not all, pathways of drug metabolism. Evidence in humans of impaired metabolism first emanated from observations of reduced non-renal clearance in patients with chronic renal insufficiency. Acute renal impairment may not have as severe a detrimental effect on drug metabolism [44], although uremic toxins have been shown to modulate CYP isozyme activity *in vitro*. Based on studies of the elimination rates of many drugs in renal failure and the classification of drugs by their pathways of elimination, it appears that most drugs are metabolized by oxidation followed by conjugation. Oxidations appear to be normal, or in some cases, somewhat faster in renal failure than in normal subjects. Glucuronide conjugation, the most frequent conjugation also appears to be normal, as does sulfate conjugation. Acetylation, on the other hand appears to be slowed. The hydrolyses of peptides are slowed, possibly because a substantial amount of peptide hydrolysis occurs in the kidney. Ester hydrolysis is also slowed [45, 46].

Although studies have shown that hepatic drug metabolism is predominantly decreased in patients with end stage renal disease, prediction of the effect of renal impairment on drug metabolism is difficult. One reason for this is that the rates of metabolism for most drugs vary so widely from person to person because of genetic factors [47, 48] that a disease state like renal failure only makes a small contribution, if any, to the interindividual variation in drug metabolism rates.

One drug whose rate of metabolism is increased in renal failure is phenytoin. Since this drug also has decreased protein binding, low total plasma drug levels are associated with normal "therapeutic" concentrations in plasma water. The net result is that, on average, patients in renal failure need higher than average doses of phenytoin to achieve the desired intensity of effect. They achieve this effect with a lower than average serum phenytoin level but an average free drug level in plasma water. Figure 2 shows the desired levels of phenytoin in serum to correct for the decreased protein binding in renal failure [49].

Excretion

One consequence of drug metabolism in renal failure is the accumulation of drug metabolites that are normally rapidly excreted. If these metabolites are pharmacologically inactive, their build-up will have no consequences. However, if the metabolites do have activity, even if only weakly active, their build-up can lead to pharmacologic effects that may only occur in patients with renal insufficiency and may not be recognized as drug effects. A number of drugs have active metabolites and have been reviewed by Drayer [50]. Two examples are procainamide that has an active metabolite, acetylprocainamide, that contributes to the antiarrhythmic or toxic activity of procainamide therapy in patients with renal insufficiency [51] and meperidine whose active metabolite, normeperidine, causes seizures in patients when the normeperidine levels get very high. These very high levels only occur in patients with renal insufficiency [52, 53].

Drug excretion is generally considered to fall in proportion to the fall in glomerular filtration rate. Dettli was the first to propose the concept that the non-renal

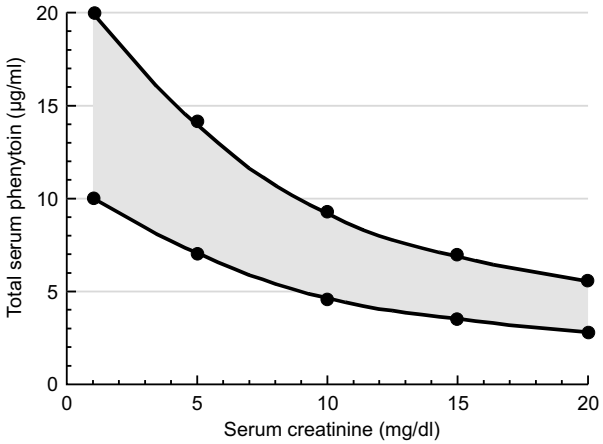

Figure 2. *Calculated values of total serum phenytoin (DPH) concentration that will produce a concentration of phenytoin in plasma water equal to that in metabolically normal patients when their total serum phenytoin concentration is 10-20 µg/ml.*

elimination rates of drugs were not affected by renal failure [54]. By measuring the total body clearance of a drug and its renal clearance in normal subjects, the calculated difference is the non-renal clearance. Total body clearance of a drug can be measured by measuring the steady state drug level during a constant infusion of the drug (clearance is infusion rate divided by steady state level) or the area under the drug level versus time curve after a single dose (clearance is dose divided by area under the drug level versus time curve). The non-renal clearance is considered constant while the renal clearance falls in proportion to the fall in GFR. One can then calculate the dose of the drug for a patient with any degree of renal function from data obtained from normal subjects. First, take the fraction of non-renal clearance divided by the total body clearance and multiply this by the usual dose in metabolically normal subjects. The result is the dose for anuric patients. The difference between this dose and the usual dose for normals is the amount of the usual dose that is excreted by the kidneys. This amount times the patient's GFR divided by the normal GFR (120 ml/min for a normal size adult) is the amount of drug to replace that amount excreted by the kidneys. Thus, the calculated dose for a patient with any degree of renal insufficiency is the sum of the anuric person's dose plus the dose to replace the amount renally excreted. This dose is given at the usual dose interval. The advantage of this concept and calculation is that one can estimate the dose of a drug for a person with any degree of renal failure using only data from normal individuals. The weakness is in the assumptions that are made: that non-renal clearance is unchanged in uremia, that active metabolites do not accumulate and produce effects, and that there is no change in the tissue sensitivity to the drug in uremia. While these assumptions are true for many drugs, they are not true for all. One should consider the pharmacology of each specific drug and its metabolites to estimate the validity of these assumptions. More common is the problem of a patient undergoing an extracorporeal procedure such as dialysis that adds another pathway of drug elimination. The amount of drug eliminated by this pathway should be added to the amount of drug eliminated by the other pathways to determine the new dose.

The concept of Dettli was first used to reduce the dose of a drug and keep the interval between doses the same as for metabolically normal people. Another way is to use the concept to determine the total daily dose of a drug and then have each individual dose the same as in normals but prolong the interval between doses. Either method will work and often a mixture of the two, reducing the dose a bit and prolonging the interval a bit, will be the most practical approach. Because slowed drug excretion prolongs the drug half life, one cannot reproduce the exact drug level versus time curve of a normal person in a patient with renal failure no matter how one adjusts the dose and interval between doses. One can reproduce the overall drug exposure (area under the drug level versus time curve) or the peak level and trough level but not both the levels and the total exposure. If the peak and trough levels are wanted, the total exposure will be increased. If total exposure is wanted, then peak and/or trough levels will be changed. One has to determine which is desired and recognize that the other will be changed.

Tissue sensitivity

Individualizing drug dosage to compensate for changed pharmacokinetic factors in patients with renal failure will not address changes in tissue sensitivity to drugs that occur in renal failure. Some effects, such as lack of sensitivity to diuretics or to the cholecalciferol form of vitamin D, are direct results of the diseased kidney being the site for the drug to act or be activated. Renal failure also produces changes in the body that modify the sensitivity of other tissues to some drugs. Some classic examples include increased sensitivity to thiopental anesthesia [55] and decreased sensitivity of skeletal muscle to insulin [56]. Because it is so much easier to measure drug levels than the intensity of drug effects, most studies of drugs in renal failure are kinetic studies only. Studies relating drug level or even dose to intensity of effect are hard to accomplish and therefore uncommon. In general, individualizing dose to correct for abnormal kinetics will correct for most of the variations in dose-response and only leave the residual variation in sensitivity to the drug.

Conclusions

In the past, renal failure was a clear predisposing cause for an increased incidence of adverse drug reactions. Systematic studies of drug kinetics and drug sen-

sitivity in renal failure have shown how patients with renal failure differ from metabolically normal patients. Generalizations developed from these studies have lead to the principles that enable one to individualize drug therapy for azotemic patients. Using these principles, one can make the response to drugs more predictable for the patient with renal failure. And more predictable means safer.

References

1. Yasuda K, Lan LB, Sanglard D, Furuya K, Schuetz JD, Schuetz EG. Interaction of cytochrome P-450 3A inhibitors with P-glycoprotein. J Pharmacol Exp Ther 2002; 303: 323-332.
2. Cummins CL, Jacobsen W, Benet LZ. Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. J Pharmacol Exp Ther 2002; 300: 1036-1045.
3. Dresser GK, Schwarz UI, Wilkinson GR, Kim RB. Coordinate induction of both cytochrome P-4503A and MDR1 by St John's wort in healthy subjects. Clin Pharmacol Ther 2003; 73: 41-50.
4. Fleming I. Cytochrome P-450 enzymes in vascular homeostasis. Circulation Res 2001; 89: 753-762.
5. Nebert DW, Russell DW. Clinical importance of the cytochromes P-450. Lancet 2002; 360: 1155-1162.
6. Yu Z, Huse LM, Adler P, Graham L, Ma J, Zeldin DC, Kroetz DL. Increased CYP2J expression and epoxyeicosatrienoic acid formation in spontaneously hypertensive rat kidney. Mol Pharmacol 2000; 57: 1011-1020.
7. Holla VR, Makita K, Zaphiropoulos PG, Capdevila JH. The kidney cytochrome P-450 2C23 arachidonic acid epoxygenase is upregulated during dietary salt loading. J Clin Invest 1999; 104: 751-760.
8. Renton KW. Alteration of drug biotransformation and elimination during infection and inflammation. Pharmacol Ther 2001; 92: 147-163.
9. Mancinelli LM, Frassetto L, Floren LC, Dressler D, Carrier S, Bekersky I, Benet LZ, Christians U. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. Clin Pharmacol Ther 2001; 69: 24-31.
10. Weinshilboum R. Inheritance and drug response. New Engl J Med 2003; 348: 529-537.
11. Tanigawara Y. Role of P-glycoprotein in drug disposition. Ther Drug Monit 2000; 22: 137-140.
12. Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res 2001; 42: 1007-1017.
13. Stouch TR, Gudmundsson O. Progress in understanding the structure-activity relationships of P-glycoprotein. Adv Drug Deliv Rev 2002; 54: 315-328.
14. Benet LZ, Cummins CL. The drug efflux-metabolism alliance: biochemical aspects. Adv Drug Deliv Rev 2001; 50 (suppl 1): S3-S11.
15. Lin JH, Yamazaki M. Role of p-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinetics 2003; 42: 59-98.
16. Kullak-Ublick GA, Beuers U, Paumgartner G. Hepatobiliary transport. J Hepatol 2000; 32 (suppl 1): 3-18.
17. Fricker G, Miller DS. Relevance of multidrug resistance proteins for intestinal drug absorption *in vitro* and *in vivo*. Pharmacol Toxicol 2002; 90: 5-13.
18. Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A, Ohdo S, Ohtani H, Sawada Y, Higuchi S, Otsubo K. Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. Clin Pharmacol Ther 2002; 72: 209-219.
19. Siegmund W, Ludwig K, Giessmann T, Dazert P, Schroeder E, Sperker B, Warzok R, Kroemer HK, Cascorbi I. The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. Clin Pharmacol Ther 2002; 72: 572-583.
20. Johne A, Kopke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Brinkmann U, Eichelbaum M, Brockmoller J, Cascorbi I, Roots I. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. Clin Pharmacol Ther 2002; 72: 584-294.
21. Cummins CL, Wu CY, Benet LZ. Sex-related differences in the clearance of cytochrome P-450 3A4 substrates may be caused by P-glycoprotein. Clin Pharmacol Ther 2002; 72: 474-489.
22. Masereeuw R, Terlouw SA, van Aubel RA, Russel FG, Miller DS. Endothelin B receptor-mediated regulation of ATP-driven drug secretion in renal proximal tubule. Mol Pharmacol 2000; 57(1): 59-67.
23. Terlouw SA, Masereeuw R, Russel FG, Miller DS. Nephrotoxicants induce endothelin release and signaling in renal proximal tubules: effect on drug efflux. Mol Pharmacol 2001; 59(6): 1433-1440.
24. Miller DS. Xenobiotic export pumps, endothelin signaling, and tubular nephrotoxicants - a case of molecular hijacking. J Biochem Mol Toxicol 2002; 16(3): 121-127.

25. Jalava KM, Partanen J, Neuvonen PJ. Itraconazole decreases renal clearance of digoxin. Ther Drug Monit 1997; 19(6): 609-613.
26. Wakasugi H, Yano I, Ito T, Hashida T, Futami T, Nohara R, Sasayama S, Inui K. Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. Clin Pharmacol Ther 1998; 64(1): 123-128.
27. del Moral RG, Andujar M, Ramirez C, Gomez-Morales M, Masseroli M, Aguilar M, Olmo A, Arrebola F, Guillen M, Garcia-Chicano MJ, Nogales FF, O'Valle F. Chronic cyclosporin A nephrotoxicity, P-glycoprotein overexpression, and relationships with intrarenal angiotensin II deposits. Am J Pathol 1997; 151(6): 1705-1714.
28. Miller DS, Fricker G, Drewe J. p-Glycoprotein-mediated transport of a fluorescent rapamycin derivative in renal proximal tubule. J Pharmacol Exp Ther 1997; 282(1): 440-444.
29. Gutmann H, Fricker G, Drewe J, Toeroek M, Miller DS. Interactions of HIV protease inhibitors with ATP-dependent drug export proteins. Mol Pharmacol 1999; 56(2): 383-389.
30. Koziolek MJ, Riess R, Geiger H, Thevenod F, Hauser IA. Expression of multidrug resistance P-glycoprotein in kidney allografts from cyclosporine A-treated patients. Kidney Int 2001; 60(1): 156-166.
31. Hall SD, Thummel KE, Watkins PB, Lown KS, Benet LZ, Paine MF, Mayo RR, Turgeon DK, Bailey DG, Fontana RJ, Wrighton SA. Molecular and physical mechanisms of first-pass extraction. Drug Metab Dispos 1999; 27(2): 161-166.
32. Doherty MM, Charman WN. The mucosa of the small intestine: how clinically relevant as an organ of drug metabolism ? Clin Pharmacokinet 2002; 41(4): 235-253.
33. Barone GW, Gurley BJ, Ketel BL, Abul-Ezz SR. Herbal supplements: a potential for drug interactions in transplant recipients. Transplantation 2001; 71: 239-241.
34. Muscari A, Puddu GM, Puddu P. Lipid-lowering drugs: are adverse effects predictable and reversible ? Cardiology 2002; 97: 115-121.
35. Vlahakos DV, Manginas A, Chilidou D, Zamanika C, Alilvizatos PA. Intraconazole-induced rhabdomyolysis and acute renal failure in a heart transplant recipient treatad with simvastatin and cyclosporine. Transplantation 2002; 73: 1962-1964.
36. Kane GC, Lipsky JJ. Drug-grapefruit juice interactions. Mayo Clin Proc 2000; 75: 933-942.
37. Flockhart DA, Tanus-Santos JE. Implications of cytochrome P450 interactions when prescribing medication for hypertension. Arch Intern Med 2002; 162: 405-412.
38. Craig RM, Murphy P, Gibson TP, Quintanilla A, Chao C, Cochrane C, Patterson A, Atkinson AJ. Kinetic analysis of D-xylose absorption in normal subjects and in patients with chronic renal failure. J Lab Clin Med 1983; 101: 496-506.
39. Kunihara M, Nagai J, Murakami T, Takano M. Renal excretion of rhodamine 123, a P-glycoprotein substrate, in rats with glycerol-induced acute renal failure. J Pharm Pharmacol 1998; 50(10): 1161-1165.
40. Huang ZH, Murakami T, Okochi A, Yumoto R, Nagai J, Takano M. Expression and function of P-glycoprotein in rats with glycerol-induced acute renal failure. Eur J Pharmacol 2000; 406(3): 453-460.
41. Veau C, Leroy C, Banide H, Auchere D, Tardivel S, Farinotti R, Lacour B. Effect of chronic renal failure on the expression and function of rat intestinal P-glycoprotein in drug excretion. Nephrol Dial Transplant 2001; 16(8): 1607-1614.
42. Laouari D, Yang R, Veau C, Blanke I, Friedlander G. Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure. Am J Physiol Renal Physiol 2001; 280(4): F636-F645.
43. Boobis SW. Alteration of plasma albumin in relation to decreased drug binding in uremia. Clin Pharmacol Ther 1978; 22: 147-153.
44. Matzke GR, Frye RF. Drug administration in patients with renal insufficiency. Drug Safety 1997; 16: 205-231.
45. Reidenberg MM. The biotransformation of drugs in renal failure. Am J Med 1977; 62: 482-485.
46. Gibson TP. Renal disease and drug metabolism: an overview. Am J Kidney Dis 1986; 8: 7-17.
47. Wood AJJ, Zhou HH. Ethnic differences in drug disposition and responsiveness. Clin Pharmacokinetics 1991; 20: 350-373.
48. Brosen L. Recent developments in hepatic drug oxidation. Clin Pharmacokinet 1990; 18: 220-239.
49. Reidenberg MM, Affrime M. Influence of disease on binding of drugs to plasma proteins. Ann NY Acad Sci 1973; 226: 115-126.
50. Drayer DE. Pharmacologically active drug metabolites: therapeutic and toxic activities, plasma and urine data in man, accumulation in renal failure. Clin Pharmacokinet 1976; 1: 426-443.
51. Drayer DE, Lowenthal DT, Woosley RL, Nies AS, Schwartz A, Reidenberg MM. Cumulation of N-acetylprocainamide, an active metabolite of procainamide, in patients with impaired renal function. Clin Pharmacol Ther 1977; 22: 63-69.
52. Szeto HH, Inturrisi CE, Houde R, Saal S, Cheigh J, Reidenberg MM. Accumulation of normeperidine, an active metabolite of meperidine, in patients with renal failure or cancer. Ann Intern Med 1977; 86: 738-741.
53. Kaiko RF, Foley KM, Grabinski PY, Heidrich G, Rogers AG, Inturrisi CE, Reidenberg MM. Central nervous system excitatory effects of meperidine in cancer patients. Ann Neurology 1983; 13: 180-185.
54. Dettli L, Spring P, Ryter S. Multiple dose kinetics and drug dosage in patients with kidney disease. Acta Pharmacol Toxicol 1971; 29 (suppl 3): 211-224.

55. Dundee JW, Richards RK. Effect of azotemia upon the action of intravenous barbiturate anesthesia. Anesthesiology 1954; 15: 333-346.
56. Mak RH, DeFronzo RA. Glucose and insulin metabolism in uremia. Nephron 1992; 61(4): 377-382.
57. Hall SD, Thummel KE, Watkins PB, Lown KS, Benet LZ, Paine MF, Mayo RR, Turgeon DK, Bailey DG, Fontana RJ, Wrighton SA. Molecular and physical mechanisms of first-pass extraction. Drug Metab Dispos 1999; 27(2): 161-166.
58. Lo A, Burckart GJ. P-glycoprotein and drug therapy in organ transplantation. J Clin Pharmacol 1999; 39(10): 995-1005.
59. van Gelder T, Klupp J, Sawamoto T, Christians U, Morris RE. ATP-binding cassette transporters and calcineurin inhibitors: potential clinical implications. Transplant Proc 2001; 33(3): 2420-2421.

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Drug dosage in renal failure

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Introduction

Evolution within the field of dialysis and advances in surgical procedures providing for superior access for shunt placement have made it possible to treat patients with end-stage renal disease with dialysis therapy for more than 50 years. Improved pharmacotherapy of pre- and post- dialysis has also contributed to these remarkable advancements. Drug therapy is also evolving. Health care provider for patients with renal diseases needs to understand the latest drug therapy and ensure appropriateness of therapy in each individual patient [1].

Renal insufficiency and dialysis alter the pharmacokinetics and pharmacodynamics of most commonly used drugs. Most common, an average of eight different classes of drugs per patient are prescribed in patients with renal failure. In comparison to the general population, patients with renal insufficiency experience significantly more adverse drug reactions. Therefore, clinicians should be familiar with the pharmacokinetic behavior of each agent and of the impact of renal fail-

M.E. De Broe, G.A. Porter, W.M. Bennett & G.A. Verpooten (Editors). Clinical Nephrotoxins, 2nd Ed., 667-679

ure on the drug elimination process. A particular area of concern is that many patients with renal insufficiency are elderly which in itself may effect drug disposition. Most therapeutic agents or their metabolites are completely or partially eliminated by the kidneys. In patients with renal insufficiency both metabolism and elimination is impaired, therefore, these patients are at a greater risk of adverse drug reactions or drug toxicity. Because of co-morbid conditions, most patients with advanced renal diseases require multiple medications for the treatment of hypertension, hyperlipidemia, hyperuricemia and congestive heart failure. Patients with chronic renal failure are at a greater risk of drug-drug interactions. Finally, depending on various factors such as the size of the drug molecule and degree of protein binding, a significant amount of drug removal may occur during dialysis. Most drug dosages may be adjusted based on plasma therapeutic concentrations (Table 1). To prevent toxicity and optimize efficacy, it is critical that these factors be taken into account and appropriate dosage adjustments made when prescribing drugs for dialysis patients [2-12]. This chapter discusses the pharmacological principles for prescribing drugs in this population and provides specific dosage recommendations (Tables 2-7).

Principles of pharmacokinetics in uremia

Pharmacokinetics is the study of drug behavior (absorption, distribution, metabolism and elimination) in the body. The ability of the body to remove a drug is called clearance. Clearance indicates the intrinsic ability of the body to decrease plasma drug concentration. The three major processes effecting drug clearance are metabolism by the liver, metabolism by the gastrointestinal tract (cytochrome P-450 and P-glycoproteins) or elimination and metabolism by the kidney. At steady state, the overall rate of clearance is equal to rate of drug absorption [1]. The important elements of a drug's pharmacokinetics are shown in Figure 1.

All important pharmacokinetic parameters, such as drug absorption, volume of distribution, protein-protein binding, and drug metabolism must be taken into account when dose modifications are made in uremic patients. For example, gastroparesis in diabetic patients, slow gastric emptying time and edema of gastrointestinal tract in patients with advanced renal failure may affect drug absorption. Iron preparations and phosphate binders may also alter drug absorption [2].

Absorption

Following oral drug administration, only a certain proportion of the drug is absorbed reaching systemic circulation (F or bioavailability). The percentage of a drug dose that appears in the systemic circulation following oral administration compared with the intravenous route for the same drug defines its bioavailability. In general, drugs given by the intravenous route reach the central compartment directly and usually have a more rapid onset of action. Drugs given by other routes must pass through a series of biologic membranes before entering the systemic circulation. For many drugs, only a fraction of the administered dose may reach the circulation to exert any pharmacodynamic effect [3]. Chronic renal failure may influence drug absorption and bioavailability. The dissolution rate, chemical forms, rout of administration, the gastrointestinal stability and dosage form may alter drug's bioavailability. Bioavailability only indicates the extent of drug absorption not the rate of drug absorption. Drugs can be highly bound to plasma proteins (e.g. aspirin) or unbound (free active moiety). Only the free or unbound concentration of the drug interacts with specific receptors at the site of pharmacologic action. The liver can either metabolize drugs in the 'first pass' as the drug is absorbed into the portal circulation, or later when the drug is delivered to the liver via the systemic blood flow prior to reaching systemic circulation. First pass metabolism can significantly reduce the rate and extent of drug absorption. Most often, gastric pH is high due to the use of antacids or anti-

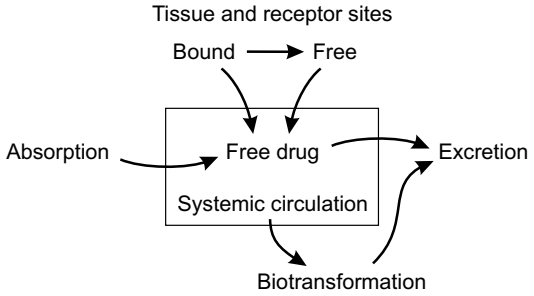

Figure 1. *Interrelationship of absorption, distribution biotransformation and excretion.*

Table 1. Therapeutic drug monitoring (TDM).

Drug Name	Therapeutic range	When to draw sample	How often to draw levels
Aminoglycosides (Conventional Dosing) Gentamicin & Tobramycin: Amikacin:	 Peak: 5-8 mg/L. Trough: 0.5-2 mg/L Peak: 20-30 mg/L. Trough: <10mg/L	Trough: immediately prior to dose Peak: 30 min after a 30min infusion	Check P & T with 3rd dose For therapy less than 72h, levels not necessary. Repeat drug levels weekly or if renal function changes
Aminoglycosides (24 hour Dosing)	0.5 - 3 mg/L	Obtain random drug level 12 hours after dose	After initial dose. Repeat drug level in 1 week or if renal function changes
Carbamazepine	4-12 μg/ml	Trough: immediately prior to dosing	Check 2 to 4 days after first dose or change in dose
Cyclosporine	150-400 ng/ml	Trough: immediately prior to dosing	Daily for first week, then weekly.
Digoxin	0.8 - 2.0 ng/ml	12 hours after maintenance dose	5-7 days after first dose for patients with normal renal and hepatic function; 15-20 days in anephric patients
Lidocaine	1 - 5 μg/ml	8 hours after IV infusion started or changed	
Lithium	Acute: 0.8-1.2 mEq/L Chronic: 0.6-0.8 mEq/L	Trough: Before AM dose at least 12 hours since last dose	
Phenobarbital	15-40 μg/ml	Trough: immediately prior to dosing	Check 2 weeks after first dose or change in dose. Follow-up level in 1 to 2 months
Phenytoin Free Phenytoin	10-20μg/ml 1-2μg/ml	Trough: immediately prior to dosing	5-7 days after first dose or after change in dose
Procainamide NAPA (n-acetyl procainamide) a procainamide metabolite	4-10 μg/ml Peak: 8 μg/ml. Trough: 4 μg/ml 10-30 μg/ml	Trough: immediately prior to next dose or 12-18 hours after starting or changing an infusion. Draw with procainamide sample	
Quinidine	1-5 μg/ml	Trough: immediately prior to next dose	
Sirolimus	10-20 ng/dl	Trough: immediately prior to next dose	
Tacrolimus (FK-506)	10-15 ng/ml	Trough: immediately prior to next dose	
Theophylline PO or Aminophylline IV	15-20 μg/ml	Trough: immediately prior to next dose	
Valproic Acid (divalproex sodium)	40-100 μg/ml	Trough: immediately prior to next dose	Check 2 to 4 days after first dose or change in dose
Vancomycin	Peak: 25-40 mg/L Trough: 5-15 mg/L	Trough: immediately prior to dose Peak: 60 min after a 60min infusion	With 3rd dose (when initially starting therapy, or after each dosage adjustment). For therapy less than 72h, levels not necessary. Repeat drug levels if renal function changes

ulcer medications that may result in decreased absorption of medications requiring an acid milieu. Aluminum- or calcium-containing antacids may also form non-absorbable chelation products with certain drugs, such as digoxin or tetracycline and impair these agents's absorption [4-6].

Volume of distribution

Following drug absorption, individual drugs distribute throughout the body in a characteristic manner. The apparent volume of distribution (V_d) is the quantity of drug in the body (L/kg body weight) divided by the plasma concentration at steady state. Volume distribution also represents the amount of water that is needed for a drug to dissolve to reach an observed plasma concentration. Therefore, lipophilic agents or drugs with high tissue binding capacity most commonly have a large volume of distribution. In contrast, drugs with high circulating protein binding or water-soluble drugs have a small volume of distribution. Drugs that are largely confined to the intravascular compartment usually have a volume of distribution less then 0.2 L/kg. Uremia, edema and renal failure may alter the volume of distribution of most commonly used agents in patients with renal insufficiency [7-9]. Changes in volume of distribution are usually not clinically significant except for those drugs which have a small volume of distribution under normal circumstances (i.e., >0.7 L/kg).

Protein binding

Unbound or free drugs are pharmacologically active. Therefore, the degree of protein binding is an important issue in adjustment of drug dosing in renal failure. Low plasma albumin or increase in plasma albumin can potentially increase the pharmacodynamic effects of highly bound drugs. Organic acids usually have a single binding site on albumin whereas organic bases tend to have multiple binding sites and their behavior in the presence of increasing renal insufficiency is less predictable. In general, acidic drugs have reduced plasma protein binding in patients with renal failure; this reduction is attributable to a combination of decreased albumin concentration and a reduction in albumin affinity, which is, in turn, influenced by either structural changes in the albumin molecule or accumulation of competing endogenous inhibitors of protein binding. For some agents with high protein binding, the reduced sites or decreased plasma protein can cause potentially important pharmacologic consequences. For example in patients with renal failure, the free plasma concentration of phenytoin increases from 0.1 to 0.35. Therefore, the observed plasma concentration of 4 mg/L is comparable to 10-15 mg/L in patients with normal renal function. Finally only unbound or free drugs are available for drug metabolism or excretion. Uremia may decreases binding capacity of most drugs and result in increased metabolism in patients with renal failure. For any given drug therapeutic concentration (bound plus unbound), the proportion of free or active drug is increased. It is more desirable to obtain free drug plasma concentrations in patients with renal failure [10-13].

Eliminations

The presence of progressive renal insufficiency affects most body biochemical processes including drug biotransformation. In addition, some drugs have pharmacologically active metabolites, which, although unimportant in patients with normal renal function, may accumulate in patients with renal insufficiency causing adverse drug reactions [13-17]. Some of these pharmacologically active metabolites may account for the high incidence of adverse drug reactions in patients with renal failure. Some of the best-known examples of this phenomenon are the accumulation of pharmacologically active metabolites of meperidine causing seizures, nitrofurantoin causing peripheral neuropathy and morphine sulfate causing excess respiratory depression. The metabolic biotransformation of drugs to another more water-soluble chemical moiety also may be altered in uremia. In patients with renal failure, chemical reduction, acetylating, ester or peptide hydrolysis may be delayed, whereas metabolism by hepatic microsomal oxidation is usually normal. Drug elimination rate is usually expressed as elimination half-life (t_2). Drug half-life is the time required for the plasma concentration to decrease by 50%. The half-life is dependent upon V_d and clearance (renal, hepatic, or other) as expressed by the formula:

$$t_2 = 0.693 \times V_d/\text{clearance}$$

For drugs eliminated primarily intact through the kidneys, as the renal clearance decreases, t_2 will in-

crease (assuming that V_d is unchanged). It should be noted that active drug metabolites may also be excreted by the kidney and therefore have a prolonged half-life in renal failure.

Dosing regimens

Most drug or their metabolites that are normally excreted unchanged by the kidney will require dosage modification in advanced renal failure. The loading dose of a drug will stay the same unless the V_d is significantly altered. The maintenance regimen may be modified by the interval extension method or dosage reduction. The interval extension method utilizes the same dose at greater intervals and is useful for drugs with long half-lives. The dosage reduction method reduces the dosage and leaves the interval between doses unchanged. This method generally leads to more constant serum levels. Therapeutic drug monitoring is a useful method in guiding drug therapy and preventing toxicity. Interpretation of drug levels must be made in light of the amount of drug given; the time elapsed since the last dose, and the route of administration and clinical scenario of the patients [14-18].

References

1. Winters ME, Basic clinical pharmacokinetics. Winter ME, Applied Therapeutic Inc. Vancouver, WA 1994.
2. Zhang Y. Benet LZ. The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. Clinical Pharmacokinetics. 40(3):159-68, 2001
3. Olyaei AJ, Bennett WM. "The Effect of Renal Failure on Drug Handling". Oxford Textbook of Critical Care, 1996.
4. Benedetti P. de Lalla F. Antibiotic therapy in acute renal failure. Contributions to Nephrology. (132):136-45, 2001.
5. Olyaei AJ, deMattos AM, Bennett WM. "Prescribing Drugs in Renal Failure". Brenner and Rector's, The Kidney, 2000.
6. Olyaei AJ, deMattos AM, Bennett WM. "Drug-drug Interaction and most Commonly Used Drug in Transplant Recipients" . Primer on Transplantation, American Society of Transplantation. 2000
7. Olyaei AJ " Drug Dosing in Renal Failure " in Treatment Strategies In Nephrology and Hypertension. By Bennett 1st edition. PocketMedicine.com Inc 2001
8. Olyaei AJ, deMattos AM, Bennett WM "Principle of Drug Usage in Dialysis Patients" Dialysis Therapy, Hanley & Belfus Inc. Medical Publishers. May 2001
9. Olyaei AJ, deMattos, Bennett WM, Drug usage in dialysis patients. In: Nissenson AR, Fine RN, Gentile DE, eds. Clinical dialysis. Norwalk, NJ: Appleton and Lange; 2001.
10. Bennett WM. Guide to drug dosage in renal failure. In: Speight TM, Holford N, eds. Avery's drug treatment, 4th edn. Auckland: ADIS International; 1997:1725–92.
11. Beauchamp D, Labrecque G. Aminoglycoside nephrotoxicity: do time and frequency of administration matter?. Current Opinion in Critical Care 2001:7: 401-408.
12. Livornese LL, Jr., Slavin D, Benz RL, Ingerman MJ, Santoro J. Use of antibacterial agents in renal failure. Infectious Disease Clinics of North America 2001:15: 983-1002.
13. Chertow GM, Lee J, Kuperman GJ et al. Guided medication dosing for inpatients with renal insufficiency. JAMA 2001:286: 2839-2844.
14. Bugge JF. Pharmacokinetics and drug dosing adjustments during continuous venovenous hemofiltration or hemodiafiltration in critically ill patients. Acta Anaesthesiologica Scandinavica 2001:45: 929-934.
15. Izzedine H, Launay-Vacher V, Baumelou A, Deray G. An appraisal of antiretroviral drugs in hemodialysis. [Review] [47 refs]. Kidney International 2001:60: 821-830.
16. Subach RA, Marx MA. Drug dosing in acute renal failure: the role of renal replacement therapy in altering drug pharmacokinetics. Advances in Renal Replacement Therapy 1998:5: 141-147.
17. Joy MS, Matzke GR, Armstrong DK, Marx MA, Zarowitz BJ. A primer on continuous renal replacement therapy for critically ill patients. Annals of Pharmacotherapy 1998:32: 362-375.
18. Joos B, Schmidli M, Keusch G. Pharmacokinetics of antimicrobial agents in anuric patients during continuous venovenous haemofiltration. Nephrology Dialysis Transplantation 1996:11: 1582-1585.

Table 2. Antimicrobial agents.

Drugs normal doses		% of renal excretion	Dosage adjustment in renal failure: GFR: >50	10-50	<10	Comments
AMINOGLYCOSIDES						Nephrotoxic, ototoxic, may prolong the neuromuscular blockade effect of muscle relaxants. Check level post-HD
Gentamicin	2 mg/kg q8hrs	95%	60-90% q12hrs	30-70% q24hrs	20-30% q24hrs	Peak 6-8, Trough <2
Tobramicin	2 mg/kg q8hrs	95%	''	''	''	Peak 6-8, Trough <2
Netilmicin	2 mg/kg q8hrs	95%	''	''	''	Peak 6-8, Trough <2
Amikacin	7.5 mg/kg q12hrs	95%	''	''	''	Peak 20-30, Trough <5
CEPHALOSPORIN						Coagulation abnormalities, transitory elevation of BUN, rash and serum sickness like syndrome
Oral Cephalosporin						
Cefaclor	250-500 mg tid	70%	100%	100%	50%	
Cefadroxil	500 to 1 g bid	80%	100%	100%	50%	
Cefixime	200 to 400 mg q12h	85%	100%	100%	50%	
Cefpodoxime	200 mg q12hrs	30%	100%	100%	100%	
Ceftibuten	400 mg q24hrs	70%	100%	100%	50%	
Cefuroxime	250-500 mg tid	90%	100%	100%	100%	
Cephalexin	250-500 mg tid	95%	100%	100%	100%	
Cephradine	250-500 mg tid	100%	100%	100%	50%	
IV Cephalosporin						
Cefamandol	1-2 g IV q6-8hrs	100%	q6hrs	q8hrs	q12hrs	
Cefazolin	1-2 g IV q8hrs	80%	q8hrs	q12hrs	q12-24hrs	
Cefepime	1-2 g IV q8hrs	85%	q8-12hrs	q12hrs	q24hrs	
Cefmetazole	1-2 g IV q8hrs	85%	q8hrs	q12hrs	q24hrs	
Cefixime	200 mg q12hr	50%	q12hrs	q24hrs	q24hrs	
Cefoperazone	1-2 g IV q12hrs	20%	No renal adjustment is required			
Cefotaxime	1-2 g IV q6-8hrs	60%	q8hrs	q12hrs	q12-24hrs	
Cefotetan	1-2 g IV q12hrs	75%	q12hrs	q12-24hrs	q24hrs	
Cefoxitin	1-2 g IV q6hrs	80%	q6hrs	q8-12hrs	q12hrs	
Cefpodoxime	200-400 mg po q12hrs	30%	q12hrs	q12-24hrs	q24hrs	Three times a week in dialysis patients
Ceftazidime	1-2 g IV q8hrs	70%	q8hrs	q12hrs	q24hrs	
Ceftriaxone	1-2 g IV q24hrs	50%	No renal adjustment is required			
Cefuroxime	750-1.5 g IV q8hrs	90%	q8hrs	q8-12hrs	q12-24hrs	
PENICILLIN						Bleeding abnormalities, hypersensitivity
Oral Penicillin						
Amoxicillin	500 mg po tid	60%	100%	100%	50-75%	
Ampicillin	500 mg po q6hrs	60%	100%	100%	50-75%	
Dicloxacillin	250-500 mg po q6hrs	50%	100%	100%	50-75%	
Penicillin V	250-500 mg po q6hrs	70%	100%	100%	50-75%	

Table 2 continued.

	Drugs normal doses	% of renal excretion	Dosage adjustment in renal failure: GFR: >50	10-50	<10	Comments
IV Penicillin						
Ampicillin	1-2 g IV q6hrs	60%	q6hrs	q8hrs	q12hrs	
Nafcillin	1-2 g IV q4hrs	35%	No renal adjustment is required			
Penicillin G	2-3 million Units IV q4hrs	70%	q4-6hrs	q6hrs	q8hrs	
Piperacillin	3-4 g IV q4-6hrs		No renal adjustment is required			
Ticarcillin/ clavulanate	3.1 g IV q4-6hrs		100%	3.1 g q8hrs	3.1 g q12hrs	
Piperacillin/ tazobactam	3.375 g IV q6-8hrs		100%	2.25 g q6hrs	2.25 g q8hrs	
QUINOLONES						Photosensitivity, Food, dairy products, tube feeding and Al (OH)3 may decrease the absorption of quinolones
Ciprofloxacin	200-400 mg IV q24hrs	60%	q12hrs	q12-24hrs	q24hrs	
Gatifloxacin	400 mg po/IV q24hrs	88%	100%	50%	50%	
Levofloxacin	500 mg po qd	70%	100%	50%	50%	
Moxifloxacin	400 mg qd	20%	No renal adjustment is required			
Norfloxacin	400 mg po q12hrs	30%	q12hrs	q12-24hrs	q24hrs	
Ofloxacin	200-400 mg po q12hrs	70%	q12hrs	q12-24hrs	q24hrs	
MISCELLANEOUS AGENTS						
Azithromycin	250-500 mg po qd	6%	No renal adjustment is required			NO drug-drug interaction with CSA/KF
Clarithromycin	500 mg po bid	20%	No renal adjustment is required			Pseudomembranous colitis
Clindamycin	150-450 mg po tid	10%	No renal adjustment is required			Increase CSA/FK level,
Dirithromycin	500 mg po qd		No renal adjustment is required			
Erythromycin	250-500 mg po qid	15%	No renal adjustment is required			Increase CSA/FK level, avoid in transplant patients
Imipenem/ Cilastatin	250-500 mg IV q6hrs	50%	500 mg q8hrs	250-500 q8-12hrs	250 mg q12hrs	Seizure
Linezolid	400-600 mg IV/PO q12hrs	30%	q12hrs	q12hrs	q12hrs	Thrombocytopenia
Meropenem	1 g IV q8hrs	65%	1 g q8hrs	0.5-1 g q12hrs	0.5-1 g q24hrs	
Metronidazole	500 mg IV q6hrs	20%	No renal adjustment is required			Peripheral neuropathy, increase LFTs, disulfiram reaction with alcoholic beverages
Pentamidine	4 mg/kg/day	5%	q24hrs	q24hrs	q48hrs	Inhalation may cause bronchospasm, IV administration may cause hypotension, hypoglycemia and nephrotoxicity
Rifampin	300-600 mg po qd	20%	No renal adjustment is required			Decrease CSA/FK level
Trimethoprim/ Sulfamethoxazole	DS po q12hrs	70%	q12hrs	q12hrs	q24hrs	Increase serum creatinine
Vancomycin	1 g IV q12hrs	90%	q12hrs	q24-36 hrs	q48-72 hrs	Nephrotoxic, ototoxic, may prolong the neuromuscular blockade effect of muscle relaxants. Peak 30, trough 5-10
Vancomycin oral	125-250 mg po qid	0%	100%	100%	100%	Oral vancomycin is indicated only for the treatment of C. diff

Table 2 continued.

Drugs normal doses		% of renal excretion	Dosage adjustment in renal failure: GFR: >50	10-50	<10	Comments
ANTIFUNGAL AGENTS						
Amphotericin B	0.5 mg-1.5 mg/kg/day	<1%	No renal adjustment is required			Nephrotoxic, infusion related reactions, give 250 cc NS before each dose
Amphotec	4-6 mg/kg/day	<1%	No renal adjustment is required			
Abelcet	5 mg/kg/day	<1%	No renal adjustment is required			
AmBisome	3-5 mg/kg/day	<1%	No renal adjustment is required			Less nephrotoxic
Azoles and other antifungals						Increase CSA/FK level
Fluconazole	200-800 mg IV qd/bid	70%	100%	100%	50%	
Itraconazole	200 mg q12hrs	35%	100%	100%	50%	Poor oral absorption
Ketoconazole	200-400 mg po qd	15%	100%	100%	100%	Hepatotoxic
Miconazole	1200-3600 mg/day	1%	100%	100%	100%	
Terbinafine	250 mg po qd	< 1%	100%	100%	100%	CHF & edema
Voriconazole	200 mg bid		100%	100%	Avoid	Ocular toxicity
ANTIRETROVIRAL AGENTS						
Abacavir	300 mg q12hrs	<1%	100%	100%	100%	
Amprenavir	1200 mg q12hrs		100%	100%	100%	
Delavirdine	400 mg q8hrs	<5%	100%	100%	100%	
Didanosine	200 q12hrs	18%	100%	200 mg qd	100 mg qd	Pancreatitis
Efavirenz	600 mg qd	<1%	100%	100%	100%	
Indinavir	800 mg q8hrs	<20%	100%	100%	100%	May cause nephrolithiasis Require adequate hydration
Lamivudine	150 mg bid	71%	100%	100-150 mg qd	25-50 mg qd	
Lopinavir/ ritonavir	400/100 mg q12hrs	< 3%	100%	100%	100%	
Nelfinavir	750 mg q8hrs	< 1%	100%	100%	100%	
Nevirapine	200 mg q12hrs	< 5%	100%	100%	100%	
Ritonavir	600 mg q12hrs		100%	100%	100%	
Saquinavir	200 mg q8hr	< 1%	100%	100%	100%	Take with meals
Stavudine	40 mg q12hrs	40%	100%	20 mg q12hrs	20 mg q24hrs	
Zalcitabine	0.75 mg q8hrs	70%	100%	0.75 mg q12hrs	0.75 mg q24hrs	Neuropathy
Zidovudine	100 mg q4hrs	14%	100%	100 mg q6hrs	100 mg q8hrs	Pancytopenia

Table 2 continued.

Drugs normal doses		% of renal excretion	Dosage adjustment in renal failure: GFR: >50	10-50	<10	Comments
ANTIVIRAL AGENTS						
Amantadine	100-200 mg q12hrs	90%	100%	50%	25%	
Famciclovir	250-500 mg po bid to tid	60%	q8hrs	q12hrs	q24hrs	VZV: 500 mg po tidHSV: 250 po bid
Foscarnet	40-80 mg IV q8hrs	85%	40-20 mg q8-24 hrs according to ClCr			Nephrotoxic, neurotoxic, hypocalcemia, hypophosphatemia, hypomagnesemia and hypokalemia
Ganciclovir IV	5 mg/kg q12hrs	95%	q12hrs	q24hrs	2.5 mg/kg qd	Granulocytopenia and thrombocytopenia
Ganciclovir PO	1000 mg po tid	95%	1000 mg tid	1000 mg bid	1000 mg qd	Oral ganciclovir should be used ONLY for prevention of CMV infection. Always use IV ganciclovir for the treatment of CMV infection
Valganciclovir	450 mg po bid	95%	q12hrs	q24hrs	Mon, Wed and Fri	Oral vanciclovir can be used for the treatment of mild to moderate CMV infection
Lamivudine	150 mg po bid	80%	q12hrs	Qq24hrs	50 mg q24hrs	For hepatitis B
Ribavirin	500-600 mg q12hrs	30%	100%	100%	50%	Hemolytic uremic syndrome
Rimantadine	100 mg po bid	25%	100%	100%	50%	
Oseltamivir	75 mg po bid	99%	100%	100%	50%	
Valacyclovir	500-1000 mg q8hrs	50%	100%	50%	25%	Thrombotic thrombocytopenic purpura/ hemolytic uremic syndrome Avoid in transplant recipients
Zanamivir	2 puffs bid x 5 days	4-17%	100%	100%	100%	

Table 3. Cardiovascular agents.

	Normal doses		% of renal excretion	Dosage adjustment in renal failure: GFR:			Comments
	Starting dose	Maximum dose		>50	10-50	<10	
ACE-INHIBITORS							Hyperkalemia, acute renal failure, angioedema, rash, cough, anemia and liver toxicity
Benazepril	10 mg qd	80 mg qd	20%	100%	75%	25-50%	
Captopril	6.25-25 mg po tid	100 mg tid	35%	100%	75%	50%	
Enalapril	5 mg qd	20 mg bid	45%	100%	75%	50%	
Fosinopril	10 mg po qd	40 mg bid	20%	100%	100%	75%	
Lisinopril	2.5 mg qd	20 mg bid	80%	100%	50-75%	25-50%	
Ramipril	2.5 mg qd	10 bid	15%	100%	50-75%	25-50%	
Trandolapril	1-2 mg qd	4 mg qd					
ANGIOTENSIN II RECEPTORS ANTAGONISTS							Hyperkalemia, angioedema (less common than ACE-inhibitors)
Losartan	50 mg qd	100 mg qd	13%	100%	100%	100%	
Valsartan	80 mg qd	160 mg bid	7%	100%	100%	100%	
Candesartan	16 mg qd	32 mg qd	33%	100%	100%	50%	
Irbesartan	150 mg qd	300 mg qd	20%	100%	100%	100%	
BETA BLOCKERS							Decrease HDL, mask symptoms of hypoglycemia, bronchospasm, fatigue, insomnia, depression and sexual dysfunction
Atenolol	25 mg qd	100 mg qd	90%	100%	75%	50%	
Carvedilol	3.125 mg po tid	25 mg tid	2%	100%	100%	100%	
Esmolol (IV only)	50 μg/kg/min	300 μg/kg/min	10%	100%	100%	100%	
Labetalol	50 mg po bid	400 mg bid	5%	100%	100%	100%	
Nadolol	80 mg qd	160 mg bid	90%	100%	50%	25%	
Propranolol	40?160 mg tid	320 mg/day	<5%	100%	100%	100%	
Sotalol	80 bid	160 mg bid	70%	100%	50%	25-50%	
CALCIUM CHANNEL BLOCKERS							Dihydropyridine: headache, ankle edema, gingival hyperplasia and flushing; Non-dihydropyridine: bradycardia, constipation, gingival hyperplasia and AV block
Amlodipine	2.5 po qd	10 mg qd	10%	100%	100%	100%	
Diltiazem	30 mg tid	90 mg tid	10%	100%	100%	100%	
Felodipine	5 mg po bid	20 mg qd	1%	100%	100%	100%	
Isradipine	5 mg po bid	10 mg bid	<5%	100%	100%	100%	
Nicardipine	20 mg po tid	30 mg po tid	<1%	100%	100%	100%	
Nifedipine XL	30 qd	90 mg bid	10%	100%	100%	100%	Avoid short-acting nifedipine formulation
Nimodipine			10%	100%	100%	100%	
Nisoldipine	20 mg qd	30 mg bid	10%	100%	100%	100%	
Verapamil	40 mg tid	240 mg/day	10%	100%	100%	100%	

Table 3 continued.

	Normal doses		% of	Dosage adjustment in renal failure: GFR:			Comments
	Starting dose	Maximum dose	renal excretion	>50	10-50	<10	
DIURETICS							Hypokalemia/hyperkalemia (potassium sparing agents), hyperuricemia, hyperglycemia, hypomagnesemia, increase serum cholesterol.
Acetazolamide	125 mg po tid	500 mg po tid	90%	100%	50%	Avoid	
Amiloride	5 mg po qd	10 mg po qd	50%	100%	100%	Avoid	
Bumetanide	1-2 mg po qd	2-4 mg po qd	35%	100%	100%	100%	
Ethacrynic Acid	50 mg po qd	100 mg po bid	20%	100%	100%	100%	
Furosemide	40-80 mg po qd	120 mg po tid	70%	100%	100%	100%	
Metolazone	2.5 mg po qd	10 mg po bid	70%	100%	100%	100%	
Spironolactone	100 mg po qd	300 mg po qd	25%	100%	100%	Avoid	
Torsemide	5 mg po bid	20 mg qd	25%	100%	100%	100%	
MISCELLANEOUS AGENTS							
Clonidine	0.1 po bid/tid	1.2 mg/ day	45%	100%	100%	100%	Sexual dysfunction, dizziness, postal hypotension
Digoxin	0.125 mg qod/qd	0.25 mg po qd	25%	100%	100%	100%	
Hydralazine	10 mg po qid	100 mg po qid	25%	100%	100%	100%	Lupus-like reaction
Minoxidil	2.5 mg po bid	10 mg po bid	20%	100%	100%	100%	Pericardial effusion, fluid retention, hypertrichosis and tachycardia
Nitroprusside	1 μg/kg/min	10 μg/kg/min	<10%	100%	100%	100%	Cyanide toxicity
Amrinone	5 μg/kg/min	10 μg/kg/min	25%	100%	100%	100%	
Dobutamine	2.5 μg/kg/min	15 μg/kg/min	10%	100%	100%	100%	
Milrinone	0.375 μg/kg/min	0.75 μg/kg/min		100%	100%	100%	

Table 4. Anti-lipidemic agents.

	Normal doses		% of	Dosage adjustment in renal failure: GFR:			Comments
	Starting dose	Maximum dose	renal excretion	>50	10-50	<10	
Atorvastatin	10 mg/day	80 mg/day	<2%	100%	100%	100%	Liver dysfunction, myalgia and rhabdomyolysis with CSA/FK
Cholestyramine	4 g bid	24 g/day	None	100%	100%	100%	Schedule CSA/FK 3 hrs before the dose, N/V and constipation
Colestipol	5 gbid	30 g/day	None	100%	100%	100%	Schedule CSA/FK 3 hrs before the dose, N/V and constipation
Fluvastatin	20 mg daily	80 mg/day	<1%	100%	100%	100%	Liver dysfunction, myalgia and rhabdomyolysis with CSA/FK
Gemfibrozil	600 bid	600 bid	None	100%	100%	100%	Hyperglycemia, rhabdomyolysis, elevation of LFTs
Lovastatin	5 mg daily	20 mg/day	None	100%	100%	100%	Liver dysfunction, myalgia and rhabdomyolysis with CSA/FK
Pravastatin	10-40 mg daily	80 mg/day	<10%	100%	100%	100%	Liver dysfunction, myalgia and rhabdomyolysis with CSA/FK
Simvastatin	5-20 mg daily	20 mg/day	13%	100%	100%	100%	Liver dysfunction, myalgia and rhabdomyolysis with CSA/FK

Table 5. Anti-platelets and anti-coagulation agents.

	Normal doses		% of	Dosage adjustment in renal failure: GFR:			Comments
	Starting dose	Maximum dose	renal excretion	>50	10-50	<10	
Aspirin	81 mg/day	325 mg/day	10%	100%	100%	100%	GI irritation and bleeding tendency
Clopidogrel	75 mg/day	75 mg/day	50%	100%	100%	100%	
Dalteparin	2,500 units Sq /day	5,000 units Sq /day	Unknown	100%	100%	100%	
Enoxaparin	20 mg/day	30 mg bid	8%	100%	100%	50%	1 mg/kg q12hrs for treatment of DVT. Check anti-factor Xa activity 4 hours after second dose in patients with renal dysfunction. There are some evidence of drug accumulation in renal failure.
Ticlopidine	250 mg bid	250 mg bid	2%	100%	100%	100%	Decrease CSA level and may cause severe neutropenia & thrombocytopenia
Warfarin	5 mg/day	Adjust per INR	<1%	100%	100%	100%	Monitor INR very closely. Start at 5 mg/day. 1 mg Vit. K IV over 30 minutes or 2.5-5 mg po can be used to normalize INR.

Table 6. Gastrointestinal agents.

	Normal doses		% of	Dosage adjustment in renal failure: GFR:			Comments
	Starting dose	Maximum dose	renal excretion	>50	10-50	<10	
ANTI-ULCER AGENTS							
Cimetidine	300mg po tid	800 mg po bid	60%	100%	75%	25%	Multiple drug-drug interactions; beta blockers, sulfonylurea, theophylline, warfarin, etc
Famotidine	20 mg po bid	40 mg po bid	70%	100%	75%	25%	Headache, fatigue, thrombocytopenia, alopecia
Lansoprazole	15 mg po qd	30 mg bid	None	100%	100%	100%	Headache, diarrhea
Nizatidine	150 mg po bid	300 mg po bid	20%	100%	75%	25%	Headache, fatigue, thrombocytopenia, alopecia
Omeprazole	20 mg po qd	40 mg po bid	None	100%	100%	100%	Headache, diarrhea
Rabeprazole	20 mg po qd	40 mg po bid	None	100%	100%	100%	Headache, diarrhea
Pantaprazole	40 mg po qd	80 mg po bid	None	100%	100%	100%	Headache, diarrhea
Ranitidine	150 mg po bid	300 mg po bid	80%	100%	75%	25%	Headache, fatigue, thrombocytopenia, alopecia
Cisapride	10 mg po tid	20 mg qid	5%	100%	100%	50-75%	Avoid with Azole Antifungal, Macrolide antibiotics and other P450 IIIA-4 inhibitors
Metoclopramide	10 mg po tid	30 mg po qid	15%	100%	100%	50-75%	Increase CSA and tacrolimus blood concentration, neurotoxic
Misoprostol	100 μg po bid	200 μg po qid		100%	100%	100%	Diarrhea, N/VAbortifacient agent
Sucralfate	1 gm po qid	1 gm po qid	None	100%	100%	100%	Constipation, decrease absorption of MMF

Table 7. Hypoglycemic agents.

	Normal doses		% of	Dosage adjustment in renal failure: GFR:			Comments
	Starting dose	Maximum dose	renal excretion	>50	10-50	<10	
Acrobose	25 mg tid	100 mg tid	35%	100%	50%	Avoid	Abdominal pain, N/V and Flatulence
Glimepiride	1 mg qd	8 mg qd	60%	100%	50%	50%	
Glipizide	5 mg qd	20 mg bid	80%	100%	50%	50%	
Glyburide	2.5 mg qd	10 mg bid	50%	100%	50%	Avoid	
Metformin	500 mg bid	2,550 mg/day (bid or tid)	95%	100%	Avoid	Avoid	Lactic acidosis
Pioglitazone	15 mg qd	45 mg qd	3%	100%	100%	100%	Hepatotoxic. Edema
Repaglinide	0.5-1 mg	4 mg tid	8%	100%	100%	100%	
Rosiglitazone	4 mg qd	8 mg qd	3%	100%	100%	100%	Hepatotoxic. Edema, increase LDL

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LIST OF ABBREVIATIONS

AA	aristolochic acid
AAP	alanine aminopeptidase
AAS	atomic absorption spectrometry
ABC	ATP-binding cassette
ABCD	amphotericin B colloidal dispersion
ABLC	amphotericin B in lipid complex
ACAM	N-cadherin
ACE	angiotensin converting enzyme
ACEI	angiotensin converting enzyme inhibitor
ACGIH	American Conference of Governmental Industrial Hygienists
AcP	acid phosphate
ADH	antidiuretic hormone
ADP	adenosine diphosphate
ADR	adverse drug reaction
AER	adverse event reaction
AIDS	acquired immunodeficiency syndrome
AIN	acute interstitial nephritis
ALB	albumin
α_1-AG	α_1-acid glycoprotein
α_1-m	α_1-microglobulin
ALT	alanine aminotransferase
AmB	amphotericin B
AMP	adenosine monophosphate
ANCA	anti-neutrophil cytoplasmic antibody
ANDA	abbreviated new drug application
ANF	atrial natriuretic factor
ANT	adenine nucleotide translocator
ANZDATA	Australian and New Zealand Dialysis and Transplant Registry
AP	alkaline phosphatase
APA	aminopeptidase 1 (angiotensinase)
APC	antigen presenting cell
APN	aminopeptidase N
AQP	aquaporin
ARF	acute renal failure
AST	aspartate aminotransferase
ATN	acute tubular necrosis
ATP	adenosine triphosphate
AVP	arginine vasopressin
BBA	brush border antigen
BBM	brush border membrane
BBMV	brush border membrane vesicle
β_1-m	β_1-microglobulin
bFGF	basic fibroblast growth factor
BID	twice daily
BN	Brown-Norway
BP	blood pressure
BSA	bovine serum albumin
BUN	blood urea nitrogen
BW	body weight
CA	carbonic anhydrase
cADPR	cyclic ADP-ribose
Calc	calcitonin
CAM	cell adhesion molecule
cAMP	cyclic adenosine monophosphate
CC	cytochemistry
CCB	calcium channel blocker
CCD	cortical collecting duct
Ccr	creatinine clearance
CD	collecting duct
CD-IC	collecting duct intercalated cell
CdMT	cadmium-metallothionein
CD-PC	collecting duct principal cell

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CG	density gradient centrifugation
CHD	coronary heart disease
CHF	congestive heart failure
CHN	Chinese herb nephropathy
CI	confidence interval
CM	contrast-media
CMIN	contrast-media induced nephropathy
CMV	cytomegalovirus
CNS	central nervous system
CNT	connecting tubule
COX	cyclooxygenase
CPH	cephaloridine
CPK	creatinine phosphokinase
CRF	chronic renal failure
CsA	cyclosporine A
CSF	colony-stimulating factor
CSFL	cerebrospinal fluid
CT	computer tomography
C_{urea}	urea clearance
Cx	clearance of a marker
CYP	cytochrome P450
dA-AAI	7(desoxyadenosin-N^6-yl) aristolactam I
dA-AAII	7(deoxyadenosin-N^6-yl) aristolactam II
DAC	diacylglycerol
DCT	distal convoluted tubule
DCVC	dichlorovinylcysteine
dDAVP	1-desamino-8-D-arginine-vasopressin
DDT	dichlorodiphenyltrichloroethane
DES	diethyl stilbesterol
DEVD-CHO	Asp-Glu-Val-Asp-aldehyde
DFO	desferoxamine
dG-AAI	7(deoxyguanosine-N^2-yl) aristolactam I
DHG	dehydrogenase
DHP	vitamin D binding protein
DISC	death inducing signaling complex
DMEM	Dulbecco's modified Eagle medium
DMPC	dimyristoyl phosphatidylcholine
DMPG	dimyristoyl phosphatidylglycerol
DMPS	dimercaptopropane 1 sulphonate
DMSA	dimercaptosuccinic acid
DMSO	dimethylsulfoxide
DMTU	dimethylthiourea
DPCPX	selective adenosine A1 receptor antagonist
DPP	dipeptidyl peptidase
DTL	descending thin limb
DTPA	diethylenetriaminepentaacetic acid
ecNOS	endothelial nitric oxide synthase
EDRF	endothelium-derived relaxing factor
EDTA	ethylenediamine tetraacetic acid
EDTA	European Dialysis and Transplant Association
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EMA	epithelial membrane antigen
eNOS	endothelial nitric oxide synthase
ERK	extracellular regulated kinase
ERPF	effective renal plasma flow
ESRD	end-stage renal disease
ET	endothelin
ET-1	endothelin-1
ETA	endothelin A
ETB	endothelin B
FACS	fluorescence-activated cell sorting
FAD	flavin adenine dinucleotide
FAK	focal adhesion kinase
FAT	focal adhesion targeting
FCS	fetal calf serum
FDA	Food and Drug Administration
FE	fractional excretion
FE_{urea}	fractional excretion of urea
FF	filtration fraction
FGS	focal glomerulosclerosis
FITC	fluorescein isothiocyanate
FKBP	FK-binding protein
FMN	flavin mononucleotide
FSGS	focal segmental glomerulosclerosis
G6PD	glucose 6 phosphate dehydrogenase
GC	gas chromatography
GFR	glomerular filtration rate
GI	gastrointestinal
GLDH	glutamate dehydrogenase
GMP	guanosine monophosphate
GN	glomerulonephritis
GP	glycoprotein
GSC	glomerular sieving coefficient
GSH	glutathione
GSSG	glutathione disulfide
GST	glutathione-S-transferase
GT	glutamyl transferase
H&E	hematoxylin and eosin
HCTZ	hydrochlorothiazide
HCV	hepatitis C virus
HDL	high-density lipoprotein
HETE	hydroxyeicosatetraenoic acid
HHV	human herpes virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HMW	high molecular weight
HO	heme oxygenase
HPT	human proximal tubular cells
HSP	heat shock protein
HUS	hemolytic uremic syndrome
HUVEC	human umbilical vein endothelial cells
IAP	intestinal alkaline phosphatase
IARC	International Agency for Research on Cancer
IBD	inflammatory bowel disease
IC	information component
ICAD	inhibitor of caspase-activates Dnase
ICAM	intercellular cell adhesion molecule
ICC	immunocytochemistry
ICD	International Classification of Diseases

ICU	intensive care unit
IEG	immediate early gene response
IFN	interferon
Ig	immunoglobulin
IGF	insulin-like growth factor
IL	interleukin
IM	intramuscular
iNOS	inducible nitric oxide synthase
IP3	inositol 3,4,5 triphosphate
IPRK	isolated perfused rat kidney
ISOM	inner stripe outer medulla
IV	intravenous
IVP	intravenous pyelography
JCAHO	Joint Commission on Accreditation of Healthcare Organizations
JGA	juxtaglomerular apparatus
JNK	c-Jun N-terminal kinase
kD	kilodalton
KIM	kidney injury molecule
L-Amph	amphotericin B liposome
LAP	leucine aminopeptidase
LD50	lethal dose for 50%
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LEHD-CHO	Leu-Glu-His-Asp-aldehyde
LFA	lymphocyte function-associated antigen
LMW	low molecular weight
L-NAME	N-nitro-l-arginine methyl ester
LPS	lipopolysaccharide
LR	likelihood ratio
LT	leukotriene
LX	lipoxin
mAb	monoclonal antibody
MACS	magnetic cell separation
magn.	magnification
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
MCD	medullary collecting duct
MCP	monocyte chemoattractant protein
MD	macula densa
MDA	malondialdehyde
MDMA	methylenedioxymethamphetamine
MDR	multidrug resistance
MEK	MAP kinase kinase
MHC	major histocompatibility complex
MMP	matrix metalloproteinases
mPDS	methylprednisolone
MPGN	membranoproliferative glomerulonephritis
MPO	myeloperoxidase
MPT	mitochondrial permeability transition
MR	magnetic resonance
Mr	molecular weight
MRP	multidrug resistance-associated protein
MRS	magnetic resonance spectroscopy
MRSA	methicillin-resistant Staphylococcus aureus
MT	metallothionein
MTAL	medullary thick ascending limb
MTT	methylthiotetrazole
NAA	neutron activation analysis
NADC	Na dependent α-ketoglutarate cotransporter
NADPH	nicotinamide adenine dinucleotide phosphate
NAG	N-acetyl-β-D-glucosaminidase
Na-K-ATPase	sodium-potassium-ATPase
NAME	nitric oxide synthase inhibitor
NAPA	N-acetyl procainamide
NAPAP	N-acetyl-p-aminophenol
NCAM	neural cell adhesion molecule
NCX	Na^{+}-Ca^{++} exchanger
NDA	New Drug Application
NDMA	N-methyl-D-aspartate
NEP	neutral endopeptidase
NIP	NF-AT interacting protein
NMN	N-methylnicotinamid
NMTT	N-methyl-tetrazole-thiol
NO	nitric oxide
NOS	nitric oxide synthase
NPT	sodium-dependent phosphate transporter
NRF	nuclear respiratory factors
NRTI	nucleoside analogue reverse transcriptase inhibitor
NSA	neuron specific enolase
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OAT	organic anion transporter
OCT	organic cation transporter
OCTN	organic cation/carnitine transporter
OFR	oxygen-derived free radicals
OKT3	anti-CD3 monoclonal antibody
OM	outer medulla
OR	odds ratio
OSOM	outer stripe outer medulla
OTA	ochratoxin
PAH	para-aminohippurate
PAS	periodic acid Schiff
PAM	periodic acid methenamine
PBMC	peripheral blood mononuclear cells
PC	Pneumocyctis carinii
PCE	perchloroethylene
PCOP	plasma colloid osmotic pressure
PCP	Pneumocyctis carinii pneumonia
PCSA	planar cell surface area
PCT	proximal convoluted tubule
PDGF	platelet derived growth factor
PEEP	positive end-expiratory pressure
PEPCK	phosphenol pyruvate carboxy-kinase
PEPT	peptide cotransporter
PG	prostaglandin
PGC	PPAR-gamma-coactivator
PGP	P-glycoprotein
PIP	phosphatidylinositide 4,5 biphosphate

PK	protein kinase
PKB	protein kinase B
PL	phospholipase
PMA	phorbol myrastate acetate
pmp	per million population
PPAR	peroxisome proliferator-activated receptor
PR3	proteinase 3
PSS	progressive systemic sclerosis
PST	proximal straight tubule
PT	proximal tubular cells
PTFE	polymeric tetrafluoroethylene
PTH	parathyroid hormone
PTK	protein tyrosine kinase
QD	once daily
RA	rheumatoid arthritis
RANTES	regulated on activation, normal T-cell expressed and secreted
RAP	receptor-associated protein
RAS	renin-angiotensin system
RBF	renal blood flow
RCT	randomized clinical trial
RIA	radio immunoassay
ROC	receiver-operating characteristic
ROS	reactive oxygen species
RPF	renal plasma flow
RPGN	rapidly progressive glomerulonephritis
RR	relative risk
RTE	renal tubular epithelial cells
RVR	renal vascular resistance
RXR	retenoic orphan receptor
S-	serum-
SAPK	stress-activated protein kinase
SAT	sulfate-oxalate exchanger
SBP	systolic blood pressure
Scr	serum creatinine
SDS-PAGE	sodium dodecyl sulphate - polyacrylamide gel electrophoresis
SEM	standard error of the mean
SHR	spontaneously hypertensive rats
SLC	Na^+/Li^+ countertransporter
SLE	systemic lupus erythematosis
SMZ	sulfamethoxazole
SNGFR	single nephron glomerular filtration rate
SNS	sympathetic nervous system
SSc	systemic sclerosis
t_2	elimination half-life
T3	triiodothyronin
TAL	thick ascending limb
T-bet	T-box expressed in T-cells
TBM	tubular basement membrane
TCA	trichloroacetic acid
TCR	T-cell receptor
TDM	therapeutic drug monitoring
TEA	tetraethylammonium
TER	transepithelial resistance
TFR	transferrin
TGF	transforming growth factor
Th	T-helper cell
THP	Tamm-Horsfall protein
TID	trice daily
TLV	threshold limit value
TMA	thrombotic microangiopathic anemia
TMP	trimethoprim
TNAP	tissue non-specific alkaline phosphatase
TNF	tumor necrosis factor
TQ	triple quantum
TRP	tubular reabsorption of phosphorus
TSC	thiazide sensitive Na^+-Cl^- cotransporter
TSH	thyroid-stimulating hormone
TTP	thrombotic thrombocytopenic purpura
TTR	transthryetin
TUNEL	terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP nick end-labeling assay
TxA2	thromboxane A2
TxB2	thromboxane B2
U-	urinary
UP:Ucr	urine protein to creatinine ratio
USRDS	United States Renal Data System
VC	vasoconstriction
VCAM	vascular cell adhesion molecule
VD	vasodilatation
V_d	volume of distribution
VGEF	vascular endothelial growth factor
VLA	very late antigen
VSMC	vascular smooth muscle cells
vWF	von Willebrand factor
WHO	World Health Organization
XRF	x-ray fluorescence
ZAG	zinc-α_2-globulin
ZVAD-fmk	Z-Val-Ala-Asp-fmk

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