Blood Purification

Advances in Chronic Kidney Disease 2010

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Preface

The Renal Research Institute's 12th International Conference on Dialysis, 'Advances in Chronic Kidney Disease 2010', in New Orleans emphasizes recent developments in operational areas, technology, biology of uremia and epidemiology. This year we also celebrate the 10-year anniversary of the New York-Vicenza sistership program, which provides the framework for continuous fruitful cooperation between the Renal Research Institute and Beth Israel Medical Center in New York and the Department of Nephrology, San Bortolo Hospital, Vicenza.

The current issue of *Blood Purification* incorporates many of the papers delivered at the conference.

Michael Brines outlines the potential of erythropoiesis-stimulating agents for tissue protection, which is mediated by a low-affinity erythropoietin receptor different from the one responsible for hematopoiesis.

Treatment of hypertension in dialysis patients is still far from being resolved. In their paper, Josep Redon et al. discuss special considerations for antihypertensive agents in dialysis patients, such as the high prevalence of sympathetic overactivity.

Po Shun Lee et al. describe the potential links between gelsolin, a highly prevalent circulating protein and potential risk indicator that is depleted by inflammatory mediators with low levels being reported in dialysis patients.

Salt is conceived by many as a uremic toxin and Ercan Ok reports on his year-long experience on how to successfully achieve salt restriction in dialysis patients and the outcome of this.

Although cardiac abnormalities are highly prevalent in dialysis patients, myocardial stunning did not receive much attention. Chris W. McIntyre provides novel insights into the pathophysiology of myocardial stunning and additional therapeutic targets.

Exciting new insights into blood-pressure-independent effects of aldosterone on nonepithelial tissues are the focus of the paper by Eberhard Ritz and Nadezda Kolganova.

Chronic inflammation is closely linked to cardiovascular complications of chronic kidney disease (CKD); A.E.M. Stinghen et al. review strategies to modulate immune response and thus improve the course of cardiovascular disease.

Depression is associated with impaired quality of life in CKD; Fredric O. Finkelstein et al. review screening for and therapy of clinical depression in CKD.

John Feehally draws attention to the complex interaction between ethnicity and CKD, and the varied and substantial inequities faced by ethnic minority populations in areas such as renal transplantation.

E. Schepers et al. remind us of the fact that the gut may serve as a source of uremic retention solutes and discuss the potential of intestinal therapeutic interventions to reduce uremic toxin generation and absorption.

Beth Piraino and Heena Sheth present an in-depth literature review comparing the use of automated peritoneal dialysis with Luer lock connection versus continuous ambulatory peritoneal dialysis with a disconnect system in altering the peritonitis risk.

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John R. Speakman and Klaas R. Westerterp address in an elegant analysis the 'obesity paradox' in dialysis patients; their results support the idea that relative underdialysis may be responsible for reduced survival in small patients.

Rajesh Mohandas and Mark S. Segal address the significance of endothelial progenitor cells and endothelial vesicles in CKD, in particular their potential as markers of endothelial health.

Much controversy exists on the best way to manage calcium balance in chronic dialysis patients. Based on mathematical models, Frank Gotch et al. support the view that calcium balance is best managed by adjusting dialysate calcium levels.

Congestive heart failure is among the leading causes of death worldwide, and new therapeutic avenues are investigated. Jason G. Andrade et al. review the role of peripheral ultrafiltration in the management of acute decompensated heart failure.

David Humes et al. explore the clinical use of a selective cytopheretic device to treat immunological dysregulation in acute and chronic renal failure.

Regional citrate anticoagulation (RCA) provides an alternative to systemic heparin anticoagulation, and 3 papers in this journal are dedicated to it. Heleen M. Oudemans-van Straaten reports on citrate anticoagulation for continuous renal replacement therapy. Stephan Thijssen et al. describe a novel generic mathematical model of RCA, and Balazs Szamosfalvi et al. describe a methodology to dose venous calcium infusion in RCA.

We hope that you will enjoy the wide range of papers published in this issue of *Blood Purification* and that you will consider attending next year's 13th International Conference on Dialysis, 'Advances in Chronic Kidney Disease 2011'.

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Water for Haemodialysis and Related Therapies: Recent Standards and Emerging Issues

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Key Words
Water, haemodialysis · Dialysis fluid · Waste water

Abstract
Dialysis is a well-established and widely used procedure. For a number of years, the focus has been on ensuring that water used in the preparation of dialysis fluid meets the required chemical and microbiological quality and complies with national or international standards which have recently been updated. Continued vigilance is required, in particular when new chemicals such as silver-stabilized hydrogen peroxide and chlorine dioxide are used to prevent growth of *Legionella* bacteria in hospital water systems, since residues are harmful to patients receiving dialysis. To achieve the required quality, large volumes of water are processed, and a substantial portion is sent to waste via the municipal sewer systems with little attempt to reuse such water on site. In view of concern about global warming and climate change, there is a need to adopt a more environmentally conscious attitude requiring dialysis providers to focus on this aspect of water usage.

Introduction

Awareness concerning the possible effects of chemical impurities present in water began in the 1970s, and since that time, there has been a continuous improvement in the water quality used in the preparation of dialysis fluid paralleled by the use of more complex approaches to the treatment of water. Recently, the focus has shifted to microbiological quality, to minimize patient exposure to endotoxins and bacterial fragments capable of being transferred from the water to the patient [1–4].

The pore size of the membrane appears to be less important than the thickness and the capacity of the membrane to adsorb bacterial products. Consequently, low-flux (standard) dialysis does not necessarily translate into higher microbiological safety compared to high-flux dialysis, and patients receiving standard dialysis treatment with low-flux cellulose-based membranes (thickness 6–8 μm) may be at greater risk than those treated using thicker synthetic membranes which have the capacity to adsorb bacterial endotoxin [5]. In such membranes, however, the adsorption capacity of the synthetic membranes is not infinite, and a breakthrough of pyrogenic substances can occur in the event of excessive water contamination.

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As water quality is pivotal in ensuring patient well-being and contributes to the minimization of dialysis-related complications in patients who today are predominantly elderly and suffer from a range of comorbid conditions in addition to renal disease, the operational and clinical aspects of this element of dialysis therapy are discussed in the context of recent standards and issues.

Standards for Chemical and Microbiological Purity of Water and Dialysis Fluid Used for Renal Replacement Therapy

Standards exist for water quality, dialysis fluid quality and equipment used for the preparation of the water and dialysis fluid. Those prepared by the International Standards Organization (ISO) have recently been updated. For example, it is desirable that the complete water treatment, storage and distribution system used in the dialysis unit should meet the requirements of ISO 26722:2009 'Water treatment equipment for haemodialysis applications and related therapies' and the water produced be capable of meeting the requirements of ISO 13959:2009 'Water for haemodialysis and related therapies' at the time of installation. The ISO also produces standards for the concentrates used for the preparation of dialysis fluid (ISO 13958:2009 'Concentrates for haemodialysis and related therapies'). A new document providing guidance for the preparation and quality management of fluids for haemodialysis and related therapies is in preparation and will be issued during 2010 (ISO 23500).

In addition to the ISO, a number of other groups and organizations have also produced standards and guidelines. These include the European Pharmacopoeia, the Japanese Society for Dialysis Therapy, the Canadian Standards Association and the American National Standards Institute/Association for the Advancement of Medical Instrumentation (AAMI). Within these standards, there is general agreement concerning the maximum allowable levels of inorganic chemicals with documented toxicity in haemodialysis patients such as aluminium, chloramines, copper, fluoride, lead, nitrate, sulphate and zinc, although minor differences between the standards exist [6].

There are, however, some exceptions. For example, the current edition of the European Pharmacopoeia does not explicitly specify maximum allowable levels for copper or chloramines. In the case of nitrate, the European Pharmacopoeia specifies a maximum of 2 mg/l nitrate (NO_3), compared to the AAMI standard and ISO 13959:2009 'Water for haemodialysis and related therapies' which recommend a limit of 2 mg/l of nitrate as nitrogen (N), equating to approximately 9 mg/l of NO_3. The more stringent limits can only be met using double-pass reverse osmosis water treatment systems. Currently, none of the standards and recommendations include limits for organic chemical contaminants, the rationale for this omission being that organic chemicals with specific toxicity to haemodialysis patients have not been identified and that carbon adsorption and reverse osmosis remove most organic compounds. Recently, however, there has been a reported instance of patient exposure following inadequate removal of such compounds [7].

The standards also define the microbiological quality of the product water.

Dialysis fluid is produced by the mixing of treated water, acid and bicarbonate concentrates. The microbiological contaminant levels for acid and bicarbonate concentrates are defined in ISO 13958:2009 'Concentrates for haemodialysis and related therapies'. In contrast to chemical contaminants, the required level differs; furthermore, even if the limit given in numbers is the same, the interpretation is not the same due to the different cultivation techniques specified [8].

Monitoring of Water Used in the Preparation of Dialysis Fluid

Each renal unit should have standard operating procedures in place for sampling, monitoring and recording of feed (raw, untreated water entering the water treatment plant) and product water quality. The operating procedures should include details of the procedures to be followed if prescribed limits are exceeded.

Within the USA, Centers for Medicare and Medicaid Services compliance with AAMI water standards is mandatory, with annual testing of water for chemical contaminants except for chlorine/chloramine which is required to be tested at the beginning of each treatment day prior to patients initiating treatment and again prior to the beginning of each patient shift. If there are no set patient shifts, testing should be performed approximately every 4 hours. For home installations, it may be impractical to maintain a monthly testing programme, and to ensure adequate patient safety, the dialysis machine should be fitted with point-of-use filtration.

The absence of any type of bacteriostat in the water following treatment makes it susceptible to bacterial contamination downstream of the water treatment plant.

Such contamination may be further enhanced by stagnant areas within the distribution network as well as irregular cleaning, all of which contribute to the development of a biofilm which may also be present in the dialysate pathway of the proportioning system, particularly when non-sterile liquid bicarbonate concentrate is used. Whilst the daily disinfection of dialysis machines is standard, the disinfection procedures in respect of water treatment systems and the distribution loop for the treated water have received much less attention. Furthermore, disinfection is often reactive rather than proactive. To minimize the development and growth of a biofilm, a strict strategy of proactive preventive measures should be followed. Using such an approach, it is possible to maintain microbiological activity at low levels for a long time. At facilities run and managed by the Renal Research Institute, using a combination of membrane filtration and a rigorous quality control programme, water quality which is lower than set by the current AAMI standards and close to those recommended by the European Renal Association Best Practice Guidelines has been routinely achieved. Elements of this approach include the regular validation of the reverse osmosis performance to ensure that it meets the required standards in respect of chemical quality; the additional installation of a 0.05-μm hollow-fibre polysulphone filter (Fibercor; Minntech Corp., Minneapolis, Minn., USA) to filter water entering the distribution loop, the installation of Diasafe filters (Fresenius Medical Care, Lexington, Mass., USA) on each dialysis machine and a weekly microbiological monitoring of the distribution system as well as a rolling monthly monitoring for all dialysis machines have resulted in the routine production of water and dialysis fluid containing 20 CFU/ml bacteria and <0.03 EU/ml of endotoxin. In addition to these measures, a routine heat disinfection using hot water (≥80°C) for a minimum of 120 min is performed every 2 weeks in some facilities. The use of heat instead of the more commonly used chemicals negates the need to monitor for chemical residues in the distribution loop and allows immediate use of the system once it has cooled to ambient temperature. Furthermore, as the heat treatment is linked to the heat cleaning of the dialysis machines, the link tubing between the machine and the distribution loop is also treated.

Whilst this approach has been a proactive prospective one, there are problems in retrofitting these newer technologies, as the current standard polyvinyl chloride distribution loop must be replaced with materials such as chlorinated polyvinyl chloride, cross-linked polyethylene, Teflon, stainless steel or polyvinylidene fluoride as polyvinyl chloride cannot withstand the high temperatures coupled with the pressures the systems operate under. Polyvinylidene fluoride or cross-linked polyethylene piping is suitable for use with hot water and can be welded, thereby further reducing the potential for bacterial colonization of joints. Cross-linked polyethylene tubing has proven to be very cost-effective as it comes in large rolls and can be installed without any joints other than at the patient stations or at connections to technical equipment or solution mixing equipment.

Emerging Issues

New Chemicals

There is increasing use of compounds such as silver-stabilized hydrogen peroxide and chlorine dioxide to prevent growth of *Legionella* bacteria in hospital water systems. The renal water treatment plant including carbon filters and a reverse osmosis plant does not remove the hydrogen peroxide, thereby exposing patients using the renal unit for haemodialysis to potentially high levels of hydrogen peroxide resulting in exposed patients developing haemolysis [9].

With regard to chlorine dioxide, there is currently no guidance on the control and monitoring of chlorine dioxide in water for dialysis. Confirmation that the standard test used to monitor chlorine and chloramines gives an accurate measure of the levels of chlorine dioxide and its breakdown products (chlorite and chlorates) is needed, as is data on the carbon filter empty bed contact time required for effective removal.

In the bid to minimize the effect of such chemicals, it is desirable that newly built renal units have a direct feed water supply, separate from that of the hospital water supply, and in existing systems where the water supply is via the hospital, there should be awareness of the potential risks that may ensue from the introduction of chemicals into the hospital water supply by both renal unit and hospital engineering staff. Introduction of chemicals into the water should not be undertaken without prior consultation with renal services.

Conservation of Water

The average dialysis patient undergoing 3 times weekly dialysis treatment for 4 h uses about 18,000 litres of dialysis fluid. In addition, for each litre of usable water to

make up the dialysis fluid, up to 70% of the water entering the water treatment system may be sent to the drain as part of the reverse osmosis treatment it undergoes. Questions are beginning to be raised as to whether some of this water may be reused [10]. With regard to conservation and reuse of water, two aspects need to be considered: first, can less water be used during the treatment, and second, can water rejected by the reverse osmosis and the spent dialysis fluid be reused?

Theoretical considerations relating haemodialyser performance to blood and dialysis fluid flow rates were first established by Renkin [11] in the late 1950s. The relationship of the curve for solute transport and blood flow rate at a constant dialysate flow rate, or dialysate flow rate and a constant blood flow rate, are similar. Clearance increases with dialysate flow; however, at a certain point, the cost of dialysate fluid will no longer be compensated by the benefit of improved efficiency or shorter treatment time. A theoretical study by Sigdell and Tersteegen [12] indicated that the practical upper limit of the dialysate flow is twice the blood flow rate; beyond this, the improvement in solute transport for small molecules is minimal. The clearance of middle or large molecules is however less dependent on the flow rates within the dialyser, and subsequently, Polaschegg and Peter [13] suggested $Q_D = 1.5 \times Q_B$ as a reasonable compromise. This type of approach is also suitable for automatic control [14].

Water treatment and the used dialysis fluid generate two very different types of water. The efficiency of the widely used reverse osmosis systems is dependent upon the nature of the feed water and temperature. Temperature has an inverse effect on product flow through the membrane: a high temperature increases product flow, a low temperature decreases product flow, and up to 70% of the feed water may be rejected in the process; this can be reduced by the use of a dual-pass system in which the rejected water passes through the reverse osmosis process before being discarded to waste. The optimization of the efficiency of reverse osmosis systems is complex and outside the scope of this article. In most dialysis units, the waste component of reverse osmosis-treated water passes to the drain. This water contains high concentrations of dissolved solutes and is unsuitable for use as drinking water; it can nevertheless be diverted to a recovery tank for use in non-drinking applications [15]. The recycling of used dialysis fluid remains in its infancy possibly because of concerns that such fluid may cause environmental bacterial or viral contamination. Tarrass et al. [16] performed an analysis of the potential for reusing such water and calculated that both the rejected water and dialysis fluid discharge in the USA correspond to around 27 gigalitres, a volume that is sufficient to provide the yearly requirement for a conurbation with a population of 175,000.

Conclusions

Dialysis is now a well-established and widely used procedure. For a number of years, the focus has been on ensuring that water used in the preparation of dialysis fluid meets the required chemical and microbiological quality and complies with national or international standards. This has been achieved, but continued vigilance, particularly when new chemicals such as those to minimize bacterial proliferation are used, is required.

To achieve the required quality, large volumes of water are processed with a substantial portion sent to waste via the municipal sewer systems. Currently, there is little attempt to find alternate uses for such water locally. Some reuse off site is beginning. For example, 20% of treated sewage from Melbourne's two major sewage treatment plants will be recycled by 2010 to conserve water resources [17].

The next challenge in respect of water used in dialysis will be a more 'green' and environmentally friendly approach to both reverse osmosis rejected water and spent dialysis fluid.

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The Therapeutic Potential of Erythropoiesis-Stimulating Agents for Tissue Protection: A Tale of Two Receptors

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Key Words

Erythropoietin · Cytokines · Inflammation · Innate immune response · Ischemia · Trauma · Cytoprotection · Apoptosis · Necrosis · Wound healing

Abstract

Erythropoietin (EPO) is a well-known therapeutic protein employed widely in the treatment of anemia. Over the past decade, abundant evidence has shown that in addition to its systemic role in the regulation of plasma pO_2 by modulating erythrocyte numbers, EPO is also a cytoprotective molecule made locally in response to injury or metabolic stress. Many studies have shown beneficial effects of EPO administration in reducing damage caused by ischemia-reperfusion, trauma, cytotoxicity, infection and inflammation in a variety of organs and tissues. Notably, the receptor mediating the nonerythropoietic effects of EPO differs from the one responsible for hematopoiesis. The tissue-protective receptor exhibits a lower affinity for EPO and is a heteromer consisting of EPO receptor monomers in association with the common receptor that is also employed by granulocyte macrophage colony-stimulating factor, interleukin 3, and interleukin 5. This heteromeric receptor is expressed immediately following injury, whereas EPO production is delayed. Thus, early administration of EPO can dramatically reduce the deleterious components of the local inflammatory cascade. However, a high dose of EPO is required and this also stimulates the bone marrow to produce highly reactive platelets and activates the vascular endothelium into a prothrombotic state. To circumvent these undesirable effects, the EPO molecule has been successfully altered to selectively eliminate erythropoietic and prothrombotic potencies, while preserving tissue-protective activities. Very recently, small peptide mimetics have been developed that recapitulate the tissue-protective activities of EPO. Nonerythropoietic tissue-protective molecules hold high promise in a wide variety of acute and chronic diseases.

The Stereotypic Injury Response

Multicellular organisms react to localized injury or metabolic stress by mounting a stereotypic innate immune response that culminates in programmed cell death within and surrounding the lesion [for a review, see 1]. This cascade (fig. 1) is triggered by the molecular signatures of specific pathogens as well as a variety of 'alarm' signals produced by cells within and around the injury site [2]. These molecules ignite a self-amplifying cascade driven by the production of proinflammatory cytokines, e.g. tumor necrosis factor (TNF) α, that form a decreasing gradient around the injury site. Proinflam-

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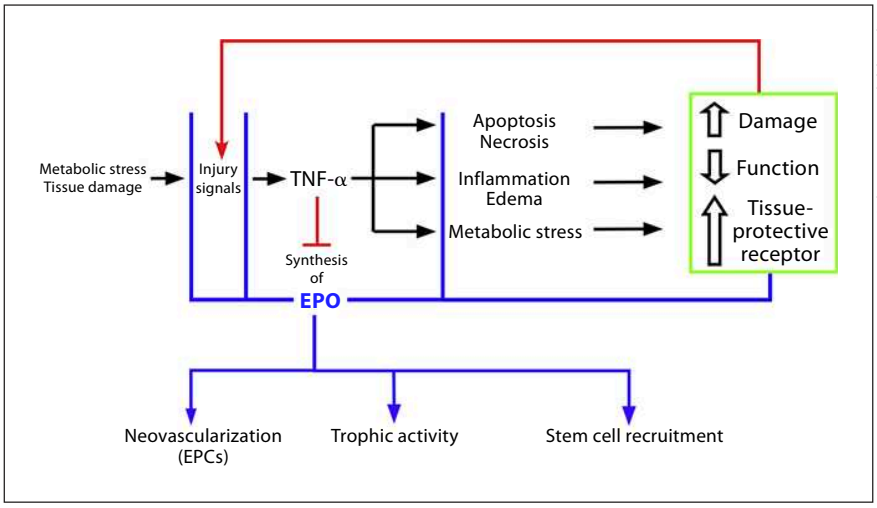

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Fig. 1. EPO antagonizes proinflammatory cytokines and promotes healing and restoration of function following injury. Metabolic stress and tissue damage produce local injury signals that elicit the release of proinflammatory cytokines, e.g. TNF-α, that drive self-amplifying processes including inflammation, metabolic stress, edema and ultimately necrosis or apoptosis of nearby cells. Increases in damage and decreases in function further amplify injury signals in a positive-feedback manner. Additionally, proinflammatory cytokines stimulate the upregulation of a counterregulatory tissue-protective receptor in cells adjacent to the epicenter. EPO is produced locally at the periphery of the injury and antagonizes TNF-α, breaking the self-amplifying cycle. However, as TNF-α inhibits synthesis of EPO in a concentration-dependent manner, a relative deficiency of EPO exists in the vicinity of potentially viable bystander cells. Administration of exogenous rhEPO (or other erythropoiesis-stimulating agents) rectifies the EPO deficiency and is protective. In addition to its effects in acute injury, EPO also plays critical roles in the healing and restorative phase, e.g. fostering revascularization of damaged tissue by recruitment of endothelial progenitor cells (EPCs). EPO is also trophic with respect to the regrowth of neuronal processes and the mobilization of tissue-specific stem cells for the repopulation of damaged regions.

matory factors activate further damage by stimulating the release of cytotoxic compounds, as well as by recruiting immune-competent cells into the vicinity of the evolving lesion. Typically, the resulting lesion is not confined entirely to the proximity of the necrotic region, and the pathology is correspondingly amplified with extensive damage of normal tissues within the penumbral region.

Until recently, it was unknown how this positive-feedback inflammatory cascade remains localized around the injury site. Work performed over the last decade has identified locally produced erythropoietin (EPO) as the principal negative modulator of this system. Many cell types have been identified that produce EPO when stressed, including the ubiquitous capillary endothelial cell. However, similar to the suppression of EPO by circulating proinflammatory cytokines in the anemia of chronic disease [3], proinflammatory cytokines suppress locally produced EPO in a concentration-dependent manner. Therefore, EPO production only occurs at the periphery of a lesion [4] where proinflammatory cytokine concentrations are low, even though potentially viable cells typically exist close to the lesion center. In a converse manner, EPO also suppresses TNF-α production in a concentration-dependent manner [5, reviewed in 6]. The ultimate lesion size is thus determined by the relative 'balance' of these cytokines with mutually counterregulatory activities. In contrast to EPO, expression of the receptor for EPO (EPOR) is strongly stimulated by proinflammatory cytokines, particularly under conditions of metabolic stress [7]. Therefore, potentially viable cells surrounding the lesion center express EPOR, and if EPO is present, they can be rescued from apoptosis caused by inflammation.

In addition to the characteristic spatial differences in cytokine and receptor expression, in which proinflammatory molecules predominate centrally and EPO peripherally, there is also a characteristic temporal profile in the activation of the innate immune response. Immediately following damage, proinflammatory cytokines

are produced. Diffusion of these molecules to nearby intact cells then subsequently stimulates an expression of EPOR that plateaus within 6–8 h. In contrast, EPO expression is further delayed, peaking only at 12 h or beyond [4]. This temporal pattern determines the characteristic delay of hours in the evolution of programmed cell death within the lesion penumbra. The delay in EPO production therefore provides a 'window of opportunity' for therapeutic intervention. This mismatch between EPOR and EPO expression provides the rationale for the administration of exogenous EPO which, upon reaching the injury site in adequate amounts, will trigger anti-apoptotic protein production following binding to the heteromeric receptor.

EPO as a Tissue Protectant

Since the mid-1990s, the tissue-protective effects of recombinant human EPO (rhEPO), as well as other erythropoiesis-stimulating agents, have been explored using a multitude of preclinical disease models. One well-examined type of injury in many tissues is that caused by ischemia and ischemia-reperfusion. The first studies focused on EPO-EPOR expression in the brain [8, 9] were followed by rodent models of transient or permanent ischemic brain injury [10, 11]. Because early workers assumed that the large rhEPO protein could not cross the blood-brain barrier, rhEPO was injected directly into the injury site. As predicted by the innate immune response model described above, exogenous EPO exhibited a dramatic effect by rescuing cells within the 'penumbra' surrounding the lesion (where EPOR is expressed by stressed neurons, but EPO production is low). Also as expected, rhEPO had no effect within the central, necrotic injury zone, but dramatically reduced proinflammatory cytokine production [12]. In confirmation of the tissue-protective role of endogenous, locally produced EPO, neutralization by administration of a soluble EPOR produced a greatly increased volume of injured tissue. However, further study also showed that direct, intrathecal injection was not necessary, as peripherally-administered EPO crossed the blood-brain barrier in concentrations adequate for tissue protection [13]. These experiments additionally showed that the beneficial effects of EPO were not limited to acute ischemic brain damage, but that it was also treatment for blunt cortical trauma, neurotoxin exposure, as well as the more chronic course of experimental autoimmune encephalitis, a model of multiple sclerosis [13].

In addition to the brain, virtually every tissue/organ that has been evaluated has been reported to benefit from EPO administration following ischemia-reperfusion injury. For example, a number of workers have studied the effects of EPO given prior to or after ischemia-reperfusion injury of the kidney [for a review, see 14]. As only one specific example, Vesey et al. [15] showed that high concentrations of rhEPO (200 IU/ml) completely blocked apoptosis and stimulated proliferation of cultured human proximal tubular cells exposed to 1% oxygen for 16 h, followed by a return to normoxia for 24 h. In contrast, lower concentrations of rhEPO within the hematopoietic range were not effective. In parallel in vivo experiments, rats receiving high-dose rhEPO (5,000 IU/kg) via the intraperitoneal route 30 min before a unilateral or bilateral 30-min renal artery occlusion exhibited renal function superior to control when evaluated 24 h later. Other workers have found EPO beneficial in a wide variety of renal injury models including cisplatin and radiocontrast toxicity, ureteral obstruction and diabetic nephropathy, among others [for reviews, see 6, 16].

Potential Problems in the Use of rhEPO for Tissue Protection

Similar to other paracrine-autocrine systems, the local concentrations of EPO required for tissue-protective effects are much higher than for its circulating (hormonal) effects. In its erythropoietic role, a stable, low-picomolar concentration of EPO is required to prevent the programmed cell death of continuously-produced erythrocyte precursors. In contrast, EPO in the low nanomolar range is required, but only briefly, to trigger the tissue-protective receptor. In rodent stroke models, for example, the minimum effective dose of rhEPO is approximately 450 IU/kg body weight, i.e. 1–2 orders of magnitude greater than required to maximally stimulate erythropoiesis. Additionally, a short-lived ligand is equally effective: desialylated rhEPO has a 2-min circulating half-life, yet provides neuroprotection equivalent to rhEPO with a half-life of 5–6 h [17].

The large difference between concentrations required for the different biological effects of EPO normally assures the absence of cross-talk between the two endogenous EPO systems. However, to effectively treat injured tissue using exogenous rhEPO, large parenteral doses are required. These will unavoidably activate hematopoiesis, as well as establish a strong prothrombotic milieu (see, for example, the observation of increases in thromboses re-

lated to erythropoiesis-related agents in cancer patients treated for anemia [18]). Another concern, less worrisome for the acute use of EPO in the setting of injury but of potentially major concern for chronic administration, is the possibility that EPO is a growth factor for malignancies that express the EPO homodimeric receptor [19]. rhEPO (including the engineered variants darbepoietin or continuous erythropoietin receptor activator with prolonged biological activities) are therefore less than ideal pharmaceutical agents for the potential treatment of tissue injury. How to circumvent this problem?

Another Receptor

The receptor transducing erythropoiesis was identified more than 20 years ago as consisting of a dimer of two identical receptor subunits (reviewed by Jelkmann [20]). Current data suggest that this receptor exists as a preformed unit. When EPO binds via well-described molecular interactions to each subunit [21], a conformation change of the receptor results, leading to phosphorylation of janus tyrosine kinase 2 (JAK-2) that is bound to an intracellular portion of EPOR close to the cell membrane. Phospho-JAK-2 subsequently activates multiple intracellular pathways, especially STAT-5, ultimately resulting in the production of antiapoptotic proteins and erythrocyte precursor survival [for a review, see 22].

The substantial difference between the affinity of the EPOR expressed by erythrocyte precursors and the one on nonhematopoietic cells [23] suggested that a different receptor could transduce tissue protection. Work employing a number of models showed that this receptor is heteromeric in structure, consisting of the same EPOR monomer utilized in the hematopoietic receptor, but complexed with the common β-receptor, another cytokine receptor subunit that is utilized by granulocyte macrophage colony-stimulating factor, interleukin 3 and 5 in signaling [24]. The molecular pathways underlying tissue protection are multiple and depend upon the cell and tissue type studied. Phosphorylation of JAK-2 is the usual initial step (although phospholipase C and calcium channel modulation is used by some cells), with downstream pathways including the phosphoinositide-3-kinase/protein kinase B, mitogen-activated protein kinase and the extracellular signal-related kinase systems [for reviews, see 6, 16, 25].

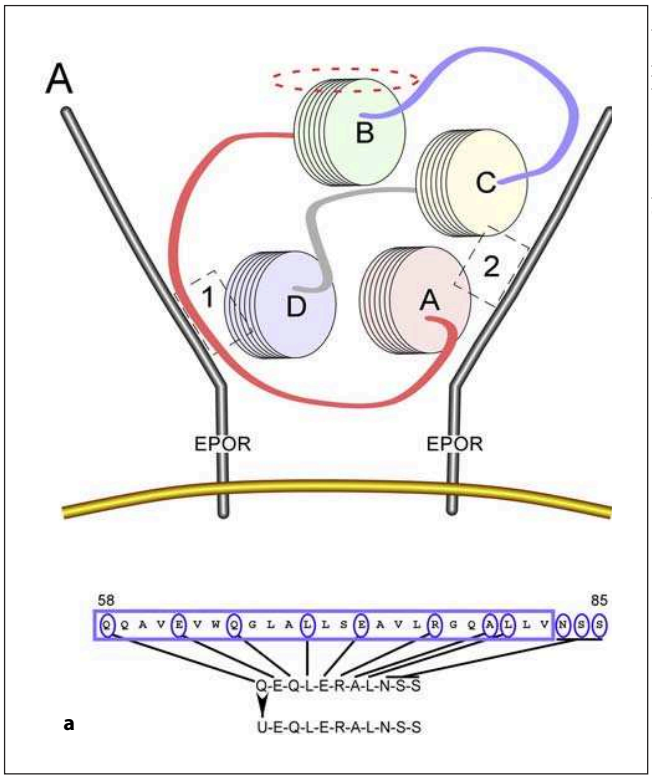
Nonerythropoietic, Tissue-Protective Molecules

The identification of an alternative EPOR for tissue protection raised the possibility that a receptor-specific ligand could be developed. Prior work by numerous groups has provided a detailed understanding of which regions of the EPO molecule bind to the EPOR homodimer and trigger erythropoiesis [for a review, see 24]. Numerous chemical modifications or amino acid substitutions within these critical EPO-binding regions have been described that abolish hematopoietic signaling. As one example among many, the multiple positively charged lysine residues that exist within the two binding sites can be converted into the neutral amino acid homocitrulline by carbamoylation. As previously known, EPO so modified (carbamoyl EPO; CEPO) is not erythropoietic [26]. In contrast, CEPO is a potent tissue protectant, as has been demonstrated using models of nervous system injury [27, 28] or kidney injury [29], among others.

CEPO is similar to rhEPO in terms of half-life (approx. 4 h after intravenous administration) and biological activities in the tissue-protective arena [27]. However, from a pharmaceutical perspective, CEPO is also a biological product that requires a complicated (and therefore expensive) production line. Additionally, to obtain CEPO of a uniform, high purity, the reaction yield is only in the order of about 50% and moreover the finished product, like rhEPO, requires cold storage to assure stability. For these reasons, it was desirable to seek small molecular alternatives useful for tissue protection.

EPO, a member of the type 1 cytokine superfamily, is a globular protein due to the self-assembly of its 4 α-helices A–D [20]. Three of the helices (A, C and D) participate in the two binding sites within the hematopoietic EPOR homodimer (fig. 2a). This observation suggested that the remaining helix might be involved in tissue protection. Synthesis of peptides derived from helix B and testing of these in a variety of preclinical models, e.g. stroke, indeed confirmed that helix B peptides are tissue protective [24]. In sum, small peptides devoid of hematopoietic activity can recapitulate the biological activities of EPO in tissue protection.

An additional refinement was also suggested by the relative rigidity of the EPO molecule: only one surface region of helix B is exposed in aqueous solution (fig. 2a). The amino acids within this region are presumed to interact with the tissue-protective receptor. If so, a peptide consisting of a linearized version of the surface amino acids of helix B would also be tissue-protective. Testing in a wide variety of preclinical models has confirmed that



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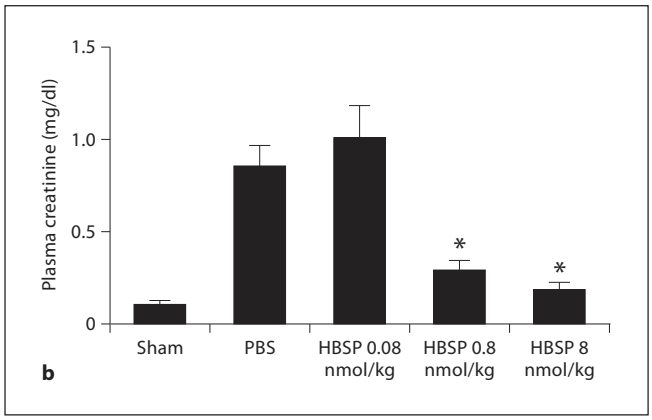

Fig. 2. EPO binds to a homodimeric receptor to mediate erythropoiesis, but also contains a region that binds to a tissue-protective receptor isoform. **a** Helix B contains the tissue-protective motif of EPO. The 4 α-helices of EPO associate via hydrophobic interactions, forming a globular molecule. Binding of EPO to the homodimer occurs via interaction at two binding sites (dashed squares 1 and 2), involving portions of helices A, C and D. In contrast, helix B faces away from the binding region of the homodimer. Modification of specific amino acids within binding sites 1 and 2 produces a modified EPO molecule that can no longer interact with the receptor and is therefore not erythropoietic. However, the helix B portion of the altered EPO molecule can nonetheless interact with the tissue-protective receptor and prevent injury. Finally, amino acids located on the surface portion of helix B (dashed ellipse) are cytoprotective when synthesized as a linear peptide (below). **b** Helix B surface peptide is protective in a murine kidney ischemia-reperfusion model. Mice (n = 12 for each group) were subjected to a sham procedure or renal ischemia-reperfusion injury: bilateral renal pedicle occlusion for 30 min; saline or helix B surface peptide (HBSP, i.p., 1 min, 6 h and 12 h into reperfusion). A dose-dependent protective effect of HBSP is evident in the plasma creatinine levels as a biochemical marker of renal dysfunction. Data represent means ± SEM and were analyzed by a one-way ANOVA followed by Dunnett's test for multiple comparisons; $p < 0.05$ versus renal ischemia-reperfusion group (figure and data previously published in Brines et al. [24]).

helix B surface peptide is indeed cytoprotective [24]. As only one example of peptide-mediated tissue protection (fig. 2b), anesthetized mice were subjected to 30 min of bilateral renal artery and vein occlusion followed by 24 h of reperfusion. Animals were administered either saline or helix B surface peptide at various concentrations via the intraperitoneal route, and renal function and injury were assessed the next day. The results showed a dose-dependent preservation of renal function as well as a marked reduction in tissue injury, as assessed by plasma aspartate aminotransferase [24].

Conclusion

Endogenous EPO has only recently been recognized as a major component which limits the spread of inflammation and damage. The significant time delay following injury in the production of EPO provides the rationale for the use of exogenous rhEPO. Although numerous studies have shown that exogenous erythropoiesis-stimulating agents can provide impressive tissue protection and regeneration in a wide variety of tissues and injury models, these compounds unavoidably activate the hematopoiet-

ic and thrombotic systems. The identification of a specific EPOR isoform involved in tissue protection stimulated the successful molecular engineering of the EPO molecule to produce selective tissue-protective ligands, e.g. CEPO. The recent development of small peptides that trigger the same protective responses suggest that small, orally active mimetics will likely be identified in the future. Because the injury response is highly stereotypic irrespective of etiology, tissue-protective compounds hold high promise for therapy of a very wide variety of acute and chronic diseases.

Acknowledgements

Over the past years, a very large body of work concerning EPO has accumulated which cannot be comprehensively assessed in this short review. I apologize in advance to my many colleagues whose work I cannot discuss here. The development of nonerythropoietic molecules was carried out with my colleagues Anthony Cerami, Carla Hand, Thomas Sager, Marcel Leist, Lars Torup, Jens Gerwien, Pietro Ghezzi, Thomas Coleman, Michael Yamin and Xiao-Wen Xie. I also thank Michael Yamin for reading the manuscript and his valuable suggestions.

Conflict of Interest

M.B. has an equity stake in Warren Pharmaceuticals which is developing nonerythropoietic tissue-protective molecules for clinical use.

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Special Considerations for Antihypertensive Agents in Dialysis Patients

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Key Words
Hypertension · Antihypertensive drugs · Dialysis · Chronic kidney disease stage 5

Abstract
Hypertension is present in most patients with end-stage renal disease and likely contributes to the premature cardiovascular disease in dialysis patients. Previous practice guidelines have recommended that, in patients on chronic dialysis, blood pressure (BP) should be reduced below 130/80 mm Hg. This is based on opinions but not strong evidence, since no concrete information exists about which BP values should be the parameter to follow and which should be the target BP values. The majority of the antihypertensive agents can be used in this population, but the pharmacokinetics altered by the impaired kidney function and dialyzability influence the appropriate dosage as well as the time and frequency of administration. Combination therapy using multiple agents is often necessary. Because of the prevalence of overactivity of the renin-angiotensin-aldosterone system and sympathetic tone as well as the high calcium influx in vascular smooth muscle cells in dialysis patients, drugs acting in these three specific systems may potentially have additional cardioprotective benefits beyond their BP-lowering effect. Thus, antihypertensive regimens should preferably be based on these classes of drugs, alone or in combination. Other antihypertensive drug classes can play a complementary role.

Introduction

Hypertension, as defined in the general population, is present in most patients with end-stage renal disease and probably contributes to premature cardiovascular diseases in this population [1]. Consequently, control is recommended in the attempt to reduce the cardiovascular disease burden, although complications associated with these treatments are not uncommon as the pathogenesis of hypertension in end-stage renal disease is complex and multiple mechanisms are likely involved in the blood pressure (BP) dysregulation. Thus, antihypertensive treatment is sometimes not an easy task, and refractory hypertension is common [2]. Understanding the treatment targets, intra- and interdialytic BP behavior and pharmacokinetic properties of antihypertensive drugs in impaired kidney function and during dialysis is the key to achieve success in BP treatment. Furthermore, potential additional cardioprotective benefits of the antihyper-

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tensive drugs, beyond their BP-lowering effects, need also to be considered. In the following sections, key issues about the use of antihypertensive drugs in dialysis are reviewed.

Beneficial Impact of Antihypertensive Drugs in Cardiovascular Risk

Practice guideline recommendations [3–5] stated that in patients on chronic dialysis, BP should be reduced below 130/80 mm Hg. This is based largely on opinions, but not on sound evidence, since unfortunately no solid information exists about the BP parameters that should be followed and the target values of these parameters that would yield the best clinical outcome.

In fact, there are very few randomized trials on the impact of antihypertensive drugs on hard clinical outcomes in dialysis patients, and all of them have been pooled in two recently published meta-analyses [6, 7]. The analysis performed by Heerspink et al. [6] included 8 randomized trials, 1 published only in abstract form, 5 of which examined renin-angiotensin-aldosterone system (RAAS) blockade, 2 examined β-blockers (βBs) and 1 examined a calcium channel blocker (CCB). Data for 1,679 patients and 495 cardiovascular events were provided. BP-lowering treatment was associated with a significant reduction of cardiovascular events (29%), all-cause mortality (20%) and cardiovascular mortality (29%), for a systolic/diastolic BP difference versus control (not using these antihypertensive agents) of –4.5/–2.3 mm Hg. Regretfully, no information was provided about the absolute BP values achieved with treatment, although the fact that statistically significant beneficial effects were observed only in the subgroup of patients with hypertension and not in the normotensive subgroup may suggest that BP values achieved by treatment were not particularly low.

The analysis performed by Agarwal and Sinha [7] included 5 randomized trials, which were a subset of the one performed by Heerspink et al. [6]. Although the main results are similar to the previous one, these latter authors stressed that beneficial effects of antihypertensive medications were markedly diminished and became statistically nonsignificant when normotensive subjects were included. The matter is further complicated by the extensive use in these studies of RAAS blockers, which are thought to possess specific cardioprotective and renoprotective properties that make the effect attributable to BP reductions more difficult to unravel.

The results of these meta-analyses need to be interpreted with caution. Beside limitations such as heterogeneity of individual study designs and publication bias, causality cannot be inferred from the association between the decrease in BP and beneficial clinical effects, in part because no BP goals were predefined in these trials. Adequately powered randomized trials specifically designed to compare the effects of different BP goals on clinical outcomes are necessary to guide clinical management decisions. However, the inherent difficulties to these large randomized studies and the complexity in dialysis patients, including the large variations in inter- and intradialytic BP values, make this a difficult task. In the meantime, inference from studies performed on hypertensive patients in earlier stages of chronic kidney disease (CKD) or the non-CKD population with individualized clinical assessment is a reasonable approach.

BP-Lowering Effect

While important, nonpharmacological treatments, such as fluid removal, are often insufficient to achieve the presumed target BP levels in dialysis patients. Multiple antihypertensive drugs are often necessary [2]. Because of this, emphasis on a single first line of drugs to be used is not very useful. Nevertheless, there are many conditions for which evidence supports the use of one drug over others, either as initial treatment or as a part of a combination.

Four major classes of antihypertensive agents are suitable for the initiation and maintenance of antihypertensive treatment, alone or in combination. These are CCBs, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers and βBs. In addition, α-adrenergic blockers, central-acting agents and direct vasodilatory drugs can also be useful. Loop diuretics can be used in subjects with residual kidney function, with more than 300 ml daily of urinary output, usually a short period of time after starting hemodialysis. Finally, the recently introduced direct renin inhibitors are also of interest although no information on this class of drugs has been available for patients on dialysis up to now [8]. Whether or not one class of drug results in greater BP reduction compared to other classes in dialysis patients has not been fully explored.

The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and of the European Society of Cardiology (ESH/ESC) has provided recommendations for the use of combination

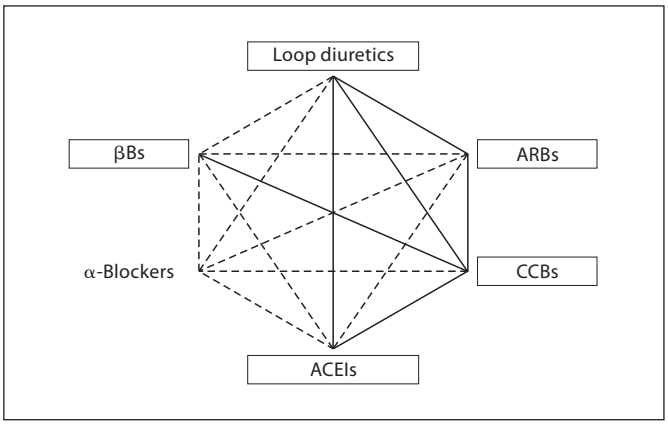

Fig. 1. Recommendations for combining antihypertensive drugs according to the ESH/ESC guidelines [5]. The most recommended are those connected by solid lines. For dialysis patients who do not respond to diuretics, dialytic fluid removal should substitute loop diuretics. Caution should be exercised when combining CCBs and βBs in patients who are prone to develop bradycardia or heart block. ARBs = Angiotensin receptor blockers.

drug therapy [5]. This schema is presented in figure 1. The unique characteristics and the complexity of treatment in dialysis patients do not favor the use of single-pill combination drugs.

When using antihypertensive agents in the dialysis patient, recommendations for antihypertensive treatment in the non-CKD population generally apply [4, 5]. Long-acting antihypertensive drugs which cover the 24 h with once-a-day administration are recommended to improve adherence to treatment [9]. In addition, several questions should be addressed: can the full dose of the drug be used or does it need to be reduced because of impaired kidney clearance of the drug? Is the drug removable by hemodialysis or peritoneal dialysis? Is a supplemental dose necessary after dialysis? Finally, are there special conditions that favor one drug or another? These questions have been discussed in detail in recent publications [10, 11].

Dose Reduction

The necessity or not to reduce the dose of the drug in dialysis patients largely depends on the metabolism and excretion route and capacity of the drug (fig. 2). Not only do drug classes differ in their pharmacokinetic patterns from each other, clinically relevant intraclass differences also exist. All loop diuretics, CCBs, angiotensin receptor blockers and α-adrenergic blockers can be administered at full dose and at time intervals as in the non-CKD population. This generalization is based on considerations of plasma drug levels. However, considerations for efficacy may necessitate the change in dose. For example, a much larger dose of loop diuretics may be required to achieve diuresis in the dialysis patients. In contrast, the dose should be reduced by at least 50%, or the dosing interval should be at least doubled, for the majority of the ACEIs. Only fosinopril and benazepril allow use with a modest reduction of dose of approximately 25%, because they are largely metabolized in the liver.

The situation of the other antihypertensive classes is more heterogeneous. Consider, for example, βBs, 1 of the 4 main antihypertensive classes. While the doses of acebutolol, atenolol, betaxolol, carvedilol, nadolol and sotalol need to be reduced at least by half, all the others can maintain the full dose. Among the central-acting agents, in contrast to clonidine and guanabenz, the doses of guanethidine and methyldopa need to be reduced, while reserpine should be avoided. Hydralazine is the only direct vasodilatory drug that requires dose reduction. The usage and dosing of direct renin inhibitors should await specific data on this drug in dialysis patients.

Removal and Supplement for Dialysis

The extent to which a drug is affected by dialysis is determined by several physicochemical characteristics of the drugs. These include molecular weight, plasma protein binding, volume of distribution and intercompartmental transfer, water solubility and plasma clearance by the body, besides the technical aspects of the dialysis procedure such as characteristics of the dialysis membrane, blood and dialysate flow rates, convective versus diffusive mode of solute transport, and treatment time [10]. The removal of drugs during dialysis is the result of this complex interplay of conditions. The extent of this removal, in turn, determines whether supplemental dosing is necessary during or following dialysis. Interactions of a specific hemodialysis membrane, the AN69 membrane, with coagulation proteins in the presence of ACEIs can produce anaphylactoid reactions, as a result of the generation and accumulation of vasoactive bradykinin in the plasma [12].

In general, antihypertensive drugs are not removed significantly by hemodialysis, with the exception of ACEIs, some βBs (acebutolol, atenolol, nadolol, sotalol), methyldopa and hydralazine (fig. 3). In these cases, the use of long-acting drugs 3 times a week after each hemodialysis session is a good option for promoting drug ad-

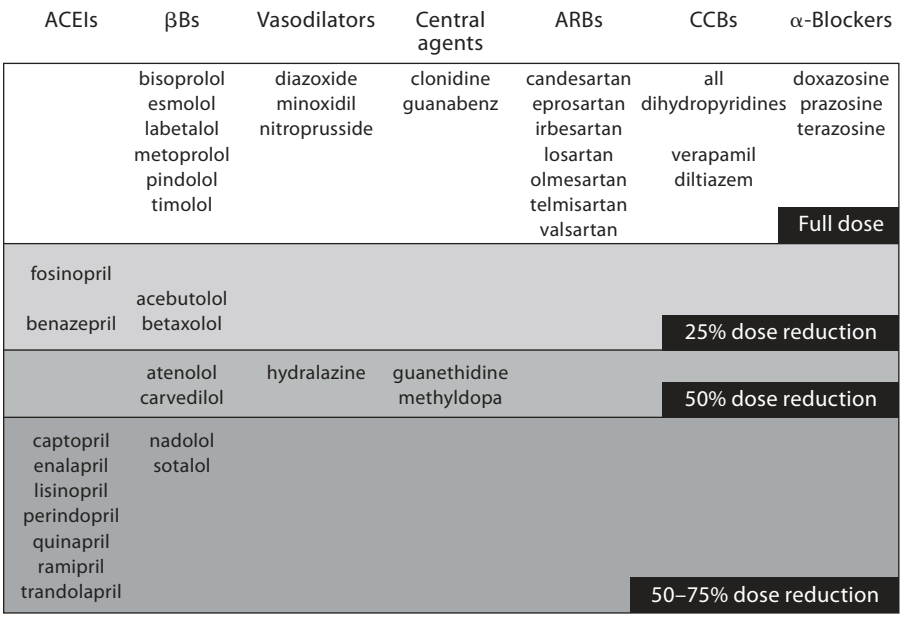

Fig. 2. Dose recommendations for the antihypertensive drugs used in dialysis patients. ARBs = Angiotensin receptor blockers.

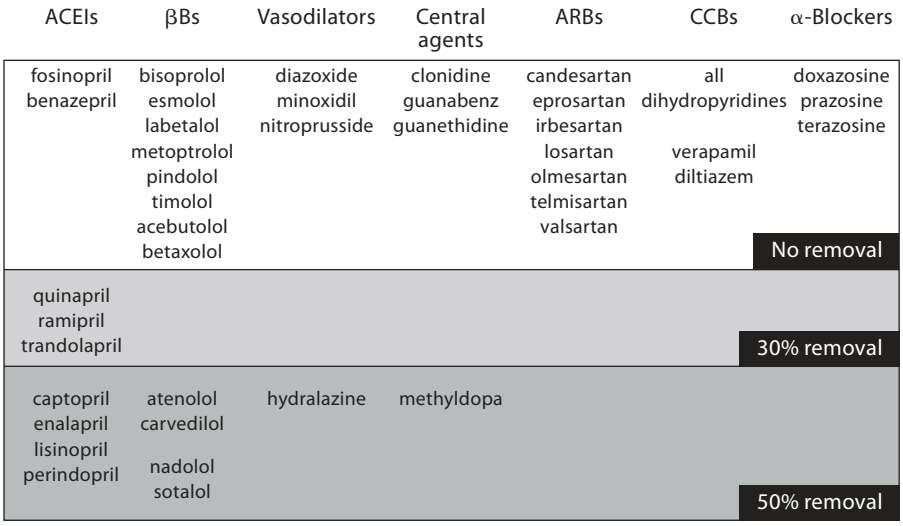

Fig. 3. Removal of antihypertensive drugs by hemodialysis. Drugs in the shaded areas require a supplementary dose after dialysis. The magnitude of removal varies depending on the efficiency and duration of the dialysis session. Removal also occurs on peritoneal dialysis. ARBs = Angiotensin receptor blockers.

herence. In peritoneal dialysis, only methyldopa and hydralazine are the major drugs that are dialyzable. These drugs, especially methyldopa, are not commonly used nowadays.

Special Conditions

Clinical characteristics of individual patients can affect the choice of the antihypertensive drugs, either favoring or discouraging their use, as in the case of hypertensive patients not on chronic dialysis. For example, the presence of coronary heart disease or congestive heart failure is a compelling indication for βBs, while concomitant peripheral vascular disease or bronchial hyperreactivity discourages their use. The venodilatory property of furosemide can be beneficial in congestive heart failure. Persistent hyperkalemia can limit the use of drugs that block the RAAS or the βBs, even in dialysis patients.

A complete list of reasons for favoring or for avoiding the use of each antihypertensive class was listed in the

ESH/ESC guidelines published in 2007 [5]. Recommendations of specific drug classes for the treatment of cardiovascular diseases should be taken with caution in dialysis patients [13–15], because of the paucity of solid evidence-based data supporting their efficacies.

Potential Effects beyond BP Reduction

A large burden of cardiovascular disease exists in the dialysis patients. End-stage renal disease is associated with a 10- to 20-fold increased risk of cardiovascular mortality, compared with age- and sex-matched controls without CKD [1]. Cardiac deaths are extremely common in dialysis patients and account for the majority of cardiovascular deaths [16]. Coronary heart disease, vascular calcification and uremic cardiomyopathy probably underlie the majority of these cardiac deaths [17]. Thus, cardioprotective properties of antihypertensive drugs, beyond their BP-lowering effect, should be taken into account. Drugs blocking the RAAS and βBs and CCBs may confer potential additional benefits, since overactivity of the RAAS (as reflected by increased plasma renin activity), increased levels of sympathetic activity (as reflected by elevated plasma levels of norepinephrine and neuropeptide Y and reduced heart rate variability) and intracellular calcium overload are common in dialysis patients. Furthermore, sympathetic overactivity has been associated with increased cardiovascular mortality in hemodialysis patients [18, 19].

Antihypertensive drugs also have effects on other putative cardiovascular risk factors that are frequently present in dialysis patients. Hypertriglyceridemia and glucose intolerance can be worsened by the use of βBs, while CCBs [20] and drugs blocking the RAAS [21] exert a beneficial or at least a neutral effect.

However, the potential additional benefits of these classes of drugs are inferior to their BP-lowering effects in hypertensive subjects not on dialysis [22–24]. In contrast, subjects with BP values in the 'normal range' can gain benefits with the use of these antihypertensive drugs, but excessive BP reduction should be avoided in frail patients in which a 'J curve' relationship between BP and mortality is observed. Drug-induced electrolyte disorder is an additional concern in dialysis subjects who are prone to develop hyperkalemia.

Despite the uncertainty about the additional non-BP-related benefits, RAAS blockers, βBs and CCBs, either used alone or in combination, should be strongly considered as antihypertensive agents in dialysis subjects.

Conclusions

Antihypertensive drugs are commonly used in dialysis patients, often based on the belief that lowering BP reduces morbidity and improves survival, despite the fact that no solid information is available about when antihypertensive treatment should be initiated and what the goals of treatment should be. Nonpharmacological treatments, although important, are usually insufficient to control BP in dialysis patients, and multiple antihypertensive drugs are often necessary. The majority of the antihypertensive agents can be used in dialysis patients, but their pharmacokinetics and dialyzability should be considered. Combination drug therapy offers additive antihypertensive activity and often reduction in side effects, but a single-pill combination is ideal because of the complexity of dialysis patients. The potential benefits of some antihypertensive drugs beyond their BP-lowering effect should be considered, although many uncertainties still exist concerning dialysis patients. Balancing the potential benefits and risks in individual patients is warranted.

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The Potential Role of Plasma Gelsolin in Dialysis-Related Protein-Energy Wasting

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Key Words
Gelsolin · Protein-energy malnutrition · Wasting syndrome · Kidney failure · Renal dialysis

Abstract
Protein-energy wasting (PEW) is increasingly recognized to be a prevalent and significant contributor to the clinical deterioration of patients with chronic kidney disease (CKD). While factors reflecting various aspects of PEW correlate with outcomes in CKD, the mechanism linking PEW and CKD outcomes is not completely understood. Plasma gelsolin (pGSN) is a highly abundant circulating protein that is depleted by inflammatory mediators and mainly produced by muscles. Recent data documenting the prognostic ability of low pGSN levels in hemodialysis patients suggests that circulating pGSN levels incorporate the degree of systemic inflammation and muscle wasting. Therefore, pGSN deficiency appears to be a powerful biomarker and a potential therapeutic target in CKD patients.

Despite much effort, the mortality rate for patients on hemodialysis (HD) remains unacceptably high [1]. While several clinical and laboratory factors have been linked to adverse outcomes, protein-energy wasting (PEW) is a common phenomenon associated with increased mortality and morbidity among patients undergoing HD [2–4]. Muscle wasting, protein catabolism and persistent inflammation are some of the central characteristics of PEW [3]. Biochemical and physiological indicators, such as low serum albumin levels, low body mass index, high circulating cytokines and elevated white blood cell counts, are also associated with PEW and have prognostic value in HD [3, 4]. The biology underlying the association between PEW and HD is incompletely understood, but a uremia-induced increase in protein degradation by the proteasome-ubiquitin system has been implicated [5].

Plasma gelsolin (pGSN) is a secreted high-molecular-weight (83 kD calculated, 93 kD by SDS-PAGE) actin-binding protein that normally circulates at 190–300 mg/l and is mainly produced and secreted by myocytes [6–9]. pGSN differs from its cytoplasmic isoform (cytoplasmic GSN) transcriptionally, structurally and compartmentally [7, 10, 11]. Cytoplasmic GSN participates in actin dynamics exclusively in the intracellular compartment, while pGSN is actively secreted into the extracellular space. Failure to distinguish the two isoforms has led to much confusion in the literature.

As pGSN avidly binds actin, some have suggested that it may be part of an extracellular actin-scavenging system [12]. However, emerging data demonstrate that pGSN also binds and modulates several endogenously produced bioactive lipids, such as platelet-activating factor and ly-

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sophosphatidic acid [13]. pGSN has also been reported to bind and neutralize exogenous bacterial lipid mediators such as lipopolysaccharide and lipoteichoic acid, suggesting that pGSN may play a role in innate immunity [14, 15]. Indeed, pGSN appears to localize inflammation and prevent systemic escape of proinflammatory lipids. Consistent with this hypothesis, low pGSN levels have been documented in settings of acute tissue injury or inflammation, such as trauma, burns, postoperative states, chemotherapy and sepsis [16–19] and, more importantly, blood pGSN levels are inversely related to clinical outcomes in these conditions. These findings suggest that pGSN levels may be a useful sensor for systemic inflammation. pGSN depletion is thought to be secondary to extracellular actin exposure, locally or systemically [8, 18, 20, 21]. Because of the rapidity with which pGSN declines, decreased protein production is unlikely to play a significant role in acute illness.

While clinical trials have yet to be conducted to examine the therapeutic potential of pGSN replacement in humans, data from several animal models are promising. Exogenous pGSN administration confers a survival benefit while suppressing proinflammatory cytokines in animal models of sepsis [22]. The same exogenous therapy also improves physiological parameters in models of burns [23] and acute lung injury [24]. Thus, pGSN deficiency appears to be a potentially modifiable risk factor in these acute diseases. pGSN's role in chronic conditions remained unexplored until recently when Osborn et al. [21] reported low pGSN levels in patients with rheumatoid arthritis. This finding suggests that ongoing local inflammation may also cause a detectable drop in circulating pGSN. As pGSN appears to integrate immunity, acute and chronic inflammation and muscle mass, it has the potential as a useful biomarker for patients on HD, where alterations in these factors have been implicated in clinical outcomes.

We recently reported a case-control study examining the association between pGSN levels obtained at initiation of HD and clinical outcomes [8]. We found that pGSN levels in patients initiating HD were about half of those in healthy individuals. Furthermore, these levels were inversely associated with 1-year mortality. Patients in the lowest tertile of pGSN levels had a greater than 3-fold increase in 1-year mortality compared to the highest tertile (95% CI = 1.2–9.4), after controlling for parameters such as age, gender, albumin, body mass index, creatinine, high-sensitivity C-reactive protein and white blood cell count. Notably, we detected circulating actin in 69% of patients, suggesting ongoing tissue/cellular damage. The presence of 'actinemia' added prognostic information: patients with low pGSN levels and measurable circulating actin had a 9.8-fold increase in mortality (95% CI = 2.9–33.5) compared to those with higher pGSN levels and undetectable circulating actin. That relationship was even more impressive in patients with catheter-based access, where the odds ratio for 1-year mortality rose to 25.9 (95% CI = 4.3–157). We concluded that pGSN levels with or without actinemia have a significant prognostic value in HD patients.

There are likely several factors leading to decreased pGSN levels in HD patients. As in acute diseases, chronic inflammation in HD patients appears to deplete pGSN, as evidenced by an inverse relationship between pGSN and high-sensitivity C-reactive protein. Ongoing tissue damage leading to actinemia, possibly from the HD treatment itself, leads to actin-pGSN complexes which are cleared more rapidly than free pGSN [25]. Finally, pGSN levels correlated with creatinine, suggesting that decreased muscle mass may be a contributing cause of low levels of pGSN. pGSN levels therefore reflect several elements of PEW: an individual's muscle mass, ongoing inflammation and tissue damage.

Our findings, if confirmed to be generalizable, suggest that pGSN can potentially be a useful clinical biomarker for patients initiating HD. For example, those patients with low pGSN should avoid vascular catheter access and may be preferentially considered for renal transplantation. Efforts to improve PEW state by nutritional supplementation or pharmacological intervention could be monitored by changes in pGSN levels. Since recombinant pGSN is available, and early studies suggest that it is safe to administer, pGSN replacement therapy deserves future consideration and evaluation in HD patients.

Conflict of Interest

P.S.L. and R.T. are named as co-inventors on a recently filed provisional patent filed by the Harvard Hospitals for the use of GSN in renal failure subjects. P.S.L. is an equity holder in Critical Biological Corporation, a private company that is currently developing recombinant GSN for potential use in sepsis.

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How to Successfully Achieve Salt Restriction in Dialysis Patients? What Are the Outcomes?

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Key Words
Salt restriction · Dialysis · Blood pressure · Volume control

Abstract
Despite the fact that dietary salt restriction is the most logical measure to prevent accumulation of salt and water in patients without renal function, it is not applied in most dialysis centers. In this review, the reasons for this unlucky development are analyzed. First, it appears that many dialysis patients are slightly overhydrated, but this is often not noticed and, if so, the deleterious effects in the long run are not appreciated. These consist not only of 'drug-resistant' hypertension, but also dilatation of the cardiac compartments leading to preventable cardiovascular events. Second, there are practical reasons why salt restriction is neglected. It is very difficult to buy salt-poor food. Salt consumption is an addiction, which can be overcome, but time and efforts are needed to achieve that. Suggestions are made how to reach that goal. Finally, examples are given how cardiac damage (often considered irreversible) can be improved or even cured by a 'volume control' strategy, whose crucial part is serious salt restriction.

When the kidney fails, the dialysis doctor has to take over its functions. Roughly speaking, these can be categorized into (1) removal of unwanted and potentially harmful metabolites and (2) volume control. The latter mainly consists in removal of extracellular fluid by ultrafiltration. Because modern man consumes excessive and unnatural amounts of salt, this forces him to drink an equivalent amount of water that leads to an expansion in the extracellular volume and also the blood volume. Depending on the eating habits, this volume excess ranges between 2 and 5 liters between two dialysis sessions. It goes without saying that removal of such an amount within a few hours by ultrafiltration will cause circulatory instability which often prevents complete correction. Thus, the patient will remain more or less overhydrated and suffer from hypertension, so-called heart failure and cardiovascular damage [1]. Even if the 'dry weight' is reached during a dialysis session, the patient will be 'volume expanded' most of the time between the sessions. The potential harm of this 'harmonica effect' has never been investigated. The most logical solution of this problem is evidently dietary salt restriction. Hegstrom et al. [2] convincingly showed that it prevents blood pressure rise and that, moreover, it can correct even severe hypertension. Unfortunately, this simple truth has been neglected for a very long time by the dialysis world.

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On the other hand, it is not easy to achieve successful dietary salt restriction, the necessity of which is no longer doubted. It is clear that just telling the patient to consume salt-poor food does not work. Let me analyze with you the reasons why it is so difficult to achieve this goal.

Although it is mainly a practical problem, first of all it is crucial that the dialysis physician is convinced of the deleterious effect of even a little bit of overhydration. However, it is also necessary that he (or she) has a basic knowledge of salt and water physiology. Many doctors seem to believe that volume excess results from drinking too much water, because they blame their patients for it. Indeed, they drink too much, but the reason for it is salt consumption. That causes thirst, which is an irresistible urge. Thus, the patient will not comply, feels guilty and looses confidence. But worst of all, the attention of the dialysis team is diverted from the real culprit: salt. Yet, *Kidney International* accepted a paper [3] of respected nephrologists investigating how to prevent thirst without mentioning salt. While it is theoretically possible that inappropriate thirst would contribute to volume retention, it should cause hyponatremia. This has not been documented to my knowledge. Anyhow, it is sufficient to tell our patients not to drink more than the feeling of thirst indicates.

Salt consumption is a habit with all the characteristics of a mild addiction. The reason is that salt sensing of the tongue adapts to salt. If a person eats salt-free food for some time, the same solution which was previously found to be tasteless will then be sensed as very salty. Of course, the reverse is also true. The time requested for this adaptation is several weeks to months. If they sometimes consume salted food, the adaptation may not occur. Therefore we should help the patient to overcome that difficult period. It is very strange that in some dialysis units, 'normal' meals are provided to the patients in the erroneous idea that bad constituents will be washed out anyhow. In this way the patients will never adapt their taste for salt.

How can we achieve these goals? Of course we should explain to the patient all these aspects mentioned above, but telling it once is not enough. Repeating these pieces of advice, which are not easy to understand, will take time and effort. These educational talks should be started early, even before the start of dialysis treatment. The longer a bad habit lasts, the more difficult it will be to stop. The patient will not be aware of the harmful effects of salt and may conclude that it is not so important after all. This issue should be explained also to the spouse and to the family.

It is important that dietary advice should not be complicated. We suggest focusing on salt restriction and the danger of high potassium.

It is important to realize that the problem concerns the whole dialysis community. In particular the nurses can be more influential than the doctor. Dialysis patients form a community. They meet each other several times a week and talk and share their problems. Therefore, it is useful to organize common information and discussion sessions, as regularly performed in our practice.

Now let us suppose you have convinced your patients. But how will they get their salt-poor food? This is a real problem. Because not adding salt to their cooking and not using the salt shaker is absolutely not sufficient. Depending on the local culture, the largest part of the salt intake comes from the food they buy in the shops and supermarkets [4]. In the USA it may be 80–90%. In particular, bread may contain a lot of salt. It may not be easy to find salt-free bread or other salt-poor food in several countries. Thus, the patients and their families have to spend a lot of effort to find or prepare themselves salt-poor meals. Here the dialysis personnel may be of much help with advice and coordination.

What Will Be the Benefits of Such a Policy? Are These Efforts Really Worthwhile? Can We Reach the Same Results with Some Drugs?

Apart from Scribner's experience, which has been forgotten, several centers in the world have published very favorable results of strict volume control [5, 6]. It must be admitted that although salt restriction was the key of their approach, some of them also applied longer dialysis sessions and more persistent ultrafiltration. The following outcomes can be reached.

First, normal blood pressure was achieved without the use of antihypertensive drugs in around 90% of the patients. We should realize that there is no linear relationship between the degree of volume excess and the level of blood pressure. Most importantly, an elevated blood pressure does not normalize immediately when a normal volume is reached and needs time to decrease. This is the famous 'lag phenomenon' so well described by Charra et al. [7]. Despite treatment with multiple drugs, hypertension is not adequately controlled in most centers where salt restriction is not applied, because these drugs cannot get rid of the main cause: volume excess.

The second benefit concerns the condition of the heart. In the first months after starting dialysis, many authors reported a very frequent occurrence of 'heart failure' [1, 8]. In fact, this is not failure but lung edema from sudden

volume overload and can also happen with a normal heart. It is never encountered in centers with good volume control.

When the (often not suspected) volume excess persists, the lymphatics of the lung adapt and edema is no longer seen [9], but the combined increase in pressure and volume load cause left ventricular hypertrophy. This is a well-known risk factor for cardiac arrhythmias and coronary events. In contrast to patients with essential hypertension, in whom antihypertensive drug treatment causes regression of left ventricular hypertrophy, in dialysis patients it does not decrease as long as volume excess persists. However, it can be markedly improved by volume reduction without drugs [10, 11].

This latter fact once more points to the importance of 'volume' as a risk factor independent of blood pressure. Echocardiographic examination of the heart shows slight to moderate dilatation of the left atrium end ventricle in most dialysis patients. This often goes unnoticed because it does not cause complaints. If dilatation goes on, in the long run valvular regurgitation, extreme cardiomegaly, drop in ejection fraction and subnormal blood pressure may ensue. This dilated cardiomyopathy has unfortunately been called 'uremic'. This term suggests that it is caused by uremic toxins, and many authors consider it to be irreversible. However, it can be markedly improved and sometimes even cured by severe salt restriction and ultrafiltration [12, 13].

The final proof of the benefit of salt restriction should of course be a decrease in mortality [6]. Several investigators have indeed shown a very low mortality in the patients thus treated. However, they have been criticized with the argument that their 'case mix' was different from other series and that there was no control group. The reasons for this omission are evident: first, the physician has a moral dilemma, for he should deprive patients from a treatment that he knows to be beneficial, until their mortality reaches the statistically required number. Second, there is also a practical difficulty: as I explained before, effective salt restriction is a matter of the whole dialysis community. It is not possible to pursue two different strategies in the same center. We recently tried to solve that problem by changing the current strategy of 3 centers to salt restriction and using the results of 3 adjacent centers in the south of Turkey, which continued in the conventional way as control. The results confirmed the advantages of salt restriction [Ozkahya et al., unpubl. data]. But this setup was not ideal either.

In conclusion, salt restriction is the most logical step to prevent hypertension and cardiovascular determination in dialysis patients. Application of this strategy is not easy and requires time and effort from the dialysis team. Basic physiological principles should not be neglected. Marked improvement of cardiac disturbances can also be achieved with a volume control strategy.

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Haemodialysis-Induced Myocardial Stunning in Chronic Kidney Disease – A New Aspect of Cardiovascular Disease

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Key Words
Myocardial stunning · Haemodialysis · Heart failure · Cardiovascular disease

Abstract
Chronic haemodialysis (HD) patients are already primed by a large number of structural and functional peripheral vascular and cardiac abnormalities to experience demand myocardial ischaemia. Transient myocardial ischaemia may lead to left ventricular (LV) dysfunction that can persist after the return of normal perfusion. This prolonged dysfunction is known as myocardial stunning. Repetitive episodes of ischaemia can be cumulative and have been shown to lead to prolonged LV dysfunction (in patients with ischaemic heart disease). Conventional HD itself is a sufficient cardiovascular functional stressor to precipitate such recurrent ischaemic insults, leading to myocardial functional and structural changes, eventually resulting in fixed systolic dysfunction and heart failure (conferring a dismal prognosis for patients undergoing dialysis). Furthermore these same haemodynamic insults may also adversely affect other vascular beds in other vulnerable organ systems, driving an even wider range of pathophysiological processes. A variety of therapeutic manoeuvres aimed at improving the haemodynamic tolerability of treatment have been shown to reduce acute dialysis-induced myocardial ischaemia. This article aims to give an appreciation of the possibility that modification of the dialysis treatment to improve tolerability of therapy may have the potential to provide us with additional therapeutic targets, to reduce currently excessive rates of cardiovascular morbidity and mortality.

Introduction

It is well recognised that dialysis patients display hugely elevated rates of cardiac mortality [1]. It is also becoming appreciated that this rate of cardiovascular attrition is not driven by the same variety of risk factors or pathophysiological processes that are important in the general population [2]. Classical complicated atherosclerotic disease appears not to be the predominant mode of death in haemodialysis (HD) patients. This increase in cardiovascular mortality is driven by a combination of sudden cardiac death and heart failure. The development or aggravation of heart failure is associated with a particularly low survival [3].

It has long been suspected that myocardial ischaemia may be precipitated by HD. Short intermittent HD treatments exert significant haemodynamic effects, and 20–

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30% of treatments are additionally complicated by episodes of significant intradialytic hypotension (IDH) [4]. In conjunction with this, HD patients are particularly susceptible to myocardial ischaemia through a variety of mechanisms involving myocardial small-vessel changes, coronary atheroma, defective vasoregulation and reduced peripheral arterial compliance [5].

In patients with coronary artery disease, but without chronic kidney disease, transient myocardial ischaemia may lead to left ventricular (LV) dysfunction that can persist after the return of normal perfusion. This prolonged dysfunction is known as myocardial stunning [6]. Repetitive episodes of ischaemia can be cumulative and have been shown to lead to prolonged LV dysfunction. Myocardial stunning has been well described, in the non-dialysis patient population, as a causative mechanism for heart failure [7]. Repeated episodes of myocardial ischaemia lead to a spectrum of disease encompassing myocardial stunning to myocardial hibernation and ending in myocardial remodelling and scarring, with irreversible loss of contractile function [8]. There is evidence to suggest that hibernating myocardium is still highly vulnerable to increases in demand or reductions in oxygen supply, such as further haemodynamic stress during HD. Therefore, ongoing recurrent episodes of ischaemia precipitated by HD may have further negative consequences on this adaptive balance, leading to further myocardial injury and eventual non-viable myocardium with irreversible reduction in LV function.

This article aims to review the current developing evidence base to support the contention that dialysis-related cardiac injury is common, cumulative and directly involved in the poor outcomes characteristic in this patient group. Furthermore, this article will introduce the concept that other vulnerable vascular beds with defective vasoregulation may also be susceptible to significant episodic dialysis-related ischaemia, and that this may play a part in driving systemic inflammation and widespread organ damage.

Myocardial Ischaemic Potential in HD Patients

HD patients are particularly susceptible to myocardial ischaemia. This is a result of large-vessel and microcirculatory changes resulting in a reduced coronary flow reserve. This determines the ability to increase blood flow to the myocardium during increased demand. In part this may be due to LV hypertrophy, present in up to around 75% of patients on dialysis, which reduces coronary flow reserve and is associated with a myocyte-capillary mismatch. Increased peripheral artery stiffness is also recognised to have an adverse effect on myocardial perfusion and reduces the ischaemic threshold [9]. Therefore, LV hypertrophy in tandem with increased vascular stiffness may lead to a propensity to reduced myocardial blood flow (MBF), and particularly subendocardial MBF.

HD patients characteristically exhibit defective blood pressure control in the face of ultrafiltration requirements, in part due to impaired baroreflex sensitivity. In patients with low baroreflex sensitivity values, this lack of effective vasoregulation results in a tight association over a dialysis session between change in relative blood volume and change in cardiac output. In HD patients with intact baroreflex sensitivity the fall in relative blood volume has no association at all with change in cardiac contractile performance. This lack of a fully intact baroreflex arc leaves cardiac performance excessively dependent on intravascular volume [10], in patients where tissue flow/perfusion has become excessively dependent on pressure.

Dialysis-Induced Ischaemia and Myocardial Stunning

Initial evidence of HD-induced myocardial ischaemia has previously come from ECG-based studies, augmented by some limited isotopic perfusion imaging work and observations relating to humoral biomarkers of cardiac injury [11].

We have previously utilised $H_2{}^{15}O$ positron emission tomography to measure MBF during dialysis to demonstrate that HD precipitates reductions in MBF to levels at peak dialytic stress, consistent with the development of myocardial ischaemia. This was true even in the absence of angiographically significant coronary disease. The same study also confirmed that HD-induced segmental LV dysfunction (measured echocardiographically) correlated with matched reduction in segmental MBF [12].

HD is capable of inducing subclinical myocardial ischaemia, and this phenomenon is related to ultrafiltration and haemodynamic instability. We have studied a cohort of 70 prevalent HD patients. HD-induced myocardial stunning was assessed utilising serial intradialytic echocardiography to evaluate the extent and severity of HD-induced cardiac injury, as manifested by the development and subsequent recovery of regional wall motion abnormalities (RWMAs) [13]. HD-induced myocardial stun-

ning occurred in around two thirds of patients. In multivariate analysis, intradialytic reduction in blood pressure (BP) and ultrafiltration volume both independently determined the propensity to suffer HD-induced cardiac injury. The only other associated factors of significance from this model were patient age and cardial troponin T (cTnT) level (predialysis levels being around 3 times higher in affected patients). These 4 factors displaced all other standard biochemical/haematological, historical and dialysis-treatment-based variables Presumably, this additional effect of ultrafiltration volumes, over and above effects on BP, relates to potential haemoconcentration with increasing microcirculatory shear stress and reduced microperfusion leading to myocardial ischaemia.

Additional series of studies have further elucidated the importance of these two key factors.

Haemodynamic Stability

The importance of a dialysis-induced reduction in BP in the pathogenesis of HD-induced ischaemic injury is illustrated by a series of studies of modification of dialysis technique to assist in the maintenance of BP, without the alteration of ultrafiltration volume removal. In the first of these studies, we compared standard bicarbonate HD with a biofeedback technique that responded to significant declines in relative blood volume by temporarily reducing the ultrafiltration rate and increasing dialysate conductivity [14]. This was done within defined limits to ensure total fluid and sodium depuration was unaffected. In the second study in a different group of stable chronic HD patients, we compared standard dialysis with dialysate temperatures of 37°C and cooled dialysate at 35°C [15]. This latter intervention, well recognised to reduce IDH, has the advantages that it is extremely simple to perform, is available on all dialysis monitors and does not incur additional treatment costs, although the long-term tolerability of such a significant reduction in dialysate temperature may represent a problem. These studies contained small numbers of patients and were relatively short-term. In both studies, a significant number of new RWMAs occurred during standard dialysis. By improving mean BP and reducing IDH episodes with either biofeedback dialysis (BFD) or reduced-temperature dialysis, a significant reduction in the number of new RWMAs was observed.

In addition to segmental changes we also observed a higher overall LV ejection fraction, with both BFD and cool dialysis. It may be that either the higher mean BP or the reduction in IDH was responsible for the reduction in the incidence of RWMAs, although it is also conceivable that the effects of both of these factors were synergistic, with IDH that occurs at a lower mean BP potentially having a greater detrimental affect on myocardial perfusion. In a study of intradialytic MBF based on positron emission tomography, use of BFD resulted in reduced instability only in the later part of HD, and this was associated with a significantly better recovery of MBF after HD [12], supporting the contention that at least with BFD the beneficial changes with respect to MBF are linked to maintenance of intradialytic BP.

The relationship between BP and HD-induced stunning is further reinforced by an additional study which we performed to look at the effects of serial reduction in dialysate temperature. Twelve dialysis patients entered a randomised cross-over study to compare the development of LV RWMAs at 37°C (HD_{37}), isothermic body temperature (HD_{iso}) and isothermic – 0.5°C ($HD_{iso-0.5}$), to allow assessment of the 'dose response' between temperature and cardiovascular effects. Significantly more new RWMAs developed at a dialysate temperature of 37°C, compared to HD_{iso}. BP was higher during dialysis at HD_{iso} compared to HD_{37}, maintained predominantly by increased peripheral vascular resistance. Reduction in dialysate temperature below the isothermic point was associated with occurrence of adverse symptoms, but without any additional benefit to haemodynamic tolerability or cardiac injury [16].

Ultrafiltration Volume and Rate

To study further the previously identified relationship between ultrafiltration volume/rate and dialysis-induced cardiac injury, we elected to investigate the effects of more regular and longer HD schedules. We aimed to identify differences in the occurrence and severity of myocardial stunning in stable patients receiving the current spectrum of available quotidian HD regimes. Four patient groups were studied: conventional in-centre HD 3 times per week (CHD3, n = 12), short daily HD 5–6 times per week, both in centre (CSD, n = 12) and at home (HSD, n = 12), and nocturnal home HD (HN, n = 10). The groups were matched for age and dialysis vintage. Ultrafiltration volumes and intradialytic systolic BP reduction were both significantly lower in home-based frequent therapies. HD-induced myocardial stunning was ubiquitous in CHD3 patients. The proportion of patients exhibiting dialysis-induced RWMAs was reduced significantly with increasing dialysis intensity (CHD3 > CSD > HSD > HN). More frequent HD (HSD and HN) was associated with fewer RWMAs and lower high-sensitivity C-reactive protein levels than CHD3. The ultrafiltration rate

correlated strongly with the number of RWMAs. This study demonstrated for the first time that more frequent HD regimes are associated with less myocardial stunning compared to conventional HD [17].

Role of Large-Vessel Coronary Artery Disease in Dialysis-Induced Myocardial Stunning

There are convincing data that a combination of microcirculatory dysfunction, ischaemic potential of reduced peripheral arterial compliance, in addition to the other non-atheromatous consequences of the uraemic milieu are capable of allowing dialysis-induced injury to occur in the absence of conventional epicardial coronary artery disease. Firstly, our initial study of MBF during HD was performed in patients who had first been subjected to coronary angiography. Despite the absence of any significant disease, those patients still exhibited a myocardial ischaemic response to HD. Secondly, to acquire a patient group with uraemic cardiovascular disturbances, but in the absence of conventional cardiovascular risk factors, we studied a cohort of paediatric HD patients. The dialysis treatments were characterised by particularly large ultrafiltration requirements and significant relative dialysis-induced hypotension. A high proportion of the children (11/12) exhibited evidence of acute dialysis-induced myocardial stunning, with some biochemical evidence of cardiac injury [18].

Further indirect evidence that the recurrent myocardial ischaemia experienced during HD is not a result of classical recurrent plaque instability/rupture is provided by the study of free pregnancy-associated plasma protein A (fPAPP-A). This is a marker of atheromatous plaque instability and is a promising indicator of ischaemic myocardial stress prior to development of myocardial necrosis. fPAPP-A levels in acute coronary syndromes predict adverse cardiovascular outcomes. We studied 130 prevalent patients from two HD centres (in Finland and the UK) to investigate the relationship of fPAPP-A to HD-induced cardiac injury. A subset of 64 patients underwent serial echocardiography (predialysis, peak stress and postdialysis measurements) to assess HD-induced myocardial stunning. Pre-HD fPAPP-A was markedly elevated in the HD patients as compared to normal controls, and this was associated with greatly increased markers of systemic inflammation, cTnT, and longer dialysis vintage. This was consistent with the contention that established HD patients have a high plaque burden. However, there was no relationship with the presence of stunning, suggesting that coronary plaque instability is not a critical factor in the pathogenesis of HD-induced ischaemic cardiac injury, in the majority of patients [19].

Long-Term Consequences of Recurrent HD-Induced Ischaemic Injury

Twelve-month follow-up of HD patients, initially studied with dialysis-based echocardiography to identify those suffering from HD-induced cardiac injury, revealed significant effects on cardiac structure, function and patient survival. Those patients who did not develop HD-induced myocardial stunning by 1 year of follow-up had experienced only one significant cardiac event, no change in segmental shortening fraction, no reduction in overall LV ejection fraction and 100% survival. This was in stark contrast to the group characterised by the development of dialysis-induced myocardial stunning, where 28% of the patients had died. In those patients who survived to the 1-year follow-up there had been a rough halving of shortening fraction of those ventricular segments identified as suffering dialysis-based RWMA and a reduced overall LV ejection fraction (at rest and at peak during HD) by around 10% (absolute) in conjunction with an attendant significant increase in cTnT levels [13].

After a years' follow-up, intradialytic BP was significantly lower in patients who showed stunned BP during dialysis at the baseline assessment. This was in contrast to patients who did not exhibit dialysis-induced myocardial stunning and who had identical haemodynamic tolerability of HD at both ends of the study period. Given the underlying vulnerability of hibernating myocardium to increases in demand, coupled with decreased coronary flow reserve in HD patients, it may be that this adaptive process actually leads to further segmental injury by exacerbating intradialytic instability. This may be one of the reasons why prevalence of heart failure is so high and survival so poor in HD patients who start to develop myocardial contractile dysfunction.

Survival may also be determined by the incidence of intradialytic and postdialytic ventricular arrhythmias. In 40 patients serial echocardiography was augmented with 12-lead Holter recording to capture pre-, intra- and postdialytic ECG changes over a 48-hour period. Dialysis-induced myocardial stunning was associated with an increased rate of intradialytic and postdialytic ventricular arrhythmias [20]. This suggests that this phenomenon may be important in both of the interlinked major iden-

tifiable causes of cardiovascular mortality in HD patients (sudden cardiac death and heart failure).

Left atrial volume (LAV) has previously been shown to predict cardiovascular risk in HD patients. LAV is commonly driven by intravascular volume overload and progressive diastolic dysfunction. When we examined the association of LAV indexed to height$^{2.7}$ (LAVI) and RWMAs, we discovered that LAVI was increased by a factor of 2 in the 60% of patients who developed myocardial stunning. LAVI significantly correlated with number of RWMAs and chronological age. The strongest predictor of LAVI was the presence of stunning. LAVI was a better predictor of mortality than LV mass index, but both were displaced as independent determinants of mortality with the addition of myocardial stunning. These data suggest that the previously reported association of increased LAV and reduced survival may be mediated by an increased propensity to recurrent HD-induced ischaemia-based cardiac injury, with its attendant consequences [21].

Potential Effects of Haemodynamic Perturbation on Other Vascular Beds

The possibility of other critical vascular beds being potentially affected by systemic perturbation of BP and volume status, during dialysis resulting in reduced perfusion, has received little attention. The brain is one such potential bed. There are several pathologies described in dialysis patients including silent cerebral infarct [22], cerebral atrophy [23] and leuko-araiosis. The mechanisms for the progressive development of ischaemia-based white matter lesions remain to be elucidated further, but might be important given the progressive loss of cognitive function attendant on sequential cerebrovascular insults.

The gut is also a high-flow vascular bed. Translocation of endotoxin across the gut wall is well described in severe heart failure [24] and occurs both in the setting of shock and portal hypertension with severe hepatic impairment. Endotoxinaemia is associated with a wide range of well-recognised pathophysiological biological processes as well as being a profoundly pro-inflammatory stimulus. We have also been examining the possibility of 'gut stunning' in HD patients. Circulating endotoxin levels are around 1,000 times greater than in patients without chronic kidney disease, with roughly a quadrupling of serum levels after initiation of HD as compared to stable chronic kidney disease stage 5 [25]. These elevated levels correlate with intradialytic instability, systemic inflammation and cTnT levels in patients with dialysis-induced myocardial stunning [26]. The possibility that this phenomenon may be an important integrating component in the pathophysiology of inflammation, malnutrition and adverse cardiovascular outcomes awaits further study.

Conclusions

The procedure of HD exerts significant acute stress upon the cardiovascular system. There is an increasing body of evidence to suggest that subclinical ischaemia is precipitated by dialysis and that this is a common phenomenon. Episodes of ischaemia may potentially have a role in the development of cardiac failure and as a trigger for arrhythmias. Furthermore this same process of dysregulated blood flow under the stress of HD to a variety of vascular beds may be an important element in the development of such poor outcomes in HD patients manifested in a variety of body systems. Therefore, reducing the acute impact of dialysis on the cardiovascular system would seem to be a desirable therapeutic target, with the realistic prospect of providing a plethora of previously unappreciated therapeutic targets amenable to treatments designed to improve the subjective and objective tolerability of dialysis.

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Aldosterone in Uremia – Beyond Blood Pressure

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Key Words
Aldosterone · Salt · Blood pressure · Endothelial cell function · Dialysis

Abstract
Aldosterone was in the past considered only as a prohypertensinogenic agent. It has recently become clear that apart from the classical endocrine action, i.e. causing blood pressure elevation as a result of salt retention, aldosterone has numerous blood-pressure-independent actions on nonepithelial tissue. Under conditions of high salt concentration, aldosterone is injurious to the kidney, heart and vasculature. Of particular interest are recent observations that aldosterone is a permissive factor for the effect of minor increases in plasma sodium concentration on endothelial cell dysfunction. Despite surprising effects of aldosterone blockade on blood pressure of anuric dialysis patients, the potential role of mineralocorticoid receptor blockade in dialysis patients is currently unclear and requires controlled investigation to define the risk of potential hazards, specifically hyperkalemia.

Introduction

Since the classical paper of Conn [1] in 1955, the relation of adrenal adenoma or bilateral hyperplasia to hypertension is well recognized. Recently there has been a controversy whether we are currently confronted with an unrecognized epidemic of primary aldosteronism because up to 20–30% of hypertensive patients have values above the (arbitrary) plasma aldosterone/renin ratio. The data of the Framingham study suggest that progressively higher blood pressure values and prevalence rates of hypertension are found in progressively higher quartiles of serum aldosterone concentration [2], arguing for the following hypothesis: the higher the aldosterone concentration (at least under the present conditions of high salt consumption), the greater the risk of hypertension. Animal experiments provided convincing evidence for this assumption [3]: genetic manipulation to increase the activity of aldosterone synthase was associated with normal blood pressure on a low-salt diet, but significantly increased blood pressure and target organ damage on a high-salt diet.

While sodium is a permissive factor, the blood pressure effect of aldosterone does not necessarily require changes in sodium balance. In anuric hemodialysis patients, Gross et al. [4] showed that 50 mg of spironolactone decreased systolic blood pressure by 11 mm Hg, remarkably without any hyperkalemia; this observation indicates that under these conditions aldosterone had affected blood pressure by a direct effect on vascular resistance.

Activation of Mineralocorticoid Receptor – Signals Other than Aldosterone

Animals living in salt water have no aldosterone, but a precursor of the mineralocorticoid receptor which is stimulated by glucocorticoids. This receptor was later ap-

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parently highjacked by aldosterone once animals conquered the continents with scant salt supply. The mineralocorticoid receptor can be strongly stimulated by cortisol, but this is normally prevented by prereceptor metabolism in the synaptic cleft, i.e. the conversion of cortisol to cortisone by 11β-hydroxysteroid dehydrogenase 2. Under certain conditions, cortisol is increased in the synaptic cleft, specifically during inflammation, metabolic syndrome or altered redox potential; cortisol then activates the mineralocorticoid receptor, and although the receptor is activated by the 'wrong' ligand, the downstream effects can still be successfully antagonized by mineralocorticoid receptor antagonists.

One observation is particularly relevant for the nephrologist. Visceral fat of obese individuals or animals releases a factor with the name EKODE (epoxy-keto-octadecenoic acid) [5] which activates aldosterone synthase in the adrenal cortex and stimulates aldosterone secretion independent of angiotensin II, adrenocorticotropin and K^+. Albuminuria (and presumably further renal damage) in the metabolic syndrome is caused by EKODE-stimulated aldosterone and responds to mineralocorticoid receptor blockade [6].

The emerging clinical implications of the role of aldosterone in the metabolic syndrome, renal sequelae and resistant hypertension have recently been elegantly summarized by Sowers et al. [7].

Aldosterone Action – Beyond the Classical Actions

The classical concept proposed that the transcription of aldosterone requires endocrine actions on vectorial transepithelial transport of transport epithelia, e.g. the distal nephron, colon, salivary gland or sweat gland.

It has become clear that the endocrine genomic effects of aldosterone are not its only actions. Elegant experiments provide clear evidence for nongenomic effects, amongst others also in the pre- and postglomerular arterioles of the kidney [8].

Apart from the classical effects on ephithelial cells as targets, aldosterone exerts also nonclassical effects on interstitial tissues, specifically endothelial cells and fibroblasts [9] which are involved in fibrosis of the heart, kidney and vessels.

The various aspects of interaction between aldosterone and the cardiovascular system have recently been characterized as 'the good, the bad and the ugly' [10]:

– the good: sodium retention, avoiding hypotension;
– the bad: under high-sodium conditions, persistent hypertension and blood-pressure-dependent organ damage;
– the ugly: in a permissive milieu (e.g. inflammation), even at normal aldosterone concentrations, blood-pressure-independent target organ damage through inflammation, profibrotic pathways, oxidative stress, neural factor κB, activator protein 1, nicotinamide adenine dinucleotide phosphate oxidase, intercellular adhesion molecule, vascular cell adhesion molecule …

The human relevance of this is impressively illustrated by a recent study (LURIC trial) on subjects with coronary heart disease where aldosterone concentrations within the normal range were related to increasing hazard ratios of cardiovascular mortality.

These considerations prompted a recent comment in 'Renal aspirin: will all patients with chronic kidney disease one day take spironolactone?' [11].

Aldosterone – Blood-Pressure-Independent Target Organ Damage

In the past, nephrologists were concerned only about sodium balance. Sodium concentration was thought to be nearly constant and minor changes irrelevant. That the sodium concentration is not irrelevant was shown in patients with essential hypertension in whom a minor but highly significant elevation of plasma Na^+ concentration was found [12]. In animal experiments, such minor elevations have been shown to increase sodium concentration in the cerebrospinal fluid [13] promoting the synthesis of cardiotonic steroids (i.e. digitalis-analogous endogenous steroids), increased blood pressure [14] and caused blood-pressure-independent cardiomyopathy and accelerated progression of kidney disease [15].

Recent studies of Oberleithner et al. [16] have carried this one step further: raising the extracellular sodium concentration from 135 to 160 mmol/l caused a gradual increase in endothelial stiffness, reduced nitric oxide production and caused endothelial cell shape change. The presence of aldosterone was permissive for this effect. This effect is mediated via amiloride-sensitive sodium channels [17]. From the perspective of the dialysis patient, it may therefore be quite relevant to watch not only the sodium balance and its impact on 'dry weight', but also the sodium concentration, one determinant of which is the dialysate sodium concentration. Arterial stiffness is a known complication of uremia. Of interest are therefore observations in essential hypertension. Al-

dosterone is a permissive factor for the above effect of Na^+ on the vasculature. In hypertensive patients, the pulse wave velocity (as a marker of arterial stiffness) responded to an aldosterone antagonist, but not to similar blood pressure lowering by a thiazide [18].

Numerous animal experiments showed that in the absence of a change in blood pressure, aldosterone receptor blockade reduced target organ damage in hypertensive animals, e.g. spontaneously hypertensive stroke-prone rats [19].

In the past, administration of spironolactone or other mineralocorticoid receptor blockers was considered to be strictly contraindicated because of the risk of hyperkalemia which is undoubtedly a serious problem [20, 21]. In the light of the above data [4], this issue requires reassessment in carefully conducted studies in dialysis patients.

Aldosterone-induced target organ damage is sodium-dependent. It is remarkable that a low-sodium diet or renal sodium loss completely prevent aldosterone-induced target organ damage, e.g. cardiac hypertrophy or cardiac fibrosis [22]. These considerations illustrate the wisdom of the nestor of our specialty, Belding Scribner, who fought until the end of his life for 'the drug-free, salt restriction, ultrafiltration method of blood pressure control' in dialysis patients [23]. Based on the above new finding, it appears possible that beyond blood pressure sodium restriction may even have major blood-pressure-independent target organ benefits [11].

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Immune Mechanisms Involved in Cardiovascular Complications of Chronic Kidney Disease

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Key Words
Chronic kidney disease, inflammation · Cardiovascular disease · Myocardiopathy · Atherosclerosis

Abstract
A sustained status of chronic inflammation is closely linked to several complications of chronic kidney disease (CKD), such as vascular degeneration, myocardial fibrosis, loss of appetite, insulin resistance, increased muscle catabolism and anemia. These consequences of a chronically activated immune system impact on the acceleration of atherosclerosis, vascular calcification and development of heart dysfunction. Recent evidence suggests that these immune-mediated consequences of uremic toxicity are not only important to stratify the risk and understand the mechanisms of disease, but also represent an important area for intervention. Thus, the aim of this brief review is to discuss the immune mechanisms behind atherosclerosis and myocardiopathy in CKD. We also display the emerging evidence that strategies focusing on modulating the immune response or reducing the generation of triggers of inflammation may represent an important tool to reduce mortality in this group of patients. Ongoing studies may generate the evidence that will translate these strategies to definitive changes in clinical practice.

Introduction

Signs of an activated immune system and elevated levels of inflammatory mediators can be observed in the early stages of chronic kidney disease (CKD) and increase with the progression of renal dysfunction. This chronic inflammatory state is closely linked to several complications of CKD, such as vascular degeneration, myocardial fibrosis, loss of appetite, insulin resistance, increased muscle catabolism and anemia. Potentially, these consequences of a chronically activated immune system impact on the acceleration of atherosclerosis, vascular calcification and development of heart dysfunction. Not surprisingly, the presence of systemic inflammation is an important predictor of poor outcome in CKD patients, and the central role of immune-mediated changes in the heart and vascular system (the main cause of mortality in CKD) has been consistently described [1]. Recent evidence suggests that these immune-mediated consequences of uremic toxicity are not only important to stratify the risk and understand the mechanisms of disease, but also represent an important area for intervention. Thus, the aim of this brief review is to discuss the immune mechanisms behind atherosclerosis and myocardiopathy in CKD. We also display the emerging evidence that shows that strategies focusing on modulating the immune re-

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sponse or reducing the generation of triggers of inflammation may represent an important tool to reduce mortality in this group of patients.

Immune Mechanisms Involved in the Development of Cardiovascular Disease in CKD

The interaction between kidney failure and the cardiovascular system, currently defined as cardiorenal syndrome, appears to be largely mediated by the immune system, which recognizes the uremic state as a continuous aggression to cells and tissues. The uremic toxins consist of heterogeneous substances, including organic compounds and peptides, with proinflammatory effects [2]. Uremic toxin accumulation, which is only partially corrected by dialysis, as well as dialysis-related factors, such as interactions between blood and dialyzer, endotoxin presence in water, access-related infections, peritoneal dialysis solutions with high glucose concentration, low pH and the presence of glucose degradation products, represent the chronic stimuli to the inflammatory response [3]. In concert these responses to multifactorial stimulation impact on the mechanisms involved in the uremic vascular degeneration and myocardiopathy, which will be reviewed in the following section.

Inflammatory Mechanisms Involved in the Vascular Changes in CKD

Vascular changes that characterize the atherosclerotic process are initiated and perpetuated by the interaction of immune cells with cells of the vessel wall. Leukocyte interactions with vascular endothelium during inflammation occur through steps involving selectin-mediated leukocyte rolling, mild adhesion mediated by adhesion molecules (vascular adhesion molecule 1 and intercellular adhesion molecule-1) and subsequent firm adhesion mediated by chemokines (particularly the monocyte chemoattractant protein 1) and interleukin-8 [4]. There is increasing evidence generated from experimental and clinical studies that these early stages of atherosclerosis are extremely important [5]. Vascular inflammatory responses, through chemotactic and haptotactic pathways, not only contribute to the growth and expansion of early lesions, but also participate in plaque destabilization, resulting in thrombotic complications associated with significant morbidity and mortality [6]. Interestingly, animal models that combine atherosclerosis-prone mice with renal dysfunction result not only in increased plaque formation, but show also increased signs of vascular inflammation, oxidative stress and calcification [7].

The endothelium is a continuous layer of cells that separates blood from the vessel wall, and, as an active and dynamic tissue, it controls many important functions, including maintenance of blood circulation and fluidity as well as regulation of vascular tone, coagulation and inflammatory responses [8]. The constant aggression of the endothelium by uremic toxins leads to modification in the endothelial cell phenotype and to endothelial dysfunction, with secretion of many proinflammatory molecules, such as tumor necrosis factor (TNF) α and C-reactive protein [9–12]. Endothelial cell dysfunction can be evaluated by measuring indices such as flow-mediated vasodilation and indirectly by measuring circulating markers of endothelial cell dysfunction such as monocyte chemoattractant protein 1, vascular adhesion molecule 1 and intercellular adhesion molecule 1 [13] and markers of processes that are known to interfere with endothelial cell function, e.g. oxidative stress, microinflammation, adipokine abnormalities such as low serum adiponectin levels, vascular calcification and others [14]. Finally, prospective data strongly suggest that endothelial activation and vascular inflammation occur early in the atherosclerotic process and predict cardiovascular events in the general [15] and CKD [16] populations. Anti-inflammatory strategies to reduce vascular inflammation will be reviewed later in this article.

Inflammation and Myocardiopathy

Left ventricular hypertrophy (LVH) is the most frequent cardiac alteration in end-stage renal disease (ESRD) patients, documented in 75–80% of patients undergoing dialysis therapy, and is an independent risk factor for survival [17]. The pathogenic factors involved in the generation of LVH in CKD and ESRD patients are diverse and complex, involving afterload-related factors (elevated systolic and diastolic arterial hypertension), preload-related factors (expansion of intravascular volume, anemia) and non-afterload- or preload-related factors [18, 19]. The links between these triggers and myocardial changes are largely mediated by the immune system.

LVH is characterized not only by an increased myocardial fiber mass, but also by interstitial fibrosis [20], and many abnormalities such as cardiomyocyte hypertrophy, myocardial fibrosis and thickening of intramural arteries and arterioles are constant findings in heart biopsies and necropsy studies in CKD patients with LVH [21]. Processes seemingly unrelated to both afterload or preload, such as activation of the mammalian target of

rapamycin pathway and those related to the parathyroid hormone-vitamin D-phosphate axis, microinflammation and oxidative stress, are also emerging as important in the production of LVH and cardiac fibrosis in patients with CKD and ESRD [19]. Activation of the intracardiac renin-angiotensin system appears to be critically involved in this pathway, and both angiotensin II and aldosterone can also be involved in myocardial cell hypertrophy and fibrosis independent of afterload [22]. These phenomena can lead to a progressive impairment in contractility and a stiffening of the myocardial wall, leading to systolic and diastolic dysfunction and ultimately to dilated cardiomyopathy and diastolic and/or systolic congestive heart failure [23].

These nonhemodynamic/volume-related factors represent potential new targets for treatment directed at modifying LVH and its clinical consequences. The cardiovascular system is one of the most important targets of the inflammatory activation, so cardiovascular risk stratification is desirable in the clinical management of CKD patients, and biomarkers such as inflammatory cytokines are increasingly used in these patients.

Inflammatory cytokines may play an important role in the pathogenesis of myocardial damage in CKD patients, determining a depressant effect on the myocardium and inducing ventricular dysfunction [24]. Circulating levels of TNF-α and interleukin 6 are increased in patients with heart failure and may promote apoptosis [25]. Additionally, TNF-α may induce acute cardiac dysfunction mediated by nitric oxide, and, moreover, the observation that failing hearts express elevated levels of TNF-α suggests that overexpression of this cytokine may be one of several different maladaptive mechanisms responsible for the progressive cardiac decompensation that occurs in advanced heart failure [26]. There is clinical evidence revealing an excess of cytokines such as cardiotrophin 1 and transforming growth factor β_1 in CKD. Mechanical overload imposed on the myocardium is the initial stimulation to production of transforming growth factor β_1, which may further increase the response to anemia-related myocardial hypoxia [27].

Patients with fluid overload, such as patients with congestive heart failure, present signs of systemic inflammation that reduce when the disease is compensated [28]. This inflammatory state appears to be associated with an altered gut barrier permeability that occurs as a consequence of the edema, allowing the translocation of macromolecules including endotoxins into the circulation, such as lipopolysaccharides [29]. As a consequence of the presence of circulating endotoxins, the immune system may be activated, generating a chronic inflammatory status, potentially working as a drive to cardiovascular disease [30].

CKD and ESRD patients have a high prevalence of vitamin D deficiency [31] that is characterized by low levels of 25-hydroxyvitamin D and frequently low levels of 1,25-dihydroxyvitamin D, the hormonal form of this vitamin, and there is significant evidence suggesting beneficial cardiovascular effects of vitamin D therapy in uremia [32]. Recent findings from several large observational studies [33] have suggested that the benefits of vitamin D receptor activators may extend beyond the traditional parathyroid hormone-lowering effect and could result in direct cardiovascular and metabolic benefits. Vitamin D may play a role in the inflammatory response, modulating production of cytokines involved in calcification and atheroma formation [34], upregulating anti-inflammatory molecules and, also, modulating the expression of tissue matrix metalloproteinases. Indeed, vitamin D can act as a negative endocrine regulator of renin-angiotensin synthesis [4] and reduces cardiac hypertrophy, all processes that may be directly related to cardiovascular disease in CKD. Additionally, vitamin D seems to play a crucial role in the organization of cardiac tissue, regulating intracellular calcium levels, maturation, differentiation and proliferation of cardiac cells [35].

Inflammation-Focused Targets for Intervention Aiming to Reduce Cardiovascular Mortality in CKD

There are two potential therapeutic approaches using inflammation as a target that may result in cardiovascular benefits in CKD patients: pharmacological manipulation of cell response and reduction of source of ligands. There is an increasing number of studies analyzing the potential impact of these strategies, which will be reviewed in the following section.

Pharmacological Manipulation of Cell Response

Although pharmacological interventions in dialysis patients frequently result in negative findings [36, 37], some randomized and controlled (or not) clinical trials with cardiovascular endpoints reveal interesting aspects in the area of inflammation. First, two classes of the renin-angiotensin system (angiotensin-converting enzyme inhibitor and angiotensin II receptor blockers, antihypertensive agents with proven anti-inflammatory activity) have been tested in clinical trials on a dialysis population [38, 39]. The results of a randomized trial using fo-

sinopril [38] showed a slight benefit for this agent in comparison to placebo. In another randomized trial with a small number of patients [39], candesartan significantly reduced cardiovascular events and mortality in patients on chronic maintenance hemodialysis. More recently, Suzuki et al. [40] verified that treatment with an angiotensin receptor blocker was independently associated with reduced fatal and nonfatal cardiovascular disease events in a hemodialysis population, although the large effect may be a spurious finding because of the small sample size of that trial.

In observational studies, statin (another drug with anti-inflammatory effects) users had lower mortality than non-statin-using hemodialysis patients [41], results that were not confirmed by randomized controlled trials in ESRD hemodialysis patients [36, 42], even with substantial reductions in low-density lipoprotein. Although the treatment with rosuvastatin had no significant effect on the composite primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke [42], there was a reduction in mean high-sensitivity C-reactive protein, an important inflammatory biomarker in CKD patients. In the AURORA trial [42], it was noticeable that inflammation at the baseline was one of the most important risk factors for mortality when the population was treated as a cohort, which points to the need of trials looking at the effect of statins in a select group of patients with inflammation.

Renal dysfunction is frequently associated with oxidative stress, which may be an interesting target for therapeutic interventions. In this field, some clinical trials emerged with positive results. One of them investigated the effect of high-dose vitamin E supplementation on cardiovascular disease outcomes in hemodialysis patients with preexisting cardiovascular disease. After a median follow-up of 519 days, the use of vitamin E was associated with reduced cardiovascular disease endpoints and myocardial infarction [43]. Another antioxidant agent, acetylcysteine, a thiol-containing antioxidant, had its effects observed in a randomized controlled trial in hemodialysis patients. Fatal and nonfatal cardiovascular endpoints, as well as mortality were attenuated by acetylcysteine use [44].

The circulating levels of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 can potentially influence the activity of many tissues and cells that have a vitamin D receptor and have no function in regulating calcium homeostasis and bone health. These include, among others, the cardiomyocytes, active T and B lymphocytes, mononuclear and endothelial cells [45]. As discussed, dialysis patients on different types of activated vitamin D and analogs [33] have a survival advantage, probably related to the systemic activation of vitamin D receptors, acting as a negative endocrine regulator of renin-angiotensin synthesis [4] and inflammation and reducing cardiac hypertrophy [32].

LVH in CKD and ESRD is frequently related to myocardial fibrosis, by mechanisms that are not directly related to hypertension or fluid overload. A potential and promising target for intervention in this situation is the mammalian target of rapamycin pathway, overexpression of which may be related to myocardial fibrosis in such patients. In experimental models [19], cardiac hypertrophy in uremic mice was reduced by rapamycin use and not by reduction of blood pressure, suggesting that uremic cardiomyopathy is mediated, at least partially, by activation of a pathway that involves the mammalian target of rapamycin pathway.

Reduction of Source of Ligands

Immune aspects related to membrane biocompatibility in dialysis patients are frequently the object of clinical research. In hemodialysis patients, the use of high-flux hemodialysis membranes was associated with better survival, specifically in the subgroup of patients with systemic inflammation or malnutrition (seric albumin $\leq$4.0 g/dl), and in diabetic patients. In peritoneal dialysis, the bioincompatibility of peritoneal dialysis fluids has been attributed to low pH, lactate, glucose, glucose degradation products and osmolality. In a retrospective observational study in peritoneal dialysis patients [46], the treatment with a novel biocompatible peritoneal dialysis fluid with low glucose degradation product concentration and neutral pH confers a significant survival advantage, even after adjustment for age, gender and diabetes status. The exact mechanisms for such a survival advantage could not be determined, but reduction of inflammation may be an important factor.

Periodontal disease is associated with cardiovascular disease, may work as an occult source of chronic inflammation and is thought to accelerate systemic atherosclerosis. In a recent clinical study in hemodialysis, patients with moderate-to-severe disease compared to those with mild or no periodontal disease had a significant association with death from cardiovascular causes. Intervention trials to determine if treating periodontitis (and other hidden infections) reduces cardiovascular disease mortality in dialysis patients are desirable.

Endotoxemia has recently been related to fluid overload and systemic inflammation in CKD nondialysis [30]

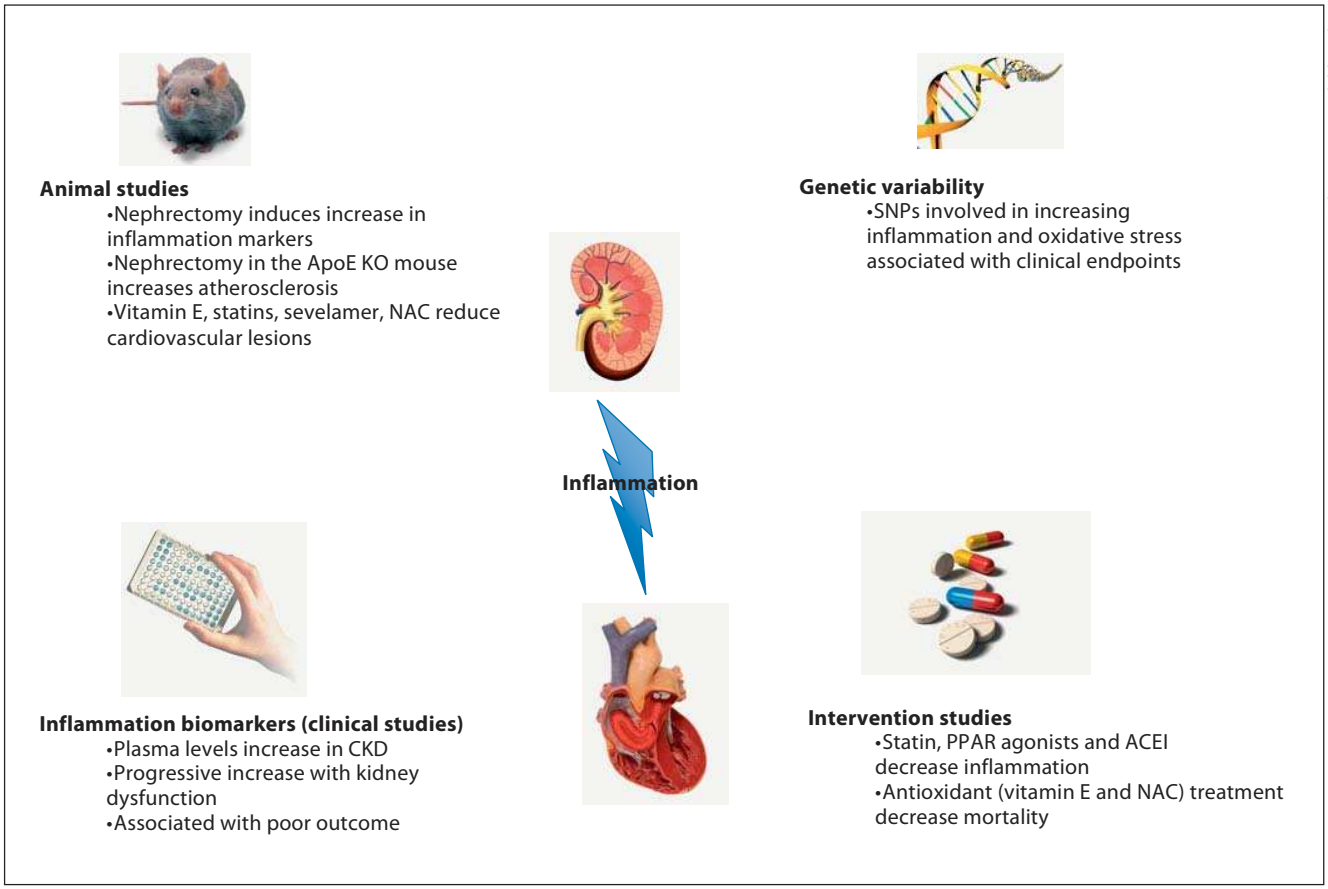

Fig. 1. Immune mechanisms involved in the interaction between kidney dysfunction and cardiovascular complications analyzed using different investigation strategies. ApoE = Apolipoprotein E; NAC = N-acetylcysteine; SNPs = single-nucleotide polymorphisms; PPAR = peroxisome proliferator-activated receptor; ACEI = angiotensin-converting enzyme inhibitor.

and in peritoneal dialysis patients [47]. Although the drug had been created to be used as a phosphate binder, recently sevelamer hydrochloride showed a potential endotoxin-binding effect in the intestinal lumen, reducing systemic inflammation in an experimental model [48]. Other potential pleiotropic effects of sevelamer that could have cardiovascular impact include a lipid-lowering action and reduction in C-reactive protein levels [49]. Recently, Stinghen et al. [50] demonstrated in a clinical study that sevelamer treatment leads to a decrease in C-reactive protein levels, which was accompanied by a parallel decrease in endotoxemia, suggesting that endotoxemia may contribute to the systemic inflammation in hemodialysis patients, which was partially reduced by the use of sevelamer.

Conclusions

In summary, there is strong evidence derived from animal models, cellular and molecular studies linking inflammation to cardiovascular alterations in CKD (fig. 1). Also, several inflammation biomarkers are related to disease state, degree of kidney dysfunction and prediction of cardiovascular events and mortality. Emerging evidence is showing that the reduction of mortality in CKD patients can be achieved with anti-inflammatory strategies. Ongoing studies may generate the evidence that will translate these strategies to definitive changes in clinical practice.

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An Approach to Addressing Depression in Patients with Chronic Kidney Disease

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Key Words
Depression · Chronic kidney disease · End-stage renal disease · Dialysis · Health-related quality of life

Abstract
Depressive symptoms and clinical depression are commonly noted in patients with end-stage renal disease and chronic kidney disease (not on dialysis). This association is important since depressive symptoms have been associated with both an impaired quality of life and increased morbidity and mortality. It is, therefore, important to develop strategies to screen patients with chronic kidney disease for depression and to develop strategies to treat clinical depression in this group of patients.

Recently, there has been a dramatic increase in interest in the health-related quality of life (HRQoL) of patients with chronic kidney disease (CKD) [1–6]. This reflects in part the clear association between various HRQoL measures and outcomes of patients with CKD and end-stage renal disease (ESRD) [1–5, 7, 8]. In addition, there is recognition that HRQoL is a valid outcome measure for trials involving CKD and ESRD patients [9, 10].

It is now mandated in the USA, as part of the conditions of coverage by the Center for Medicare Services, that all patients with ESRD receiving dialysis therapy have assessments of their HRQoL performed annually or more often if clinically indicated and that efforts be made to address impairments of HRQoL [11]. The HRQoL assessment tool recommended by the Center for Medicare Services is the KDQoL-36, which uses the SF-12 plus 24 additional questions defining the domains of burden of kidney disease, effects of kidney disease on daily life, and symptoms/problems of kidney disease. Results are reported in 16 cells – 2 for gender, 2 for diabetes (present or absent), and 4 for age (<45, 45–64, 65–74 and >74 years) – with standardized results defined for these groups in previous investigations.

Depression, the most common psychological problem encountered in ESRD and CKD patients, impacts on a variety of HRQoL measurements [2, 8, 12]. Patients with clinical depression will have reductions in scores on various HRQoL assessments, including both mental and physical scores, since depressive symptoms include both somatic (e.g. tiredness, fatigue, sleep disturbance, loss of appetite) as well as psychological domains (e.g. depressed mood, loss of interest) [13].

The frequency of clinical depression and the impact of depression on outcomes of ESRD patients has been well documented [2, 12, 13]. Over 25% of prevalent dialysis patients have clinical depression. Depression can be screened for by standardized questionnaires, which have been well validated in CKD and ESRD patients [2, 12, 14,

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15]. However, the diagnosis of clinical depression can only be made with direct interviewing using standard DSM-IV criteria [12]. However, if appropriate cut-off scores are utilized, screening instruments such as the Beck Depression Inventory (BDI) and Patient Health Questionnaire-9 (PHQ-9) can be useful in selecting those patients who should be interviewed. BDI scores of 16 or greater and PHQ-9 scores of 10 or greater have a sensitivity and specificity for diagnosing clinical depression of about 90% in ESRD patients [12, 15].

The importance of diagnosing depression in the ESRD patient is underscored by the association of both clinical depression (diagnosed by direct patient interviews) and depressive symptoms (diagnosed by patient-reported questionnaires) and morbidity and mortality [2, 3, 8, 16]. For example, data from the Dialysis Outcomes and Practice Patterns Study suggest that patients with scores on the Center for Epidemiological Studies Depression questionnaire of 15–30 have a nearly twofold greater risk of death than patients with scores of 0–4 after correction for a variety of variables [3]. And, a recent study by Hedayati et al. [2] using direct patient interviews and DSM-IV criteria to diagnose clinical depression indicated that 26.5% of hemodialysis patients had clinical depression and that these patients had a twofold greater risk of death or hospitalization than patients who were not clinically depressed.

Recent studies have extended the observations concerning depression to CKD patients not on dialysis. These studies have suggested that clinical depression occurs in about 21% of CKD patients with stages 2–5 [13]. Independent variables associated with clinical depression included diabetes mellitus, comorbid psychiatric illness and history of drug or alcohol abuse [13, 14]. The incidence of depression did not vary with CKD stage. BDI scores of 11 or greater had a sensitivity and specificity of about 90% for diagnosing clinical depression, suggesting that questionnaires can be helpful to screen patients who should have diagnostic interviews [14]. Although the relationships between depression and hospitalizations and mortality were not examined in these studies, the relationships between clinical depression and depressive symptoms in a variety of medical illnesses are now well documented [17–19]. This is particularly true for cardiovascular diseases. In fact, an association between depression and mortality has been observed in a study of a cohort of 374 patients admitted to the hospital with congestive heart failure [20]. Documenting the relationship between depression and medical outcomes in CKD patients will be important to confirm in future studies.

The treatment of depression in ESRD and CKD patients presents special challenges. Given the high incidence of clinical depression in these patients and the clear association between HRQoL and depression, it is important that effective strategies to treat depression be reviewed and appropriate programs implemented [12]. Given the studies cited above concerning the high sensitivity and specificity of patient-reported symptoms (with the BDI and PHQ-9), it is reasonable to screen patients with these (or similar) instruments. Patients with high scores (or patients with particular clinical findings suggesting depression) should then be interviewed by appropriate personnel – primarily the social worker in collaboration with the nephrologist, nurse, liaison psychiatrist or psychologist – to establish a diagnosis of clinical depression.

Once the diagnosis is established, potential treatment options need to be reviewed by the patient care team and a plan formulated that is tailored to the needs of the individual patient. It is important to review the dialysis or CKD treatment regimen in terms of an examination of medications and adequacy of care. A variety of medications, such as antihypertensive drugs, can have negative impacts on psychosocial functioning; these need to be carefully reviewed. Hemoglobin levels should be maintained at 11–12 g/dl, since lower levels can negatively impact on various HRQoL measures [21]. For dialysis patients, the dialysis treatment regimen should be reviewed. Of note are the recent reports indicating that more frequent hemodialysis can result in a significant reduction in depressive symptoms at 4 and 12 months after initiation of more frequent therapy [22, 23]. In addition, the shortened recovery time after more frequent hemodialysis has been associated with improved HRQoL measures [24, 25].

As in clinically depressed patients without kidney disease, psychological counseling (individual, marital and/or family) and antidepressant medications should be considered on an individual basis. Stress and anxiety need to be evaluated, since they are commonly noted in ESRD patients and their relationship with depression is well documented [26]. Recent reports of a positive impact of cognitive behavioral therapy on BDI and HRQoL scores in a randomized trial in a cohort of hemodialysis patients is of interest [27].

In addition to individual issues, marital and family issues for CKD patients have been emphasized as significant factors contributing to psychosocial problems and therefore deserve careful attention [28–30]. The social support network for CKD patients needs to be explored

since disruptions of these networks have been described that have been associated with depressive symptoms [31]. Involvement of community resources for patients with depression should be considered. And, attention needs to be paid to the caregiver, particularly for the ESRD patient; the ongoing burden on the caregiver in terms of providing support for the chronically ill patient has not received enough attention [32].

Given the magnitude of the problem of depression in CKD patients, nontraditional treatments of depression need to be studied. For example, two recent papers have emphasized the importance of exercise programs in alleviating depressive symptoms; interestingly, these can be done intradialytically [33, 34]. Addressing sleep problems, pain management, meditation, muscle relaxation and other alternative therapies, such as music therapy, need to be explored [12, 35].

In conclusion, clinical depression is emerging as a common and significant problem in both CKD and ESRD patients, impacting on both HRQoL and medical outcomes. It is important, therefore, to develop systematic approaches to screening patients for depression, diagnosing clinical depression and then planning treatment strategies for those patients with clinical depression. It will be important to monitor the impact of these treatment programs on the quality of life and medical outcomes of CKD and ESRD patients with clinical depression.

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Ethnicity and Renal Replacement Therapy

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Key Words
Ethnic minorities · Renal replacement therapy · Haemodialysis · Peritoneal dialysis · Renal transplantation

Abstract
There are significant ethnic variations in the incidence of kidney disease. White European populations appear to be uniquely protected compared to increased incidences of end-stage renal disease in indigenous and migrant ethnic minority populations. This increase is partly explained by a high prevalence of diabetic nephropathy, but there is also an increased susceptibility to a range of other renal diseases. The relative contributions of genetic, environmental and fetal environmental factors to this susceptibility are not yet well understood. Strategies for early detection and management of chronic kidney disease to delay progression are particularly critical in countries where access to renal replacement therapy (RRT) is restricted. In developed countries with wide availability of RRT, resources to provide dialysis will need to be increased in regions with substantial minority populations. There is apparently counterintuitive evidence that survival on dialysis is increased in many minority populations. Access to renal transplantation, both from deceased and living donors, is also restricted in many minority populations, and graft survival is often inferior. Analysis of the explanations for these differences is complex because of the many confounding factors (for example cultural, social and economic) which typically cosegregate with ethnicity. Nevertheless, reduction of the varied and substantial inequities faced by ethnic minority populations with kidney disease is an important responsibility for the renal community.

Introduction

People from indigenous or migrant ethnic minority populations have increased susceptibility to chronic kidney disease (CKD). This offers many challenges:

- for researchers in understanding the complex processes which underlie this susceptibility;
- for clinicians seeking to prevent or delay the progression of CKD in an individual patient, and optimising renal replacement therapy (RRT) when it is unavoidable;
- for those responsible for organising, delivering and meeting the cost of the increased demands for RRT in areas serving minority populations.

While it is pragmatically necessary to make broad categories of ethnicity to purposes of epidemiology and clinical research in this field, it is also important to appreciate that within any grouping such as African or South Asian there are populations with enormous variety of culture, religion, education and socio-economic circumstances which makes the interpretation of causal relationships complex.

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Epidemiology

Migration of populations across the world continues to increase. This has led to an increasing variety of ethnic minority populations in many countries, some of which also have minority indigenous populations. Minority populations are often geographically localised. For example, ethnic minorities are 9% of the UK population, but in the city of Leicester, the population originating from South Asia has rapidly increased to more than 50% of those living within the city boundary.

Such minority populations present challenges to health care. Even if they had no variations in disease prevalence, substantial language and cultural variations can provide additional demands on the health care system, and these are most difficult in long-term conditions such as CKD. But in fact, there are many disease variations in minority populations, not least an increased incidence of type 2 diabetes and progressive CKD. Furthermore, recent migrant populations typically have a younger age distribution than the indigenous population so that diseases such as CKD, which increase in incidence with increasing age, will at first be underrepresented compared to the eventual population burden as the minority population ages.

The incidence of CKD is increased in many ethnic minority populations. There is a 3- to 4-fold increase in the incidence of end-stage renal disease (ESRD) in South Asian and African Caribbean populations in the UK [1], and there are similar increases in African Americans, Hispanics and Native Americans in the USA [2]. Other populations at increased risk include Aborigines, Maoris and Pacific Islanders in Australasia. Most studies have used the onset of ESRD and acceptance for RRT as a means of case ascertainment, but this approach might underestimate the true incidence if patients have inequity of access to RRT. It is common to emphasise the increased susceptibility to ESRD in these ethnic minority populations; but perhaps we should instead consider that the white European population seems uniquely at low risk of renal disease. This decreased risk could have a genetic basis or may reflect environmental factors, such as the very gradual urbanisation in most Caucasian European populations compared to the very rapid recent urbanisation in many developing countries.

In more homogeneous populations, the incidence of ESRD is even more strikingly increased. While the Pima Indians have a specific susceptibility to diabetic nephropathy [3], Native Americans of the Zuni Pueblo tribe – with an incidence of ESRD 18 times that of white Americans – have a broader susceptibility to CKD including diabetic nephropathy and glomerulonephritis [4]. The Aboriginal population in Tiwi Island, Northern Territory, where obesity and type 2 diabetes are endemic, has an ESRD incidence of more than 2,000/million population/year; comprising only 26% of the population of Northern Territory, Aborigines are 96% of those undergoing RRT [5].

All these ethnic minority populations at increased risk of CKD share a marked increase in susceptibility to type 2 diabetes and an increased susceptibility among diabetics to ESRD. For example, data from Leicester, UK, indicated a 13-fold increase in the risk of a South Asian diabetic developing ESRD compared to a white diabetic [6], the Pima Indians have a risk of ESRD 14 times that of the white US diabetic population [7], and there is also a marked increase in African American type 2 diabetics compared to whites. In many populations severe hypertension adds to the risk for ESRD. Current predictions suggest an increase in the prevalence of type 2 diabetes of at least 30% above current levels by the year 2025 in developed countries, but 2- to 3-fold increases in prevalence in developing countries during the same period [8]. This predicted epidemic of ESRD in people with diabetes will be uncontainable unless effective preventive strategies are developed.

Studies using requirement for RRT for case ascertainment will not distinguish susceptibility to the development of renal disease from an increased risk of progression to ESRD once nephropathy is established. In hypertensive blacks, there is long-established evidence of an increased progression risk despite equivalent blood pressure control [9]. In CKD of other aetiologies, evidence increasingly points to the increased risk of progression as the predominant explanation for increased ESRD. A US birth cohort analysis showed no difference between blacks and whites in the prevalence of CKD, yet 5 years later in the same cohort, a near 5-fold increased risk of ESRD among the blacks was apparent [10]. Recent UK data also show no difference in the prevalence of early CKD between white and South Asian populations despite the marked excess of ESRD in South Asians.

Pathogenesis

Genetic susceptibility to renal diseases and their progression, susceptibility provoked by environmental factors, susceptibility induced by the effects of fetal environment and socio-economic influences all can contribute to susceptibility to CKD in ethnic minority populations [11].

One particular challenge is separating the influence of ethnicity from that of socio-economic disadvantage, two major factors which so often cosegregate. Socio-economic disadvantage is itself associated with an increased risk of ESRD [12], a complex interaction that might directly influence renal damage, be associated with damaging health behaviours or influence the quality of health care of those with kidney disease.

Preventing Progression to ESRD

There is much work needed to unravel the contributions of these various pathogenic mechanisms, but in the meantime there are opportunities to prevent CKD in high-risk populations and to detect CKD early, intervening to delay its progression. If such opportunities are grasped, they may not only benefit the individual, but offer significant health system economies if they avoid the cost of RRT, and of course are critically important in health economies where RRT is unavailable or unaffordable.

But such opportunities to turn the tide of ESRD will not be grasped if those at risk are not engaged by the health care system or are unresponsive to our recommendations. Minority populations may lack the awareness or health education to engage, and programmes which approach these problems must be designed with cultural sensitivity.

In the USA, considerable evidence points to blacks having inferior access to health care. Blacks report receiving less information about health care and express more dissatisfaction with their treatment [13]. Fewer black hypertensive patients have their blood pressure checked at least annually. These differences are reported at all income levels, although blood pressure control might be more unsatisfactory among blacks living in inner cities [14]. The only similar study in the UK shows a more reassuring trend for earlier referral of blacks with CKD compared to whites [15].

Outcome of RRT in Ethnic Minority Populations

In health care settings where RRT is available, it is important that RRT be delivered with equity in ethnic minority populations. The proper assessment of equity requires evaluation of mortality, morbidity and quality of life, if care is to be optimised.

In the USA, mortality in patients receiving RRT (adjusted for socio-economic factors and comorbidity) is consistently lower in ethnic minorities [16]. The UK Renal Registry reports a significant reduction in 1-year mortality for blacks and South Asians undergoing RRT [17]. In Hong Kong, survival of Chinese patients undergoing peritoneal dialysis (PD) is significantly better than that reported in other large PD studies involving predominantly white populations [18]. The reasons for these discrepant outcomes are likely to be complex; ethnicity itself might not be the explanation, and many other factors might introduce bias. For example, 80% of all dialysis patients in Hong Kong are undergoing PD compared to the predominance of haemodialysis in the USA. A low patient acceptance might also alter the case mix; only 54% of Hong Kong Chinese identified as suitable for PD agreed to receive the treatment [19]. A low transplantation rate, as in Hong Kong, might also lead to younger, fitter patients remaining on PD, thus improving the cohort outcome. The improved survival may also reflect survival advantage, the individuals with the highest risk having not survived to commence RRT.

Improved survival on haemodialysis is found in African Americans despite other factors expected to reduce survival, including non-adherence to treatment regimens, increased use of dialysis catheters and higher requirements for erythropoietin [20].

Renal Transplantation

Registry data from several parts of the world including the UK and the USA show lower rates of transplantation in ethnic minorities which may represent inequity of access. Ethnic minorities are overrepresented in dialysis programmes but less likely to be listed for deceased-donor transplantation, wait longer and are less likely actually to be transplanted [21]. Ethnic minority populations are more likely to receive poorly matched kidneys, since they are underrepresented among deceased donors. Graft survival is also reduced in minorities in the USA, and in both blacks and South Asians in the UK [21, 22]. But the most disadvantaged patients of course are eligible patients who are never transplanted, whether with a deceased-donor or living-donor kidney.

Promotion of living-donor transplantation is a key element in strategies for minimizing inequity of access to transplantation. A study from Baltimore, USA, showed that a decade of active encouragement of volunteerism led to the same living-donor transplant rate in blacks as whites, with equivalent patient and graft survival, and median waiting times below the national average [23]. In-

novative approaches to living-donor transplantation, for example strategies aimed at transplanting across AB0 incompatibility barriers, might also assume special importance. US physicians, when asked why ethnic minority patients were referred less frequently for transplantation, most commonly cited the lack of a potential live donor, patient preference, comorbidity or concern that patients did not complete necessary evaluations [24]. While living-donor transplantation is the optimal RRT for many patients, donor selection needs special care in populations which typically have a high incidence of familial renal disease.

The shortage of deceased donors from ethnic minority populations is a continuing concern. In the UK, South Asians and blacks only contribute 1.5% of the total donor pool yet comprise 6% of the general population and 19% of patients on the deceased-donor transplant waiting list. The reasons underlying this shortfall have been extensively explored with these communities, and programmes are in place to improve donor rates by awareness and education, although progress is slow.

An adverse effect of the relative difficulties in identifying appropriate deceased donors for minority populations has been the surge of 'transplant tourism' for those with resources to 'buy' a living-unrelated-donor kidney, a practice condemned by the international renal community through the Istanbul Declaration [25].

Quality of Life under RRT

Tools for the evaluation of health-related quality of life are available for renal disease. However, these have typically been developed and tested in majority populations and might not be sensitive to the cultural, religious and social distinctions in ethnic minorities. Important differences might also emerge when questionnaire-based quality-of-life assessment relies on the use of health professionals, interpreters and family members. These potential biases will not be infrequent, even in stable migrant populations, in which older people do not read or write in English or even in their native language.

Nevertheless, South Asians in the UK reported inferior quality of life compared to whites both on PD and haemodialysis, and following renal transplantation [26]. However, health-related quality of life in the Dialysis Outcomes and Practice Patterns Study was higher in ethnic minority patients [27]. The prominence of religious belief as a coping mechanism has been suggested as a possible explanation for this finding [28]; alternatively, in the US health care setting, a narrowing of the gap in access to good-quality health care after the initiation of RRT might alter perception of quality of life.

Public Health Implications

Although exploration of the mechanisms that underlie susceptibility to renal disease in these populations is of the utmost importance for the future, there is a pressing worldwide health care challenge that will accelerate at a faster pace than fundamental research can head off. In the UK, the overall age-adjusted relative risk remains more than 3-fold in both the South Asian and African Caribbean populations, and this risk might be increasing. The younger age distribution of the ethnic minority population means even larger future increases in the demand for RRT. Estimates of need are based on the presumption that current acceptance rates are a true reflection of demand; this might not be the case if there is inequity of access to health care. Vigorous advocacy must continue if resources sufficient to deliver the required standards of care are to be made available where populations include large ethnic minorities. Indeed it must be recognised that the delivery of high-quality health care that is linguistically and culturally adequate for ethnic minorities will likely require additional resources compared to the resources needed for majority populations. Although intensive efforts at augmenting transplantation rates from both deceased and living donors must continue, a disproportionate requirement for dialysis facilities is unavoidable. In parallel, programmes for screening and early intervention must be introduced if the future RRT burden is to be restrained [29]. Priority must also be given to the long-term goals of understanding genetic and other factors influencing the susceptibility of ethnic populations to CKD if truly effective preventative strategies are to be achieved.

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The Gut: The Forgotten Organ in Uremia?

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Key Words
Gut · Uremia · Uremic retention solutes · *p*-Cresol

Abstract
Part of the uremic retention solutes are generated in the intestine, but this option is rarely discussed in the literature. In this publication, we describe consecutively the role of the intestine in generating uremic retention solutes, the pathophysiological importance of the generated solutes and therapeutic options that are inspired by this knowledge. Apart from its role as a route via which uremic toxins or their precursors enter the body, the intestine also acts as an active player by presenting more precursors for fermentation due to disturbances in assimilation caused by uremia, followed by alterations in further processing related to changes in the composition of the fermenting flora. Many of the toxins generated or introduced into the body via the intestine (advanced glycation end products, indoles, phenols) play an active role in vascular damage. Intestinal therapeutic interventions that could help decrease solute concentration are restriction of dietary intake, however at the expense of increasing the risk of malnutrition, rerouting of intestinal metabolism by administration of prebiotics or probiotics and/or the administration of active sorbents such as AST-120 (Kremezin®).

Introduction

When the glomerular filtration rate decreases, the concentration of retained solutes [1] depends on the interference between removal, which in most cases is decreased, and a variable degree of generation [2] (fig. 1). A substantial part of this generation process is regulated in the intestine. In spite of its importance, this option is rarely taken into account in reviews of uremic toxicity and/or in the development of therapies.

Pathophysiological Elements

Uremic solute generation per se is ruled by several mechanisms (fig. 1): endogenous production by often endocrine processes without interference of intestinal absorption as is the case for most peptides; for the purines uric acid or xanthine, intestinal absorption plays a role only after excessive intake of nutrients containing large amounts of nucleotides as precursors (i.e. organ meat, sweetbread); intake of exogenous toxins via the gastrointestinal tract as food components which remain entirely or partially unaffected by digestion (e.g. advanced glycation end products, AGEs); intake of exogenous products via the gastrointestinal tract, digestion in the intestine and uptake of the digested end product followed by further metabolic modification in the body; if the absorbed compound is not further modified by metabolism and since it is conceivable that the digested end product is a

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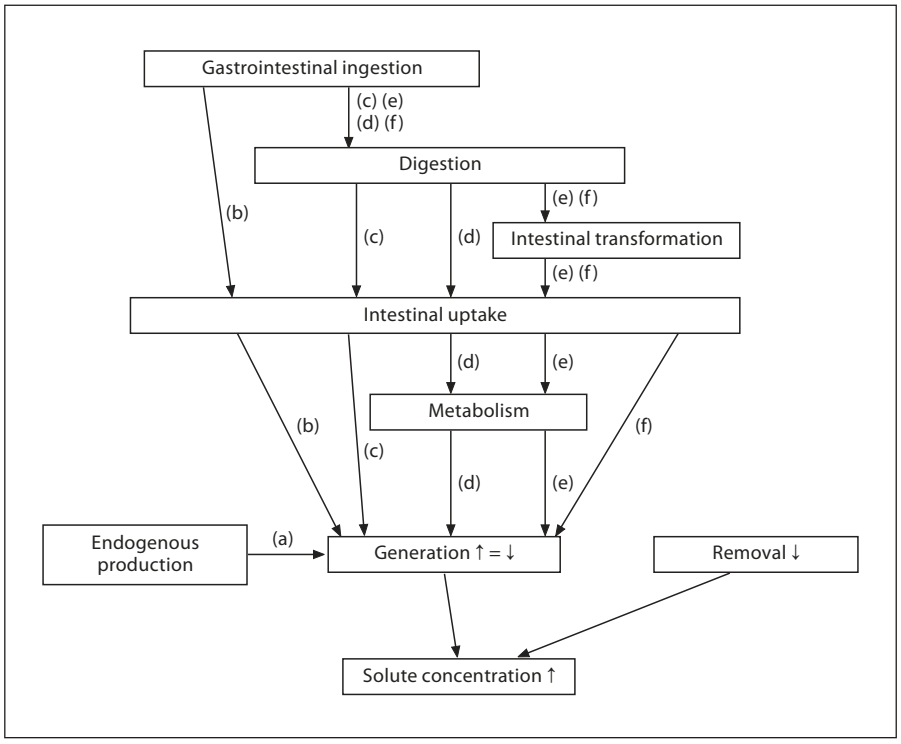

Fig. 1. Different mechanisms for the generation of uremic toxins: a = endogenous production without any interference of the intestine; b = gastrointestinal ingestion and immediate intestinal uptake of the compound as such; c = gastrointestinal ingestion followed by digestion and uptake; d = gastrointestinal ingestion followed by digestion, uptake and finally metabolic modification in the body; e = ingestion, digestion, intestinal transformation, uptake and then additional modification in the body; f = ingestion, digestion, intestinal transformation and uptake without further metabolic modification in the body; ↑ = increased versus normal; = = unmodified versus normal; ↓ = decreased versus normal; in uremia, depending on the molecule and the metabolic condition of the patient, the three options are possible for generation.

nutrient (e.g. amino acids), it is generally not noxious as such; it becomes only a toxin upon further modification (e.g. some of the guanidine compounds); intake of exogenous products via the gastrointestinal tract, digestion in the intestine followed by metabolic transformation by the intestinal enzymes or bacteria into toxins, only then uptake of this product and often further metabolic modification in the body (e.g. phenols and indoles).

Sources of these uremic toxins might not only be pure nutrients, but also food preservatives (benzoic acid generating phenols) [3], flavor correctors (pennyroyal oil generating *p*-cresol) [4], environmental toxins (toluene generating phenol) [5], alternative therapeutic agents or psychedelic drugs (menthofuran generating *p*-cresol) [6].

In addition, the concentration of uremic solutes in the gut may also be influenced by excretion into the intestine, e.g. by the gall bladder. A further contributing factor is a shift in the composition of the intestinal flora due to the uremic condition, favoring overgrowth of bacteria producing toxic compounds (fig. 2) [7]. Metabolism of peptides and proteins by anaerobic germs (putrefaction) generates phenols and indoles [8]. When those microbes are killed, a decrease in fecal and urinary excretion of phenolic and aromatic substances ensues [9]. In addition, changes in assimilation (digestion plus absorption) of proteins make more substrate available for fermentation [10, 11], as illustrated by the higher daily urinary *p*-cresol excretion in subjects with a glomerular filtration rate of <30 ml/min compared to those with ≥ 60 ml/min [11]. This results in a higher generation rate in chronic kidney disease than in normal subjects, even if the patients with renal failure are neither catabolic nor anabolic.

Some compounds might be neither metabolized nor absorbed, so that they ultimately leave the intestinal tract unmodified; this is the case with resistant starches, cellulose or gums. Such compounds can of course have no direct biological or toxic impact, but they can still affect toxicity indirectly by modifying the constitution or function of the intestinal flora (see section below on therapeutic options).

Examples of Intestinally Generated Uremic Toxins

Advanced Glycation End Products

AGEs are typically retained in older subjects, diabetics and patients with kidney failure. In diabetes, a large part of the generation of AGEs is attributed to the glycation of proteins, peptides and amino acids due to excess glucose.

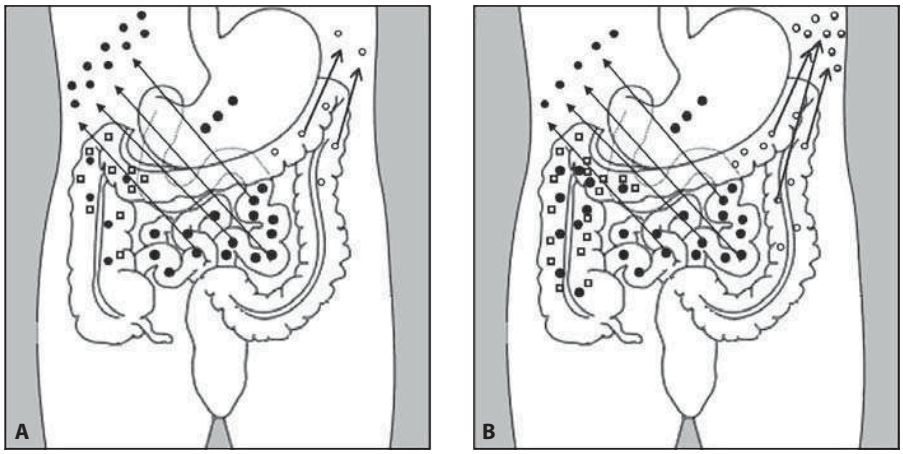

Fig. 2. Fermentation processes under normal conditions (**A**) and in uremia (**B**). **A** Under normal conditions, compounds (e.g. proteins) are ingested and digested (e.g. to amino acids; black dots); these compounds are partially absorbed via the small intestine; the rest moves to the large intestine where they come into contact with the intestinal flora (open squares), which transforms the molecules into other compounds (e.g. *p*-cresol; open circles); part of these are absorbed as well. **B** In uremia, digestion is the same but absorption of the primary molecules (black dots) is hampered so that more substrate enters the large intestine, where also a larger amount of transforming bacteria are present (open squares); the result is more generation of end product (open circles) of which also more is absorbed.

Other sources are posttranslational modifications due to microinflammation and oxidation which play a role in diabetics, uremics as well as in the elderly.

The third major source of AGEs are food products which have been processed by heating [12]. Several studies have demonstrated that nutritional ingestion of AGEs increases their concentration, both in nondiabetics [13] and in diabetics [13, 14].

More importantly, those studies also showed that nutritional AGEs conveyed some of the deleterious pathophysiological effects of these compounds such as inflammation [12] or endothelial dysfunction [13].

Phenols

Intestinal fermentation of the amino acids phenylalanine and tyrosine generates *p*-cresol, phenol [15] and very likely also phenylacetic acid as well as other phenols.

For a long time it has been thought that after its generation *p*-cresol was absorbed as such by the intestine and then distributed over the body, since upon analysis the molecule was found in serum of subjects with normal and disturbed renal function [16]. Only recently has it become clear that after its absorption, *p*-cresol is conjugated in the intestinal wall to *p*-cresylsulfate as well as to *p*-cresylglucuronide, while what remains of *p*-cresol after transfer into the portal vein is modified further on to *p*-cresylglucuronate in the liver, leaving very little or no remnant *p*-cresol present in the body (fig. 3). Although no similar data about conjugation of other phenols is available, it is conceivable that some of these (e.g. phenol itself) may be conjugated as well.

The reason why *p*-cresol was considered for a long time as a major phenolic compound in the body is attributable to an artifact created by the preparation of the samples for analysis. Until a few years ago, virtually all determination methods used deproteinization by acidification as a first step, causing hydrolysis of the *p*-cresol conjugates. Deproteinization without acidification left *p*-cresylsulfate intact, with virtually no detectable *p*-cresol [17].

Indoles

Similar to phenol, indole is generated by the bacterial flora, now with tryptophan as the mother compound. The main indole detected in uremics is indoxylsulfate, but this sulfated conjugate resists acidic hydrolysis, in contrast to *p*-cresylsulfate.

The phenolic and indolic conjugates are not necessarily generated by the same metabolic process, as suggested by the diverging metabolic behavior of the two compounds under identical clinical conditions [18].

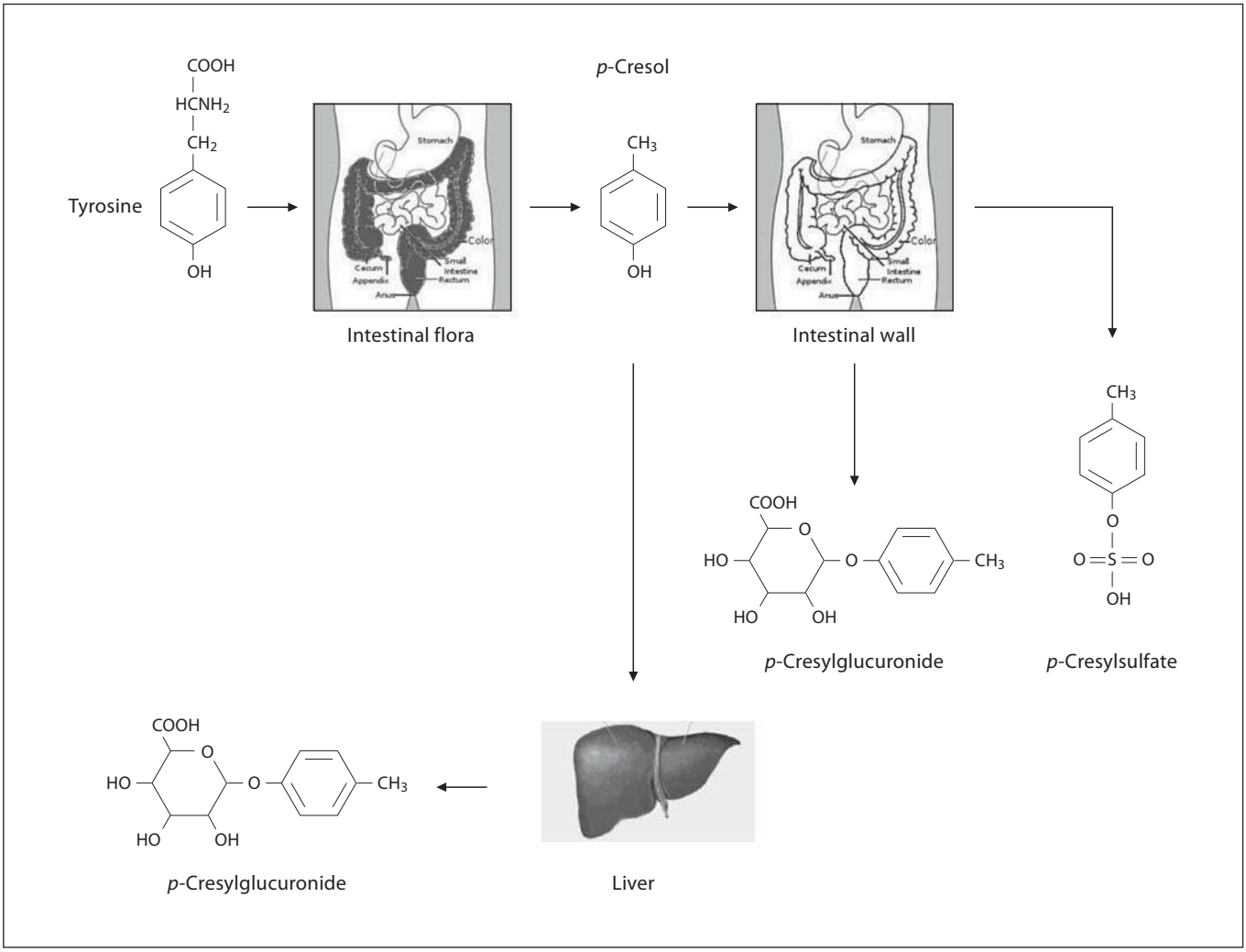

Fig. 3. Metabolic pathways involved in the transformation of tyrosine to *p*-cresylsulfate and *p*-cresylglucuronide.

Biological Impact

Although it is not the intention to present an in-depth review of the biological impact of the discussed compounds, a brief summary of their potential toxicity will allow the reader to realize that influencing concentration, e.g. by changing intestinal generation or absorption, might have clinical benefits.

Advanced Glycation End Products

AGEs have been associated with inflammation, oxidation, leukocyte stimulation and endothelial dysfunction [12, 13, 19]. Many early in vitro studies showing those effects have, however, been performed with artificially prepared AGEs [20] of which it was uncertain whether they were structurally related to the compounds retained in uremia. More recently, also the proinflammatory impact of AGEs present in dialysis patients has been demonstrated [19].

Up to now only a limited number of studies has addressed the relation between AGE concentration and outcome. One study paradoxically found the best outcomes in the group with the highest AGE concentration, possibly pointing to an overriding effect of nutritional status which at the same time improves survival and supplies extra AGEs [21]. Other studies demonstrated a direct correlation between mortality and concentration of low-molecular-weight AGEs [22] and skin autofluorescence, a presumed indicator of skin AGE content [23].

Table 1. Intestinal approaches to decrease supplementation of toxin

Method	Target molecule	Outcome
Diet	AGEs, phenol, indoles	decrease concentration
Prebiotics		
Gum arabic fiber	urea	decrease concentration
Oligofructose-enriched inulin	*p*-cresol	decrease concentration
Lactulose	*p*-cresol	decrease concentration
Resistant starch	phenol	decrease concentration
Probiotics		
Urease-positive bacteria	urea	decrease concentration
Lactobacillus	urea, *p*-cresol	decrease concentration
Bifidobacterium	*p*-cresol	decrease concentration
Lactic acid bacteria	*p*-cresol, phenols indoles	decrease concentration
Sorbents		
AST-120	*p*-cresol, indoles	survival; preservation of renal function

Phenols

p-Cresylsulfate has a proinflammatory impact on monocytes and lymphocytes [24]. To the best of our knowledge, this is the only study as of today demonstrating a biological impact for this compound.

Several observational studies show a relationship between *p*-cresol and overall mortality [25], cardiovascular disease [26], infectious complications [27] and uremic symptoms [28]. As all these data were obtained with analytical methodology applying acidification, it is conceivable that those findings on *p*-cresol can be extrapolated to the real main retention product, *p*-cresylsulfate. Interventional trials showing a positive impact of decreasing concentration of phenols are not available.

In contrast to *p*-cresylsulfate, the mother compound *p*-cresol is a strong inhibitor of leukocyte response [24, 29]. Although it is unlikely that *p*-cresol exerts this effect on the immune cells throughout the body, because of the absence of direct contact, such an immunosuppressive effect might be at play in the gut-associated lymphoid tissue, which is very likely in more direct contact with the intestinal content, and an important element in shaping the immune response within the rest of the body [8].

Indoles

A host of in vitro and in vivo animal studies point to indoxylsulfate as causing inflammation [30], endothelial dysfunction [31] and disturbances of bone metabolism [32]. In addition, it has repeatedly been associated with loss of residual renal function [33], by itself a factor with strong impact on outcome [34]. Inhibition of intestinal absorption of indoxylsulfate by the sorbent AST-120 (Kremezin®) has been associated with a postponement of the start of dialysis [35] and, if applied before the start of dialysis, with better outcomes once dialysis was undertaken [36]. Although controlled, these studies have been performed in small populations so that they need confirmation.

In summary, the set of uremic toxins selected for this discussion are all characterized by in vivo and/or in vitro toxicity to the cardiovascular system, one of the main clinical problems haunting the uremic population today [37]. Evidence is not solid enough, however, to allow firm recommendations about the necessity of their removal.

Therapeutic Possibilities

Therapeutic modalities (table 1) implicating intestinal factors should especially be considered in view of the difficult removal of the relevant compounds even with the most efficient dialysis therapies [38, 39].

Type of Therapy

Restriction of the dietary intake of AGEs results in a decrease in concentration [14]. If this intervention results in less dietary protein intake, it may however result in malnutrition. Also intestinal generation of phenols and indoles is dependent on dietary protein intake [40, 41]. Since proteins are almost the only source of these molecules, the threat of malnutrition, if one intervenes via dietary protein restriction, is even more likely here.

Therefore, it seems more appealing to change generation via other strategies. A first option aims at modifying

the intestinal flora to refrain generation of toxins, either by prebiotics [42–45], which are nondigestible compounds beneficially modifying the composition and/or function of the intestinal flora, or by probiotics, which are bacteria administered as food components or supplements providing specific benefits themselves [7, 43, 44, 46–48].

Finally, oral sorbents may be administered to bind solutes and prevent their intestinal absorption. To the best of our knowledge, AST-120 (Kremezin®) is the only therapeutic sorbent of the solutes under discussion, with an impact on outcome parameters shown in a number of studies [35, 36]. Especially the absorptive capacity of AST-120 on indoxylsulfate has been emphasized, although also *p*-cresol is absorbed [49].

Target Molecules

Several studies aim at reducing urea alone [42, 47, 48]. In as far as urea per se is a relatively inert uremic solute, strategies aimed only at urea removal [47, 48] are less appealing than therapies reducing total nitrogen load, including urea [42]; the latter approach supposedly affects toxicity more globally and less selectively.

Several approaches directly reduce *p*-cresol [7, 43, 44], phenol [7, 45] and/or indoxylsulfate [7].

Outcomes

All studies with probiotics and prebiotics evaluate the impact on solute concentration in serum or on their fecal or urinary excretion, which should be considered only as surrogate endpoints. All studies mentioned above show a decrease in concentration [7, 42–48]. The studies with AST-120 obviously affect the concentration of indoxylsulfate and *p*-cresol [49, 50] but show in a few publications also an impact on the timing of the start of dialysis [35] and on survival [36] as hard(er) endpoints; however, data are not convincing enough to allow definite conclusions.

Hemodialysis versus Peritoneal Dialysis

While removal of *p*-cresol and indoxylsulfate is markedly higher with hemodialysis than with peritoneal dialysis [18, 51, 52], serum concentration in peritoneal dialysis patients is paradoxically low [51–53]. These discrepancies cannot be explained by differences in protein intake so that other causes should be considered; some of these may be related to the intestine: intestinal blood losses, laxative use, digestive transit or intake of binding agents such as phosphate binders or potassium-binding resins [51]. These data suggest that uremic toxin supplementation may differ based on intestinal factors, even if no direct intervention via diet, probiotics or prebiotics has taken place.

Conclusions

The intestine is a major source of uremic toxin generation and/or uptake. Some of the toxins involved (AGEs, phenols, indoles) have a substantial biological impact. Many of these effects are related to vascular damage. Administration of prebiotics and probiotics as well as of sorbents (AST-120) decreases their concentration. Studies showing that such a decrease has a positive impact on outcome are, however, scarce and need confirmation.

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Phosphate Elimination in Modalities of Hemodialysis and Peritoneal Dialysis

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Key Words
Hyperphosphatemia · Phosphate elimination · Hemodialysis · Peritoneal dialysis

Abstract
Hyperphosphatemia is highly prevalent in hemodialysis (HD) and peritoneal dialysis (PD) patients and is a major risk factor for cardiovascular mortality. Elimination of inorganic phosphate by dialysis is a cornerstone of the management of hyperphosphatemia. Phosphate clearance during HD is affected by various factors of dialysis prescription, such as blood and dialysate flow rate, dialyzer membrane surface area and ultrafiltration volume. Phosphate mass removal can be improved by hemodiafiltration, increased dialysis frequencies and extended treatment times. Short daily or extended daily or 3 times weekly nocturnal HD allow higher phosphate mass removal and potentially complete discontinuation of phosphate binder medication. In PD, phosphate mass removal appears to be correlated with peritoneal creatinine but not urea clearance. In hyperphosphatemic PD patients, the decision on the optimal PD modality should be based on peritoneal creatinine and ideally also on peritoneal phosphate transport characteristics.

Introduction

Both hemodialysis (HD) and peritoneal dialysis (PD) patients are at an increased risk for cardiovascular mortality. Besides classical cardiovascular risk factors, hyperphosphatemia has been identified as one of the most important risk factors for mortality [1, 2]. Hyperphosphatemia differs from many other cardiovascular risk factors in one important aspect: it appears potentially well controllable. However, since hyperphosphatemia is still widely prevalent among HD and PD patients worldwide, there is a lot of room for improvement in the strategies currently applied to manage hyperphosphatemia. These are based on three principles, namely dietary phosphorus restriction, use of phosphate binder substances and phosphate elimination by one of several dialysis modalities. An innovative approach to the integrative use of the first two principles, i.e., patient self-adjustment of phosphate binder dose in relation to eye-estimated meal phosphorus content, has been discussed recently by the author [3]. This review will focus on strategies to optimize the removal of inorganic phosphate (P_i) by the various modalities of HD and PD.

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HD Modalities

Based on its molecular weight of 96 Da alone, P_i clearly falls into the category of water-soluble low-molecular-weight uremic toxins. However, due to its hydrophilic characteristics, the phosphate molecule is surrounded by an aqueous cover, which considerably increases the effective molecular weight. Phosphate is mainly distributed in the intracellular space with a slow intra-/extracellular solute transfer rate and with a distribution volume which is assumed to be equal to total body water. It needs to be stressed that in contrast to urea, phosphate is not freely diffusible across cell membranes and about 5% of circulating phosphate has been shown to be a component of sodium, calcium and magnesium salts. All these factors contribute to the fact that the elimination characteristics of phosphate in HD and PD are dissimilar to those of urea and other small-molecular-weight toxins and much more similar to those of typical middle molecules [4].

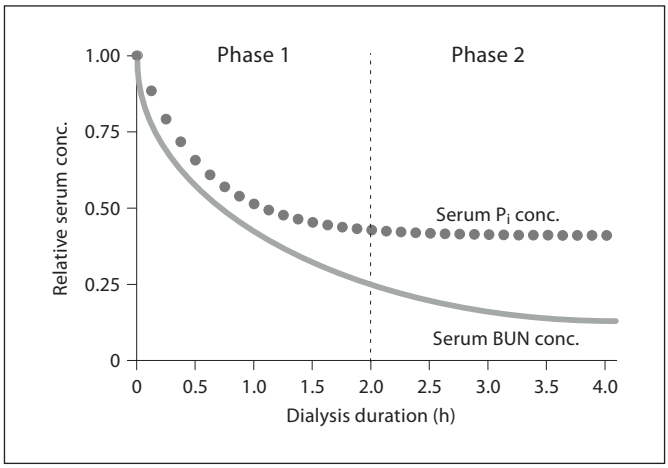

Fig. 1. Comparison of intradialytic phosphate and blood urea nitrogen (BUN) kinetics. Serum P_i concentration sharply drops during the first phase of dialysis (phase 1) and, after reduction of serum P_i to about 40% of predialysis levels, stabilizes throughout the rest of the treatment (phase 2). In contrast, BUN levels steadily decline during dialysis without reaching a plateau.

Phosphate Kinetics during HD

The kinetics of intradialytic phosphate removal differ significantly from classic urea kinetics. During HD, blood urea nitrogen concentrations continuously decline and, following a short rebound period immediately after termination of the treatment, steadily return to predialysis values in relation to protein intake and endogenous urea generation during the interdialytic interval. Intradialytic plasma P_i kinetics show a characteristic 2-phase pattern (fig. 1): the first phase is determined by a relatively steep decline of plasma P_i levels and lasts for about 2–2.5 h after start of the treatment. This is followed by the second phase, during which plasma P_i levels do not further decline or even slightly increase towards the end of the dialysis session. This characteristic stabilization of serum P_i levels despite ongoing P_i elimination reproducibly occurs when plasma P_i levels have dropped to about 40–50% of baseline predialysis levels. Within a couple of hours after termination of dialysis, plasma P_i levels rebound to almost predialysis values [5, 6]. These kinetics suggest that during the first phase of dialysis predominantly the P_i available in the extracellular plasma compartment is removed, while during the second phase P_i removal occurs from the intracellular space with the rate of change in plasma P_i levels determined by the rate of P_i transfer from one or more intracellular compartments to the plasma compartment [7]. The strong postdialytic P_i rebound is representative for this transfer rate. It can be concluded from these kinetics, that the P_i mass removal *rate* (P_i mass removed per time unit) is highest during the first phase of HD. On the other hand, since the P_i diffusion gradient between plasma and dialysate is maintained stable during the second phase of treatment, the *absolute* P_i mass removed during the second phase may be higher than during the first phase, depending on the duration of the second treatment phase. This stands in contrast to urea kinetics, where fractional urea mass removal during dialysis steadily declines due to the constant reduction of the concentration gradient across the dialyzer membrane. With these characteristics of intradialytic phosphate kinetics in mind, several strategies to optimize HD P_i removal can be formulated:

(i) optimizing dialysis prescription to maximize P_i removal with conventional 3 times a week HD regimens;

(ii) increasing dialysis frequency with shorter treatment times (short daily HD, SDHD); with this strategy a focus is put on the first phase of phosphate kinetics;

(iii) extending dialysis treatment time (nocturnal HD, NHD, with variable frequency); with this approach, the constant P_i mass removal during the second phase of dialysis is maximized.

Table 1. Weekly P_i mass removal with various HD and PD treatment modalities

Modality and ref.	P_i mass removal mg/week	Dialysis schedule	Flow rates ml/min	Treatment specifications
Hemodialysis				
HD high-flux [8]	2,356 ± 864	3 × 230 min	Q_B: 323 ± 22 Q_D: 500	UFV 1.8 ± 0.8 liters
HD + passive muscle activity [9]	PCM: 3,515 ± 945 TEMS: 3,591 ± 795	3 × 195–240 min	Q_B: 300–400 Q_D: 600	S-P_i 5.1 ± 0.9 mg/dl
HD – double dialyzer [10]	2,970 mg	3 × 4 h	Q_B: 350–400 Q_D: 800	F80A or F160 dialyzers S-P_i 5.3 mg/dl
Postdilution HDF [11]	3,570 ± 270 mg	3 × 4 h	Q_B: 315–345 Q_D: 500	F8 dialyzer, MSA 1.8 m^2 Q_{UF} 25–35 ml/min
Mixed-dilution HDF [12]	975 ± 272 mg/Tx (2,975 mg/week)	231 ± 18 min	Q_B: 385 ± 20 Q_D: 625 ± 16	Q_{UF} 181 ± 12 ml/min MSA 1.8 m^2
SDHD [13]	2,452 ± 720 mg	6 × 3 h	Q_B: 400 Q_D: 800	high-flux dialyzer S-P_i 4.2 mg/dl
NHD [14]	8,000 ± 2,800	6 × 6–8 h	Q_B: 150–300 Q_D: 300	high-flux dialyzer F80
	P_i mass removal mg/week	Dwell time h	Flow rates ml/min	Treatment specifications
Peritoneal dialysis				
APD, CCPD [8]	2,739 ± 1,042	18.5 ± 7.3	–	DV 13.2 ± 3.5 liters ex. 5.5 ± 1.1 S-P_i 5.0 ± 1.4 mg/dl
CAPD [8]	2,790 ± 1,022	24.0	–	DV 10.5 ± 2.1 liters ex. 4.2 ± 0.5 S-P_i 4.2 ± 0.9 mg/dl

HDF = Hemodiafiltration; APD = automated PD; CCPD = continuous cycling PD; CAPD = continuous ambulatory PD; Tx = treatment; PCM = passive cycling movements; TEMS = transcutaneous electrical muscle stimulation; Q_B = blood flow rate; Q_D = dialysate flow rate; S-P_i = serum P_i concentration; Q_{UF} = ultrafiltrate flow rate; MSA = membrane surface area; DV = peritoneal fluid drainage volume; UFV = ultrafiltration volume; ex. = number of PD fluid exchanges.

Conventional HD Regimens

With plasma P_i levels at the end of dialysis reaching about 40% of predialysis levels, it is evident that the absolute phosphate mass removed per treatment depends mainly on predialysis P_i levels. For a standard HD treatment of 240 min duration, an average phosphate mass removal of 700–900 mg has been reported. This amounts to a weekly P_i removal of 2,100–2,700 mg with a conventional 3 × 4 h HD regimen (table 1). Considering a daily dietary phosphorus consumption of 1,000 mg with a gastrointestinal absorption rate of 70%, the weekly P_i burden of roughly 5,000 mg is inadequately removed by conventional HD strategies. Phosphate removal with conventional HD regimens can be maximized by the means described below.

Dialyzer Membrane and Surface Area

For any dialyzer membrane P_i clearance is generally lower than urea clearance. This is due to a higher diffusive resistance for phosphate in full blood, with blood cells acting as diffusion barrier. From specification sheets it appears that low-flux membranes have a lower P_i clearance than high-flux membranes, but studies have shown that low-flux and high-flux membranes apparently do not differ if corrected for membrane surface area [5]. Membrane surface area itself has a potentially important impact on phosphate mass removal. In a recent study in 18 patients over a period of 6 weeks, doubling of membrane area by the use of 2 dialyzers in parallel (with blood flow equally split between the dialyzers) resulted in a 1.34 mg/dl decline in predialysis serum P_i levels compared with conventional HD [10]. The somehow surprising ob-

servation that absolute phosphate mass removal was not different from standard dialysis was explained by hypothetical larger phosphate adsorption to dialyzer membranes. It may be concluded that, in order to optimize P_i removal, a dialyzer with a large membrane surface area should be used.

Blood Flow Rate and Dialysate Flow Rate

For any substance, dialyzer clearance depends on the effective blood flow rate (Q_B). However, in contrast to urea and potassium removal, increasing Q_B to >250–300 ml/min has only limited effects on P_i removal [15]. On the other hand, raising the dialysate flow rate (Q_D) from 300 to 500 ml/min is associated with a 10% increase in P_i clearance [16]. A rise of Q_D from 500 to 800 ml/min apparently does not significantly increase P_i clearance [10].

Hematocrit

The dialyzer clearance of any substance also depends on the effective distribution volume within the bloodstream. While for urea this is total blood water and for creatinine it is plasma water volume and 61% of erythrocyte volume, for phosphate it is plasma water volume alone. Since plasma water volume depends on hematocrit and albumin levels, dialyzer P_i clearance declines with increasing hematocrit [16]. Therefore, in hyperphosphatemic patients with high predialysis hematocrit, mixed-dilution hemodiafiltration (HDF) may be a better alternative than conventional HD (see below).

Choice of Anticoagulant

Even with the use of heparin, unnoticed clotting of dialyzer fibers and membrane pores often occurs. This subtle clotting, while not interfering with the completion of the treatment, reduces the efficiency of dialytic removal of phosphate and other solutes. A recent study indicates that acidifying bicarbonate-based dialysate with citrate instead of acetate may be associated with increased solute removal including phosphate. Possible explanations include the local anticoagulant activity of dialysate citrate at the site of the dialyzer, thereby preventing the clogging of larger pores and maintaining effective dialyzer surface area throughout the treatment [17].

Physical Activity

Physical activity before or during HD increases muscle perfusion and facilitates blood/tissue equilibration for urea, phosphate and other solutes. Predialytic or intradialytic short active physical activity has been shown to increase P_i mass removal by 6–9% [18]. Repetitive muscle activity may further enhance P_i removal substantially. Intradialytic motor-driven passive leg cycling movements or transcutaneous electrical muscle stimulation of both thighs and calves increased P_i removal by 31% (1,172 ± 315 vs. 895 ± 202 mg) and 34% (1,197 ± 265 vs. 895 ± 202 mg), respectively. This translates into an increase in weekly P_i removal from 2.6 to 3.5 g with passive leg cycling movements and to 3.6 g with transcutaneous electrical muscle stimulation, effects similar in size to an additional HD treatment per week (table 1) [9].

Hemodiafiltration

Online HDF allows a greater fraction of uremic middle molecules and phosphate to be removed by convection. HDF can be delivered in a predilution or postdilution mode. The latter may cause hemoconcentration and an increase in blood viscosity resulting in increased diffusive resistance for phosphate, high transmembrane pressure, loss of membrane permeability and eventually filter clotting. Predilution HDF, in contrast, is associated with reduced efficiency due to dilution of the solute concentration gradient across the membrane. In direct comparison, postdilution HDF has been shown to be more effective than predilution HDF, and an increase in P_i removal by 30–40% has been reported for postdilution HDF by several groups [11, 19]. Mixed-dilution HDF is a relatively new modality where blood dilution is split between pre- and postfiltering and which was developed to achieve the best possible rheological and hydraulic conditions within the dialyzer and to optimize the fluid exchange rate and convective solute removal. However, a significant advantage of mixed-dilution over postdilution HDF in regard of P_i removal still needs to be demonstrated [12]. Mixed dilution may be of special advantage in patients with high predialysis hematocrit and an increased risk of filter clotting with postdilution HDF due to hemoconcentration.

Increased Dialysis Frequency

Increasing dialysis frequency from 3 to 5 or 6 times per week appears an attractive alternative treatment schedule which may be associated with an increased quality of life and potentially better outcome and phosphate control [20]. Increased dialysis frequencies with shorter treatment times take advantage of the high P_i mass removal rate during the first phase of dialysis.

Short Daily HD

The concept of SDHD offers more frequent dialysis at shorter treatment duration. Effective control of phosphate levels, however, depends on the duration of each HD session. With a regimen of 6 × 2–2.5 h, only about 400–500 g of phosphate are removed per session. In almost all studies on SDHD with treatment durations between 2.0 and 2.5 h, patients had to continue to take phosphate binders [20, 21]. In order to achieve phosphate control without the use of phosphate binders, longer treatment duration, such as 6 × 3 h, may be necessary. This was demonstrated in a study by Ayus et al. [13] where 77 patients were treated with either conventional 3 times weekly dialysis (3 × 4 h; n = 51) or with SDHD (6 × 3 h; n = 26). Total weekly P_i removal was 56% greater in the SDHD group, and serum phosphate levels decreased from 6.3 ± 2.57 to 4.0 ± 1.19 mg/dl. Phosphate binder medication was withdrawn in 73% of the SDHD group. Improved phosphate removal and a reduction in phosphate binder dose have also been reported for short daily HDF (6 × 2–2.5 h) [22].

Extended Treatment Times

With extended treatment times, the benefits of the sustained blood/dialysate concentration gradient during the second phase of dialysis are maximized. Extending weekly dialysis time from 12 (3 × 4) to 15 (3 × 5) h without changing treatment Kt/V significantly increases phosphate mass removal by 13% [18]. Eloot et al. [23] have recently demonstrated that applying even longer treatment times of 6 and 8 h without increasing urea Kt/V resulted in increased phosphate mass removal compared to conventional treatment times of 4 h. This is in line with intradialytic phosphate kinetics discussed above and the advantage of the sustained phosphate diffusion gradient during the second phase of dialysis. It appears that with longer dialysis times, phosphate is removed more efficiently from deeper body compartments.

Nocturnal HD

With NHD, treatment times are even further extended. Several small studies report the effects on P_i removal by NHD with treatment times of up to 8 h of varying frequency. Pierratos et al. [24] demonstrated in a study comparing 3 × 4 h standard HD with 6 × 8 h NHD (with Q_D of 100 ml/min) significantly improved P_i elimination despite unchanged urea mass removal. In addition, prescription of phosphate binders was completely stopped despite significantly increased dietary protein and phosphorus intake[24]. Daily NHD with higher Q_D would even further increase phosphate removal, which may then be well in excess of daily dietary phosphorus intake [25]. In a recent randomized controlled trial of 52 patients comparing NHD 6 times weekly with conventional HD 3 times weekly, NHD was significantly more effective at lowering serum phosphate levels. Average predialysis serum phosphate levels were reduced from 5.5 ± 1.5 to 4.4 ± 1.7 mg/dl, and phosphate binder medication was reduced or completely discontinued in 73% of patients [26]. NHD with a frequency of only 3 times a week, such as in-center 3 times weekly NHD, has also been shown to improve phosphate management. In a retrospective study of 39 patients switched from 3 times weekly conventional HD to 3 times weekly in-center NHD, median phosphate values decreased from 5.9 to 3.7 mg/dl, and the mean daily dose of phosphate binders declined from 6.2 to 4.9 pills/day [27]. Data on phosphate removal are not available from these two recent studies. Nevertheless, all published data on extended treatment times demonstrate the advantage of long treatment times for adequate P_i removal.

PD Modalities

Similar to HD patients, hyperphosphatemia is highly prevalent in PD patients and is strongly associated with overall and cardiovascular mortality [28, 29]. Although detailed knowledge about the kinetics of phosphate removal appears essential for optimizing phosphate removal in PD, peritoneal P_i clearance has not been well studied in the past. This may be due to the fact that, in general, ion transport across the peritoneal membrane is complex and influenced by a whole multitude of factors, which are difficult to take into account and even more difficult to control. Among the various cations and anions subjected to transmembranous peritoneal transport, phosphate has been studied only in a minority of cases. Even in a recently published excellent comprehensive review of the principles of fluid and ion transport across the peritoneal membrane in continuous ambulatory PD (CAPD), phosphate transport only plays a minor role [30]. According to the simplified 3-pore model of peritoneal clearance, the peritoneal membrane consists of a large number of small endothelial pores of a radius of 43 Å in the peritoneal capillaries, which account for 99.7% of the total surface area available to small solute diffusion. Less than 0.01% of the total number of pores is represented by large pores of a radius of 250 Å, across which proteins are transported by convection. The rest mainly consists of

water-conductive ultrapores (aquaporin 1) which are responsible for the osmotic water flow during the first few hours of the dwell but do not contribute to solute removal. On top of that, transmembranous active phosphate transporters exist in endothelial cells. When discussing transmembrane phosphate elimination in PD, several aspects of phosphate specific to PD need to be considered: the phosphate anion is negatively charged and so are capillary walls and interstitial matrix. About 15–20% of phosphate is bound to proteins and about 5% is complexed to other ions. Furthermore, because phosphate is not freely diffusible through cell membranes, the diffusion resistance at the capillary vessel membrane is higher than for highly diffusible substances like urea. While the molecular weight of phosphate (96 Da) lies right between those of urea (60 Da) and creatinine (13 Da), the molecular radius of phosphate (2.8 Å) is closer to that of creatinine (3.0 Å) than urea (1.8 Å). Finally, the hydrophilic phosphate molecule is surrounded by an aqueous cover which increases the effective molecular weight. Taken together, phosphate transport across the capillary walls into the peritoneal cavity is influenced by osmotic, chemical and electrical gradients, as well as by transmembranous active phosphate transporters and thus is more complex than peritoneal urea or creatinine transport [4, 30]. Principally, diffusion occurs through the small endothelial pores, while convective phosphate transport occurs through the larger pores.

Peritoneal and Renal Phosphate Clearance versus Creatinine and Urea Clearance

Peritoneal P_i clearance in average PD patients is considerably lower than clearance of small water-soluble substances. In a direct comparison, peritoneal P_i clearance (35.9 ± 2.7 l/week/1.73 m^2) was reported to be about 20% lower than creatinine clearance (45.3 ± 3.5) and almost 50% lower than urea clearance (66.6 ± 4.8) but about 8-fold higher than β_2-microglobulin clearance (4.7 ± 0.5) [4]. Various clinical studies have demonstrated that peritoneal P_i removal is more closely related to creatinine than to urea removal [31, 32]. In a retrospective analysis of urea, creatinine and phosphate clearances in 129 PD patients, a strong correlation between peritoneal P_i clearance and creatinine clearance was observed. In a multivariate regression analysis, peritoneal creatinine clearance, but not peritoneal urea Kt/V, was independently associated with peritoneal P_i clearance, indicating that peritoneal creatinine clearance may serve as a surrogate marker for peritoneal P_i clearance [31].

Renal clearance, on the other hand, has a higher relative impact on total clearance for P_i than for urea and creatinine [4]. At the start of dialysis therapy, residual renal function may contribute up to 65% of total P_i clearance, which declines over time. Interestingly, the loss of renal P_i clearance may be compensated for by an increase in peritoneal P_i clearance due to 3 potential mechanisms: first, prescription of higher dialysate volume; second, structural changes in the peritoneal membrane with increased transport of small substances caused by the exposure to dialysis fluid components, and third, an increase in 24-hour ultrafiltration volume with concomitantly increased convective P_i removal [33].

Influence of Membrane Transport Characteristics on Peritoneal Phosphate Elimination

Only recently has it been shown that membrane transport characteristics influence peritoneal P_i clearance. This is not a surprise considering the association between peritoneal P_i and creatinine clearance and the fact that membrane transport types are characterized by peritoneal creatinine equilibration kinetics [34]. Some studies have reported that patients in the low-average and low membrane transport categories have lower peritoneal P_i clearance than those in the high-average or high membrane transport categories [31, 32].

Other studies, however, question the validity of the peritoneal membrane transport category as a predictor of peritoneal P_i handling. In children on PD, the association of the 2- and 4-hour dialysate-to-plasma (D/P) ratio for P_i with the D/P ratio for creatinine was strong, while the association of peritoneal P_i clearance with the D/P ratio of creatinine was much weaker. From these data the authors concluded that creatinine is an inadequate surrogate marker of peritoneal P_i transport and argued for an individual assessment of peritoneal P_i handling. They also proposed the definition of an individual peritoneal membrane P_i transport status by defining a D2/P of P_i <0.27 and D4/P of P_i <0.41 as low P_i transport status [35]. No other studies have so far tried to define peritoneal membrane P_i transport status, but those studies appear to be urgently needed.

Phosphate Elimination in Various PD Modalities

Independently of the membrane transport category, weekly average P_i removal in adults with CAPD has been reported to be around 70 mmol (2,170 mg) with an inlet volume of 4×2 liters and 105 mmol (3,250 mg) with an inlet volume of 4×3 liters of daily PD fluid [36]. In a cross-sectional comparative study between automated

PD and CAPD, weekly total P_i mass removal was similar with 2,790 mg in CAPD and 2,739 mg in automated PD (table 1) [8]. Phosphate mass removal in CAPD and continuous cycling PD (CCPD), however, appears to be affected by membrane transport type. For patients in the high-average, low-average and low membrane transport categories, peritoneal P_i clearance is higher with CAPD than with CCPD, while creatinine clearance does not differ. For patients in the high category of membrane transport, in contrast, no difference between CAPD and CCPD was observed [31].

Based on currently available data, the following recommendations for the improvement of P_i removal can be made.

(i) For patients in the high type membrane category, phosphate elimination may be viewed as similar to the clearance of small-molecular-weight solutes. Phosphate mass removal in these patients may benefit from automated PD or CCPD with high nighttime fluid turnover and high number of cycles. However, it should be kept in mind that the diffusion of uremic toxins through the peritoneal membrane resembles a parabola and that if dialysate flow is increased beyond the individual turning point of the parabola, clearances may decrease, as the time spent for inflow and outflow becomes a significant part of the entire treatment period, which is less effective for diffusion [37]. The shape of the parabola for P_i elimination may be markedly different from that for creatinine.

(ii) For high average, low average and low membrane category type patients, peritoneal P_i clearance much more resembles the removal of middle molecules and small proteins which generally need longer dwell times to diffuse from peritoneal capillaries into the peritoneal cavity and do not depend on the number of exchanges of PD fluid. Phosphate mass removal in these patient groups will be higher with CAPD than with CCPD and may be further optimized with increasing dialysate outlet volume without reducing dwell time by using larger PD fluid volumes and higher dialysate glucose concentration.

(iii) All patient groups, independently of the PD modality used, may benefit from increased convective peritoneal P_i clearance through higher ultrafiltration rates. This is achieved by the use of higher dialysate glucose concentration or the use of icodextrin with or without shorter dwell times. However, no detailed studies on the effects of increased ultrafiltration rates on peritoneal P_i clearance are available.

These recommendations are based on the assumption that peritoneal membrane transport characteristics are predictive of peritoneal P_i removal. However, as pointed out earlier, peritoneal P_i clearance may follow its own characteristics, different from those of creatinine [35]. If that is the case, PD prescription needs to be adapted to the individual P_i-based and not only to the creatinine-based membrane transport types. Future studies should explore peritoneal P_i transport types in more detail. In hyperphosphatemic PD patients, the decision on the optimal PD modality should not solely be based on urea Kt/V but mainly on peritoneal creatinine and especially peritoneal phosphate transport characteristics.

In conclusion, comparing the various PD and conventional HD modalities, it can be stated that all remove about the same amount of phosphate per week (table 1) [31]. However, with all these HD and PD modalities, weekly phosphate removal is inadequate in relation to weekly dietary phosphate intake, and prescription of phosphate binders remains an essential component of phosphate management. The daily phosphate binder tablet burden can be reduced by optimizing the dialysis prescription for PD and HD. Modern HD treatment schedules with higher dialysis frequencies or extended treatment times, such as SDHD, or daily or 3 times weekly NHD, allow higher phosphate mass removal and potentially complete discontinuation of phosphate binder medication.

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Peritonitis – Does Peritoneal Dialysis Modality Make a Difference?

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Key Words
Peritoneal dialysis · Cycler peritoneal dialysis · Peritonitis

Abstract
Background/Aims: Peritonitis remains a significant problem for patients on peritoneal dialysis (PD). There is a certain amount of controversy as to whether peritoneal modality is itself a risk factor for peritonitis, with one modality higher than another. ***Methods:*** A literature review was done (August 2009) searching under 'peritoneal dialysis', 'peritonitis' and 'modality' to find all articles related to the topic. The highest-quality articles were extracted for review. ***Results:*** Two randomized controlled trials (RCTs) done with disconnect systems for continuous ambulatory PD (CAPD) and Luer lock connections for automated PD (APD) showed important decrements in peritonitis rate on APD compared to CAPD. The variation of peritonitis rates in studies comparing peritonitis on continuous cycling PD (CCPD) and CAPD may relate to the difference in connection type for APD in Europe (Luer lock) and North America (spike) and to differing prescriptions, including in some cases midday exchanges on APD and in other cases a dry abdomen on APD. The variation in peritonitis rates from center to center is marked. In many studies sufficient details regarding the connectology and the prescription, both of which may impact on peritonitis risk, are absent. ***Conclusion:*** At the present time, the best data suggest that use of APD with Luer lock connections versus CAPD with a disconnect system results in a reduction in peritonitis risk. More studies are needed on this important topic, particularly the possible advantage of initiating PD with a dry day in those with residual kidney function. This question would be best studied with an RCT comparing peritonitis rates in three groups of patients, i.e. those initiating dialysis on CCPD, CAPD and APD with a dry day.

Peritonitis remains a significant problem in peritoneal dialysis (PD). A recent analysis of the Australian/New Zealand database showed that peritonitis was a significant risk factor for death in PD patients [1]. Every effort is needed to continue to reduce peritonitis rates. Diaz-Buxo et al. [2] were among the first to suggest that the reduced risk of peritonitis with continuous cycling PD (CCPD) might be an attraction of this modality. They and colleagues reported a peritonitis rate of 0.48 episodes/year at risk in 150 CCPD patients in 1986, an era in PD when most were seeing much higher peritonitis rates [2]. Theoretically, because automated PD (APD) is generally associated with fewer connections (for example 2 bags of 5–6 liters may be used with each nighttime setup), the risk of contamination with APD might be lower. Spiking connection systems have been shown in studies of continuous ambulatory PD (CAPD) patients to be associated with significantly more peritonitis than Y set or twin bag disconnect systems [3]. Therefore, a CCPD system that uses a Luer lock connection might result in less contami-

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nation risk than a CCPD system that uses a spiking system for connection; this has not been studied but seems a logical extrapolation of CAPD studies of connectology. The longer day dwell of CCPD leads to a longer time during which peritoneal immune function is restored, again possibly decreasing the peritonitis risk [4–8]. Studies examining the peritonitis risk with APD compared to CAPD give mixed results, likely at least in part because of different connectology for CCPD in different studies. The peritonitis risk on CCPD versus CAPD is explored in this review.

Modality and Immune Status

There is increasing concern and interest in studying the peritoneal membrane to increase our understanding of the profound changes that can occur in this delicate structure over time in patients on PD. The mesothelial cells that line the peritoneum are critical and important participants in both chronic and acute inflammatory processes within the peritoneum [4]. Glucose, glucose degradation products, advanced glycation end-products and peritonitis all influence the immunology and subsequent dysfunction of the peritoneal membrane [4]. Glucose degradation products lead to apoptosis of mesothelial cells and are more important than glucose exposure, lactate exposure and low pH of standard dialysis fluid [5]. Transformed cells result in peritoneal neoangiogenesis and fibrosis, with concomitant peritoneal dysfunction over time [4].

De Fijter et al. [6] studied peritoneal macrophage function and effluent opsonic activity in patients on CAPD and CCPD. Important changes were seen with longer (15 h) compared to shorter dwell times (4 h). Peritoneal total white cell concentration increased by more than twofold with the longer dwell time, and peritoneal macrophage uptake of *Staphylococcus aureus* was better, as well as the ability of the macrophages to mount a respiratory burst. Mean effluent IgG also increased with the longer dwell time [6]. The long dwell in CCPD leads to a fivefold increased concentration of effluent white blood cells and peritoneal macrophages [7]. The 'dry day' after APD leads to an increased concentration of peritoneal macrophages compared to the patient on CCPD at the end of the long dwell. The nighttime dialysis (6 exchanges of 2 liters of dialysis fluid over 10–12 h) resulted in impaired cytokine response to lipopolysaccharides [7]. Peritoneal macrophages with tidal PD (initial fill of 2 liters with two subsequent exchanges of 50% tidal cycle over 3 h) versus CCPD (2 liters fill and drain, 2 cycles over 3 h) with a crossover design were better able to phagocytose *Escherichia coli* compared to the macrophages from the CCPD prescription [8]. Loss of cells and IgG were similar (controlling for the amount of dialysis fluid used and treatment time). These early results suggest that manipulation of the peritoneal prescription in patients using a cycler might lead to better immune function and less peritonitis.

Comparison of CAPD and CCPD Peritonitis Rates

Studies comparing peritonitis on APD and CAPD with a disconnect system are shown in table 1 [9–20]. The best study to date comparing peritonitis in CAPD using the Y set with 3–5 exchanges of 2 liters to CCPD (PAC-X; Baxter Healthcare, Deerfield, Ill., USA) using 4–5 nocturnal cycles and 1 daytime exchange of 2 liters was a randomized controlled trial (RCT) of patients recruited before starting dialysis, and therefore de novo ESRD patients [9]. There were 41 patients in each arm. Recruitment was from January 1988 to August 1991, and follow-up was to August 1992. The two groups were well matched. The peritonitis rates were 0.94 and 0.51 episodes/year at risk in the CAPD and CCPD groups, respectively (a difference of 0.43 episodes/year at risk, 95% confidence interval of 0.1–0.8, $p = 0.03$). Exit site infections were identical in the two groups (0.38 episodes/year at risk), and therefore it is most likely that the difference in peritonitis rates is due to contamination and perhaps differences in peritoneal immune function. Based on the prescription, the CCPD patients were likely doing 2 connections/day (both at night when attaching to the cycler) and 1 disconnect, while the CAPD patients were doing 3–5 connections and disconnections. There are many strengths to this study including the study design (randomized, initiation into the study at the start of dialysis, careful data analysis) and the results are convincing. However, the study was done with a cycler that is no longer in use.

Rodriguez-Carmona et al. [15] compared peritonitis in Spanish patients on APD and CAPD. This was an observational study comparing 213 patients beginning CAPD (Y set) and 115 patients beginning APD (Pacxtra and Home Choice, Baxter) between 1989 and 1998. The APD patients were treated with CAPD for a minimum of 2 months before converting to APD. Two of the APD patients had no daytime exchange, 5 were on tidal PD, and some of the remaining patients had an additional manual day exchange. The center had in place a *Staphylococcus aureus* nasal screening protocol and treated carriage with

Table 1. Comparison of peritonitis rates on CAPD and CCPD

Study	Patients	Study design	Location	Peritonitis rate, episodes/year at risk	
				CAPD disconnect	CCPD
Holley et al. [9], 1990	36	case control	USA	0.5	0.3
Korbet et al. [10], 1993	146	retrospective	USA	1.8	0.6
De Fijter et al. [11], 1994	82	randomized	Europe	0.94	0.51
Viglino et al. [12], 1995	104	retrospective	Europe	0.25	0.32
Golper et al. [13], 1996	1,930[1]	registry	USA	0.61	0.78
Troidle et al. [14], 1998	345	retrospective	USA	1.15	1.2
Rodriguez-Carmona et al. [15], 1999	348	observational	Europe	0.64	0.31
Yishak et al. [16], 2001	198	registry	USA	0.55	0.57
Huang et al. [17], 2001	212	retrospective	Asia	0.27	0.15
Kavanagh et al. [18], 2004	1,205	registry	UK	0.65	0.59
Bro et al. [19], 2009	34	randomized	Europe	0.31	0.17
Akman et al. [20], 2009	132	observational	Turkey	0.77	0.78

[1] Total patients on PD and all connection types.

intranasal mupirocin. The peritonitis rates were 0.64 versus 0.31 episodes/year at risk in CAPD and APD, respectively. Exit site infection rates were similar (0.23 and 0.20 episodes/year at risk, respectively) and much lower than in the Dutch study, probably due to the mupirocin protocol. While details of the prescription are not given, again, as with the Dutch study, it is very probable that the APD patients were doing fewer connections and disconnections (less risk for contamination) compared to the CAPD patients; the difference in peritonitis cannot be attributed to exit site infections. While details on the connectology of the Home Choice cycler are not provided, in Europe the Home Choice cycler is designed to use a Luer lock connection [pers. commun.].

A second randomized trial of APD and CAPD was done in 3 Danish PD units [19]. In this study design, all patients were first on CAPD a minimum of 1 month and only high and high-average transporters were included in the randomization. The follow-up was for only 6 months. APD was done using the Home Choice cycler (Baxter), which in Europe had a Luer lock connectology. Because of inclusion and exclusion criteria, only 34 patients of 118 on PD in the 3 units were eligible, and only 25 completed the study and were included in the evaluation. Infectious complications were secondary outcomes. The peritonitis rates were 0.31 versus 0.17 episodes/year at risk in the CAPD and APD groups, respectively. Exit site infection rates were similar in the two groups and low (0.15 and 0.17 episodes/year at risk). The small size of the study, restricted recruitment due to entry criteria as well as the short follow-up limit the interpretability of the results, which, however, are consistent with the other two previously described studies.

In 2001 we reported our results from the Pittsburgh PD Registry collected from multiple centers on peritonitis rates in patients on APD and CAPD between 1990 and 2000 (198 patients on 5,893 years at risk) [16]. All data were collected prospectively. The peritonitis rates were 0.55 and 0.57 episodes/year at risk for CAPD and APD. Details on the type of cycler used and connectology at the various centers were not provided. In a single-center study from New Haven, peritonitis rates were also similar for CAPD and APD, although the rates were much higher than in the Pittsburgh PD Registry [14, 16]. Although details on the CCPD connectology are not given, it is likely that in this center the machine was the Home Choice cycler (Baxter), which in the USA involved spiking of dialysis bags. At the unit affiliated with the University of Pittsburgh Medical Center, we subsequently trained all patients on the Home Choice cycler to do the setup using the compact assist device, and in the most recent decade (January 1999 to January 2009, n = 145 APD patients) the peritonitis rate for cycler patients was much lower at 0.31 episodes/year [unpubl. data].

Oo et al. [21] did an analysis of USRDS for incident CAPD and CCPD Medicare patients between 1994 and

Table 2. Problems in comparing APD and CAPD peritonitis rates

Paucity of RCTs
Limited data on connectology in published studies
Limited data on actual rates, and rates per organism
Almost absent data on causality of peritonitis by dialysis modality
Mixing of APD with dry day, last bag fill and midday exchange
Single center and limited patient numbers in published reports
Use of prevalent patients rather than incident patients

1997. Because of the limitations of the data collection, the authors analyzed the risk of first peritonitis episode after 9 months on PD and were not able to obtain actual peritonitis rates. Months 4–9 were used to describe the characteristics of the population (with 25% CCPD and 22.4% CAPD having at least 1 peritonitis episode during this period) and then examined the subsequent 9 months on PD for time to peritonitis. Relative risk of an episode of peritonitis included younger age, black race, diabetes, prior hospitalization, prior peritonitis, continuous hemofiltration and CCPD. Using CCPD as reference, CAPD had a relative risk of first peritonitis in the subsequent 9 months of 0.939 (95% confidence interval = 0.883–0.998, $p < 0.05$). There are many problems with this study. The types of connection for the CAPD and CCPD patients are not known, nor is it clear how many patients had a dry day on APD, the first 3 months on PD are not included, and most of all an actual peritonitis rate could not be calculated from the data.

Recently, Nessim et al. [22] analyzed predictors of peritonitis in 25 centers with 4,247 Canadian PD patients, using the Peritonitis Organism Exit Sites Tunnel Infection database (Baxter). Examining only the subset of PD patients who did not switch between PD modalities, the CAPD patients did not have a higher peritonitis rate than those undergoing APD. The overall peritonitis rate was 0.46 episodes/year at risk (all inclusive). Information on PD prescriptions and connectology was not given.

To summarize, as noted in a recent review there is a striking paucity of data, particularly RCTs with sufficient numbers of patients, comparing peritonitis in APD and CAPD [23]. In many studies the connectology is not described. The strongest evidence for a lower peritonitis risk for APD comes from the RCT of de Fijter et al. [11] which was published 15 years ago using a cycler no longer in use. An RCT comparing APD with a dry day, CCPD with a wet day and CAPD with careful description of the connectology used is much needed.

Problems with the Data on Comparing Peritonitis in CAPD and APD

As shown in table 2, there are a number of problems with the data available to compare peritonitis rates in CAPD and APD. First, there are very few RCTs, and one that has been included in previous reviews [23, 24] has only 8 patients [25]. Furthermore, the studies do not always describe either the connectology or the APD prescription. In the USA, the Home Choice cycler (Baxter) connectology is spiking, which can lead to contamination [3, 9], while in Europe the Home Choice cycler has a Luer lock connection. We know from studies in CAPD that spiking compared to Luer lock Y set systems is associated with higher peritonitis rates [23]. Therefore, it is logical to think that the spiking associated with the Baxter cycler in the USA might be associated with higher peritonitis rates due to contamination than the Baxter cycler in Europe and the UK, which uses a Luer lock connection. This problem may soon be resolved as the newer version of the Baxter cycler, soon to be released, will have a Luer lock, as opposed to spike connection.

Another problem with the studies of peritonitis on CAPD and APD is that the prescription is not always given. In Asia, CAPD is often done as 3 exchanges/day, rather than the typical American/European prescription of 4 exchanges/day. Theoretically, this might favor less peritonitis in those on 3 exchanges/day as the risk of contamination is lower and the dwell times longer (approx. 8 h). In addition, some patients on the cycler do not only a last bag fill, but a midday exchange, and this therefore increases the potential for contamination and results in shorter daytime exchange time.

A dry day may result in better peritoneal immune function although this is not entirely clear [7]. Theoretically, peritonitis rates might be lower with a dry day. In a preliminary study from our registry we found this to be the case [26]. This requires an RCT comparing peritonitis rates in patients initiated on APD with a dry day versus a wet day. Such a study has not yet been done.

Conclusion

There is a paucity of data on peritonitis in CAPD versus APD. Theoretically APD should be associated with lower peritonitis rates due to a positive effect on immune status compared to CAPD, and fewer connections, but the risk of contamination will be dependent on the type of connection (Luer lock vs. spiking). Further studies on

this topic should carefully describe the connectology used with not only CAPD, but also APD, and include the prescription (number of connections). More RCTs are needed in this area. Lastly, there is a striking variation in peritonitis from center to center, as shown in table 1 and Kavanagh et al. [18]. In the Scottish registry, representing 10 units, the peritonitis rates ranged from 0.43 to 0.89 episodes/year at risk for CAPD and from 0.31 to 0.79 episodes/year at risk for APD [18]. Clearly much needs to be done to standardize care to minimize the peritonitis risk.

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Reverse Epidemiology, Obesity and Mortality in Chronic Kidney Disease: Modelling Mortality Expectations Using Energetics

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Key Words

Reverse epidemiology · Original epidemiology · Obesity paradox · Thrifty gene hypothesis · Daily energy expenditure · Doubly labelled water · Dialysis · Wasting · Cachexia

Abstract

Background/Aims: Obesity is a predisposing factor for chronic illnesses such as type 2 diabetes, heart disease and cancer. In chronic kidney disease (CKD), the effect of obesity on mortality is reversed. Obese patients appear protected. Two ideas have been advanced to explain this 'reverse epidemiology'. First, obesity may buffer patients from wasting. Second, fat may sequester uraemic toxins leading to a systematic error in the prescription of dialysis. Our aim was to use data on the scaling of daily energy expenditure, fat and lean tissue mass to predict the pattern of variation in mortality with obesity under the contrasting hypotheses. ***Methods:*** We used data on daily energy demands measured using the doubly labelled water technique and body composition collected on a cohort of 503 individuals to model the expected impacts of wasting and fat sequestration/underdialysis on mortality. ***Results:*** A model predicting mortality due to wasting replicated the mortality pattern of the obesity paradox. However, quantitatively the beneficial effect of being fat was predicted to be much larger than that observed in the actual CKD population. Similar results were found for the fat sequestration/underdialysis hypothesis, but in this case the discrepancy was smaller. ***Discussion:*** These models tend to support the fat sequestration and underdialysis idea more than the wasting hypothesis. In part (or in whole) this may be because of inadequacies in the model construction which are currently based on rather crude assumptions. Refinement of the models may enable better tests between alternative ideas for the obesity paradox.

Background

Since the 1960s there has been an enormous increase in levels of body fatness in both the western world [1, 2] and more recently throughout developing nations [3, 4]. By the conventional definitions of obesity (body mass index, BMI >30) and overweight (BMI >25), the population in the USA that is obese has increased from under 5% in 1960 to over 25% in 2004 [1, 5]. This increase has been matched by an even greater explosion in the numbers of people that are overweight (increasing from 10 to 35%). The largest proportional increase has been in the mor-

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bidly obese (BMI >40) [6]. Obesity is a major health problem because it increases the risk for a number of chronic illnesses, the most significant of which is type 2 diabetes [7]. Matched with the increased risk of type 2 diabetes is an increased risk for cardiovascular disease [8, 9], fatty liver disease [10] and cancer [11, 12].

In marked contrast to the effects of obesity on mortality in the general population, it has become clear over the past 25 years that for patients with chronic kidney disease (CKD), and particularly those with end-stage renal disease (ESRD), who only remain alive if maintained on regular dialysis, the association between obesity and mortality risk is the opposite: fatter individuals have a lower mortality risk and therefore tend to survive longer [13–21]. This pattern has been repeatedly demonstrated in many independent studies including some cohort studies containing in excess of 400,000 individuals [22]. The effect appears to be independent of the actual cause of death, and the impact of elevated body fatness appears to be protective right up to very high BMI values (>35) that have been typically called 'morbid obesity' reflecting the normally extremely negative health effects of BMI in this range. The effect is observed in both males and females, and in Caucasians, African Americans and Hispanics. This phenomenon, whereby in some special circumstances the traditionally ascribed risk factors for mortality become protective, has been termed 'reversed epidemiology' or 'the obesity paradox' [21, 23, 24].

There has been a diversity of ideas that might explain the obesity paradox [25] including the suggestion that it may reflect only a statistical artefact or be a consequence of selection bias in those patients who survive to ESRD, since most patients with CKD will die before reaching this ultimate diagnosis [23]. The fact that the paradox reverses following transplantation [26] however suggests that it reflects a true biological phenomenon in need of explanation. Moreover, the 'obesity paradox' generates some important practical conundrums. For example, should people with ESRD be encouraged to lose weight like the rest of the population, or as a precursor to transplantation surgery, if this in fact increases rather than decreases their mortality risk?

Two ideas have emerged as dominant potential explanations of the obesity paradox in ESRD. The first suggestion is that obesity has two contrasting influences on mortality risk which are apparent over different time domains: long and short [17, 25]. The first 'traditional' epidemiological effect is that increased obesity leads to increased risk of chronic disease. Although this is called the 'traditional' epidemiological effect of obesity, in fact we have only become aware of this effect over the past 50 or so years. This is because the time domain over which this impact of obesity becomes apparent is very long (decades). We have only become aware of this negative effect of obesity in modern times because it is only with the advent of modern health care, the invention of antibiotics and the virtual elimination of death from epidemic diseases in the west that people have lived long enough for these negative impacts of obesity to become apparent. By this argument the 'original' epidemiological effect of obesity, which acts over a much shorter time domain, may in fact have been protective.

The classic embodiment of this 'original' positive epidemiology of obesity effects on mortality is the 'thrifty gene hypothesis' [27]. The 'thrifty gene' idea is that historically, human populations were exposed to cyclic periods of feast and famine. Under these conditions, it is suggested that selection would favour individuals that had genes allowing them to rapidly deposit body fat during periods of feast, because these fatter individuals would then have greater reserves to get them through the subsequent periods of famine. Although Neel [27] emphasised increased survival as the primary selective advantage, more recent studies have pointed out that a greater selective benefit may actually be that obese people could retain greater fertility during the famine periods [28].

By this argument the 'reverse epidemiology' observed in patients with CKD may in fact be a return to the 'original epidemiology' which emphasises the short-term benefits of obesity in situations of severe negative energy balance (such as famines). In effect the thrifty gene hypothesis posits that the modern epidemics of obesity and diabetes are the unfortunate consequences of embedding this previously advantageous genotype in a modern environment where food is readily available and easily obtained, allowing individuals to deposit enormous fat reserves in preparation for a famine that never comes. Perhaps then CKD is in effect the equivalent of a famine that the obese patient is prepared for, but the lean patient is not. The fact that CKD is a state of negative energy balance is supported by the observation that CKD patients very often experience profound wasting. Moreover, this idea is supported by the fact that weight loss is an independent risk factor for mortality in CKD, while weight gain is protective [15].

The second explanation of the obesity paradox is that it is in part a medical artefact based on the procedures used to prescribe dialysis treatment [19, 20, 29–33]. The specific suggestion is that uraemic toxins are generated

principally by the visceral body compartment. This compartment is relatively large in smaller individuals so the production of harmful metabolites is relatively greater in smaller individuals [33]. This effect is exacerbated by the fact that in larger individuals these metabolites may be diluted in a greater volume of body water, and probably most importantly, sequestered into fat tissue [34]. Current practice is that the dialysis dose is based on the distribution volume of urea (V) which approximates the body water volume. Specifically the dialysis dose is the intensity of dialysis K multiplied by time t divided by V (Kt/V). Clearly if uraemic toxins are produced at elevated rates in smaller individuals and in larger individuals are sequestered into fat, then smaller individuals will be relatively underdialysed [33]. It is suggested that this underdialysis may generate the differential mortality effect in ESRD, since the dialysis dose is related to mortality [35, 36].

Our curiosity in these alternative hypotheses stems from an interest in the validity of the idea that obesity once had a positive short time domain advantage, i.e. the notion of 'original epidemiology' as embodied in the 'thrifty gene hypothesis'.

In fact, we have previously shown that the thrifty gene idea has several basic flaws [37, 38]. First, if selection really had been so intense in our historical past due to the frequency of famines, and obesity was so advantageous, then it is difficult to understand why the favourable alleles for obesity have not spread through the entire population, making us all overweight or obese. Second, a detailed analysis of famine mortality reveals no supportive evidence that obese people survive and lean people die. Most mortality in famines falls on children and older people for reasons that are generally unrelated to their BMI (i.e. infectious diseases and diarrhoea) but more related to poor food choice under severe hunger – for example eating corpses. Given the reality of starvation events, it seems unlikely that there was ever a positive original epidemiology for obesity that is effective in the short time domain. This does not mean that 'reverse epidemiology' cannot be a consequence of obesity protecting against negative energy balance and wasting, only that if this is the case, this effect is likely a modern phenomenon.

Methods

In this paper we will explore some ways that these two alternative hypotheses might be distinguished using information on daily energy demands and body energy storage patterns. The wasting hypothesis suggests that fatness protects against wasting. One way to view this is that the body fat and muscle is an energy store that persons draw on when they are in negative energy balance. When this reaches a critical low limit, there is no energy left in the store and this leaves the person unable to respond if a crisis happens such as a cardiovascular event. If this idea were correct, we would predict that the bigger the stores an individual carries, the longer it would be before he/she ran out of energy – explaining the basic 'obesity paradox'. Moreover, if individuals expended energy at a higher rate, they would similarly exhaust their stores more rapidly. There is a complexity here however because bigger people carrying greater stores also expend more energy, so the exact balance between these effects is not intuitively obvious. Can we use data on levels of fat storage and energy demands to model the likely pattern of impact of body fatness on mortality under the wasting hypothesis? To evaluate the wasting hypothesis, we need to know two things: daily energy demands as a function of body weight, and the levels of fat and muscle tissue in relation to body weight.

What does the underdialysis hypothesis predict? To model this hypothesis we also need to know two things. First, how the actual prescription varies with body size, and second, what the prescription should be. The actual prescription criterion for dialysis is Kt/V, so we can easily model this if we know how V varies with body weight. Knowing what the prescription 'should be' is mired in controversy [31, 32, 39, 40]. Potential suggestions have been that dialysis should be independent of body size (Kt = constant), scaled to resting metabolic rate, scaled to high metabolic rate organ mass, scaled to surface area and scaled to the mass of visceral tissues. The merits and demerits of the alternatives have been discussed elsewhere. We see two components to this issue. One is how to predict the production of uraemic toxins in relation to body size. The second problem is the envisaged sequestration of these toxins by body fat. We propose here that uraemic toxins are likely generated in relation to daily energy demands (equivalent to average daily metabolic rate). Previous suggestions to scale dialysis to metabolic rate parameters such as resting metabolic rate have been criticised because the latter comprises many compartments of metabolism, such as from the heart and brain that likely contribute little to the production of uraemic toxins. For daily energy expenditure (DEE), the link to uraemic toxins may seem even more tenuous because DEE also comprises in large part energy expended on physical activity. Our reasoning for making this suggestion is as follows. The primary source of uraemic toxins probably stems from processed food intake, and over the long term, food intake has to be closely related to DEE. This argument is supported by the fact that when comparisons are made across different mammal species, several aspects of renal physiology such as creatinine clearance rates and glomerular filtration rates [41] scale similarly to body weight as DEE [42]. Given a model of uraemic toxin production, we also need to know how sequestration of these toxins is influenced by body fatness. The simplest way to model this is to assume a direct proportional uptake in relation to fat mass (FM). Hence the uraemic toxins in circulation that need to be cleared by dialysis should be proportional to DEE/FM. If dialysis should be proportional to DEE/FM but is in fact scaled to V, we can quantify the extent of underdialysis in relation to body weight from the ratio (DEE/FM)/V. Mortality should be directly proportional to this function.

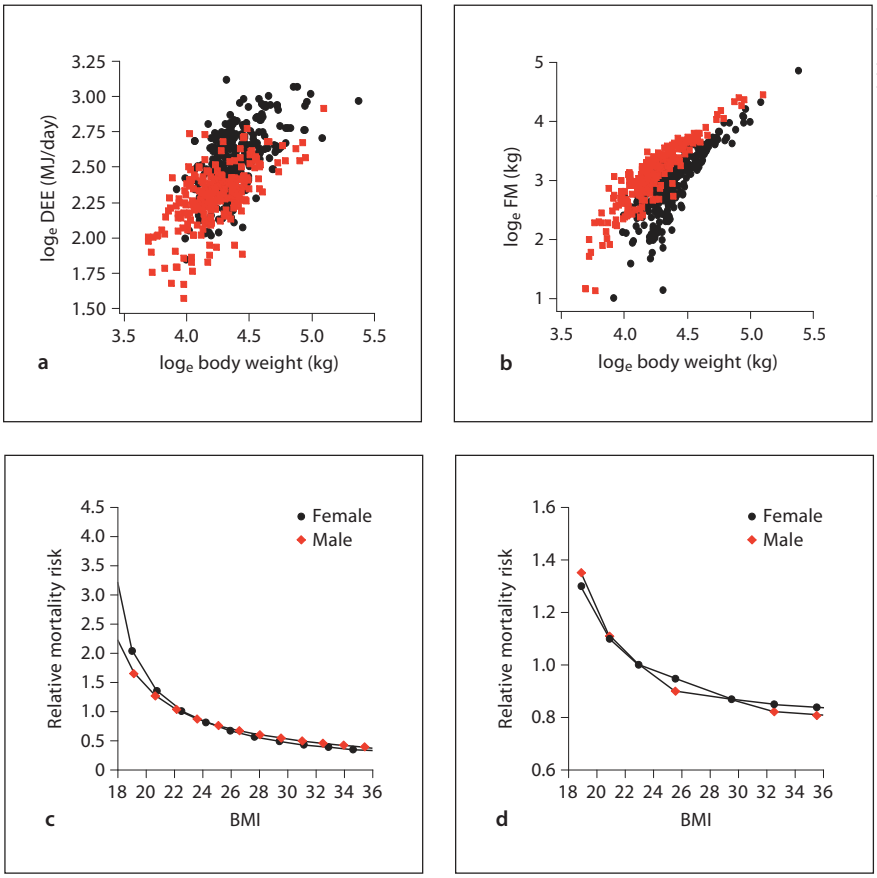

Fig. 1. a Effects of body weight and sex on DEE. Heavier individuals expend more, and at any given weight, females expend less than males. **b** Effects of body weight and sex on fat content. Heavier individuals and females store more fat. **c** Modelled effects of size on survival under a wasting model. **d** Actual pattern of mortality in CKD patients based on >400,000 subjects [22].

To construct these models that predict the exact form of the expected relations between BMI and mortality in ESRD, data were required on how daily energy demands, body water, fat and lean tissue masses all vary in relation to body weight. We used data for these traits in relation to body weight that were collected on a large cohort of individuals (n = 503) measured in Maastricht in The Netherlands using the doubly labelled water method [43]. We have utilised this cohort previously to explore aspects of daily energy demands in the context of the causality of obesity [44]. Details of the methodology used to collect the data and subject characteristics can be found in Westerterp and Speakman [44] and on the doubly labelled water method more generally in Speakman [43].

Results

The Wasting Hypothesis

Daily energy expenditure in this sample was dominated by two factors: body weight ($t = 16.8$, $p < 0.001$) and sex ($t = 10.31$, $p < 0.001$; fig. 1a). On average a female at any given body weight expends about 0.17 log$_e$ units less energy than a male of the same weight. At a median body weight of 76 kg, this difference amounts to 5 MJ of energy per day. Body fatness and body lean tissue content were also positively related to body weight ($t = 38.17$, $p < 0.001$) and sex ($t = 17.9$, $p < 0.001$; fig. 1b). This time, however, females had greater fatness than males. At the median body weight, this effect was equivalent to 9.5 kg extra fat tissue in a female, but the equivalent less in lean tissue. As individuals get heavier, the proportional contribution of fat to their body weight gets greater. Hence if an individual reduces his/her weight from 140 to 135 kg, this 5-kg weight loss comprises 4.1 kg fat and 0.9 kg lean tissue. In contrast, an individual reducing from 65 to 60 kg loses 2 kg fat and 3 kg lean tissue. Because fat contains more energy than lean tissue, the length of time that 5 kg of body weight can in theory sustain a person is much longer when they are heavier than when they are lighter.

However, this effect is potentially offset by the greater energy requirement of the larger person. To model the effects of body weight and gender on energy utilisation, we

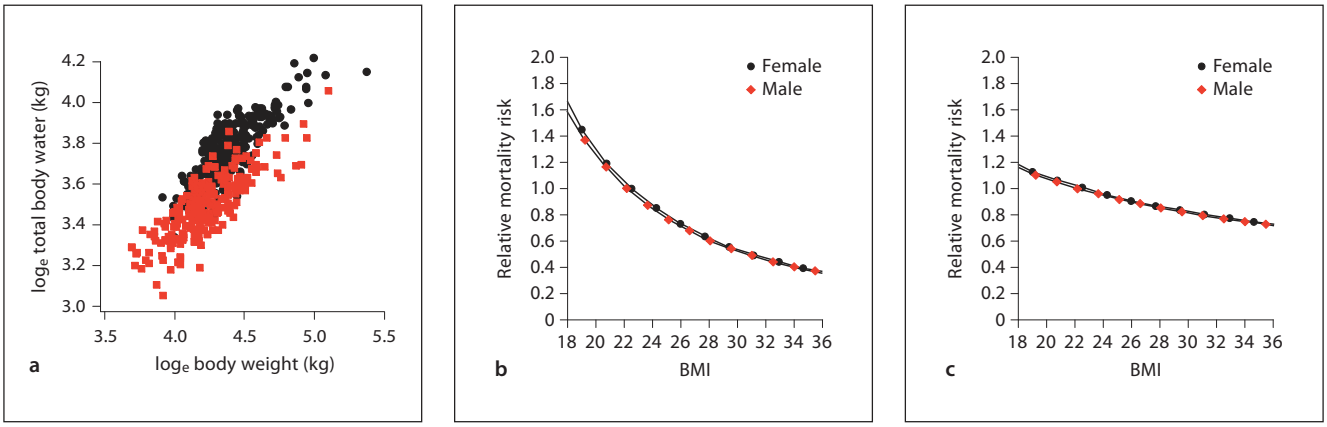

Fig. 2. a Effect of body weight on total body water. Bigger individuals and males have greater levels of total body water. **b** Modelled effect of body weight and sex on mortality under the sequestration of toxins and misprescribed dialysis hypothesis. **c** As **b** but with the relationship between fat sequestration capacity and volume non-linear.

used the regression fits relating body weight to DEE, FM and fat-free mass. We used an incremental model with a fixed lower 'fatal' body weight of 45 kg for a woman and 55 kg for a man (equivalent to BMI values of 15.5 and 16.5 in typical-height females and males, respectively). We started with a body weight of 50 kg for females and 60 kg for males and calculated how long an individual of this weight would survive given their energy reserves of fat and lean tissue at this weight and their DEE. We then asked how long a female weighing 65 kg and a male weighing 70 kg would take to decrease in weight to 60 and 65 kg, respectively, and we added this time to the time to reduce to 55 and 60 kg. We iterated this process for weights up to 130 kg for men and 120 kg for women. At each weight these times reflect the available time before an individual would reduce to the fatal weight.

To convert these survival times into mortalities we took the inverse value and using an average height of 170 cm for females and 184 cm for males we then converted this relationship into the expected protection against wasting, as a function of BMI. These mortality rates were then calculated relative to that at a BMI of 22. The resultant expected mortality curves under the wasting model are shown in figure 1c. These can be directly compared with the actual reverse epidemiology mortality curves for males and females as a function of BMI for 418,055 CKD patients [22] (fig. 1d). The similarities in the shapes of the model prediction and the observed pattern of mortality are obvious. There are however some striking differences as well. Most notably, the extent of mortality difference between the high-BMI and low-BMI individuals predicted by the model (from 0.3 at BMI = 35 to 2.0 at BMI = 19) is much greater than that observed in the actual data (from 0.8 at BMI = 35 to 1.3 at BMI = 19). Moreover, the actual data show that female mortality was significantly lower than that of males at low BMI, but higher at high BMI [22], whereas the model data show the reverse expectation, male mortality being lower at low BMI and higher at high BMI. While qualitatively successful, the model was quantitatively lacking.

The Sequestration and Underdialysis Hypothesis

Since V is approximately equal to the body water volume and this value is derived as part of the doubly labelled water technique, this was easily obtained for our cohort (fig. 2a). Like fat-free mass and FM, the body water volume was similarly dependent on both body weight ($t = 31.8$, $p < 0.001$) and sex ($t = 22.9$, $p < 0.001$). We modelled sequestration by fat assuming a linear sequestration rate in relation to fat mass (DEE/FM). We then quantified the extent of 'underdialysis' in relation to body weight from the ratio (DEE/FM)/V. If underdialysis is the problem, mortality should be directly related to this function. We made this calculation for males and females of different body weights between 45 and 120 kg for females and 55–130 kg for males using the relationship shown in figure 1a for DEE and figure 2a for V (= total body water), and then scaled this to the BMI calculated as above for the wasting hypothesis, and also normalised the data to the mortality at BMI = 22. The resultant plot of modelled

mortality as a function of BMI is shown in figure 2b. This can similarly be compared with the actual mortality plot for the obesity paradox in figure 1d [22]. This pattern appears to fit the actual mortality data slightly better than the model based on the wasting hypothesis, although again there is a discrepancy in the predicted extent of the mortality effect of obesity, and this model predicts no difference between the sexes at all.

Discussion

Overall, these analyses suggest that the pattern of mortality in relation to BMI that is observed in reverse epidemiology during ESRD fits more closely with expectations derived from the sequestration of toxins and underdialysis hypothesis than the wasting hypothesis. It is important to recognise that the usefulness of such modelling exercises depends in large part on how well the model assumptions reflect the underlying physiology. In this sense, both models are likely to be imperfect – possibly seriously so. In the wasting model, we assumed that individuals survive during ESRD basically until they waste away. This assumption places a large premium on having large energy reserves and hence generates a large predicted mortality effect between high- and low-BMI individuals. In reality, however, patients with ESRD often show wasting, but seldom die directly of it, the most frequent causes of death being cardiac events or infectious diseases. Moreover, weight loss under dialysis may not be progressive and causal of secondary complications, but a secondary effect itself following infection [45]. The phenomenon of kidney disease wasting is therefore likely much more complex than our simple model assumes. For example, patients that exercise during ESRD have a better survival probability than sedentary patients [46], while a simple interpretation of wasting energetics would predict that they should use up their energy stores more rapidly and have greater mortality. The mechanisms by which wasting might be involved, or not, in mortality events in CKD is uncertain, and if such mechanisms were established it might allow refinement of the model and ultimately provide stronger support for this hypothesis.

Our assumptions about the sequestration capacity of fat and the production of uraemic toxins may be similarly suspect. Greater knowledge of these processes would allow similar refinement of the model predictions. For example, if uraemic toxins were sequestered by fat in relation to the cube root of its mass rather than linearly, then the resultant model predictions for mortality match much more closely the actual data (fig. 2c). There is, however, no physiological justification for this assumption over the linear model used to derive the prediction in figure 2b. Perhaps this means that attempting to test between the hypotheses using this type of energetic model is a flawed approach. We prefer the interpretation that refinement of the models with better founded information may still prove valuable for evaluating alternative explanations of the obesity paradox.

Despite our models broadly supporting the sequestration and underdialysis hypothesis, two independent factors lend additional support to the wasting hypothesis. First, the obesity paradox is also apparent in CKD patient populations that are not on dialysis [47]. This might be explained by the fact that a major aspect of the underdialysis idea is based around the sequestration of toxins by fat, which will presumably also pertain before patients reach ESRD and are prescribed any dialysis treatment. Second, however, other chronic illnesses that have a 'wasting' component to them also seem to exhibit reverse epidemiology and an obesity paradox [25, 48–51]. These include chronic heart failure, chronic obstructive pulmonary disease and cancer. Clearly, in these instances, sequestration of uraemic toxins by fat and underdialysis cannot be an important factor. Perhaps wasting and separate time domains of the impact of mortality from obesity really do generate an obesity paradox, but in ESRD this effect is exacerbated by the sequestration of toxins and underdialysis effects. That is, both hypotheses may be correct and contribute to the overall effect. Comparing the magnitude of the paradox in different circumstances may illuminate their contrasting roles. Moreover, perhaps deeper understanding of these wasting diseases may provide some useful insights into the supposed 'original' epidemiology effects of obesity.

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Endothelial Progenitor Cells and Endothelial Vesicles – What Is the Significance for Patients with Chronic Kidney Disease?

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Key Words
Endothelial progenitor cells · Bone-marrow-derived progenitor cells · Circulating endothelial cells · Endothelial microvesicles · Chronic kidney disease

Abstract
Endothelial progenitor cells are cells derived from the bone marrow that circulate in the bloodstream and can exhibit phenotypic characteristics of endothelial cells. They are thought to be involved in postnatal vasculogenesis and to potentially help repair injured endothelium. Circulating endothelial cells are mature endothelial cells in the circulation, and endothelial vesicles or microparticles are thought to be derived from the membranes of endothelial cells as a result of injury or activation. Recent research has focused on using these markers of endothelial injury and repair to assess the state of endothelial health. These efforts have been hampered by lack of uniformity in methodology and terminology. Recent developments in flow cytometry techniques have allowed better characterization and definition of these cells. We review the common techniques used to identify and isolate these cells, clinical studies in patients with chronic kidney disease (CKD) where they serve as markers of endothelial health and predictors of outcome, and possible mechanisms of progenitor cell dysfunction in CKD.

Chronic kidney disease (CKD) carries a high risk of mortality, most of which is cardiovascular in origin [1]. Traditional risk factors do not explain the unusual prevalence of atherosclerosis in this high-risk group [2]. Various etiologies like oxidative stress, inflammation and endothelial dysfunction are thought to contribute to the pathogenesis of atherosclerosis in patients with CKD. The 'response to injury hypothesis' sets the endothelium as a key mechanism for the development of atherosclerosis [3].

The view of the endothelium as an inert structure that merely served to line blood vessels was formally challenged by Furchgott and Zawadzki [4] as well as others. They demonstrated that vasodilatation in response to acetylcholine occurs only in the presence of an intact endothelium. We now know that this is largely the result of endothelium-derived nitric oxide and that the endothelium secretes several vasoactive substances including prostacyclins and endothelins. The endothelium also regulates the balance between prothrombotic and antithrombotic activities. In the quiescent state, the endothelium-derived nitric oxide and prostacyclin directly inhibit platelet aggregation and thrombomodulin inactivates thrombin. In response to low shear stress and other stressors, the endothelium becomes prothrombotic, secreting platelet-activating factor and expressing thromboplastin on cell membranes. The endothelium also plays

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a crucial role in initiating and maintaining inflammation. The endothelial cells express cell adhesion molecules which regulate the binding of inflammatory cells (monocytes and specific subsets of T lymphocytes) to the endothelium. Research in the past has focused on measuring these vasoactive substances, inflammatory markers or cell adhesion molecules to assess the state of the endothelium and quantify the risk of atherosclerosis. More recently investigators have begun to use the number and function of circulating cells to gain insights into the health of the endothelium. The focus has been on bone-marrow-derived progenitor cells playing a role in repairing the endothelium as well as circulating endothelial cells (CECs) and endothelial microparticles as markers of endothelial injury or activation.

Defining and Characterizing Endothelial Progenitor Cells

In 1997, Asahara et al. [5] first demonstrated the existence of cells in the circulation that can differentiate into endothelial cells. This aroused considerable interest because of the potential for so-called endothelial progenitor cells to serve as markers of an individual's capacity to carry out repair of injured endothelium as well as the ability to use these cells in a therapeutic manner. Since then a large body of literature has accumulated concerning abnormal endothelial progenitor cell number and function in various disease states. However, lack of uniformity in terminology and methodology has caused considerable confusion in characterizing endothelial progenitor cells. Moreover what is termed an endothelial progenitor cell might not be a homogeneous population but derived from or requiring interaction of different cell sources including bone marrow, peripheral blood and even the vessel wall.

The two common methods to characterize endothelial progenitor cells are cell culture and flow cytometry. Depending on cell culture characteristics, endothelial progenitor cells have been characterized as early and late. Early progenitor cells or CFU-Hill colonies, as first described by Hill et al. [6], have been widely used in part because they have been validated as a marker for overall endothelial health and because of readily available commercial kits. These cells form colonies within a week when peripheral blood mononuclear cells are grown in angiogenic media. However, these cells have limited proliferative and angiogenic potential, and Rohde et al. [7] demonstrated that the central core of these colonies are inflammatory T cells. Sieveking et al. [8] used transwell cultures to demonstrate that the angiogenic effects of these cells may be due to paracrine effects. Yoder et al. [9] used clonal analysis to establish that the CFU-Hill colonies are of hematopoietic origin and express the common leukocyte antigen CD45 as well as the monocyte antigen CD14. Thus, these cells might be more appropriately termed 'bone-marrow-derived progenitor cells' (BMDPCs) and may be required for endothelial angiogenesis but may not directly give rise to endothelial cells. However, it is important to remember that while these cells may not be 'true endothelial progenitor cells' they may play a role in angiogenesis, and the number and function of these cells have been shown to correlate inversely with cardiovascular risk in a number of studies [6, 10].

In contrast late or outgrowth endothelial progenitor cells or endothelial outgrowth cells appear after 2–3 weeks of culture, and have enhanced proliferative and vasculogenic potential. They lack the monocyte marker CD45 but express the endothelial marker CD34. Sieveking et al. [8] used a tubulogenesis assay to demonstrate that these cells independently formed tubules and incorporate into differentiated endothelial cell tubules. These cells have been shown to be mobilized after myocardial infarction [11], can attenuate intimal hyperplasia after vascular injury and can form blood vessels in vivo in mouse models [9, 12]. These colonies are rare, methods to isolate them cumbersome, and studies linking them to cardiovascular outcomes are still lacking.

Flow cytometry, as a method of enumerating BMDPCs, has the advantage of being sensitive, reproducible, relatively easy to perform and is the method of choice to directly detect circulating progenitor cells. However, there are limitations with regard to BMDPCs – the relative rarity of these cells and the difficulty in characterizing them simply on the basis of surface antigens. Moreover these undifferentiated cells may lose or acquire other surface antigens as they mature. BMDPCs are generally characterized by markers of immaturity as well as markers of endothelial lineage. The commonly used markers of immaturity are CD34 and/or CD133. CD34 is a transmembrane sialomucin protein that is expressed on early hematopoietic cells. However, it is nonspecific and is expressed on megakaryocytes and several tumor cells. It is an important adhesion molecule, though its exact function remains largely unknown. CD133 is a pentaspan transmembrane glycoprotein selectively expressed on hematopoietic and progenitor stem cells. It binds cholesterol, though its exact function remains unknown as well.

The usual endothelial markers used are KDR/Flk-1 or vascular endothelial growth factor receptor 2, von Willebrand factor, vascular endothelial cadherin or CD146. Various groups have characterized endothelial progenitor cells as CD34+ KDR+, CD34+CD133+ and KDR+, or CD45–CD34+ as well as other combinations of these markers. Each of these has its own advantages and disadvantages. Since Asahara et al. [5] used CD34+KDR+ to identify putative endothelial progenitor cells, these markers have been used to define BMDPCs by flow cytometry. Though they have the advantage of being the only subset which has been correlated with cardiac outcomes in patients with CKD [13], we and others have found that commercially available KDR antibodies are plagued by reliability issues. Case et al. [14] included a marker of immaturity as well to define a BMDPC (CD34+KDR+CD133+) and to separate them from mature endothelial cells. However, recent studies have suggested that these cells as well may be hematopoietic precursor cells and have limited angiogenic potential. Recently several authors have used polychromatic flow cytometry to better characterize these circulating cells [15]. They identified BMDPCs as $CD31+CD34^{bright}CD45^{dim}CD133+$ cells and CECs as $CD31^{bright}CD34^{dim}CD45–CD133–$ cells. These techniques seem promising but further studies are needed to characterize the size and morphology of these cells and correlate with culture and cell isolation methods.

Flow cytometry, though useful to characterize circulating endothelial progenitor cells, does not lend itself to the rapid and efficient isolation of viable progenitor cells. To isolate these cells, protocols have utilized magnetic beads conjugated with specific antibodies against surface markers. Selecting for CD34 enriches for progenitor cells that promote angiogenesis. However, this population is inhomogeneous and includes other cells including angiogenic monocyte and macrophage progenitors.

BMDPCs as Markers of Endothelial Health and Predictors of Outcomes in CKD

CKD is characterized by reduced numbers of circulating BMDPCs as enumerated by flow cytometry, as well as decreased colony formation. Patients with CKD also show abnormal progenitor cell function including migration, adhesion and incorporation. Migration is assessed by the ability of these cells to migrate towards a potent chemotactic agent like SDF-1, adhesion by the ability to form a monolayer with mature endothelial cells and incorporation by a tube formation assay. From a vascular risk viewpoint, the functional characteristics of progenitor cells might be even more significant than the absolute numbers of such cells. These defects in progenitor cell function seem to start early in the disease. Krenning et al. [16] studied 50 patients with varying degrees of CKD and found that even stage I CKD was associated with a reduced number of circulating CD34+ cells and abnormal function and this worsened with more advanced renal failure.

Most studies in patients on hemodialysis have similarly found a reduced number and function of progenitor cells [17, 18]. Although one study did show increased progenitor cell numbers, in this study the patients were carefully selected to exclude those with diabetes and vascular disease and thus they were not representative of the typical dialysis population [19]. Even though end-stage kidney disease is associated with low BMDPC numbers and dysfunction, in patients who have been longitudinally followed, institution of dialysis seems to partially improve BMDPC number and function [20]. This is also corroborated by studies demonstrating that BMDPC numbers correlate with dialysis efficacy, as measured by Kt/V [21], and BMDPC function is improved with longer dialysis sessions such as in nocturnal hemodialysis [22]. Patients on peritoneal dialysis show similar results to those on hemodialysis, although in the one study that examined peritoneal dialysis and hemodialysis patients, the decrease in progenitor cell number was more dramatic in patients on hemodialysis [23].

Though there are several studies on BMDPC number and function in end-stage kidney disease, there are relatively few looking at outcomes. Schmidt-Lucke et al. [13] followed 77 patients on hemodialysis for a period of 10 months and found that reduced CD34+KDR+ cells conferred a hazard ratio of 3.9 for vascular events even after adjusting for all traditional risk factors. Nontraditional risk factors like coronary calcification do not seem to correlate with BMDPC number either [17]. Thus, CKD may be an independent risk factor for progenitor cell dysfunction, and this is at least in part ameliorated by renal replacement therapy and/or transplantation [24].

Mechanisms of BMDPC Dysfunction in CKD

While it is evident that progenitor cell function is impaired in CKD, the exact mechanisms for this dysfunction are unclear. Reduced BMDPC number could be due to defective mobilization from the bone marrow, shortened survival or defective proliferation. Endothelial ni-

tric oxide synthase is essential to mobilize BMDPCs from the bone marrow [25, 26]. However, CKD is associated with elevated levels of asymmetric dimethylarginine, an endogenous inhibitor of endothelial nitric oxide synthase. In addition, diabetes, a condition prevalent in CKD patients, is associated with a deficiency of nitric oxide derived from endothelial nitric oxide synthase. We have previously shown that migration defects in CD34+ cells is due to cytoskeletal alterations which can be corrected by exogenous administration of nitric oxide [27].

Another potential mechanism of BMDPC dysfunction is inflammation. Various inflammatory markers like interferon, interleukin 6 or C-reactive protein have been shown to affect BMDPC function [28, 29]. Inflammation might act in part by causing BMDPC senescence and apoptosis. Several agents including angiotensin II and oxidized low-density lipoprotein have been shown to induce apoptosis of BMDPC through oxidative stress induction and reduction of telomerase activity [30, 31]. Uremia or some of the uremic toxins like homocysteine [32] or *p*-cresol [33] may inhibit BMDPC differentiation and function. Lastly, complications of CKD such as lack of erythropoietin or secondary hyperparathyroidism may also contribute to BMDPC dysfunction.

CECs and Microvesicles

Though CECs were first reported almost 30 years ago, they have only been widely studied since the development of endothelial cell-specific monoclonal antibodies. These mature CECs are derived from the vessel wall and exhibit phenotypic endothelial markers such as von Willebrand factor, vascular endothelial cadherin or CD146. Though not clearly elucidated, potential mechanisms for the presence of these cells in the circulation include apoptosis and mechanical disruption. Several authors have reported increased numbers of CECs in response to a variety of stresses or pathological conditions. We have demonstrated that the CECs are elevated in subjects with hypertension, diabetes and on hemodialysis [34]. In a long-term follow-up study of 29 hemodialysis patients, we found that patients with elevated CECs had a 7-fold risk of vascular events compared to those with low levels of CECs [35]. The number of CECs showed no correlation with markers of inflammation or endothelial dysfunction. It has been suggested that the balance between CECs (which are indicative of endothelial injury) and BMDPCs (which can potentially repair damaged endothelium) might be more indicative of overall health of the vascular endothelium [36].

Endothelial microvesicles or microparticles are vesicles formed by the endothelial cell membrane after injury or activation. They lack a viable nucleus but express endothelial surface markers and/or cytoplasmic elements. These microparticles may also serve as a marker of endothelial injury and are elevated in numerous pathological states including hypertension, diabetes, coronary artery disease and end-stage renal disease. Circulating levels of CD144+ microparticles were found to correlate independently and inversely with flow-mediated brachial artery vasodilatation in patients with end-stage renal disease [37]. Though there are no outcome studies in kidney disease, in patients with coronary artery disease the number of annexin V+ particles has been shown to be an excellent predictor of myocardial infarction and death [38]. There has been some controversy regarding whether these microparticles are just the sequela of vascular injury or have a pathological role. Several in vitro studies have shown that microparticles have proinflammatory as well as procoagulant effects and impair the release of nitric oxide from endothelial cells [39, 40]. On the other hand, they also stimulate angiogenesis and differentiation of progenitor cells [41]. It is not certain to what extent these apparently counteracting mechanisms are involved in vivo, and certainly more research is needed in this area.

The recent application of new techniques has brought better understanding and characterization of BMDPCs, CECs and microparticles. Their role as markers of endothelial health in kidney disease and as predictors of survival and vascular events has been begun to be validated. However, their mechanistic role in postnatal vasculogenesis is still uncertain. Hopefully, the new knowledge gained will serve to effectively use these circulating cells as a predictor of health and disease and potentially as a therapeutic tool.

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Calcium Balance in Dialysis Is Best Managed by Adjusting Dialysate Calcium Guided by Kinetic Modeling of the Interrelationship between Calcium Intake, Dose of Vitamin D Analogues and the Dialysate Calcium Concentration

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Key Words

Calcium mass balance · Dialysis · Calcium absorption · Vitamin D analogues · Calcitriol · Calcium flux

Abstract

Calcium mass balance (Ca_{MB}) is determined by the difference between Ca absorbed between dialyses (Ca_{Abs}) and the Ca removed during dialysis (J_dCa^{2+}). A mathematical model to quantify (1) Ca_{Abs} as a function of Ca intake (Ca_{INT}) and the doses of vitamin D analogues, and (2) J_dCa^{2+} as a function of Ca^{2+} dialysance, the mean plasma Ca^{2+} ($_MC_{pi}Ca^{2+}$) minus dialysate Ca^{2+} ($C_{di}Ca^{2+}$), ultrafiltration rate (Q_f) and treatment time is developed in this paper. The model revealed a basic design flaw in clinical studies of Ca-based as opposed to non-Ca-based binders in that $C_{di}Ca^{2+}$ must be reduced with the Ca-based binders in order to avoid a positive Ca_{MB} relative to the non-Ca-based binders. The model was also used to analyze Ca_{MB} in 320 Renal Research Institute hemodialysis patients and showed that all patients irrespective of type of binder were in positive Ca_{MB} between dialyses (mean ± SD 160 ± 67 mg/day) with current doses of vitamin D analogues prescribed. Calculation of the optimal $C_{di}Ca^{2+}$ for the 320 Renal Research Institute patients revealed that in virtually all instances, the $C_{di}Ca^{2+}$ required for neutral Ca_{MB}, where Ca removal during dialysis was equal to Ca accumulation between dialyses, was less than 2.50 mEq/l and averaged about 2.00 mEq/l. This sharply contradicts the recent KDIGO (Kidney Disease: Improving Global Outcomes) Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease – Mineral and Bone Disorder, that suggests a $C_{di}Ca^{2+}$ of 2.5–3.0 mEq/l. Review of the KDIGO work group discussions shows that this discrepancy stems from the unwarranted work group assumption that intradialytic Ca_{MB} is zero. We strongly believe that this guideline for dialysate Ca^{2+} is inappropriate and should be modified to more realistically reflect the needs of dialysis patients.

Introduction

Patients undergoing hemodialysis (HD) frequently develop widespread medial arterial calcification which is strongly associated with adverse cardiovascular outcomes and is widely considered to be associated with excess body content of calcium (Ca) and phosphorus (P)

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[1–4]. Despite numerous large-scale multinational studies of mineral abnormalities in HD, there are no consensus guidelines for the integrated prescription of dietary Ca and P, phosphate binders, dialysate Ca, vitamin D analogues and calcimimetics to HD patients. The optimal management of mineral metabolism in HD patients should include consideration of the effects of the total therapeutic regimen on Ca mass balance (Ca_{MB}) and P mass balance in addition to changes in serum Ca and P, bone histology and parathyroid hormone. None of the many studies of Ca-based versus non-Ca-based phosphate binders have included consideration of Ca_{MB} with respect to prescribed dialysate Ca^{2+} concentration ($C_{di}Ca^{2+}$), the dose of vitamin D analogues and Ca intake (Ca_{INT}). There continue to be high incidences of hyper- and hypocalcemia, hyper- and hypophosphatemia, high and low parathyroid hormone levels and bone disorders with adynamic bone disease and secondary hyperparathyroidism. P overload is readily recognized from increased plasma concentration as this waste solute accumulates in body water. It has long been recognized that body overload of Ca can occur in the absence of elevated serum Ca [3, 4], which further emphasizes the need to quantify Ca_{MB} in these patients which in turn requires reliable estimates of net Ca absorption (or loss) between dialyses and net Ca removal (or accumulation) during dialyses.

Determinants of Solute Mass Balance

The primary purpose of HD therapy is to regulate the body content of solutes and water that are normally excreted by the kidneys. Virtually the only substance for which we regularly attempt to achieve zero mass balance in HD is H_2O, as rather crudely measured by body weight changes. Equally important would it be to achieve zero Na mass balance, but that is not generally attempted. Ca^{2+} and P are two very important solutes regulated by the kidneys and HD, but Ca_{MB} has completely escaped clinical attention.

The elements of the Ca_{MB} equation in HD are very simple:

$$Ca_{MB} = \text{Ca input from diet and binders} - \text{Ca removal by dialysis.} \quad (1)$$

Examination of equation 1 shows that to satisfy the requirement that $Ca_{MB} = 0$, we must be able to show that

$$\text{Ca input from diet and binders} = \text{Ca removal during dialyses.} \quad (2)$$

The reported clinical studies of 'Ca_{MB}' over the past 15 years have invariably provided measurements of net Ca flux during dialysis without attempting to estimate Ca accumulation between dialyses [5–9] and therefore satisfy only half of equation 1. Assessment of the control of Ca and P body content requires quantitative analysis of flux of these solutes over the complete dialysis cycle (each interdialytic interval and the immediately following dialytic interval). This obvious fundamental goal of neutral mass balance for Ca and P has virtually never been considered in the innumerable clinical studies of mineral metabolism in HD therapy over the past 10–15 years, and the recently published KDIGO (Kidney Diasease: Improving Global Outcomes) Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention and Treatment of Chronic Kidney Disease – Mineral and Bone Disorder [10] suggested that $C_{di}Ca^{2+}$ was not based on any quantitative consideration of Ca_{MB}.

Calcium Mass Balance

Ca_{MB} can be described with general mathematical notation as:

$$Ca_{MB} = \text{Ca accumulation} - \text{Ca removal}$$
$$Ca_{MB} = Ca_{Fabs} \cdot (Ca_{Diet} + Ca_{Bi}) - J_{Dial}Ca, \quad (3)$$

where Ca_{Fabs} is the fractional absorption of ingested dietary Ca (Ca_{Diet}) and P binder Ca (Ca_{Bi}), and $J_{Dial}Ca$ is the sum of diffusive and convective or total dialyzer flux of Ca during dialysis. Equation 3 is not sufficiently detailed to calculate Ca accumulation and Ca removal which requires incorporation of quantitative descriptions of the mechanisms underlying Ca_{Fabs} and $J_{Dial}Ca$.

Calcium Absorption

The term Ca_{Fabs} is of enormous clinical importance because control of Ca absorption in the gastrointestinal tract plays a major role in Ca_{MB}. The coefficient for Ca_{Fabs} in equation 3 has been shown to be a logarithmic function of the plasma concentration of calcitriol [dihydroxyvitamin D_3 (C_pD_32OH)] and Ca_{INT} as reported elsewhere [11–14]. The mathematical relationships between Ca_{INT}, Ca absorbed between dialyses (Ca_{Abs}) and C_pD_32OH are shown in figure 1. The family of logarithmic functions depicted provides quantitative estimates of the magnitude of Ca absorption to be expected over a wide range of C_pD_32OH and Ca_{INT}. As noted above,

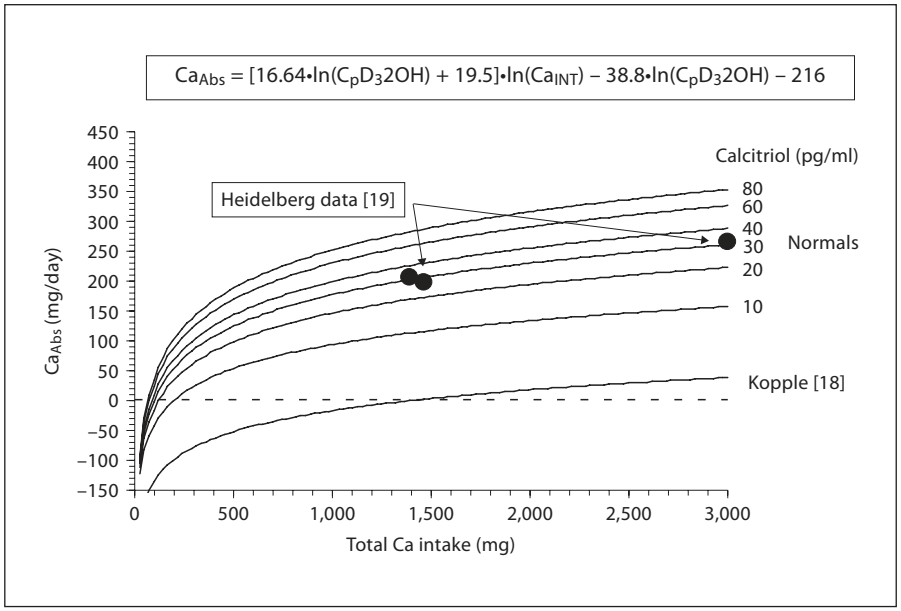

Fig. 1. A generalized model of Ca absorption as a function of (1) total Ca intake and (2) the level of plasma calcitriol. Adapted from Gotch [14], derived from Baylor Studies [15–17].

the mathematical model was derived from a series of single-meal Ca_{Abs} studies [15–17] in which Ca absorption from a single standardized meal was measured over a narrow range of Ca_{INT} of 100–1,250 mg. These were very precise studies because the bowel was emptied immediately before and 8 h after a test meal so that the large and ubiquitous confounding problem of variability in colon content of Ca complicating Ca balance studies was eliminated. The curve in figure 1 labeled 'Kopple' is plotted from a seminal study reported by Kopple and Coburn [18] showing that prior to the therapeutic advent of D_32OH, HD patients were generally in negative Ca_{MB} between dialyses, and this led to the widespread use of $C_{di}Ca$ 3.5 mEq/l to achieve a positive Ca_{MB} during dialysis.

A recent study [19] reported Ca_{Abs} over a range of intake of 1,200–3,000 mg/day normally distributed over 3 meals per day with the results depicted as 'Heidelberg data' in figure 1. These data were obtained from normal subjects so that the coordinates of the 3 points in figure 1 indicate virtually perfect correlation with the modeled relationships, since they fall on the modeling line for normal C_pD_32OH of 30–40 pg/ml [20, 21]. The mathematical function to describe Ca_{Abs} derived from these studies is

$$Ca_{Abs} = 16.64 \cdot \ln(C_pD_32OH) + 19.5 \cdot \ln(Ca_{INT}) - 38.8 \cdot \ln(C_pD_32OH) - 216. \quad (4)$$

Pharmacokinetic and Pharmacodynamic Consideration of Vitamin D Analogue Prescription

The relationship of C_pD_32OH to declining renal function is shown in figure 2a based on reports of Martinez et al. [20, 22], which have been widely quoted as definitive in many publications. It is apparent that normal glomerular filtration rate is associated with C_pD_32OH of approximately 40 pg/ml and end-stage renal disease with 5–8 pg/ml. It seems entirely reasonable to restore C_pD_32OH to that seen with a glomerular filtration rate of about 50 ml/min which is approximately 20 pg/ml plasma concentration. This will assure that the many non-mineral-metabolism effects of the vitamin (inhibition of inflammation and others) are well maintained without significantly inhibiting parathyroid hormone secretion (also seen in fig. 2a) and will avoid high rates of Ca absorption in the gastrointestinal tract. The curve for the production rate of calcitriol (PRD_32OH) in figure 2b was computed from the steady-state relationship:

$$PRD_32OHss = K_pD_32OH \cdot C_pD_32OH, \quad (5)$$

where K_pD_32OH is the metabolic clearance of D_32OH defined as 10 ml/min from data reported by Brandi et al. [21]. To achieve C_pD_32OH of about 20 pg/ml, the curve in figure 2b shows that the continuous infusion dose would be 0.5 μg/day.

This was converted to a thrice-weekly dose by iterating the pharmacokinetic equations to steady state with V = 19

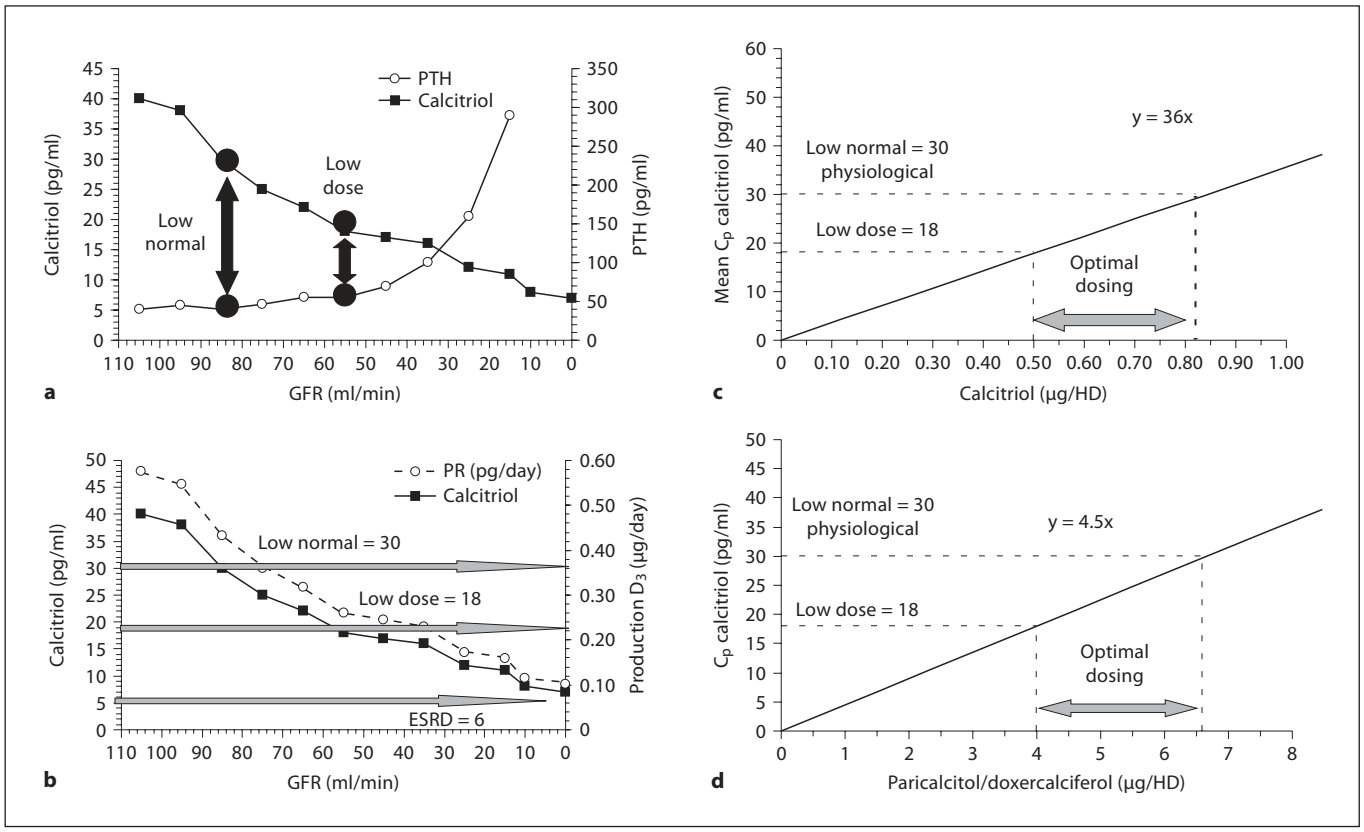

Fig. 2. Pharmacokinetics and pharmacodynamics of calcitriol and analogues of vitamin D (**a** with permission from the National Kidney Foundation). PTH = Parathyroid hormone; GFR = glomerular filtration rate; PR = production; ESRD = end-stage renal disease.

liters and K_pD_32OH 10 ml/min and finding the mean concentration defined as average at the beginning and end of each thrice-weekly interval. The results of this calculation are shown in figure 2c which shows that the mean C_pD_32OH equals 36 times the dose given thrice weekly. Since the effects of paricalcitol (Zemplar®) and doxercalciferol (Hectorol®) on Ca_{Abs} are reported to only 1/8 that of calcitriol [23], the equivalent C_pD_32OH as a function of thrice-weekly dosing with paricalcitol or doxercalciferol would have a slope of 4.5 as shown in figure 2d, and optimal thrice-weekly dosing by the definition proposed above would be approximately 0.5–0.8 µg/HD for calcitriol and 4.0–6.5 µg/HD for paricalcitol or doxercalciferol.

The relationships between C_pD_32OH and doses of calcitriol and paricalcitol/doxercalciferol in figure 2c and d can now be combined with equation 4 to calculate Ca_{Abs} as a function of Ca_{INT} and the doses of vitamin D analogues (paricalcitol, doxercalciferol), in accordance with

$$Ca_{Abs} = [16.64 \cdot \ln(36 \cdot D_32OH) + 19.5] \cdot \ln(Ca_{INT}) - 38.5 \cdot \ln(36 \cdot \text{dose } D_32OH) - 218 \quad (6)$$

and

$$Ca_{Abs} = [16.64 \cdot \ln(4.5 \cdot \text{dose paricalcitol/doxercalciferol}) + 19.5] \cdot \ln(Ca_{INT}) - 38.5 \cdot \ln(4.5 \cdot \text{dose paricalcitol/doxercalciferol}) - 218. \quad (7)$$

Net Calcium Intake

The P absorptive capacity of Ca acetate (Phoslo®) is approximately 25 mg P/pill. In the relatively alkaline bowel lumen, it can be assumed that the P is absorbed as $Ca_3(PO_4)_2$ salt. Since the Ca content of one Ca acetate pill is 167 mg, this stoichiometric relationship dictates that 50 mg of the binder Ca will form $Ca_3(PO_4)_2$ so that the net Ca available for absorption is 117 mg/pill.

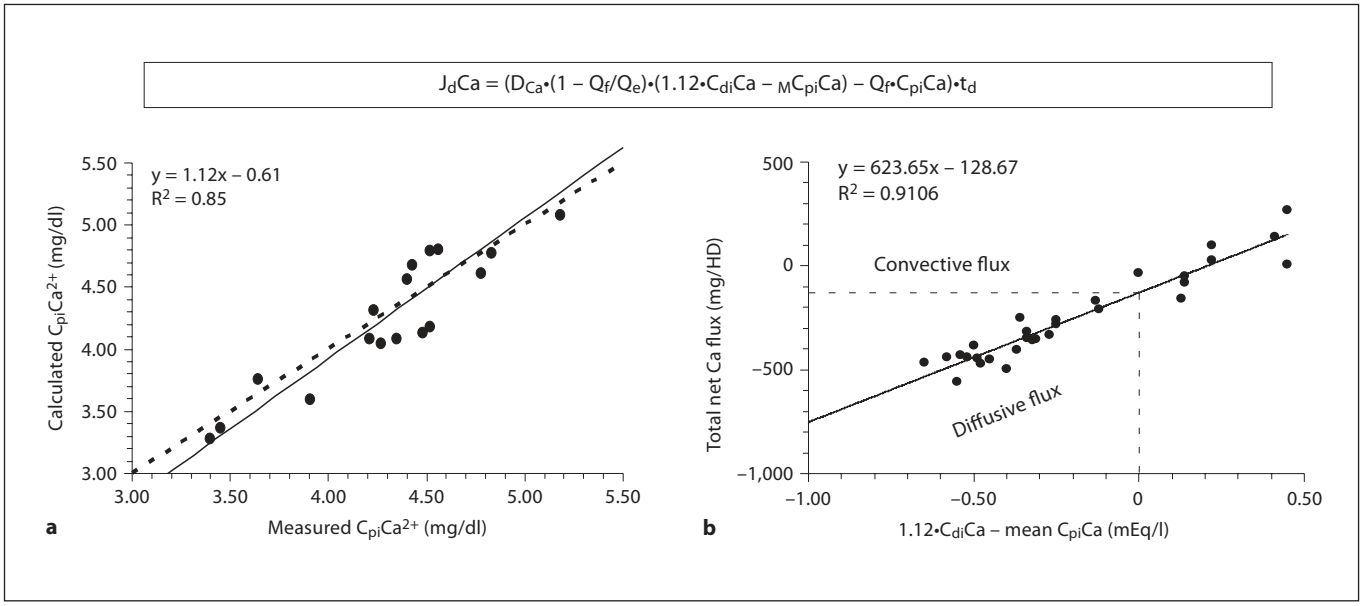

Fig. 3. Dialyzer Ca flux is a highly predictable function of Ca dialysance when the concentration gradient is defined correctly. These data were obtained with $C_{di}Ca^{2+}$ ranging from 1.50 to 3.00 mEq/l.

Calcium Removal: Dialyzer Calcium Flux

Instantaneous Ca flux (J_dCa^{2+}, mEq/min or mg/min) during dialysis is determined by: (1) the ionized Ca^{2+} concentration gradient between mean plasma inlet ($_MC_{pi}Ca^{2+}$) and dialysate inlet ($C_{di}Ca^{2+}$) of the dialyzer, i.e., ($C_{di}Ca^{2+} - C_{pi}Ca^{2+}$, mEq/l); (2) the dialysance of Ca^{2+} (D_{Ca}, l/min), and (3) the product of the ultrafiltration rate (Q_f) and $_MC_{pi}Ca^{2+}$. These parameters can be combined into a well-known transport equation [24]:

$$J_dCa^{2+} = D_{Ca}(1.12 \cdot C_{di}Ca^{2+} - {}_MC_{pi}Ca^{2+})\,(1 - Q_f/Q_e) - Q_f \cdot {}_MC_{pi}Ca^{2+}. \quad (8)$$

Ca is a very interesting solute with respect to dialyzer transport. We have found that, although only the diffusible fraction is dialyzable, the bound Ca dissociates very rapidly so that the effective driving force for diffusion is total plasma Ca [25]. The evidence for this is that mass balance is equal between change in plasma content and change in dialysate content across the dialyzer only when the change in plasma content is calculated from change in total plasma Ca.

This plasma Ca reservoir, which is twice the ionized content, results in calculated dialysance values substantially greater than plasma flow rate and hence, mass transfer area coefficient (KoA) values cannot be calculated. We derived a generalized expression to calculate KoA by defining the effective flow rate for Ca^{2+} (Q_e) as twice the plasma flow rate and found that the KoA was dependent also on the flow rate (r = 0.62, n = 30, p = 0.02) per se, such that

$$KoA_{Ca^{2+}} = 332 \cdot \ln(Q_e) - 1{,}409. \quad (9)$$

Thus, with an average blood flow rate of 400 ml/min and hematocrit of 35 ml/dl, the Q_e is 500 and KoA 650 ml/min, which results in a typical in vivo Ca^{2+} dialysance ($D_{Ca^{2+}}$) of 300 ml/min.

The reliability of J_dCa^{2+} calculation [26] is demonstrated in figure 3a. In this plot, the $C_{pi}Ca^{2+}$ calculated from the transport equation 6 is compared to measured $C_{pi}Ca^{2+}$. The $C_{pi}Ca^{2+}$ values were calculated from measurements of $C_{di}Ca^{2+}$ and $C_{do}Ca^{2+}$ (dialysate inlet and outlet concentrations of Ca^{2+}) every 10 min to determine J_dCa^{2+} and calculation of $D_{Ca^{2+}}$ from KoA and flow rates as described above with rearrangement of equation 8 to give:

$$_MC_{pi}Ca^{2+} = D_{Ca^{2+}}(1.12 \cdot C_{di}Ca^{2+}) - J_dCa^{2+}/D_{Ca^{2+}}({}_MC_{pi}Ca^{2+} + Q_f). \quad (10)$$

Note the excellent agreement indicating internal consistency of measurements and definition of transport parameters. Figure 3b shows the dependence of total flux over the whole dialysis on the inlet concentration gradi-

ent. Note that the inlet dialyzer concentration gradient is expressed as ($1.12 \cdot C_{di}Ca^{2+} - {}_{M}C_{pi}Ca^{2+}$), where 1.12 is a Donnan coefficient and ${}_{M}C_{pi}Ca^{2+}$ is the mean $C_{pi}Ca^{2+}$ during dialysis which will be further considered below. When there is a diffusion gradient with Ca^{2+} transport during dialysis, it can be anticipated that there will be some change in $C_{pi}Ca^{2+}$ during therapy which would be expected to approach the value of $C_{di}Ca^{2+}$. Consequently, in order to correctly relate flux to gradient, the mean value of ${}_{M}C_{pi}Ca^{2+}$ is required which can be calculated from consideration of the Ca^{2+} miscible pool buffer coefficient (K_{MP}).

The Miscible Calcium Pool

Equation 8 describes J_dCa^{2+} but to estimate total flux in clinical application, it is necessary to also predict the ${}_{M}C_{pi}Ca^{2+}$ during dialysis. We have previously described the buffering of changes in $C_{pi}Ca^{2+}$ during dialysis induced by diffusive removal as the amount of Ca^{2+} mobilized from $M+_{Ca}$ or sequestered in $M-_{Ca}$, the miscible Ca pool resisting the change in $C_{pi}Ca^{2+}$ [13]. This relationship was generalized conceptually as a buffer coefficient (K_{MP}) defined as

$$K_{MP} = M_{Ca}/[M_{Ca} + \Delta C_pCa^{2+} \cdot V_{ECW}], \quad (11)$$

where $[\Delta C_pCa^{2+} \cdot V_{ECW}]$ is the product of total change in plasma Ca^{2+} during dialysis multiplied by extracellular fluid volume (V_{ECW}), estimated as 1/3 of urea distribution volume.

Note that K_{MP} can vary between 1.00 ($\Delta C_pCa^{2+} \cdot V_{ECW} = 0$) and 0 ($M_{Ca} = 0$).

We have observed an average $K_{MP} = 0.70$ in Ca modeling studies to date.

From the solution of equation 9 for $\Delta C_{pi}Ca^{2+}$ and measurement of $C_{p0}Ca^{2+}$ (this is C_pCa^{2+} before dialysis) it can show that

$$C_{pt}Ca^{2+} = C_{p0}Ca^{2+} - M_{Ca^{2+}} \cdot (1 - K_{MP})/(K_{MP} \cdot VCa). \quad (12)$$

We have also observed [unpubl. data] that $C_{pi}Ca^{2+}$ approaches the $C_{pt}Ca^{2+}$ values asymptotically and integration of the mean value function results in

$${}_{M}C_pCa^{2+} = C_{pt}Ca^{2+} - [(C_{p0}Ca^{2+} - C_{pt}Ca^{2+})/0.012 \cdot t_d] \cdot [\exp(-0.012 \cdot t_d) - 1]. \quad (13)$$

Thus, for clinical use we can reliably calculate dialyzer Ca flux from equation 8 with intermediate parameters calculated from equations 12 and 13.

The Generalized Ca_{MB} Equation

Combination of equations 7 and 8 provides a general expression for Ca_{MB} which incorporates the mechanisms controlling Ca_{Abs} and $J_{Dial}Ca$ in accordance with

$$\begin{aligned} 0 &= \text{Ca accumulation} + \text{Ca removal} \\ 0 &= [(16.64 \cdot \ln[4.5 \cdot D_{\text{paricalcitol/doxercalciferol}}] \\ &+ 19.5) \cdot \ln({}_{Di}Ca + {}_{PL}Ca) \\ &- 38.8 \cdot \ln(4.5 \cdot D_{\text{paricalcitol/doxercalciferol}}) - 216] \cdot 7 \\ &+ N \cdot [D_{Ca}(1 - Q_f/Q_e) \cdot (C_{di}Ca^{2+} - {}_{M}C_pCa^{2+}) - Q_f \cdot {}_{M}C_pCa^{2+}] \cdot t_d \end{aligned} \quad (14)$$

where N = number of dialyses per week.

In equation 14, Ca removal is added to Ca accumulation because the dialyzer flux equation is written so that flux into dialysate is defined as negative relative to the blood. Generalized solutions over wide ranges of Ca_{INT}, vitamin D doses and $C_{di}Ca^{2+}$ for the Ca_{Abs} and J_dCa equations are depicted in figure 4a which shows a generalized solution of the Ca_{Abs} equation as a function of Ca_{INT}, the level of C_pD_32OH and corresponding doses of paricalcitol or doxercalciferol. Figure 4b shows the $C_{di}Ca^{2+}$ required achieving zero Ca_{MB} as a function of Ca_{Abs} calculated from the Ca_{Abs} equation with specified levels of Ca_{INT} and dose of paricalcitol or doxercalciferol as shown on the right ordinate scale. These plots are useful to examine the domains of current therapy of mineral metabolism in HD and to plan the optimal design of clinical studies of phosphate binders.

Observations on Ca_{Abs} in Current HD Therapy

We recently determined Ca and P kinetic modeling parameters on 320 HD patients in two Renal Research Institute treatment facilities. Plasma Ca and P were measured before and after dialysis on one set of routine monthly blood chemistries. The data sets included all of the urea kinetic modeling parameters and the other routine monthly chemistries. All patients were on stable, known doses of Ca acetate and stable, known intravenous doses of paricalcitol, doxercalciferol or calcitriol prescribed by the attending physicians. Spreadsheets were prepared to calculate P and Ca mass balances on this data set. The results of the calculations done with these clinical data are summarized in figure 5 where Ca_{Abs} for each patient is plotted as Ca_{INT}, which is expressed as both total intake and as diet Ca alone, and the doses of prescribed paricalcitol or doxercalciferol. There is a remarkably wide range of vitamin D analogue intakes with doses ranging from 2 μg/HD to greater than 16 μg/HD, and this in turn results in a very wide

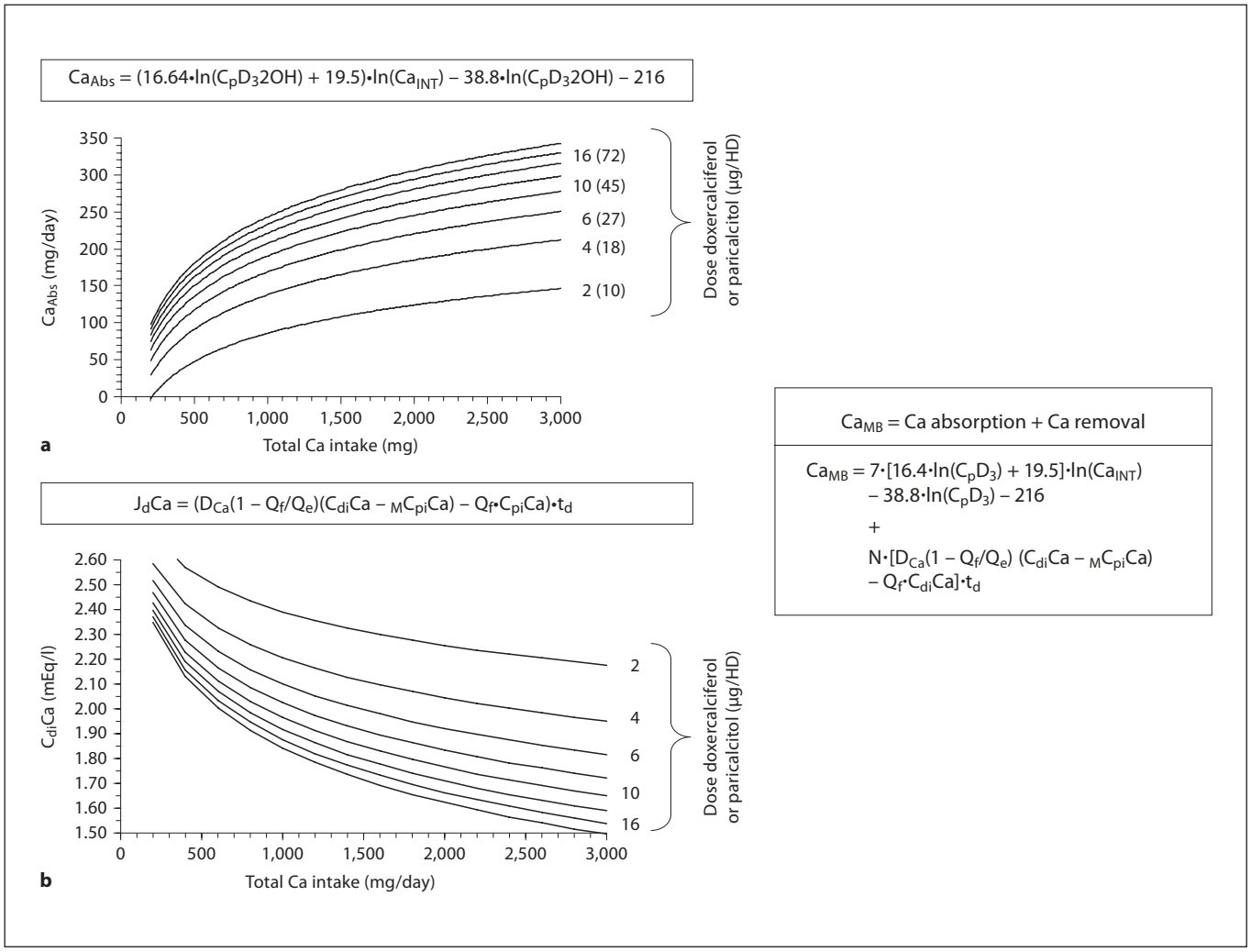

Fig. 4. The Ca kinetic model to quantify Ca_{MB} over the complete dialysis cycle. **a** Ca absorption. **b** Ca removal.

range of Ca_{Abs} in clinical practice. The mean values are 1,210 and 150 mg for Ca_{INT} and Ca_{Abs}, respectively. The doses of paricalcitol and doxercalciferol are shown in figure 6a plotted against Ca_{INT}, and in figure 6b Ca_{Abs} is plotted against Ca_{INT}. Restriction of the doses of vitamin D analogues to the recommended levels is derived in figure 2c, and the resultant marked decrease in variability in Ca_{Abs} is shown in figure 2d. Clearly avoiding high doses of these analogues is important for patients on either binder. Note that with an intake as low as 300–500 mg/day to be expected with sevelamer, Ca_{Abs} can rise to 200 mg/day with high doses of these vitamin D analogues.

Implications of the Ca_{MB} Equations for Clinical Studies of Ca and P in HD

There have been no attempts to either control or adjust the doses of vitamin D analogues as a function of Ca_{INT} in studies of Ca-containing and non-Ca-containing P binders. The consequences of this universal flaw in study design are illustrated in figure 7a where 3 patients with different levels of vitamin D therapy are shown switching back and forth between sevelamer and Ca acetate. Note that the average Ca_{INT} will vary from 500 to 1,500 mg/day on the two binders, while the Ca_{Abs} will range from 50 to 150 mg/day on sevelamer and from 150 to 250 mg/day on Ca acetate. A requirement for such

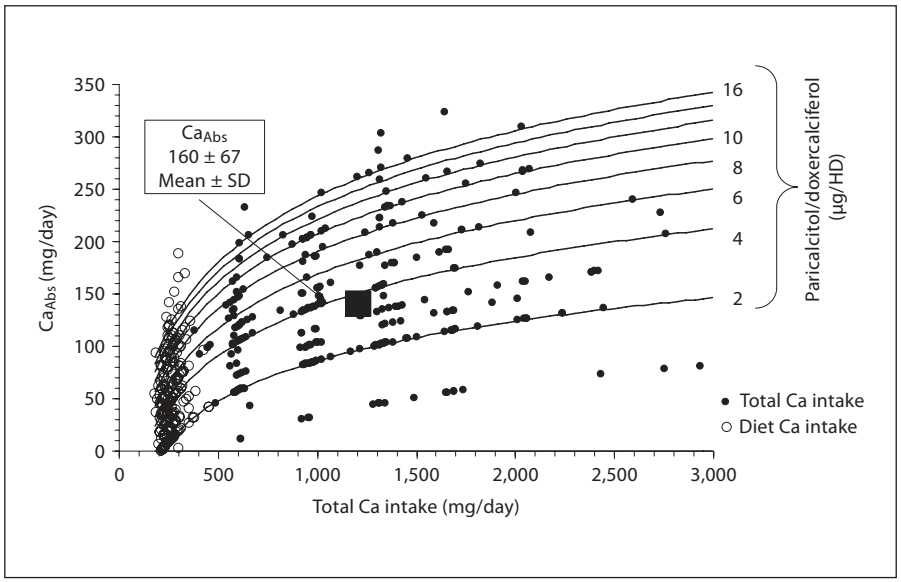

Fig. 5. A survey of Ca absorption between dialyses calculated with the Ca kinetic model in 320 FMC HD patients. Note that there is a wide range of absorption primarily dependent on the level of vitamin D analogue dosing and that all patients are in positive Ca balance between dialyses.

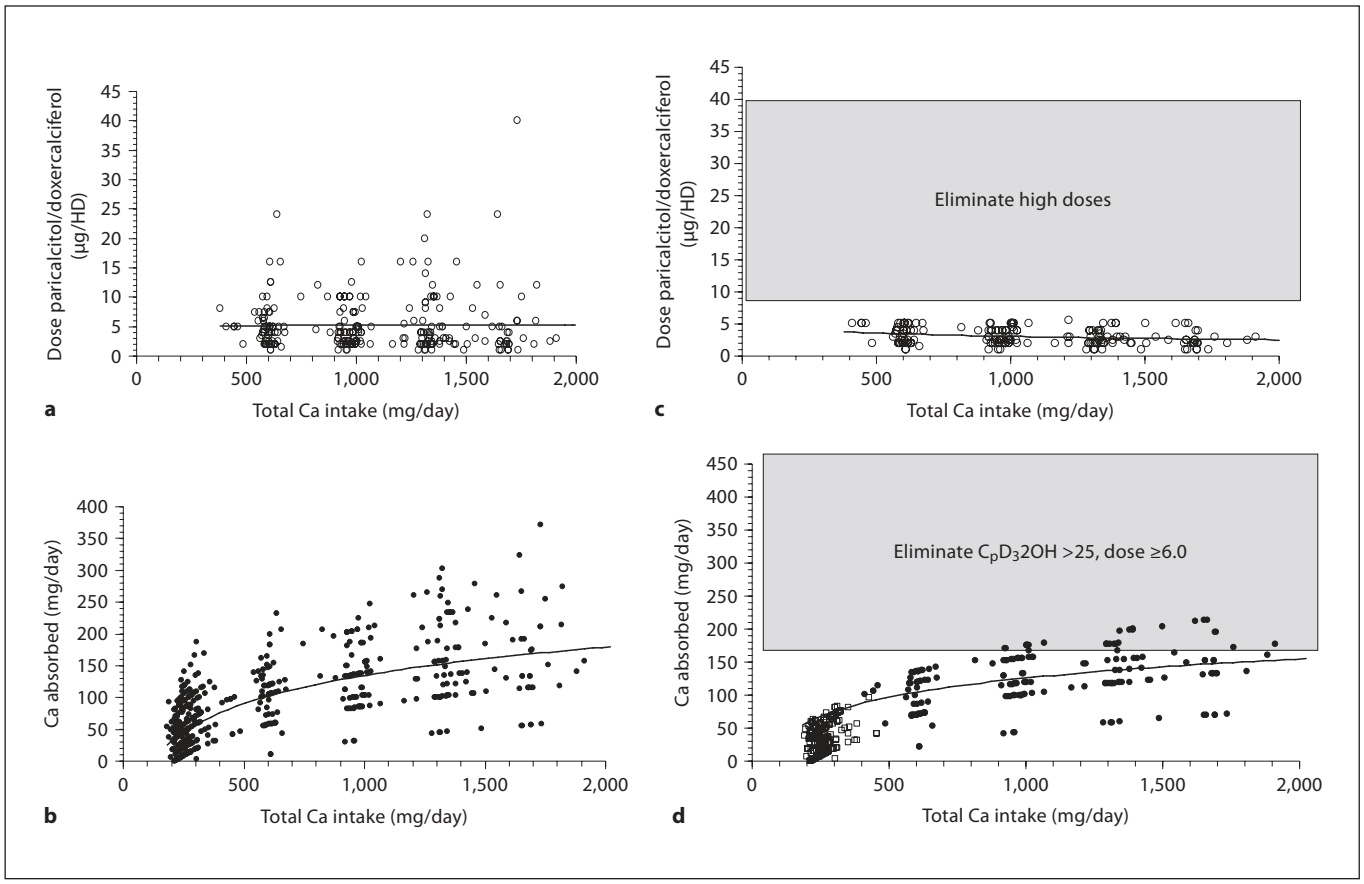

Fig. 6. Relationships between vitamin D_3 dosing and Ca absorption. Elimination of high doses of the analogues will greatly reduce the level of absorption.

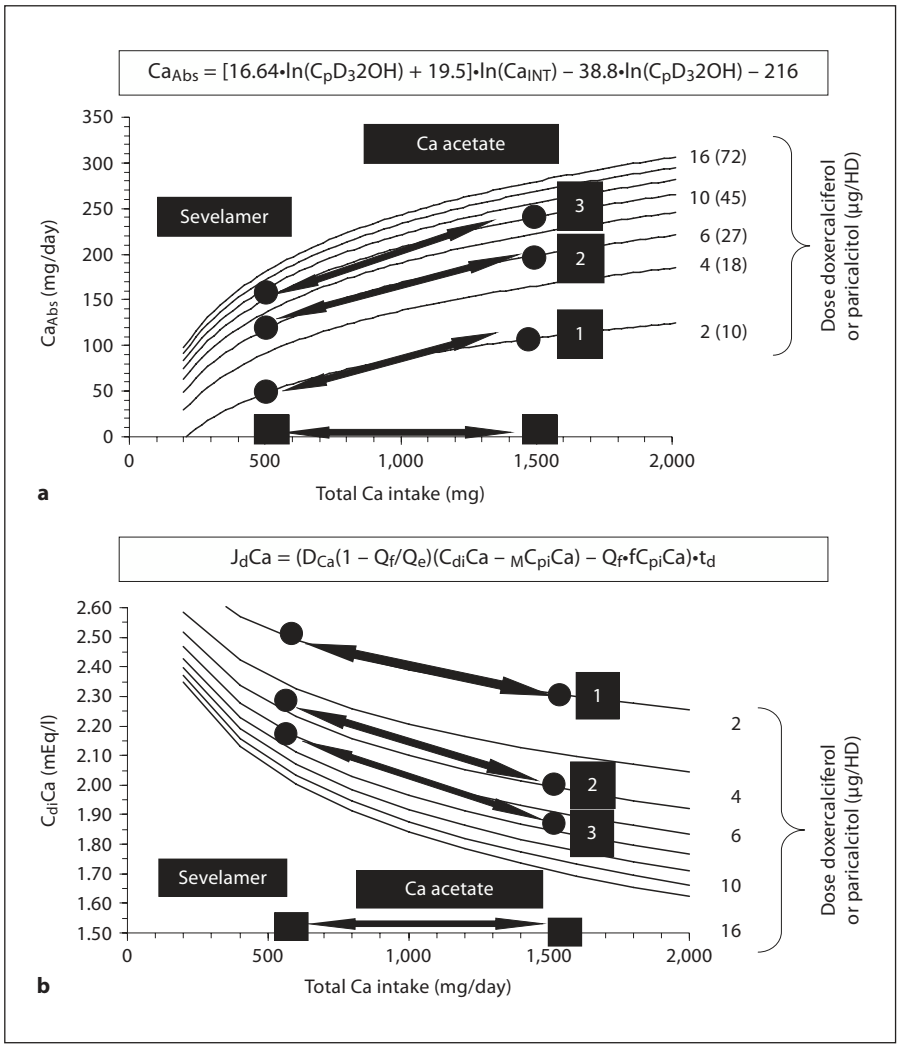

Fig. 7. a, **b** The implications of the Ca kinetic model for the design of clinical studies of P binders. **b** The J_dCa expression is solved for $C_{di}Ca^{2+}$ required to result in $Ca_{MB} = 0$ by setting $J_dCa = Ca_{Abs}$ as a function of Ca_{INT} and the dose of Zemplar/Hectorol with high-flux dialysis, $t_d = 4$ h, $Q_f = 2.5$ liters and $C_{pi}Ca^{2+} = 4.5$ mg/dl.

comparative binder studies should be to achieve neutral or zero Ca_{MB} for each patient on both binders. The same 3 patients are plotted in figure 7b where the $C_{di}Ca^{2+}$ required for neutral Ca_{MB} are depicted. In all instances, the $C_{di}Ca^{2+}$ required for neutral Ca_{MB} in the 3 examples is less than 2.5 mEq/l, and it would have to be decreased in all patients were they switched to Ca acetate. The consequences of not adjusting $C_{di}Ca^{2+}$ are shown in figure 8. Here it is assumed that a $C_{di}Ca^{2+}$ had been chosen to give neutral Ca_{MB} on sevelamer and left at the same level when the patients were switched to Ca acetate. Note that this would result in a positive Ca_{MB} with Ca acetate ranging from 60 to 100 mg/day. The range of Ca_{MB} variability in studies of binders over the past several years is further illustrated in figure 9. It can reasonably be assumed that patients chosen for binder studies would be similar to the 320 FMC patients shown again in figure 9a. Since quantitative modeling of Ca kinetics is never used to select and adjust $C_{di}Ca^{2+}$ and dose of vitamin D analogues to Ca_{INT}, the study patients would be expected to distribute in the domain defined by the broad arrow. Further, since there is no attempt to control the doses of vitamin D, variable numbers of patients will likely have substantial changes in paricalcitol or doxercalciferol doses for other clinical concerns and result in the further variability illustrated in figure 9b. It is not surprising that the studies comparing Ca-containing and non-Ca-containing binders have shown no significant differences in outcome, since none of them have included consideration of the role of $C_{di}Ca^{2+}$ and doses of vitamin D analogues on Ca_{MB} which has undoubtedly varied widely for both classes of binders.

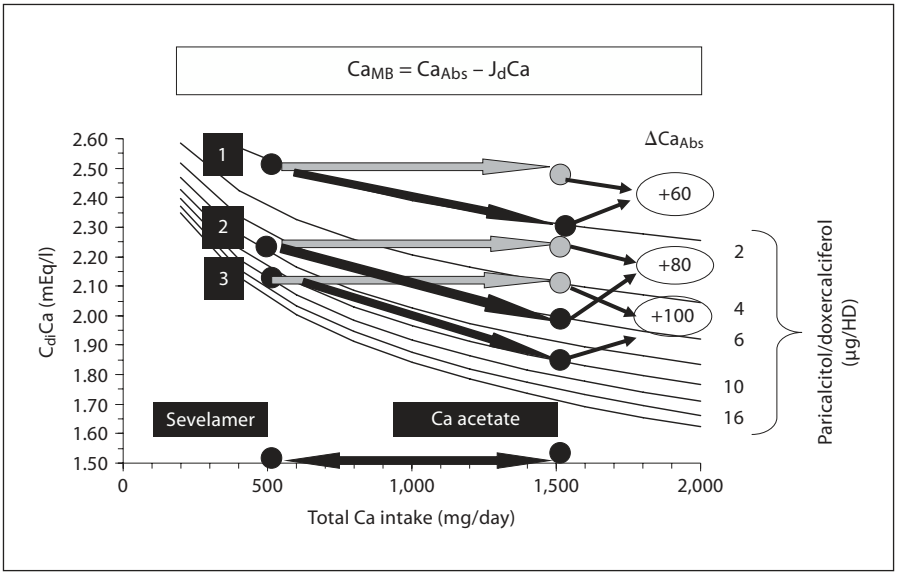

Fig. 8. The P binder studies reported in recent years can be predicted to have resulted in systematic induction of positive Ca_{MB} with the Ca-based binders studied.

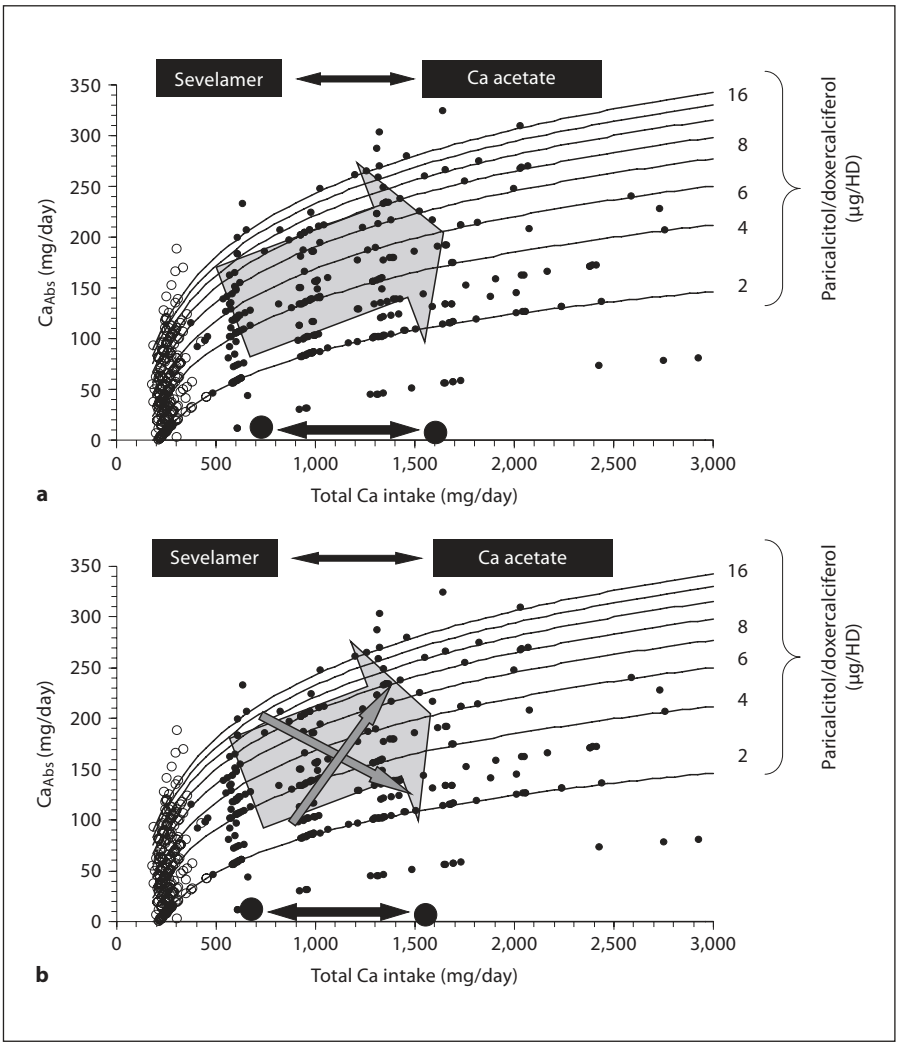

Fig. 9. An overview of the kinetic characteristics of patients likely to be recruited for comparative studies of P binders. It is predictable that there has been a wide range of Ca_{MB} in these studies confounding their clinical interpretation.

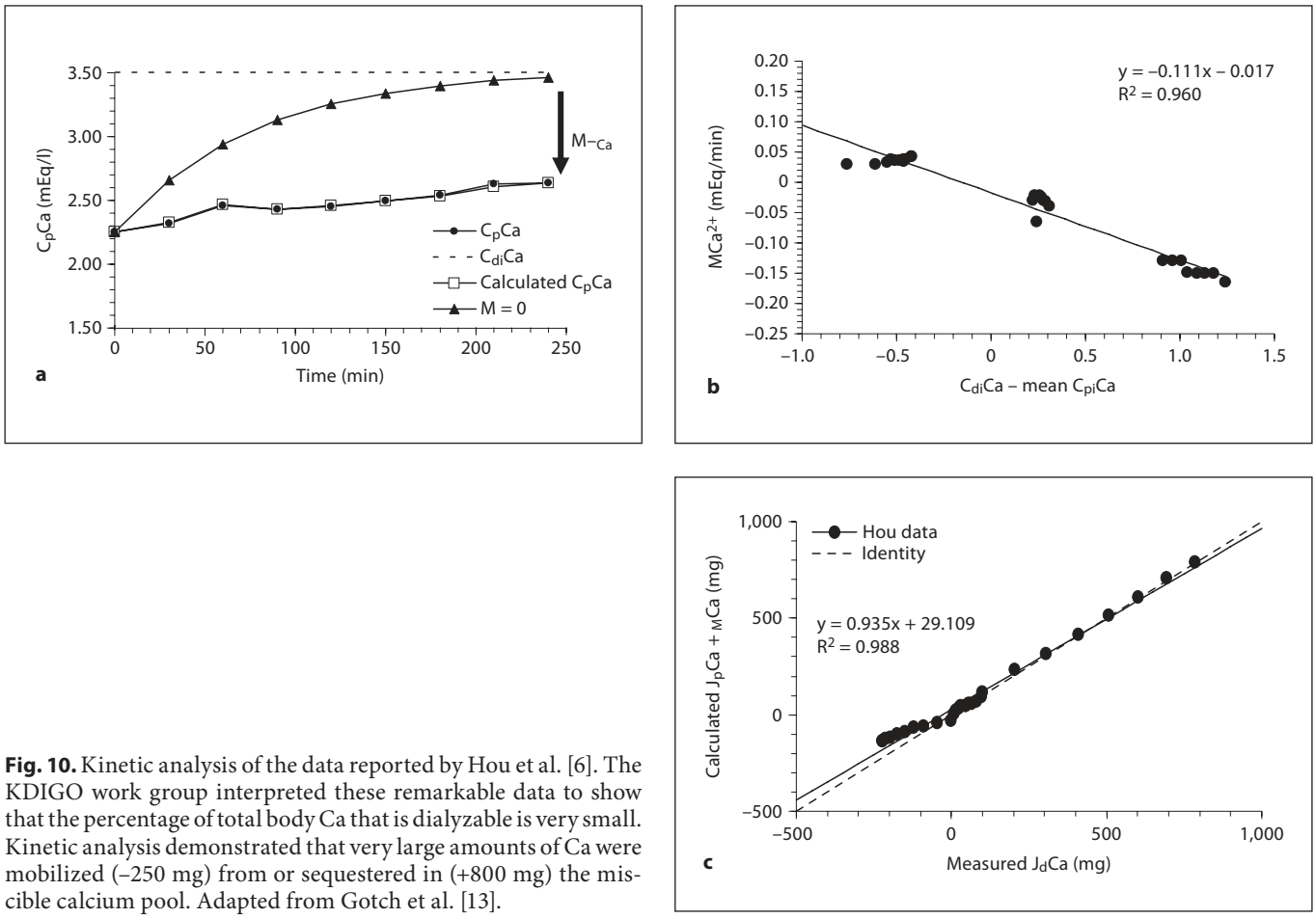

Fig. 10. Kinetic analysis of the data reported by Hou et al. [6]. The KDIGO work group interpreted these remarkable data to show that the percentage of total body Ca that is dialyzable is very small. Kinetic analysis demonstrated that very large amounts of Ca were mobilized (–250 mg) from or sequestered in (+800 mg) the miscible calcium pool. Adapted from Gotch et al. [13].

Consideration of the KDIGO Guideline for $C_{di}Ca^{2+}$

The KDIGO work group [10] recently reported that $C_{di}Ca^{2+}$ should not be outside the range of 2.50–3.00 mEq/l. This guideline is completely incompatible with the family of curves modeled in figure 4b where the maximal modeled $C_{di}Ca^{2+}$ is 2.60 mEq/l. Clearly, either the Ca kinetic model or the guideline must be wrong for such a striking inconsistency with virtually no overlap in $C_{di}Ca^{2+}$ recommendation. In an attempt to shed light on this inconsistency, the literature cited and its interpretation by the work group to support the KDIGO guideline were reviewed.

The following was emphasized in the guideline: 'The work group felt that, in general, a dialysate concentration of 1.25 mM/l (2.50 mEq/l) would be a near-neutral calcium balance for most patients.' The work group further wrote: 'The final vote on this recommendation was 16 in favor and 1 vote against (although the evidence was categorized as 2D meaning the estimate of effect is very uncertain and often will be far from the truth). The vote against was to argue that a 1.0 mM/l (2.0 mEq/l) of calcium was also helpful in some patients to reduce their positive calcium balance.' There is not a single reference cited in the work group discussion considering Ca_{MB} quantitatively as defined above by equations 1–7 and as reported in the literature [11–14]. The group cited only 5 reports of the net flux of Ca during dialysis [5–9] showing generally neutral net flux with $C_{di}Ca^{2+}$ 2.50 mEq/l and from this data concluded that $C_{di}Ca^{2+}$ 2.50 mEq/l should be the lower limit. They were overwhelmingly convinced that average neutral flux during dialysis assured neutral Ca_{MB} for all patients over the complete dialysis cycles without including any consideration of the magnitude of interdialytic Ca absorption in their discussion. The fundamental fallacy in this reasoning is shown by equation 1 above and illustrated quantitatively in figures 1 and 5 where it can be seen that virtually all patients are pre-

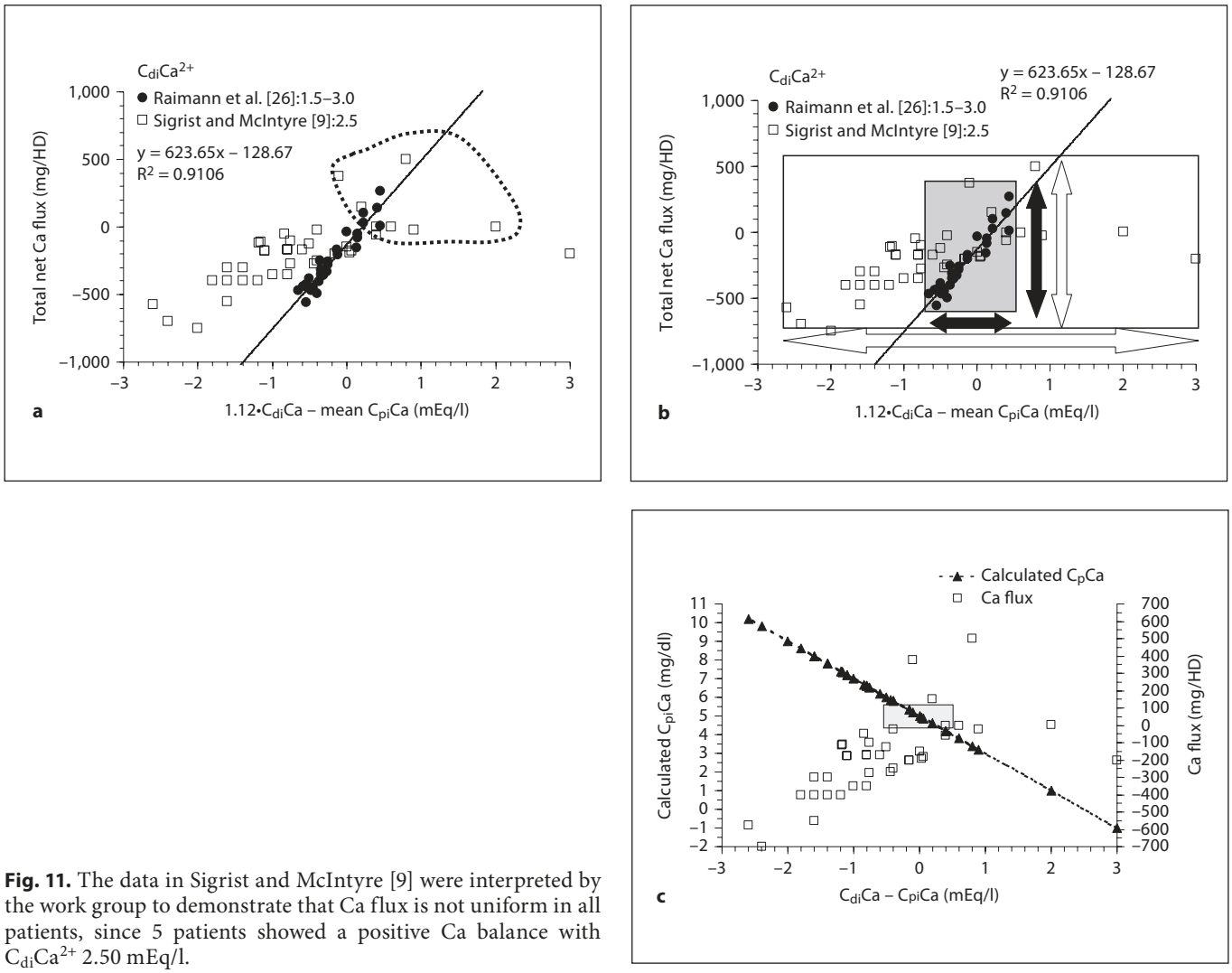

Fig. 11. The data in Sigrist and McIntyre [9] were interpreted by the work group to demonstrate that Ca flux is not uniform in all patients, since 5 patients showed a positive Ca balance with $C_{di}Ca^{2+}$ 2.50 mEq/l.

dicted to be in positive Ca_{MB} with the current management of Ca and P metabolism in HD patients.

The work group also concluded that 'the percentage of total body calcium that is dialyzable is very small' and cite the data of Hou et al. [6] in support of that conclusion. This comment clearly demonstrates that the work group did not fully appreciate the data of Hou et al. We reported a detailed kinetic analysis of this data [13] showing high rates of Ca mobilization from or sequestration in the miscible Ca pool ranging from –250 to +800 mg over the course of dialysis with $C_{di}Ca^{2+}$ 1.50 and 3.50 mEq/l (fig. 10). This kinetic analysis, which for the first time quantified the major role of the miscible Ca pool in supporting serum Ca during dialysis, was not considered by the work group. By recommending a dialysate Ca concentration between 2.5 and 3.0 mEq/l, the current KDIGO chronic kidney disease/mineral and bone disorder guidelines will result in a positive Ca balance in a large proportion of chronic HD patients. Decisions about dialysate Ca levels should be based on a quantitative understanding of Ca_{MB}, which takes interdialytic Ca absorption and intradialytic Ca removal into account. We strongly believe that a quantitative approach to dialysate Ca as suggested in this paper is warranted to prevent patients from chronic Ca overload.

The work group appeared to conclude that Ca flux during dialysis varies somehow independently of the Ca concentration gradient across the dialyzer: 'A more re-

cent study using more frequent assessments of spent dialysate found a mean calcium flux with each dialysis of –187 ± 232 mg/HD on 2.5 mEq/l dialysate. However, 6 of the 52 patients had positive calcium balance, supporting the fact that calcium flux with dialysis is not uniform in all patients.' This is a very unusual interpretation of a paper containing very unusual data. The data were presented with the Ca gradient defined as $C_{pi}Ca^{2+} - C_{di}Ca^{2+}$ which was plotted on the ordinate axis to which the dependent variable is normally assigned. Flux was calculated as $C_{do}Ca^{2+} \cdot Q_{do} - C_{di}Ca^{2+} \cdot Q_{di}$ so that when $C_{di}Ca^{2+}$ was greater than $C_{pi}Ca^{2+}$, the gradient was negative and flux was positive in the dialysate (but negative with respect to the patient). The reported data are replotted in figure 11a where the gradient is redefined as $C_{di}Ca^{2+} - C_{pi}Ca^{2+}$ on the abscissa and flux sign changed to show $C_{di}Ca^{2+} \cdot Q_{di} - C_{do}Ca^{2+} \cdot Q_{do}$ so that flux is now defined relative to the patient. The data shown in figure 2 are also plotted for comparison of flux relative to gradient in the two data sets. The data set referenced by the work group, plotted as open squares in figure 11a, was all obtained using $C_{di}Ca^{2+}$ 2.50 mEq/l while the data in figure 2 were obtained with $C_{di}Ca^{2+}$ distributed over a wide range of 1.5–3.0 mEq/l. In figure 11b, the ranges of gradient and flux are explicitly identified for the two data sets. Note that the gradient in the work group reference ranges from –3 to +3 mEq/l, whereas for the data in figure 2 the range is much smaller, from +0.25 to –0.8 mEq/l. The range of flux is fairly similar for the two data sets, ranging from about –500 to +500 mg/HD. Since the nominal $C_{di}Ca^{2+}$ was 2.5 mEq/l for all the dialyses in Sigrist and McIntyre [9], it follows that the remarkably wide range in gradient must be reflected in an extremely wide range of $C_{pi}Ca^{2+}$ or marked variability in actually measured $C_{di}Ca^{2+}$. This relationship is explored in figure 11c where it is assumed that $C_{di}Ca^{2+}$ was constant and the gradient reflected variability in $C_{pi}Ca^{2+}$. This would result in $C_{pi}Ca^{2+}$ values ranging from +11 to –3 mg/dl which of course are totally unrealistic. We believe it can be concluded that these data, which the work group interpreted to indicate patient-dependent nonuniform calcium flux relative to $C_{di}Ca^{2+}$, contain major technical errors resulting in the apparent variability in flux and are not suitable for referencing a guideline.

The work group expressed concerns about cardiac arrhythmias and decreased hemodynamic stability with lowered $C_{di}Ca^{2+}$ and cited two papers supporting these concerns [27, 28]. Neither reference addressed the issue of cardiac arrhythmias. In the paper by Maynard et al. [27], 12 patients were observed over 3 dialyses with $C_{di}Ca^{2+}$ 5.5 mg/dl and 3 dialyses with $C_{di}Ca^{2+}$ 7.5 mg/dl. There was a small but significantly greater fall in mean arterial pressure with the lower $C_{di}Ca^{2+}$, but there were no reported clinically significant hypotensive episodes with either dialysate. Since the 2 dialysates studied were 2.75 and 3.75 mEq/l, they were far above the level of 2.50 recommended by the work group as lower limit of $C_{di}Ca^{2+}$. It would seem this was a study of the pharmacological response to hypercalcemia, which is known to increase blood pressure, and has little bearing on the $C_{di}Ca^{2+}$ domain of clinical interest. In the paper by van der Sande et al. [28], 9 patients with quite severe cardiac failure were observed over 2 dialyses comprised of a 'low-Ca'-dialysate with $C_{di}Ca^{2+}$ 2.50 mEq/l and a 'high-Ca'-dialysate with $C_{di}Ca^{2+}$ 3.5 mEq/l. There was a greater fall in mean arterial pressure with the 2.50 mEq/l dialysate, but the difference was small and significantly different only at the end of the last 30 min of dialysis. Again, there were no reports of clinically significant hypotension in these patients with seriously compromised cardiac function. Thus, neither of these papers shed any light on the risk of clinically important hemodynamic instability in the $C_{di}Ca^{2+}$ range from 2.00 to 2.50 mEq/l.

In conclusion, by recommending a dialysate Ca concentration between 2.5 and 3.0 mEq/l, the current KDIGO chronic kidney disease/mineral and bone disorder guidelines [10] will result in a positive Ca balance in a large proportion of chronic HD patients. Decisions about dialysate Ca levels should be based on a quantitative understanding of Ca_{MB}, which takes interdialytic Ca absorption and intradialytic Ca removal into account. We strongly believe that a quantitative approach to dialysate Ca as suggested in this paper is warranted to prevent patients from chronic Ca overload.

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The Role of Peripheral Ultrafiltration in the Management of Acute Decompensated Heart Failure

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Key Words
Heart failure · Volume overload · Peripheral ultrafiltration

Abstract
Heart failure is a common and highly morbid condition associated with recurrent hospitalizations for disease decompensation. As such, heart failure is a growing public health concern from both a utilization and cost perspective. The current standard of care for the management of volume overload symptoms is underpinned by a diuretic-based treatment regimen. There is now increasing data to suggest that this approach may be counterproductive and result in progression of cardiac and renal disease with resultant higher mortality rates. Peripheral ultrafiltration is emerging as a viable, and in some cases preferred, option for sodium and fluid removal. Initial research trials have shown that this treatment modality results in improved clinical, biochemical and quality of life parameters. Moreover, these benefits are durable over the long term with decreased recidivism and health care utilization at 1 year. It remains unclear whether these benefits will translate into hard cardiovascular endpoints; greater adoption of this technology coupled with newer clinical trials will help researchers and clinicians identify the optimal strategy for treating symptoms of volume overload among heart failure patients.

Introduction

Heart failure is a complex clinical syndrome associated with significant morbidity and mortality. There are currently over 5 million individuals in the USA living with heart failure; however, this number is expected to increase significantly as an estimated 550,000 new cases are reported annually [1]. Despite significant progress in the management of patients with heart failure over the last two decades, the natural history of this disease is still punctuated by recurrent hospitalizations for symptom control and disease management. In contrast to the survival benefits observed with novel pharmacological and device-based therapies, the rates of hospitalization for acute decompensation have increased by 175% over the past 25 years. Heart failure remains the most common cause of hospitalization for patients over the age of 65, accounting for 3.4 million physician visits, 6.5 million inpatient hospital days and a cost in excess of 33 billion USD per year [1–3]. As such, heart failure represents a major public health concern.

As the number of hospitalizations for heart failure continues to rise, there is a desperate need for both efficient and efficacious therapies. The overwhelming majority of these hospitalizations for acute decompensations of heart failure (ADHF) are attributable to symptoms of volume overload and are characterized by the abrupt on-

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set of dyspnea secondary to elevated left ventricular filling pressures, with or without pulmonary edema [4–6]. Among patients who present with ADHF, the initial management goals include hemodynamic stabilization, support of ventilation and oxygenation, and symptom relief. Although the safety and efficacy of diuretic therapy have yet to be established in large randomized trials, their clinical use is supported by years of observational experience. As a result, diuretic therapy has become a mainstay in the management of ADHF with administration to over 90% of patients [7].

Unfortunately, the use of diuretic therapy is not without complications. As there have been no long-term studies of diuretic therapy for the treatment of heart failure or ADHF, their effect on morbidity and mortality remains largely unknown; however, observational experience suggests that high diuretic doses (≥80 mg of furosemide or equivalent per day) are associated with worsening renal function, leading to dialysis-increased hospital resource utilization, and high rates of morbidity and mortality [8–10]. Aggressive diuresis may lead to significant electrolyte derangements and intravascular volume depletion, with a resultant decrease in cardiac output and secondary neurohormonal activation.

Furthermore, it is becoming increasingly apparent that current diuretic-based strategies for the treatment of symptomatic volume overload in ADHF are either not being used effectively or do not facilitate adequate decongestion in a large proportion of patients. As such, there is increasing interest in identifying alternative therapies for the treatment of symptomatic volume overload that are clinically efficacious and cost-effective. The extracorporeal removal of fluid by ultrafiltration (UF), whether by peritoneal dialysis, intermittent hemodialysis or hemofiltration, has been explored as a possible therapeutic option for the treatment of ADHF.

UF versus Diuretic Therapy

For UF to gain prominence as a first-line therapy, it must compare favorably to diuretic strategies, the current standard of care. Diuretic therapy is thought to relieve symptomatic pulmonary congestion and volume overload by enhancing renal sodium excretion as well as reducing intracardiac filling pressures via prostaglandin-induced venodilation. Unfortunately, diuretic therapy is often associated with abrupt and prolonged periods of intravascular volume depletion due to a mismatch between the extent of diuresis and the compensatory plasma refill response. This plasma refill rate, i.e. the rate at which fluid is reabsorbed into the intravascular space from the extravascular space, is dependent on a number of factors including the serum oncotic pressure (driven largely by serum albumin), vascular permeability and myocardial function. In patients with advanced heart failure reduced oncotic pressure as well as abnormal vascular permeability and myocardial dysfunction may delay the shift of fluids from the extravascular to the intravascular space and can predispose a patient toward hypotension. This hypotension results in a further decrease in cardiac output and compensatory deleterious activation of the renin-angiotensin system, with the resultant promotion of sodium and water retention effectively negating the ability of diuretics to relieve circulatory congestion [11–13].

Moreover, diuretic therapy results in the production of hypotonic urine (average urine sodium concentration of 60 mEq/l with furosemide) [14]. This persistent sodium excess, combined with the aforementioned neurohormonal activation, results in rapid reaccumulation of water leading to recurrent congestive symptoms, worsening heart failure, and excess morbidity and mortality. As a result nearly half of all patients admitted with ADHF fail to lose weight while 21% actually gain weight during the course of their hospitalization despite aggressive diuretic therapy [5, 15]. Not surprisingly, up to 39% of patients presenting with ADHF remain symptomatic at discharge with resultant high rates of mortality and recidivism (20% 1-month and 50% 6-month readmission rates) [4–6, 16–19].

In contrast, UF has been shown to be effective at reducing the intravascular volume without adversely affecting hemodynamic parameters due to its ability to precisely control the removal of sodium and water from the intravascular space. With vigilant maintenance of the UF rate at a level that is less than or equal to the plasma refill rate, UF has been shown to effectively remove large volumes of plasma water resulting in a progressive decrease in right atrial and pulmonary wedge pressures without deleterious hemodynamic effects, as measured by systemic or pulmonary vascular resistance [20]. Likewise, the ability to precisely control the removal of sodium and water allows the ultrafiltrate extracted from serum during UF therapy to be isotonic and isonatric. As such, UF results in a more effective reduction in total body sodium content when compared to an equal volume of fluid removed from the body by diuretic therapy [21, 22]. Moreover, because solutes are free to cross the semipermeable membrane, large volumes of fluids can be removed without adversely affecting the concentration of solutes or serum electrolytes. In contrast, diuretic therapy is associ-

ated with potassium and magnesium loss; electrolyte abnormalities may predispose an already dysfunctional myocardium to malignant arrhythmias [22].

In direct contrast to diuretic therapy, UF is effective for the treatment of volume overload symptoms in all subsets of patients, including those with relative or absolute diuretic resistance or renal insufficiency. Loop diuretics directly activate the renin-angiotensin-aldosterone system via inhibition of sodium-chloride transport in the macula densa and indirectly by intravascular volume depletion [13, 23]. Regardless of mechanism, stimulation of the renin-angiotensin-aldosterone system has been associated with the development of diuretic resistance which is observed in up to 20–30% of heart failure patients despite an increasingly intensified dosing regimen. Moreover, animal models have demonstrated that administration of loop diuretics and in particular furosemide may increase myocardial aldosterone uptake, leading to enhanced myocardial fibrosis. These alterations in the neurohormonal milieu may explain the observation that furosemide therapy is associated with a shortened time to left ventricular dysfunction when compared to placebo [24]. Likewise, diuretic therapy contributes to impairment in estimated glomerular filtration rate by an unknown series of mechanisms independent of fluid volumes removed [25]. By contrast, multiple studies examining the safety and efficacy of UF have failed to demonstrate a significant change in renal function before and after UF [25–28]. In fact, mechanical fluid removal in patients with refractory volume overload may actually result in improved renal function [29]. This is especially important given that worsening renal function in patients with ADHF is a common clinical dilemma and portends a worse prognosis, especially if it occurs before euvolemia is achieved [30].

These disparate mechanisms potentially underlie the observation that high diuretic doses (greater than or equal to 80 mg furosemide or equivalent per day) are associated with worsening renal function, leading to dialysis-increased hospital resource utilization, and high rates of morbidity and mortality [8–10]. Moreover, there have been no long-term studies of diuretic therapy for the treatment of heart failure or ADHF, and, thus, its effects on morbidity and mortality are unknown.

The Evidence for UF

Although the first significant report of UF for the treatment of volume overload was made in the 1970s, it was not until 20 years later that randomized trials began to appear [31]. In 1994, Agostoni et al. [32] matched – by age, gender and peak oxygen consumption – 16 patients with NYHA functional class II–III symptoms and then randomized them to UF at 500 ml/h or an intravenous bolus of supplemental furosemide (248 mg mean dose) to a goal of removing 1.6 liters of fluid. Both therapies were successful at decreasing biventricular filling pressures at the expense of a compensatory increase in plasma renin activity, norepinephrine levels and aldosterone levels. In the diuretic group, these neurohormones remained elevated for 4 days after the procedure, triggering a prompt reaccumulation of fluid volume with subsequent elevation of ventricular filling pressures, and reoccurrence of lung congestion. Conversely, in the UF group, there was a sustained decrease in the levels of plasma renin, norepinephrine and aldosterone to below control values within the first 48 h resulting in re-equilibration of the volume status to a new lower set point. Only the UF group had an improvement in functional capacity on cardiopulmonary exercise testing [32].

While this trial established the effectiveness of extracorporeal UF for the treatment of symptomatic volume overload, the widespread adoption of central UF has been limited by the complex nature of the procedure, the need for a large-bore centrally inserted dialysis access, high flow rates and large extracorporeal blood volumes as well as the need for specialized training, equipment and monitoring. Therefore this type of central UF has largely been reserved for those patients with hemodynamic instability, diuretic resistance or diuretic intolerance due to progressive renal insufficiency.

With the recent emergence of a novel peripherally inserted UF device, the role of primary UF has been explored. The Aquadex Flex Flow™ Fluid Removal System (CHF Solutions, Minneapolis, Minn., USA) consists of a small, self-contained dual rotary occlusive extracorporeal pump connected to a console allowing for precise control of blood flow and ultrafiltrate removal rates. In contrast to conventional central UF techniques, peripheral venovenous UF (PUF) permits both fluid withdrawal and blood return through smaller (16- to 18-gauge), peripherally inserted venous catheters, thereby allowing for low flow rates and small extracorporeal blood volumes (10–40 vs. >200 ml/min and 33 vs. 75 ml with PUF vs. conventional UF, respectively). The device is designed to monitor the extracorporeal blood circuit via disposable withdrawal and infusion ultrafiltrate pressure sensors and to alert the user about abnormal conditions. Owing to simplicity of design, these new devices have the inherent advantage of not requiring the specialized nurs-

ing or critical-care monitoring associated with conventional hemodialysis or UF.

In 3 separate pilot trials, published between 2003 and 2005, PUF was shown to be a safe and effective procedure for removing large volumes of fluid in hypervolemic heart failure patients. The first of these studies, the SAFE-CHF trial, examined the feasibility and safety of rapid fluid removal via PUF [26]. In this multicenter prospective study, 21 patients with clinical volume overload, predominantly due to congestive heart failure, underwent primary PUF within 12 h of hospitalization (i.e. before any significant administration of intravenous diuretics and/or vasoactive drugs). The primary endpoint of more than 1 liter fluid removal in less than 8 h was achieved in 23 of 25 treatments. On average 2.6 ± 1 liters of ultrafiltrate (maximum 3.725 liters) were removed per treatment (treatment period 6:43 ± 1:47 h:min). Following PUF, overall patient weights decreased significantly from 91.9 ± 17.5 to 89.3 ± 17.3 kg ($p < 0.0001$) without any adverse changes in heart rate, blood pressure, electrolytes or hematocrit.

The RAPID-CHF trial was a multicenter, randomized, controlled study comparing a single 8-hour PUF session to usual diuretic therapy for hospitalized ADHF patients [27]. Compared to the 20 patients treated with diuretics, the 20 patients randomized to PUF had a greater median fluid removal after 24 and 48 h (4.65 vs. 2.84 liters with the usual care, $p = 0.001$, and 8.4 vs. 5.4 liters with the usual care, $p = 0.012$, respectively). This was associated with a significant improvement in the primary outcome of weight loss at 24 h in the PUF group (2.5 vs. 1.9 kg with the usual care, $p = 0.24$). Dyspnea and congestive heart failure symptoms were significantly improved in the PUF group at 48 h ($p = 0.039$ and $p = 0.023$, respectively) with no adverse effects on hemodynamic or renal functional parameters.

The SAFE-CHF and RAPID-CHF trials, in concert, established the feasibility, safety and efficacy of early PUF for the management of the volume overload and congestion associated with ADHF. The EUPHORIA trial went on to demonstrate that among ADHF patients with diuretic resistance or renal insufficiency a PUF-based treatment strategy, as compared to diuretics, was associated with a significantly shorter time to discharge and decreased rehospitalization at 1 year [28].

These 3 small studies were followed by the largest and most significant trial to date examining the role of PUF in the ADHF population. Investigators in the UNLOAD trial randomized 200 patients hospitalized for ADHF with evidence of volume overload to early PUF at a rate of ≤500 ml/h or standard intravenous diuretic therapy [33]. In contrast to earlier studies, renal dysfunction and/or anticipated diuretic resistance were not entry criteria. When compared to standard care with intravenous diuretics, patients assigned to PUF had greater 48-hour weight (5.0 ± 3.1 vs. 3.1 ± 3.5 kg; $p = 0.001$) and fluid losses (4.6 vs. 3.3 liters; $p = 0.001$) without adverse effect on renal function. Correspondingly, at 90 days, the group assigned to PUF comprised fewer heart failure patients rehospitalized for heart failure (18 vs. 32%; $p = 0.037$), fewer heart failure rehospitalizations (0.22 ± 0.54 vs. 0.46 ± 0.76; $p = 0.022$), fewer rehospitalization days per patient (1.4 ± 4.2 vs. 3.8 ± 8.5; $p = 0.022$) and fewer unscheduled emergency department or office visits (21 vs. 44%; $p = 0.009$) when compared to standard care. There was no difference in the mortality rate (10 vs. 13% in standard care) although the trial was not powered to detect a difference. Improvements were noted in B-type natriuretic peptide levels, NYHA class, MLWHF scores, global assessment scores and 6-min walk distance between the PUF and standard care groups.

In a subanalysis of the UNLOAD trial, PUF was compared separately to bolus intravenous diuretic regimens as well as to a continuous loop diuretic infusion. In both cases PUF resulted in greater weight loss (5.0 vs. 2.5 kg with bolus therapy and 3.6 kg with continuous infusion). The 90-day rehospitalization rates appeared lower with PUF when compared to both diuretic strategies (18 vs. 29% with bolus therapy and 39% with continuous infusion) [34].

In terms of safety endpoints, there was no difference in the proportion of patients experiencing a rise in serum creatinine >0.3 mg/dl at any of 3 separate time points (14.4 vs. 7.7% at 24 h, $p = 0.528$; 26.5 vs. 20.3% at 48 h, $p = 0.43$; 22.6 vs. 19.8% at hospital discharge, $p = 0.709$). Rates of hypotension were similar in the two groups (4 vs. 3%). There were no clinically significant changes in serum blood urea nitrogen, sodium, chloride and bicarbonate in either group, but there was less hypokalemia ($K^+ < 3.5$ mEq/l) with PUF (1 vs. 12%; $p = 0.018$). A higher incidence of bleeding was noted in the standard care arm (1 vs. 7%; $p = 0.032$).

Perhaps the most interesting and clinically compelling finding from the available evidence is that PUF-treated patients experience sustained clinical improvements up to 90 days after discharge [28, 32, 33]. Various theories have been postulated to explain the underlying mechanism by which PUF is able to facilitate a prolonged beneficial effect. These include: (1) more effective sodium and water clearance, (2) improved pulmonary vascular

resistance due to reduction of extracellular pulmonary edema, (3) enhanced norepinephrine clearance from the circulation, (4) 'resetting' of neurohormonal activation via baroreceptor-mediated reflexes and (5) direct removal of myocardial depressant factors. Furthermore since intermittent UF has been shown to restore diuretic effectiveness, it has been postulated that simply minimizing long-term diuretic exposure may result in enhanced myocardial function [35]. It remains unknown whether the sustained clinical benefits observed with PUF therapy will translate into hard cardiovascular outcomes such as improved survival.

Conclusion

Heart failure is a growing public health concern with increasing numbers of individuals being diagnosed annually. Unfortunately the clinical course is characterized by recurrent hospitalizations for acute decompensation, the vast majority of which are associated with symptomatic volume overload. Unfortunately, despite standard care with intravenous diuretics, a significant number of patients remain clinically volume overloaded at the time of hospital discharge, and, as a result, subsequent readmission rates and mortality remain high.

As the number of ADHF hospitalizations continues to rise, there is a desperate need for both efficient and efficacious therapies. Extracorporeal PUF is a safe and effective method of fluid removal shown to improve congestive symptoms, lower intracardiac filling pressures, improve cardiac output, decrease neurohormone levels, correct hyponatremia, restore diuresis and reduce diuretic requirements. Compared to conventional diuretic-based treatment, PUF facilitates quicker and more complete removal of fluid and sodium. Clinically this translates to sustained weight loss and decreased 90-day heart failure resource utilization.

In response to recent trials, the 2009 American College of Cardiology/American Heart Association guidelines have upgraded UF to a class IIa recommendation (level of evidence grade B) for patients with refractory congestion not responding to medical therapy. It should be highlighted that UF has received a higher level of evidence than standard heart failure therapies such as sodium and fluid restriction, increasing loop diuretic dose, continuous loop diuretic infusions, adjuvant diuretics (i.e. metolazone) or intravenous inotropes and vasodilators (i.e. intravenous nitroglycerin and nesiritide) [36]. As such, PUF is likely to play an increasing role in the management of ADHF patients.

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A Selective Cytopheretic Inhibitory Device to Treat the Immunological Dysregulation of Acute and Chronic Renal Failure

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Cytopheretic inhibitory device · Renal failure, acute and chronic · Immunological dysregulation

Abstract
Background: The poor outcomes in patients with acute kidney injury (AKI) and end-stage renal disease (ESRD) on chronic dialysis are due to immune dysregulation associated with these disorders. Evolving evidence suggests that the kidney, and specifically renal epithelial cells, plays an important role in the immunological response of leukocytes under disease states. ***Method:*** In this regard, the development of two therapeutic approaches utilizing renal epithelial cells and 'smart' immunomodulatory membranes has been tested in preclinical animal models and clinical trials. ***Results:*** These two approaches have been demonstrated in phase II human trials to improve the survival of intensive care unit patients with AKI and multiorgan failure. The use of a 'smart' immunomodulatory membrane is also being evaluated in a small exploratory clinical trial to assess its effects on immunoregulation in ESRD patients requiring chronic hemodialysis. ***Conclusions:*** The use of renal progenitor/stem cell therapy and/or cytopheretic membranes may result in effective treatments to alter the dysregulated immunological state of acute or chronic renal failure and improve the outcomes of these diseases.

Introduction

The kidney plays a critical role in maintaining body water and electrolyte homeostasis through excretory and regulatory functions of the glomerulus and tubule. Current dialysis therapies, including hemodialysis (HD), hemofiltration and peritoneal dialysis, replace these functions by removing waste products and excess electrolytes from blood using artificial or natural semipermeable membranes. The kidney, however, has many other roles. It is regarded as an important endocrine organ, responsible for the secretion of hormones that are critical in maintaining hemodynamics (renin, angiotension II, prostaglandins, nitric oxide, endothelin and bradykinin), red blood cell production (erythropoietin) and bone metabolism (1,25-dihydroxyvitamin D_3 or calcitriol) [1]. It synthesizes glutathione and free radical-scavenging enzymes and provides gluconeogenic and ammoniagenic capabilities [2, 3]. Catabolism of low-molecular-weight proteins, including peptide hormones, cytokines and growth factors, is also accomplished by the kidney [4].

The kidney also has a less recognized immunoregulatory function. The mammalian renal proximal tubule

[1] A list of the members of the RAD Investigator Group is given in the Appendix.

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cells are immunologically active. They are antigen-presenting cells [5] that have costimulatory molecules [6] that synthesize and process a variety of inflammatory cytokines [6, 7]. The roles of the renal tubule cells in glutathione metabolism [2], regulation of vitamin D, and production and catabolism of multiple cytokines [1] are critical to immunoregulation to maintain tissue integrity and host defense under stress conditions [8]. A growing body of evidence indicates that inflammation plays a major role in the pathophysiology of acute kidney injury (AKI) and end-stage renal disease (ESRD), so that these disorders are, to some extent, inflammatory disease processes [9].

The cause of death in AKI patients is usually the development of a systemic inflammatory response syndrome (SIRS) [10]. The exceptionally high mortality associated with SIRS is caused in part by the development of the multiple system organ failure syndrome in a subset of patients and is not ameliorated by conventional renal replacement therapy (CRRT), which treats volume overload, uremia, acidosis and electrolyte derangements [10]. The propensity of patients with AKI to develop SIRS and sepsis suggests that renal function, specifically renal tubule cell function, plays a critical immunomodulatory role in individuals under stress states [8].

Inflammatory cascades initiated by endothelial dysfunction in SIRS are further dysregulated in the setting of AKI. These cascades are evidenced by recent human studies demonstrating that the levels of the proinflammatory cytokines interleukin (IL)-6 and IL-8 in the plasma predict mortality in patients with AKI [11]. Strategies that modulate the inflammatory response provide significant beneficial effects in experimental AKI [12]. In this regard, a promising direction to improve the outcome of AKI is to better understand and interrupt the pathophysiological processes that are activated in AKI, resulting in distant multiorgan dysfunction and eventually death. Technologies directed at disrupting multiorgan dysfunction may well be the next major improvement to enhance the clinical outcome of these critically ill patients. Renal cell therapy and the development of 'smart' membranes designed to modify immunological dysregulation may provide effective new approaches to this disorder.

The disorder of ESRD is characterized by immune dysregulation presenting with clinical and biological immune deficiency despite chronic activation of immunocompetent cells, especially circulating neutrophils and monocytes [13]. This immune dysregulation develops in patients with chronic renal insufficiency prior to the initiation of chronic dialysis therapy, although dialysis causes further inflammatory activation [14]. This chronic inflammatory disease state is characterized by both neutrophil and monocyte dysfunction. Neutrophil phagocytosis and microbe killing are reduced in ESRD patients compared to normal individuals in the presence of spontaneous neutrophil and monocyte activation and reactive oxygen species generation [15–17]. High levels of proinflammatory cytokine serum levels, including IL-6, tumor necrosis factor-α and IL-1β, are found in ESRD patients, with high levels of production observed in isolated monocytes in these patients [17]. In fact, serum levels of IL-6 correlate well with IL-6 production rates of isolated monocytes from individual ESRD patients [18, 19].

The clinical ramifications of this dysregulated immune state in chronic renal insufficiency and ESRD have profound implications. The neutrophil dysfunction translates into the fact that infection is the second most common cause of death, approaching 25% of the annual mortality rate in HD patients [20]. This infection complication rate is not diminished with higher dialysis dose or high-flux membrane utilization [21]. Mortality due to sepsis occurs approximately 250-fold more commonly in these patients compared to the general population [22]. The chronic proinflammatory state, as reflected with elevated biomarkers, specifically C-reactive protein and IL-6, as well as activation of circulating leukocytes [23–26], is also highly correlated with accelerated atherosclerosis and early mortality in ESRD patients. Cardiovascular disease processes account for roughly 50% of the annual mortality rates in Europe and the USA. In fact, the discrepancy between the vascular and chronological ages of ESRD patients reflects the accelerated atherosclerotic process that develops in these patients [27].

The immune dysregulated state of chronic renal insufficiency and ESRD is also manifested in other ways. ESRD patients exhibit high rates of autoimmune diseases and neoplasia. They demonstrate cutaneous anergy in delayed hypersensitivity reactions and respond poorly to vaccination [28]. The better understanding of these immunological processes should provide insight into new approaches to treat this immunological disturbance.

Leukocytes are major contributors to the pathogenesis and progression of many clinical inflammatory disorders, including acute and chronic renal failure. Many therapeutic approaches are under investigation to limit the activation and tissue accumulation of leukocytes at sites of inflammation to minimize tissue destruction and disease progression [29, 30]. A synthetic membrane device with the ability to bind and inhibit activated leuko-

cytes along a continuous renal replacement extracorporeal circuit has been developed and is called the selective cytopheretic inhibitory device (SCD). This device incorporates a low-velocity and low-shear-force blood path around a bundled collection of dialysis hollow fibers. In conjunction with regional citrate anticoagulation, this coupling of events within the device results in the sequestration of activated leukocytes in conjunction with a low ionized-calcium environment due to citrate in the blood circuit to inhibit the release of inflammatory and destructive enzymes and cytokines from the membrane-bound neutrophils and monocytes [31, 32].

The SCD was evaluated in a porcine model of *Escherichia coli*-induced septic shock and demonstrated an ability to lower neutrophil activity (serum myeloperoxidase and CD11b cell surface expression), diminish neutrophil tissue invasion, decrease systemic capillary leak, preserve cardiac output and mean arterial pressure, and prolong survival time [31].

The development of this device arose during the preclinical and clinical evaluation of a renal tubule assist device (RAD) [33, 34] containing adult human renal tubule cells. This report presents a subset analysis of a phase IIb clinical study evaluating RAD therapy. Subsets of patients were treated with either systemic heparin or regional citrate anticoagulation and received either a sham non-cell- (SCD) or a cell-containing cartridge. With the compelling data derived from the large-animal studies with the SCD [31, 32], these subsets of patients were more carefully analyzed to compare the mortality rates in subjects treated with only sham (non-cell-containing) cartridges with systemic heparin anticoagulation or citrate regional anticoagulation. The unanticipated but compelling results are presented here and are limited to the non-cell-containing SCD subgroup.

Methods

This prospective, randomized, blinded, controlled clinical trial was conducted at 15 medical centers in the USA. The study was carried out under a corporate-sponsored (RenaMed Biologics Inc., Lincoln, R.I., USA) investigational new drug application in accordance with the Declaration of Helsinki and good clinical practice. The institutional review board at each medical center approved the protocol.

Adult male and female (nonpregnant) patients, aged 18–80 years, who required CRRT for acute renal failure (ARF) secondary to AKI in an intensive care unit (ICU) setting were enrolled in the study. Eligible patients were also required to have at least 1 nonrenal organ failure (sequential organ failure assessment, SOFA, score >2) or presence of sepsis. Exclusion criteria were irreversible brain damage, presence of organ transplant, preexisting chronic renal insufficiency (baseline serum creatinine ≥3.0 mg/dl for men or ≥2.5 mg/dl for women), chronic immunosuppression, Xigris therapy at time of randomization and do-not-resuscitate status. These criteria are more fully detailed in a previous publication [34]. SOFA scores were assessed for each patient upon enrollment.

Patients were randomly assigned to receive continuous venovenous hemofiltration (CVVH) with a noncell control sham cartridge (SCD). The extracorporeal circuit for these treatments is similar to that of the prior RAD phase IIa study [34]. Patients were anticoagulated with heparin or citrate depending upon the clinical investigator's decision or institutional protocol. If citrate regional anticoagulation was selected, citrate and calcium infusions were determined by the institution's standard practice protocols.

The SCD cartridge was a standard polysulfone hemofiltration cartridge (F40; Fresenius AG, Bad Homburg, Germany). The proprietary circuit [32] was designed to diminish the shear stress (SS) of blood along the hollow fibers, from greater than arterial SS (>30 dyn/cm^2) in the first cartridge to levels below venular SS (<1 dyn/cm^2) in the SCD cartridge, to provide an environment for leukocyte adherence [35].

The primary objective of this study was to evaluate the impact of the RAD on all-cause mortality on days 28, 60 and 90. Safety assessments included monitoring for adverse events and laboratory evaluations. An independent data safety and monitoring committee conducted unblinded and efficacy assessments at periodic intervals. Of importance, only the results of the noncell control group are presented in detail in this report.

To evaluate the interactions of the SCD with perfusing blood, the SCD extracapillary space was rinsed clear of blood and fixed intact by perfusion with 4% paraformaldehyde. Adherent cells were made permeable with 0.1% Triton-X 100 and nuclei labeled with diamidinophenylindole. Access ports were cut through the polycarbonate shell, and fibers were excised from the cartridge. Alexa Fluor 488-conjugated phalloidin was used to specifically label F-actin in cells attached to individual fibers. Fibers were etched with cyclohexanone to increase translucence of polysulfone fibers prior to imaging with a Nikon TE2000 confocal microscope. Cells adherent to the extracapillary space side of the membranes were eluted after treatment by flushing with a nonenzymatic release buffer containing 0.2% ethylenediamine tetraacetic acid. Eluted cells were centrifuged after lysis of red blood cells and stained. The number of cartridges analyzed in this manner was too small to provide comparisons between the citrate and heparin groups.

Results

Twenty-four patients were randomly assigned to the noncell cartridge group that received either systemic heparin (n = 12) or regional citrate (n = 12) anticoagulation. As detailed in table 1, baseline demographics and acuity of illness by SOFA scores were similar between the two groups. Total serum calcium levels were, on average, slightly higher (not statistically) during the SCD treatment in the citrate group than in the heparin group.

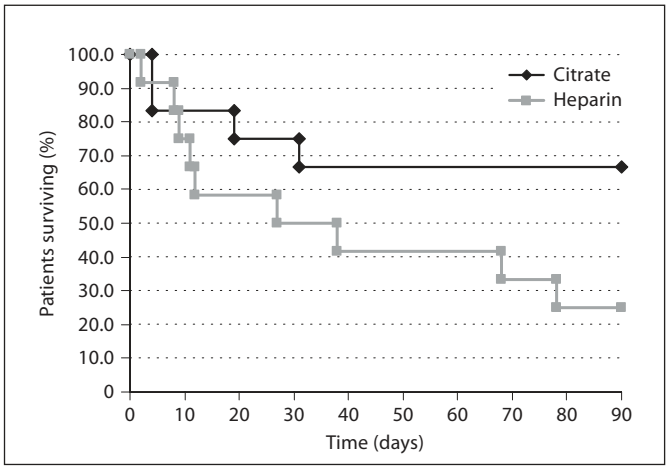

Fig. 1. Survival plot; n = 12 for each group.

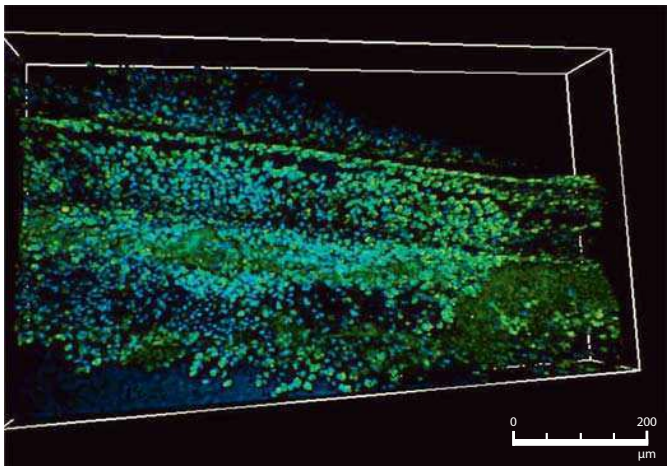

Fig. 2. Confocal micrograph of fluorescently stained leukocytes adhering to the external surface of hollow fibers in the dialysis cartridge.

Figure 1 displays the survival plot for the two subgroups. The mortality rate in the heparin patient group was 50 versus 25% in the citrate-treated group (n = 12 for each treatment arm) at 28 days and 75 versus 33% at 90 days (χ^2 <0.05). The subgroups were comparable, with similar SOFA scores, organ failure number and incidence of sepsis (table 1).

Discontinuation of the CVVH + noncell cartridge (SCD) in the AKI patients prior to 72 h was due to clotting of the perfusion circuit. In all cases, clotting originated in the CVVH system and then propagated to the noncell circuit/cartridge. The median time of blood flow patency of the circuit was 62 h. Compared to single-cartridge CRRT treatment, this time-to-failure rate is favorable [36, 37].

Device-related medical events resulted in discontinuation of 1 patient assigned to receive the SCD (4%). The reason for discontinuation of the CVVH + noncell cartridge in the heparin group was a low platelet count. Thrombocytopenia is a recognized complication of critically ill patients in the ICU. Review of the total white blood cell, neutrophil and platelet counts obtained daily during the treatment period did not show any substantive change from baseline values in either of the two groups (table 2). In fact, except for the single patient noted above, none of the patients developed absolute neutropenia or thrombocytopenia during the treatment period.

As detailed in figure 2, immunofluorescent staining of a small number of SCD cartridges after patient treatment demonstrated adherent leukocytes on the outer surface of the hemofilter membranes. Elution of cells from the extracapillary space demonstrated that >90% of the cells were neutrophils.

Table 1. SCD baseline subgroup demographics and clinical results

	Heparin (n = 12)	Citrate (n = 12)
Age, years	61.4 ± 1.4	57.7 ± 5.3
Male/female	10/2	7/5
SOFA score	13.4 ± 1.1	12.2 ± 0.9
MOF	4.17 ± 0.46	3.93 ± 0.36
Sepsis, %	58	58
28-day survival, %	50	75
90-day survival, %	25	67

MOF = Multiorgan failure number, with organ failure defined as SOFA organ-specific score ≥2.

All serious adverse events/complications during the first 28 days after randomization were reported to the Medical Monitor within 24 h in the study. Serious adverse events were reported in 52% (13 of 25 patients) receiving CVVH + noncell cartridge. The reported serious adverse events were consistent with a seriously ill ICU patient population with ARF receiving CVVH, and the data safety and monitoring committee found no consistent serious adverse event attributable to the SCD.

Table 2. White blood cell, neutrophil and platelet counts (n × 10^3)

	White blood cells				Neutrophils				Platelets			
	B	D1	D2	D3	B	D1	D2	D3	B	D1	D2	D3
Citrate												
Number	12	12	10	8	9	10	8	8	12	12	10	7
Mean ± SE	21.0 ± 3.8	20.2 ± 3.7	20.9 ± 4.7	21.7 ± 5.5	14.0 ± 2.8	15.3 ± 3.2	15.0 ± 3.5	16.4 ± 4.2	173 ± 42	137 ± 28	156 ± 34	143 ± 42
Heparin												
Number	12	12	11	8	9	7	7	7	12	12	11	8
Mean ± SE	17.2 ± 1.9	17.1 ± 1.9	16.3 ± 1.3	15.5 ± 1.8	15.6 ± 1.0	17.1 ± 2.3	14.4 ± 1.8	15.3 ± 2.5	112 ± 19	102 ± 21	93 ± 19	108 ± 21

B = Baseline; D1 = treatment day 1; D2 = treatment day 2; D3 = treatment day 3; SE = standard error. The number for each day may decline from baseline due to discontinuation of SCD treatment or data not obtained.

Discussion

In this randomized, blinded, multicenter clinical study, the addition of a second hemofiltration cartridge with an altered blood flow path to lower blood velocity and shear rates in series with a conventional CVVH circuit significantly reduced all-cause mortality at 90 days in ICU patients with AKI. This improved survival was demonstrated in the presence of regional citrate anticoagulation when compared to systemic heparin anticoagulation. The mortality differences between the citrate and heparin treatment groups could not be attributed to the acuity of illness in the two groups, since the average SOFA scores were similar for both sets of patients. Treatment with the noncell cartridge was well tolerated, without measurable effects on hematological parameters, including neutrophil and platelet counts, and with an adverse event profile expected for a seriously ill population in the ICU with AKI. The blood flow patency of the double-cartridge circuit was comparable to single-cartridge CRRT modalities. Although citrate anticoagulation in a conventional single-cartridge CRRT protocol improves filter patency compared to heparin anticoagulation [38–40], a difference in mortality rates has not been observed in most studies [39, 40], but a recent publication suggests an impact on mortality rates in selected subsets of patients [41]. The impact of this novel device design on patient survival was unanticipated when the subgroup analysis was performed and deserves further evaluation.

In this regard, current research endeavors have been predominantly nephrocentric in their focus. Major efforts are ongoing to identify biomarkers in order to diagnose AKI earlier and more accurately so that preventive measures to minimize renal injury may change patient outcome [42]. The use of stem cells to enhance the rate of recovery from AKI is also being investigated by multiple groups [43], anticipating that earlier renal recovery may lessen the morbid and mortal consequence of ARF. Although these directions of inquiry may ultimately prove useful as adjunct approaches to AKI-induced ARF, a more promising direction is to better understand and interrupt the pathophysiological processes which are activated in AKI, which results in distant-organ dysfunction, multiorgan failure and eventually death. During the maintenance phase of ARF, while HD/hemofiltration techniques are being utilized, the patient continues a downhill course into multiorgan failure. Technologies directed at disrupting these multiorgan dysfunctions may well be the next major improvement to enhance the clinical outcome of these critically ill patients. Our group has focused on two major areas of evaluation. The first is the recognition that current renal substitution therapy only provides the small solute clearance function of the kidney but not the metabolic endocrine functions of the kidney. Similar to the clinical evidence that kidney transplantation markedly prolongs survival and improves health-related quality of life compared to patients remaining on dialysis [44], the replacement of renal parenchymal cell functions in AKI may change the natural history of this disorder. A tissue engineering approach utilizing an extracorporeal renal tubule cell assist device in series to a hemofilter has had early clinical success [34]. Preclinical and clinical data suggest that the efficacy of renal cell therapy in AKI is due to an immunomodulatory role of renal epithelial cells in the excessive acute response of the innate immunological system [45–47].

A second approach is the recognition that AKI results in a profound inflammatory response state resulting in microvascular dysfunction in distant organs [12, 48]. In this regard, leukocyte activation plays a central role in

these acute inflammatory states [30]. Disruption of the activation process of circulating leukocytes may limit microvascular damage and multiorgan dysfunction. This report presents the clinical evaluation of a synthetic membrane extracorporeal device to bind and inhibit circulating leukocytes in inflammatory conditions. This SCD was developed with a proprietary design process utilizing polysulfone hollow fibers. The device and the extracorporeal blood circuit were formulated [32] to diminish the SS of blood along the hollow fibers from greater than arterial SS (>30 dyn/cm^2) in the first cartridge to levels below venular SS (<1 dyn/cm^2) in the SCD cartridge to provide an environment for leukocyte adherence [35].

The SCD was evaluated in a porcine model of *E. coli*-induced septic shock and demonstrated an ability to lower neutrophil activity (serum myeloperoxidase and CD11b cell surface expression), diminish neutrophil tissue invasion, decrease systemic capillary leak, preserve cardiac output and mean arterial pressure, and prolong survival time [31] to provide support for an immunomodulatory effect in SIRS.

The treatment approach to the immune dysregulation in CRF and ESRD has also been disappointing. Multiple interventions designed to improve survival and diminish cardiovascular events have been shown to be ineffective [49]. The recent AURORA study evaluating the effect of statin therapy to ameliorate mortality or cardiovascular events was unsuccessful in lowering the cardiovascular risk among chronic HD patients despite reductions in low-density lipoprotein cholesterol levels [50]. The potential of thalidomide, a drug with immunomodulatory and anti-inflammatory effects, has been considered along with anti-tumor necrosis factor-α-therapy [27]. So far, however, no intervention has been shown to be effective.

Rather than focusing on cytokines and cholesterol, the SCD may provide a novel approach to this immune dysregulation. Accordingly, our group has recently evaluated the SCD in a group of chronic HD patients with elevated C-reactive protein values. The SCD was placed in series with a hemodialyzer cartridge in a conventional dialysis pump system. Fifteen chronic HD patients were treated for 4 h under standard HD and SCD therapy to ameliorate leukocyte activation. Leukocyte activation parameters were assessed before, during and after treatment with flow cytometry, along with blood cytokines before and after standard and SCD dialysis therapy. During SCD treatment, multiple immunologic parameters were altered. Ongoing studies are evaluating dose-response effects and the mechanism of action of this device. This approach may be further tested in clinical studies as an innovative intervention to ameliorate the immunologic dysregulated state of ESRD and change the natural history of chronic renal failure.

Conclusion

New therapeutic approaches to alter the acute inflammatory response in AKI and the chronic proinflammatory state of ESRD present a paradigm shift in addressing the primary pathophysiology that leads to the poor outcomes of these disorders. The use of renal cell therapy and cytopheretic membranes has demonstrated early clinical success and may lead to better therapies to alter the dysregulated immunological states of renal failure and improve the clinical outcomes of these disease processes.

Appendix

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Conflicts of Interest

H.D.H. and J.T.S. are shareholders of Nephrion, Inc. (a corporate restructuring of RenaMed Biologics, Inc., which sponsored the SCD phase II clinical study), and H.D.H. is also a shareholder of Innovative BioTherapies, Inc. Nephrion and Innovative BioTherapies are biotechnology spin-out companies of the University of Michigan.

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Citrate Anticoagulation for Continuous Renal Replacement Therapy in the Critically Ill

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Key Words

Heparin · Citrate · Hemofiltration · Hemodialysis · Anticoagulation · Hemorrhage · Sepsis · Organ failure · Biocompatibility · Survival

Abstract

Background: Heparins are used for circuit anticoagulation during continuous renal replacement therapy (CRRT). Because heparins cause systemic anticoagulation, they increase the risk of bleeding. Citrate provides regional anticoagulation. Since citrate is a buffer as well, its use has metabolic consequences. The preferential use of citrate therefore remains controversial. ***Methods:*** A synthesis was performed of published studies comparing citrate to heparin for anticoagulation in CRRT with specific regard to feasibility, efficacy and safety. Search of the literature was made to explain the reported superiority of citrate. ***Results:*** Citrate provides good metabolic control if and when a well-designed protocol is strictly followed. Randomized studies report similar or longer circuit survival with citrate compared to heparin and less bleeding. The largest randomized trial up to now found that citrate was better tolerated than heparin and improved patient and kidney survival, especially in patients after surgery, with sepsis, a high degree of organ failure or younger age. Both citrate and heparin interfere with inflammation. ***Conclusion:*** During critical illness, regional anticoagulation with citrate for CRRT seems superior to heparin anticoagulation concerning tolerance and safety, mainly due to less bleeding. Whether circuit survival is better depends on the modality. In addition, citrate seems to improve patient and kidney survival. This finding needs to be confirmed. Citrate seems to confer a specific benefit in severe organ failure and sepsis. To what extent citrate protects or heparin does harm in the setting of multiple organ failure needs to be unraveled.

Introduction

To prevent clotting in the extracorporeal circuit for continuous renal replacement therapy (CRRT), anticoagulation is generally required. Heparins are the classical choice. Because heparins cause systemic anticoagulation, their main drawback is bleeding. Critically ill patients have an increased likelihood of bleeding due to recent surgery, trauma, mucosal lesions and coagulopathy. This is especially the case when heparin is administered continuously as in CRRT. Citrate anticoagulation is an alternative [1]. Citrate is administered in the extracorporeal circuit. It decreases ionized calcium by chelation. Because calcium is a cofactor in the coagulation cascade, the generation of thrombin is inhibited. Free and calcium-bound citrate is partially removed by convection or diffusion [2, 3] and partially enters the systemic circulation,

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where ionized calcium rises again due to the dilution of extracorporeal blood, the liberation of the chelated calcium when citrate is metabolized and the replacement of calcium. As a result, citrate does not confer systemic anticoagulation. Regional anticoagulation with citrate therefore seems attractive. Nevertheless, the reluctance to use citrate is still great because the intervention is difficult to understand and complex, mainly because citrate is a buffer as well.

The present contribution highlights the metabolic consequences, tolerance and limitations of citrate, its clinical feasibility, safety, efficacy and its specific benefits. Up to now, 4 randomized controlled studies comparing citrate to heparin have appeared as full papers evaluating a total of 296 patients [1–4]. I will particularly refer to our recent large randomized controlled trial comparing citrate to the low-molecular-weight heparin nadroparin for anticoagulation during continuous venovenous hemofiltration (CVVH).

Feasibility of Citrate

After the first reports of Mehta et al. [5], a wide variety of citrate systems for CRRT have been described. More than 50 hits are encountered in a Medline search. Among them are systems for continuous venovenous hemodialysis, pre- and postdilution CVVH, continuous hemodiafiltration and for different doses of CRRT (1.5–4 l/h; summarized in the electronic supplementary material of Oudemans-van Straaten et al. [6]). Studies claim superior safety, feasibility and flexibility of their system. However, none of the systems has proven its superiority. Each system has its specified composition of fluids and its own rules to titrate anticoagulation, correct metabolic acidosis or alkalosis, hypo- or hypercalcemia, depending on the modality and the solutions in use. Up to recently, citrate anticoagulation has not been incorporated in the soft- and hardware of the CRRT device, and dedicated fluids have not been certified and registered for citrate anticoagulation for CRRT.

Handling of Anticoagulation

Anticoagulation can be monitored by measuring postfilter ionized calcium, aiming at a concentration less than 0.35 mmol/l. It should be noted that changing citrate flow to optimize anticoagulation alters the buffer supply to the patient. For that reason, some protocols prefer a fixed citrate-to-blood flow ratio, thus simplifying the method and increasing metabolic stability. The *anticoagulant strength* of a citrate solution depends on its citrate concentration. Citrate solutions for postdilution CVVH/hemodialysis contain 133–1,000 mmol citrate per liter and fluids for predilution CVVH/hemodiafiltration 11–23 mmol citrate per liter [6].

Citrate and Acid-Base Control

Apart from being an anticoagulant, citrate is a buffer substrate. The amount of citrate entering the body depends on citrate dose, its concentration in the filter and its amount removed by filtration and dialysis. This amount can be easily calculated because the sieving coefficient is about 1 [7]. Citrate enters the mitochondria as citric acid, which is metabolized in the Krebs cycle, mainly in the liver, but also in skeletal muscle and renal cortex, according to the overall formula:

$$Na_3citrate + 3H_2CO_3 \rightarrow citric\ acid\ (C_6H_8O_7) + 3NaHCO_3.$$

As a consequence, anticoagulation with citrate provides sodium and bicarbonate, and the sodium and buffer content of the dialysis and replacement fluids needs to be adjusted. The *buffer strength* of the citrate solution depends on the accompanying kations, sodium and hydrogen. One mole of trisodium citrate provides 3 moles of bicarbonate. In some solutions, all kations are sodium [1]; in our solution, 10% of the kations are hydrogen (table 1) and in the ACD-A solution, 30% of them are hydrogen. Replacing sodium by hydrogen reduces buffer strength accordingly.

Safety

Safety of citrate concerns metabolic complications, consequences of user mistakes and protocol violations, and the major advantage is that citrate does not increase the patient's risk of bleeding.

Metabolic Consequences

Anticoagulation with citrate has complex metabolic consequences, which are related to the dual effects of citrate being both anticoagulant and buffer. Manipulation of citrate or blood flow, ultrafiltrate, dialysate or replacement rates and their mutual relation changes the amount of buffer substrate entering the patient's circulation. Reported derangements include metabolic alkalosis and acidosis, hyper- and hyponatremia, and hypocalcemia. The local protocol should describe how to adjust flows under

different conditions. The incidence of metabolic complications depends on the rules and flexibility of the protocol. Derangements often result from protocol violation. Training of nurses should include alertness to acid-base and calcium control, and strict compliance to the protocol. Pop-ups in the patient data management system are very helpful at the bedside.

The main risk of citrate anticoagulation consists of the uncontrolled systemic infusion of a hypertonic citrate solution causing acute and deep hypocalcemia, hypotension and possibly cardiac arrest. Treatment consists of immediate calcium infusion and discontinuation of citrate infusion. Nowadays, the safety of the method has been greatly improved by the incorporation of a citrate module in the CRRT device.

Tolerance and Limitations

Citrate tolerance depends on the amount of citrate entering the body and the capacity to metabolize citrate. Though the sieving coefficient of citrate is similar using hemofiltration or hemodialysis, dialysis can be performed at lower blood flows. Because the citrate dose is titrated to blood flow, dialysis requires a lower citrate dose, and the amount entering the body is thus lower when dialysis is used. Metabolism is diminished in conditions of liver failure or poor tissue perfusion [8, 9]. If metabolism is insufficient, citrate accumulates. Monitoring of the serum citrate concentration is not feasible in daily practice. This is no limitation because citrate is not toxic in itself. Accumulation offers adverse effects by causing hypocalcemia in the systemic circulation. Ionized calcium is monitored and corrected by calcium replacement. Citrate accumulation concomitantly increases total calcium concentration. Ionized hypocalcemia is the most sensitive indicator of citrate accumulation, but it has other causes, including insufficient replacement. The total-to-ionized calcium ratio seems more specific to detect citrate accumulation, at least regarding its side effects [10, 11]. Accumulation additionally leads to metabolic acidosis because citrate is not used as a buffer. Accumulation of citrate is therefore easily detectable with standard monitoring. A rise of the total-to-ionized calcium ratio above 2.25 should trigger the clinician to reduce the citrate dose, replace calcium and increase bicarbonate replacement as metabolic acidosis develops as well. With these safe limits, citrate accumulation causes no clinical symptoms. Because liver failure patients have an increased likelihood for bleeding, it is our policy now to give a trial of citrate, measure the calcium ratio after 2 h and reduce the dose until the ratio is in the safe range.

Table 1. Main characteristics of the citrate protocol [4]

Modality	Postdilution CVVH
CVVH dose	2–4 l/h
Blood flow	150–240 mmol/l
Citrate solution (prefilter)	Citrate 500 mmol/l Sodium 1,352 mmol/l Hydrogen 148 mmol/l
Citrate dose	3 mmol/l blood flow
Replacement fluids[1] (postfilter)	Buffer-free[2] Bicarbonate-buffered[3]
Additional supplementation of Ca/Mg chloride	Calcium 0–0.4 mmol/h[4] Magnesium 0–0.24 mmol/h

[1] Adjusted to acid-base balance. Default for 4 l/h half buffer-free, half bicarbonate-buffered and for 2 l/h pure buffer-free.

[2] Containing Na^+ 109.5 mmol/l, K^+ 2.0 mmol/l, Ca^{2+} 1.81 mmol/l, Mg^{2+} 0.52 mmol/l, Cl^- 116.2 mmol/l, lactate 3 mmol/l, glucose 1 g/l.

[3] Containing Na^+ 140 mmol/l, K^+ 2.0 mmol/l, Ca^{2+} 1.25 mmol/l, Mg^{2+} 1.00 mmol/l, Cl^- 107 mmol/l, lactate 3 mmol/l, glucose 1 g/l.

[4] Aiming at a systemic ionized calcium concentration of about 1.0 mmol/l.

In our randomized controlled trial comparing regional anticoagulation with citrate to anticoagulation with the low-molecular-weight heparin nadroparin, control of metabolic acidosis was adequate with the two methods, while unexpectedly metabolic alkalosis developed more frequently with heparin [4]. Hypo- and hypernatremia were rare and occurred less frequently and as frequently with citrate as with heparin, respectively. These results were obtained by measuring acid-base balance at a 6-hour interval, using two types of replacement fluids, one buffer-free solution and one bicarbonate-buffered solution according to a computer-driven algorithm (table 1).

The study additionally showed that critically ill patients tolerate citrate far better than heparin. After initiation of study anticoagulant, we had to discontinue nadroparin prematurely for predefined adverse events more frequently than citrate (table 2). This was the case after we had excluded 4 times more patients from randomization for anticipated adverse events to heparin (bleeding or risk of bleeding) than to citrate (Child class C liver cirrhosis; 23 vs. 6% of all CVVH patients in the study period).

Table 2. Main results of the randomized controlled trial comparing citrate to low-molecular-weight heparin anticoagulation for CVVH presented for the per-protocol patients [4]

	Citrate (n = 97)	Nadroparin (n = 103)	p value
Adverse events needing discontinuation of study anticoagulant, %	2	19	<0.001
Bleeding, %	6	16	0.08
Circuit survival time (all reasons), h	27 (13–47)	26 (15–43)	0.68
Renal recovery (all patients), %	69	52	0.02
Renal recovery (surviving patients), %	97	86	0.08
Hospital mortality, %	41 (21–51)	57 (48–62)	0.03
Three-month mortality, %	45 (35–55)	62 (53–72)	0.02

Bleeding

The primary reason to use citrate is that citrate does not increase the patient's risk of bleeding. The 4 randomized studies comparing heparin to citrate excluded patients with an increased risk of bleeding. Nevertheless, bleeding was significantly reduced in one [3] and nonsignificantly reduced in the others. In one of the studies, the relative risk of bleeding was significantly lower in the citrate patients after adjustment for severity of illness and antithrombin concentration [2]. The transfusion rate was significantly less in the citrate group of one of the randomized studies [1].

Efficacy

Circuit life is influenced by a myriad of factors; among these are the patient's acute illness, causing activation of coagulation and/or a reduced coagulation capacity, baseline platelet count, modality of CRRT (dialysis or filtration; pre- or postdilution), CRRT dose and filtration fraction, quality of the vascular access, the local protocol of disconnection and logistic events needing discontinuation of the circuit [12]. Many nonrandomized studies report longer circuit survival with citrate, but this finding is not uniform [12]. In 2 of the 4 randomized studies comparing citrate to heparin, circuit life was significantly longer with citrate [1, 2], while circuit life was similar in the other 2 [3, 4].

Factors influencing circuit life in our study were the use of postdilution hemofiltration with relatively high filtration fractions and routine disconnection after 72 h. Also, citrate dose was relatively low and not titrated to postfilter calcium. Finally, our replacement fluids contained calcium and were administered in the venous chamber, thus counteracting citrate anticoagulation and facilitating clotting at this site. Fortunately, the site of calcium replacement in the newer CRRT devices is below the venous chamber, illustrating continuous improvement of the method. Other methods use calcium-free replacement fluids and infuse calcium via a separate line. This option confers other risks.

Patient and Kidney Survival

The primary reason to perform a large randomized controlled trial comparing heparin to citrate was our concern whether citrate would be safe. Unexpectedly, our study showed a 15% absolute 3-month survival benefit for citrate on intention-to-treat analysis and a 17% absolute 3-month survival benefit in the per protocol-treated patients (table 2). Multivariate analysis found that, in addition to high age, high sequential organ failure assessment score and high transfusion rate, anticoagulation with heparin contributed to mortality. Therefore, the benefit of citrate could not be fully explained by less bleeding. Post hoc complementary subgroup analysis showed that citrate was particularly beneficial in patients after surgery (compared to medical treatment), with sepsis (compared to no sepsis), a high degree of organ failure (with equal or more than median sequential organ failure assessment points, which was 11) or younger age (less than median age, which was 73 years) [4].

In addition to improved patient survival, kidney survival was better with citrate. The difference was significant for all per-protocol patients and tended to significance for the surviving patients (table 2). Therefore, among the higher proportion of surviving patients, more patients were free from chronic dialysis in the citrate group.

Why Would Citrate Do Better than Heparin?

We can only speculate, but citrate may be beneficial, heparin may do harm, or both may play a role. Less bleeding with citrate is an important factor, but does not fully explain the benefits in our study. Post hoc analysis suggests that the lower mortality is most pronounced in inflammatory states (sepsis, severe organ failure, younger age). Several explanations can be given.

First, citrate may improve biocompatibility to the membrane. Intermittent hemodialysis with heparin anticoagulation triggers the release of inflammatory mediators from activated leukocytes, platelets and/or endothelial cells. This release occurs despite the use of so-called biocompatible membranes. Compared to heparin, citrate anticoagulation attenuates the release of myeloperoxidase, elastase, interleukin 1β, and platelet factor 4, and reduces lipid peroxidation [13–16]. Complement activation as noticed with the former cuprophane membranes is not suppressed while using citrate [13, 15]. One explanation may be that citrate downregulates the inflammation induced by foreign material by causing deep hypocalcemia in the filter modulating intracellular calcium signaling [17, 18]. Several proinflammatory actions of neutrophils are indeed calcium dependent [18].

Second, heparin may have proinflammatory effects by promoting the release of inflammatory mediators from cells. Leitienne et al. [19] showed that the proinflammatory mediator release during hemodialysis depends on heparin dose. In addition, Gritters et al. [20] found that myeloperoxidase and platelet factor 4 were released into the systemic circulation directly after the infusion of heparin before connection of the extracorporeal circuit. These and other studies suggest that inflammatory mediators may not only arise from neutrophils and platelets, but also from endothelial cells [21].

Furthermore, heparin has additional proinflammatory effects because its binding to antithrombin does not only potentiate the anticoagulant effects but additionally prohibits the anti-inflammatory actions of antithrombin [22, 23]. Furthermore, heparins bind to lipopolysaccharide-binding protein, a protein which modulates the physiological responses to endotoxin in a dose-dependent way. Heparin binding to lipopolysaccharide-binding protein facilitates the endotoxin-induced activation of monocytes, which is attenuated by serum. Heparins have clear anti-inflammatory properties as well (summarized in Cornet et al. [24]). Altogether, heparin modulates the inflammatory response in a complex way. Whether the pro- or anti-inflammatory effects of heparin supervene is not precisely known; it depends on the interplay of factors among which heparin concentration and an underlying inflammatory state seem to play a role.

Third, the citrate delivered to the systemic circulation could have metabolic effects on other organs and cells. Citrate is a readily available mitochondrial fuel; about 20 g/day are delivered in our setting. Substrate availability is a crucial regulator of the citric acid cycle, a central pathway recovering energy and maintaining redox state. In sepsis, inhibition of pyruvate dehydrogenase limits pyruvate conversion to acetyl-CoA, the main substrate of the cycle. Furthermore, the groups of Weinberg [25, 26] showed that citric acid cycle intermediates (α-ketoglutarate, malate and citrate) protect proximal tubules against and promote recovery from a sustained mitochondrial energetic deficit as occurring after hypoxia and reoxygenation by lowering the cellular burden of nonesterified fatty acids that appear to account for much of the continuing mitochondrial dysfunction. They suggest that serum concentrations of citrate reached during CRRT with citrate as an anticoagulant (0.3–0.5 mmol/l) [11] are in the range that modifies tubule cell metabolism [pers. commun.].

Conclusion

In critically ill patients with acute kidney injury, regional anticoagulation with citrate seems superior to heparin anticoagulation for CRRT concerning tolerance and safety, mainly due to less bleeding. Whether circuit survival is better depends on the modality. In addition, citrate seems to improve patient and kidney survival. This finding needs to be confirmed. Post hoc analysis suggests that this finding cannot be fully explained by less bleeding. Citrate was especially superior in patients with severe organ failure and sepsis. Both citrate and heparin interfere with inflammation. To what extent citrate protects or heparin does harm in the setting of multiple organ failure needs to be unraveled.

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A Mathematical Model of Regional Citrate Anticoagulation in Hemodialysis

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Key Words
Regional citrate anticoagulation · Citrate dialysis · Calcium · Solute kinetics

Abstract
Background/Aims: Regional citrate anticoagulation (RCA) during hemodialysis (HD) has several advantages over heparin anticoagulation, but calcium (Ca) derangements are a major concern necessitating repeated monitoring of systemic ionized Ca (Ca^{2+}). We developed a mathematical model of Ca and citrate (Ci) kinetics during RCA. ***Methods:*** Using patient- and treatment-related parameters, including pre-HD serum Ca and protein concentrations, hematocrit, blood and dialysate flow rates, dialysate composition and access recirculation, the model computes all relevant aspects of RCA based on physicochemical, biochemical and physiological principles such as chemical Ca and Ci equilibria, transmembrane solute fluxes and Ci metabolic rate. The model was validated in 17 treatments using arterial Ci infusion, Citrasate® dialysate, and no postdialyzer Ca substitution. ***Results:*** Measured and predicted systemic Ca^{2+} before HD was 1.08 ± 0.06 and 1.05 ± 0.05 mmol/l, respectively (difference –0.03 ± 0.046, 95% confidence interval, CI, –0.055 to –0.007), and at 15 min into the treatment 1.01 ± 0.05 and 1.02 ± 0.05 mmol/l, respectively (difference 0.012 ± 0.054, 95% CI –0.015 to 0.04). At 15 min, the measured and predicted predialyzer Ca^{2+} was 0.33 ± 0.06 and 0.39 ± 0.05 mmol/l, respectively (difference 0.06 ± 0.03; 95% CI 0.044–0.077), and the measured and predicted postdialyzer Ca^{2+} was 0.7 ± 0.05 and 0.61 ± 0.05 mmol/l, respectively (difference –0.09 ± 0.04; 95% CI –0.11 to –0.07). Bland-Altman analysis showed no systematic bias in these predictions. ***Conclusion:*** This novel model of RCA shows excellent accuracy in predicting systemic, pre- and postdialyzer Ca^{2+} concentrations and may prove valuable in both research and clinical applications of RCA.

Introduction

Renal replacement therapy generally requires anticoagulation of the blood to prevent clotting in the extracorporeal circuit. For this purpose, systemic anticoagulation using heparin is the method most frequently applied in clinical practice. This is associated, however, with a long list of potential complications, side effects and contraindications. Most obviously, systemic anticoagulation is not desirable in patients with active bleeding or even increased bleeding risk, such as trauma patients dialyzed after major surgery. Additionally, chronic heparinization

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may lead to side effects such as osteoporosis, hyperlipidemia and hair loss. Heparin-induced thrombocytopenia type II, although rare in chronic hemodialysis (HD) patients, is a potentially life-threatening condition that can develop after exposure to heparin.

Regional citrate (Ci) anticoagulation (RCA), meaning anticoagulation strictly confined to the extracorporeal circuit, is achieved by infusion of Ci into the arterial line. Traditionally, a calcium (Ca)-free dialysate is used, and Ca losses across the dialyzer membrane are countered by postdialyzer Ca infusion. The infused Ci forms stable complexes with ionized Ca (Ca^{2+}), which is thereby markedly decreased. Since Ca^{2+} is an indispensable cofactor in the coagulation cascade, its depletion mediates the desired anticoagulative effect. This is not a new concept. In the setting of renal replacement therapy, it was already described in the early 1960s [1]. Some of its benefits are immediately apparent: it does not increase bleeding risk and also spares the patient the other potential complications and side effects of heparin therapy. Of note, RCA confers advantages that go beyond the benefits of simply avoiding heparin administration: RCA has been shown to reduce complement activation, degranulation of granulocytes and platelets and the release of interleukin 1β, thus improving biocompatibility of the extracorporeal circuit [2–4]. The actual anticoagulative effect of RCA in the dialyzer has also been demonstrated to be superior to both unfractionated and low-molecular-weight heparin [5]. More recently, the sharp rise of heparin costs has further spurred interest in RCA as an alternative mode of anticoagulation.

The reasons that RCA, despite its advantages, does not dominate the chronic HD landscape fall into two broad categories: laboriousness and safety concerns. These are partly interrelated. Methodologically, traditional RCA requires a more complex setup involving two additional pumps (one for the arterial Ci infusion, one for the postdialyzer Ca substitution to replenish Ca losses) as well as the corresponding lines and connections to the extracorporeal circuit, which adds to the time required to set up the machine. The primary concern during RCA is an acute Ca derangement, which can potentially be life-threatening. Therefore, patients require close clinical observation and repeated measurements of systemic Ca^{2+}, again adding to the laboriousness and costs of this treatment modality. The initial flow rates of Ci and Ca infusions as well as subsequent adjustments to Ca substitution during the treatment in response to untoward shifts in systemic Ca^{2+} are guided by more or less complex algorithms, and a physician should be present in case decisions have to be made that are not covered by these algorithms. Due to this complexity, the routine administration of RCA to a large fraction of chronic HD patients has so far not been feasible.

Our goal was to develop a mathematical model of RCA that would broaden our understanding of the solute kinetics and mass balances involved in RCA, increase the comfort level of RCA administration by individualization, and guide the development of Ci anticoagulation regimens other than traditional RCA that provide benefits to the patients while maintaining feasibility for broad-scale clinical application.

Methods

Mathematical Model

The model is an extension of work done by Kozik-Jaromin [6]. Our model comprises the following 7 main components (fig. 1).

(1) Calculation of systemic Ci generation, Ci metabolism and resulting solute equilibria:
   (a) Ci generation is calculated assuming an average generation rate of 240 mg/24 h
   (b) Ci metabolism: $C_{Ci}(t) = C_0 \cdot e^{-k \cdot t}$ with k = 0.0145 min^{-1}
   (c) Solute equilibria (Ca^{2+}, protein-bound Ca, free Ci, CaCi complexes) are calculated assuming a mono-ionic milieu, using the following dissociation constants: K_{CaCi} (for CaCi complexes) = 0.776 mmol/l; K_{CaP} (for Ca-protein binding) = 11 mmol/l
(2) Calculation of solute concentration changes caused by access recirculation
(3) Calculation of required predialyzer Ci concentration and resulting solute concentrations and equilibria:
   (a) Concentration of protein-binding sites for Ca (C_B) according to protein concentration and 12 binding sites per molecule of albumin
   (b) $C_{CiT} = [-(C_{Ca^{2+}})^3 - (C_{Ca^{2+}})^2 \cdot K_{CaCi} - (C_{Ca^{2+}})^2 \cdot K_{CaP} - (C_{Ca^{2+}})^2 \cdot C_B + (C_{Ca^{2+}})^2 \cdot C_{CaT} - C_{Ca^{2+}} \cdot K_{CaCi} \cdot K_{CaP} - K_{CaCi} \cdot C_B + C_{Ca^{2+}} \cdot K_{CaCi} \cdot C_{CaT} + C_{Ca^{2+}} \cdot K_{CaP} \cdot C_{CaT} + K_{CaCi} \cdot K_{CaP} \cdot C_{CaT}]/[(C_{Ca^{2+}})^2 + C_{Ca^{2+}} \cdot K_{CaP}]$
(4) Calculation of dialysate composition with respect to free Ci, Ca^{2+}, CaCi complexes:
   (a)

$$C_{Ci\_free} = -0.5 \cdot \sqrt{0.5 \cdot \left(C_{CaT} - C_{CiT} + K_{CaCi}\right)^2 + K_{CaCi} \cdot C_{CiT}}$$

   (b)

$$C_{Ca\_free} = C_{CaT} \quad OR \quad \frac{K_{CaCi} \cdot \left(C_{CiT} - C_{Ci\_free}\right)}{C_{Ci\_free}}$$

$$\left(\textit{if citrate-containing dialysate}\right)$$

   (c)

$$C_{CaCi} = \frac{C_{Ca\_free} \cdot C_{Ci\_free}}{K_{CaCi}}$$

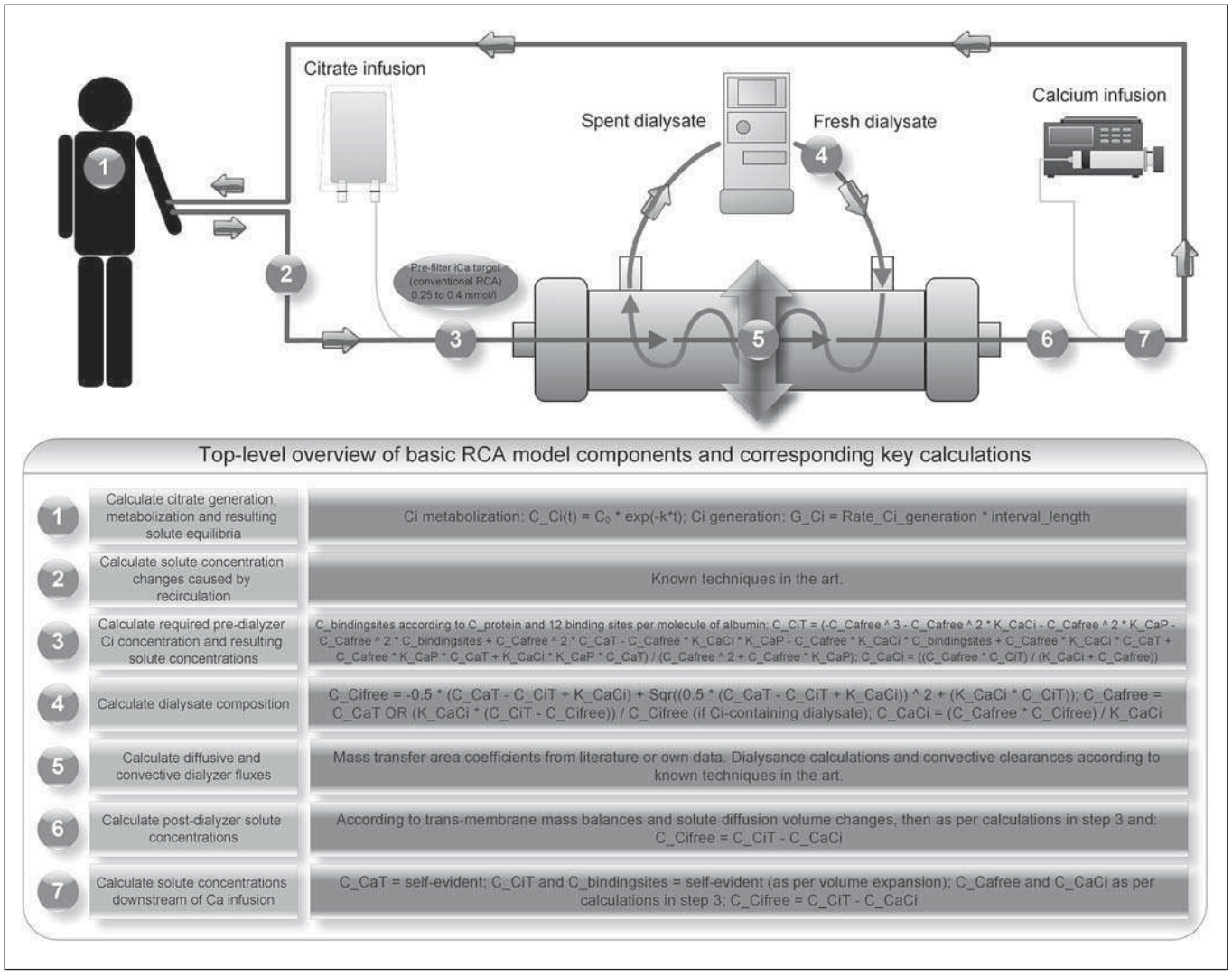


Fig. 1. Top level model overview illustrating the fundamental components of the mathematical model and the corresponding key calculations. C_bindingsites = Concentration of protein binding sites for Ca; C_CaCi = concentration of CaCi complex; C_Cafree = Ca^{2+} concentration; C_CaT = total Ca concentration; C_Ci(t) = Ci concentration at time point t; C_Cifree = free Ci concentration; C_CiT = total Ci concentration; C_protein = protein concentration; C_0 = Ci concentration at time point zero; K_CaCi = dissociation constant for CaCi complex; K_CaP = dissociation constant for Ca protein complex; see text for further details.

(5) Calculation of diffusive and convective dialyzer solute fluxes, assuming KoA_{Ca_free} = 603 ml/min; KoA_{Ci_free} = 337 ml/min; KoA_{CaCi} = 337 ml/min [6]
(6) Calculation of postdialyzer solute concentrations according to transmembrane mass balances and solute diffusion volume changes. Calculation of solute equilibria as in step 3, and $C_{Ci\_free} = C_{Ci\_total} - C_{CaCi}$
(7) Calculation of solute concentrations after Ca substitution:
  (a) Total Ca, total Ci, Ca-binding sites: self-evident (as per volume expansion)
  (b) Ca^{2+} and CaCi as per calculations in step 3
  (c) $C_{Ci\_free} = C_{CiT} - C_{CaCi}$

The entire HD treatment is modeled iteratively by performing these calculations for consecutive intervals of user-definable duration.

Model Validation

The study was approved by the Beth Israel Medical Center Institutional Review Board, and written informed consent was obtained from each subject before enrollment. Seventeen HD treatments were conducted in 8 maintenance HD patients using Ci bicarbonate dialysate (Citrasate®; Advanced Renal Technologies, Bellevue, Wash., USA; 3 mEq/l calcium, 2.4 mEq/l Ci). For one treatment only, Citrasate with 2.5 mEq/l Ca was used. No post-

dialyzer Ca substitution was performed. Total Ca, Ca^{2+} and total Ci were measured systemically, before and after dialyzer at the following time points: before HD (systemically only), at several time points throughout the treatment, and at the end of HD. Total protein and albumin were measured before dialysis. The most recent alkaline phosphatase (AP) and total parathyroid hormone (tPTH; Scantibodies Laboratory Inc., Santee, Calif., USA) were recorded. Trisodium Ci (136 mmol/l; 4%) was infused into the arterial line at various rates to result in predialyzer Ca^{2+} values of approximately 0.25–0.65 mmol/l. Blood flow rate was 350 ml/min in 4 treatments and 400 ml/min in 13 treatments; the dialysate flow rate was fixed at 500 ml/min. All subjects used Optiflux F180NR dialyzers (Fresenius Medical Care, Waltham, Mass., USA).

Measured and model-predicted systemic Ca^{2+} concentrations were compared before HD and at 15 min into the treatment. For the latter, pre-HD model predictions were adjusted to measured values. Pre- and postdialyzer comparisons between measured and predicted Ca^{2+} were performed at 15 min into the treatment.

Deviations between model-predicted and measured systemic Ca^{2+} over the entire treatment were compared for tertiles of AP and tPTH.

Statistical Analysis

Results are presented as mean ± standard deviation, unless otherwise noted. Differences between predicted and measured values were calculated as predicted – measured and were tested for significant deviation from zero by means of a 2-tailed 1-sample t test. Bland-Altman plots were generated and the underlying data analyzed for systematic bias by means of linear regression. Statistical significance was accepted for an α-level of <0.05.

Results

The study cohort consisted of 8 subjects (aged 63 ± 13.6 years, 4 males). Measured and predicted systemic Ca^{2+} at baseline (before HD) was 1.08 ± 0.06 and 1.05 ± 0.05 mmol/l, respectively (difference –0.03 ± 0.046, 95% confidence interval, CI, –0.055 to –0.007; fig. 2a), and at 15 min into the treatment 1.01 ± 0.05 and 1.02 ± 0.05 mmol/l, respectively (difference 0.012 ± 0.054, 95% CI –0.015 to 0.04; fig. 2b). At 15 min, the measured and predicted predialyzer Ca^{2+} was 0.33 ± 0.06 and 0.39 ± 0.05 mmol/l, respectively (difference 0.06 ± 0.03, 95% CI 0.044–0.077; fig. 2c). At the same time point, corresponding postdialyzer Ca^{2+} was 0.7 ± 0.05 and 0.61 ± 0.05 mmol/l, respectively (difference –0.09 ± 0.04, 95% CI –0.11 to –0.07; fig. 2d). Neither visual inspection of Bland-Altman plots nor formal analysis of the underlying data revealed any systematic bias in any of these predictions.

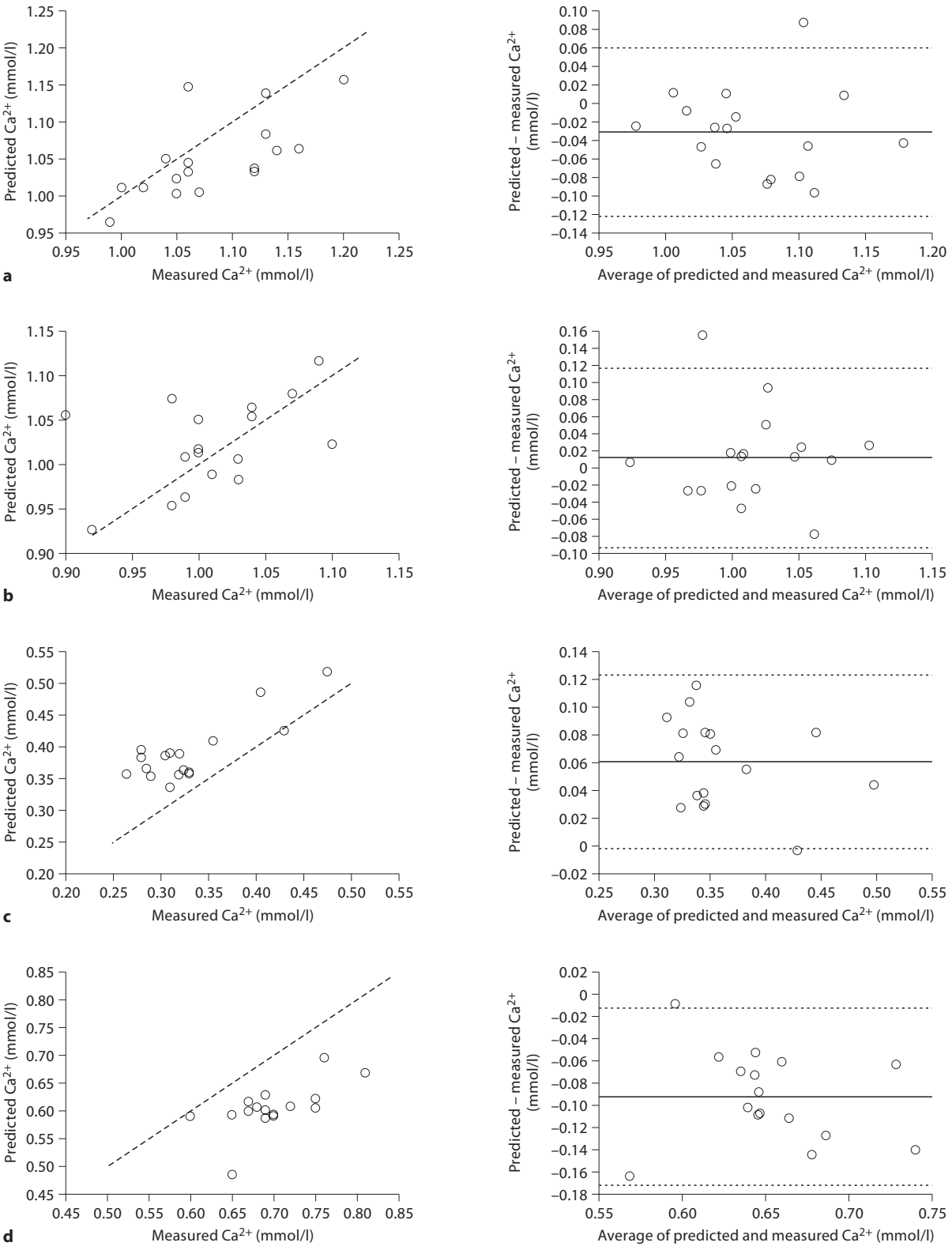
The tertile ranges for AP were 85–106 U/l (low AP), 112–143 U/l (medium AP) and 154–592 U/l (high AP). For tPTH, the tertile ranges were 258–627 pg/ml (low tPTH), 636–856 pg/ml (medium tPTH) and 916–1,287 pg/ml (high tPTH). Figure 3 shows the difference between predicted and measured systemic Ca^{2+} plotted against treatment time. Figure 3a is stratified by AP tertiles; figure 3b is stratified by tPTH tertiles. While the curves for the low and medium tertiles show no clear separation, the curves corresponding to the high-AP tertile as well as the high-tPTH tertile cluster toward the bottom of the plots, indicating that the most pronounced differences between model prediction and measured values occur in these tertiles.

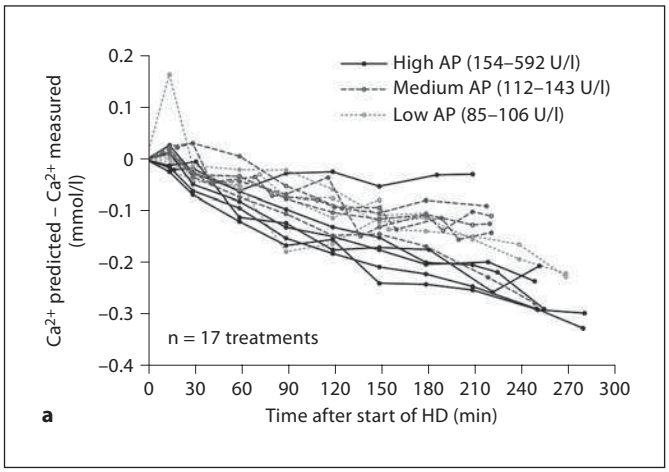
Discussion

The presented model of RCA, validated here in treatments employing Ca- and Ci-containing dialysate and no venous Ca substitution, shows good accuracy in estimating serum Ca^{2+} concentrations. The predialysis Ca^{2+} is underestimated by only 0.03 mmol/l (with the 95% CI ranging from –0.055 to –0.007), which is clinically negligible. At 15 min into the treatment, serum Ca^{2+} is overestimated by merely 0.012 mmol/l. It is worth noting that this time point covers a period of pronounced solute flux across the membrane, and it is reassuring that the model predicts these complex events and their corresponding influences on serum Ca^{2+} levels adequately. Currently, the model assumes a baseline systemic Ci concentration of 0.1 mmol/l for all treatments. It stands to reason that individualization of predialysis Ci level input will further improve model accuracy. Ci measurements are currently under way to test this hypothesis.

The pre- and postdialyzer Ca^{2+} predictions, while statistically differing from the measured values, show relatively little scatter (standard deviation of the difference 0.03 and 0.04, respectively). More importantly, however, both appear to follow almost a parallel shift from the identity line (fig. 2c, d). This underscores the validity of the underlying calculations, and it would appear that the model may readily be adjusted to correct for this shift.

Fig. 2. Comparison of predicted and measured Ca^{2+} systemically before dialysis (**a**), systemically at 15 min into the treatment (**b**), before the dialyzer (**c**) and after the dialyzer (**d**) at 15 min into the treatment. The left panel shows correlations (dotted line = line of identity), the right panel shows the corresponding Bland-Altman plots.


a
Predicted Ca2+ (mmol/l)
Measured Ca2+ (mmol/l)
Predicted – measured Ca2+ (mmol/l)
Average of predicted and measured Ca2+ (mmol/l)
b
Predicted Ca2+ (mmol/l)
Measured Ca2+ (mmol/l)
Predicted – measured Ca2+ (mmol/l)
Average of predicted and measured Ca2+ (mmol/l)
c
Predicted Ca2+ (mmol/l)
Measured Ca2+ (mmol/l)
Predicted – measured Ca2+ (mmol/l)
Average of predicted and measured Ca2+ (mmol/l)
d
Predicted Ca2+ (mmol/l)
Measured Ca2+ (mmol/l)
Predicted – measured Ca2+ (mmol/l)
Average of predicted and measured Ca2+ (mmol/l)



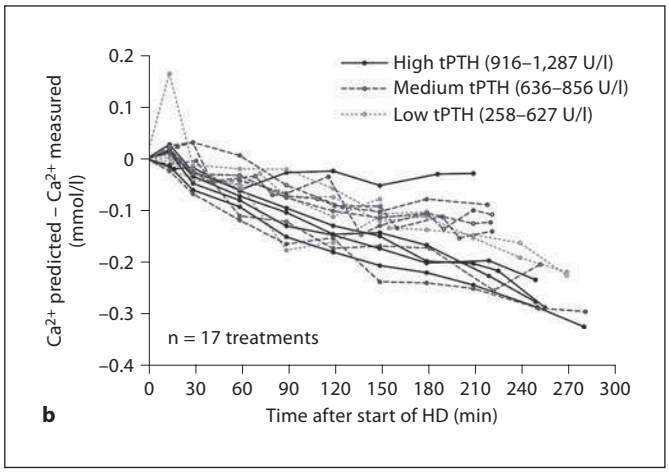


Fig. 3. Model performance over the entire course of the treatments. The difference between measured and predicted systemic Ca^{2+} (predicted – measured) is plotted on the y-axis. Results are stratified by tertiles of AP (**a**) and tPTH (**b**). See text for tertile limits. Of note, the one treatment in the high-AP and high-tPTH tertiles that does not cluster with the rest of the group is the one treatment using a dialysate with 2.5 mEq/l Ca as opposed to 3.0 mEq/l for all other treatments.

It must be noted that for the comparison at 15 min into the treatment, the baseline model predictions were adjusted to measured values in order to avoid carry-over errors and to assess how the model handles a period of pronounced Ci and Ca flux. When simulating an entire treatment en bloc, even slight deviations may add up, as can be seen in figure 3. It is also apparent that the predictions are more accurate for some treatments than for others. One major contributor to this is certainly the individual subject's capacity to buffer changes in serum Ca^{2+}. Currently, this factor is implemented in the model in the form of a term that eliminates a user-specified fraction (from 0 to 100%) of the diffusive Ca flux that occurs per iteration interval, thereby treating it as being buffered by the subject's bone. This concept has limitations primarily insofar as it does not account for changes in serum Ca^{2+} concentration that occur as a consequence of the metabolism of CaCi complexes in the liver and, hence, are not immediately related to diffusive Ca transfer across the membrane. Secondly, it is conceivable that Ca buffer capacity is not constant throughout the treatment but rather is a function of the absolute Ca^{2+} level, the rate of its change and its direction of change, as evidenced by PTH secretion being related to those factors [7]. That aside, since we do not currently have a way of estimating a subject's Ca buffer capacity, all treatments presented here were modeled using an identical buffering factor of 80%, which derives from Ca kinetic studies we are conducting at our institute [8]. The model, in its current implementation, will tend to err on the safe side, meaning it will generally predict a greater decline in serum Ca^{2+} than observed. One would expect that improved Ca buffer capacity would go along with less pronounced drops of systemic Ca^{2+} over the course of the treatment, which, in turn, would result in greater discrepancies between modeled and measured values, since for the simulations at hand, individual differences in Ca buffer capacity were not taken into account. It is conceivable that AP and tPTH, as biochemical markers of bone turnover, may provide some indication of Ca buffer capacity. As can be seen in figure 3a and b, the results in the highest tertiles of AP and tPTH, respectively, cluster toward the bottom of the plots. Following the above reasoning, this is exactly what one would expect to find, assuming that these patients are better capable of buffering changes in serum Ca^{2+} concentration than those in the middle or low tertiles. This observation leads us to believe that it will be possible to further improve the model's prediction qualities by accounting for differences in surrogates of bone turnover. A more dynamic modeling of such parameters, rather than simply applying the monthly snapshot measurements of these surrogate markers, is conceivable. Furthermore, we are currently working on a more refined approach to the general concept of implementing Ca buffer capacity that is suited for the setting of RCA and will address the shortcomings mentioned above. One limita-

tion of the current model is that only Ca is considered for forming complexes with Ci, neglecting other cations in solution. The multi-ionic milieu will be considered in a future iteration of the model.

The presented implementation of the model is very versatile in that it not only allows the simulation of traditional RCA (with arterial Ci infusion, venous Ca substitution, and Ca-free dialysate), but also grants complete freedom in the choice of dialysate composition with respect to both Ca and Ci content. Therefore, as an example, treatments without venous Ca substitution and using Ci bicarbonate dialysate containing various Ca contents may be simulated. Such regimens are of interest because they appear to increase dialyzer performance [9] and may even permit significant reductions in heparin use. Before switching an entire dialysis unit to such a regimen, the patients may be screened with the presented model for risk of developing hypocalcemia in order to identify subgroups who deserve closer monitoring or in whom the switch may not be advisable. The ultimate goal of these efforts is to devise RCA strategies that are effective in terms of anticoagulation but at the same time reduce or eliminate heparin exposure (and associated side effects), laboriousness of RCA (setup and frequent systemic Ca^{2+} monitoring) and risk for acute hypocalcemia.

We believe the presented model will be a valuable tool in research (i.e., for exploring in silico the impact of various RCA settings on solute kinetics and mass balances, and in guiding clinical research studies on RCA) as well as in actual clinical application, be it using conventional RCA or modified regimens.

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Automated Regional Citrate Anticoagulation: Technological Barriers and Possible Solutions

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Key Words

Citrate · Anticoagulation · Dialysis · Online clearance monitor · Optical hematocrit sensor · Access recirculation

Abstract

Background: Large-scale adoption of regional citrate anticoagulation (RCA) is prevented by risks of the technique as practiced traditionally. Safe RCA protocols with automated delivery on customized dialysis systems are needed. ***Methods:*** We applied kinetic analysis of solute fluxes during RCA to design a protocol for sustained low-efficiency dialysis (SLED) for critically ill patients. We used a high-flux hemodialyzer, a zero-calcium (Ca) dialysate, a dialysis machine with online clearance and access recirculation monitoring, and a separate optical hematocrit (Hct) sensor. Flow rates were Q_B = 200 ml/min for blood; Q_D = 400 ml/min for dialysate, with Na = 140 mmol/l and HCO_3 = 32 mmol/l; $Q_{citrate}$ = 400 ml/h of acid citrate dextrose A; ultrafiltration as indicated. The Q_{Ca} was infused into the return blood line, adjusted hourly based on online Hct and a <24-hour-old albumin level. ***Results:*** Using the SLED-RCA protocol in an anhepatic, ex vivo dialysis system, ionized Ca (iCa) was >1 mmol/l in the blood reservoir and <0.3 mmol/l in the blood circuit after citrate but before Ca infusion (Q_{Ca}) with normal electrolyte composition of the blood returning to the reservoir. Clinically, SLED-RCA completely abrogated clotting, without adverse electrolyte effects. The Q_{Ca} prediction algorithm maintained normal systemic iCa (0.95–1.4 mmol/l) in all patients. The high citrate extraction on the dialyzer prevented systemic citrate accumulation even in shock liver patients. Safety analysis shows that building a dialysis system for automated SLED-RCA is feasible. ***Conclusion:*** Using predictive Q_{Ca} dosing and integrating control of the infusion pumps with the dialysis machine, SLED-RCA can be near-automated today to provide a user-friendly and safe system.

Introduction

Regional citrate anticoagulation (RCA) during renal replacement therapy was introduced more than four decades ago [1]. Citrate acts by complexing with calcium (Ca), making free ionized calcium (iCa) unavailable as a cofactor for the clotting cascade. The anticoagulant effect is reversed by removal of citrate in the artificial kidney and by restoring the plasma iCa to normal levels by a Ca infusion. RCA cannot cause systemic bleeding tendency, and in a high dose, citrate is more effective than alternative anticoagulation methods [2–5], with other benefits including the suppression of platelet, white blood cell and complement activation in the extracorporeal circuit [6, 7]. A true toxicity or unavoidable adverse effect has not been discovered to date. Nevertheless, RCA has not gained widespread application outside academic hospi-

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tals [8] possibly due to the risk of severe complications with early clinical protocols [9–13], the absence of expert consensus on best approaches [14] and a lack of dialysis equipment custom-designed for safe RCA delivery. Improved RCA protocols and accumulated clinical experience have made RCA safer [15–18], but even the safest protocol can quickly lead to a life-threatening complication due to equipment malfunction (e.g. unrecognized failure of the Ca infusion pump) or human error (e.g. if the operator connects the Ca infusion prefilter and the citrate infusion postfilter). Thus, there is a need for automation of RCA with safe protocols, integrated delivery systems and online safety monitoring modules to allow broad use of the technique.

Development of Safe RCA Protocols Amenable to Automated Delivery

We performed a comprehensive kinetic analysis of citrate, sodium, bicarbonate, Ca, magnesium and phosphate fluxes during RCA with any combination of hemofiltration and/or dialysis [Szamosfalvi et al., unpubl. data]. The most important findings include:

- A high, fixed concentrated citrate-flow-to-blood-flow ratio ensures strong anticoagulant activity from the point where the citrate infusion enters the blood circuit, keeping filter performance in removing citrate stable
- A very high single-pass citrate removal on the dialyzer (>85%) is critical for safe RCA protocols amenable to automation; this prevents systemic citrate accumulation even in the complete absence of body metabolism of citrate
- When citrate (and Ca in the form of Ca-citrate complex) is cleared in the artificial kidney using Ca-free bicarbonate-based dialysis and/or replacement fluids, the low iCa level in the circuit blood and the anticoagulant effect are maintained
- Magnesium dialysance is about equal to Ca clearance, and this must be accounted for
- When only an insignificant amount of citrate reaches the patient in the venous blood return, bicarbonate mass balance is negligibly affected by the ability to metabolize citrate
- The target plasma total Ca level is defined only by the target systemic iCa and the serum albumin level when significant (>1 mmol/l) systemic citrate accumulation is prevented
- The Ca loss into the filter effluent is calculated and replaced using the patient's target plasma total Ca level, the circuit plasma flow (derived from the online optical hematocrit, Hct, sensor data and the blood flow) and the known dialyzer performance in removing the Ca-citrate complexions
- Infusing concentrated Ca into the venous limb of the blood circuit, as opposed to a separate intravenous line, eliminates the confounding effect of variable access recirculation on Ca dosing

The hallmark of safe RCA protocols developed using the above principles is that they can be tested in an ex vivo, fully heparinized sham dialysis system as shown in figure 1. A safe RCA protocol ready for automated delivery will achieve normal, >1 mmol/l iCa in the 0.5-liter blood reservoir ('patient') without citrate metabolism and <0.3 mmol/l iCa in the blood circuit at any point after the citrate and before the Ca infusion while maintaining normal electrolyte (sodium, potassium, Ca, magnesium, bicarbonate, chloride and phosphate) concentrations in the blood returning to the reservoir. Importantly, the optimal Ca infusion rate must be predictable with clinically acceptable accuracy using the above principles and without the need for frequent blood sampling.

Clinical Implementation of Safe RCA for Sustained Low-Efficiency Dialysis

Since February 2009, we have been using a novel RCA protocol for sustained low-efficiency dialysis (SLED) at the Henry Ford Hospital. We use a commercial dialysis machine equipped with an effective ionic dialysance (K_{ecn}) monitor (OLC) and a body temperature module (BTM) which measures access recirculation (in percent). A commercial optical Hct sensor is also incorporated into the system. We tested the SLED-RCA protocol in the ex vivo system as shown in figure 1 (the OLC and BTM are turned off during ex vivo modeling). The experimental prescription and the total Ca, iCa and citrate levels at various points in the dialysis circuit are shown in figure 2. During clinical SLED in the intensive care unit, the machine is set up in intermittent hemodialysis (IHD) mode for 9 h 59 min with a fixed flow: for blood Q_B = 200 ml/min, for dialysate Q_D = 400 ml/min, $Q_{citrate}$ = 400 ml/h (acid citrate dextrose A, ACDA, 116 mmol/l citrate), Q_{Ca} = 70–160 ml/h (136 mmol/l $CaCl_2$ in 0.9% normal saline; the rate is selected hourly from a table based on the most recent serum albumin and the hourly optical Hct value) and $Q_{net\ ultrafiltration}$ = 0–500 ml/h. As opposed to

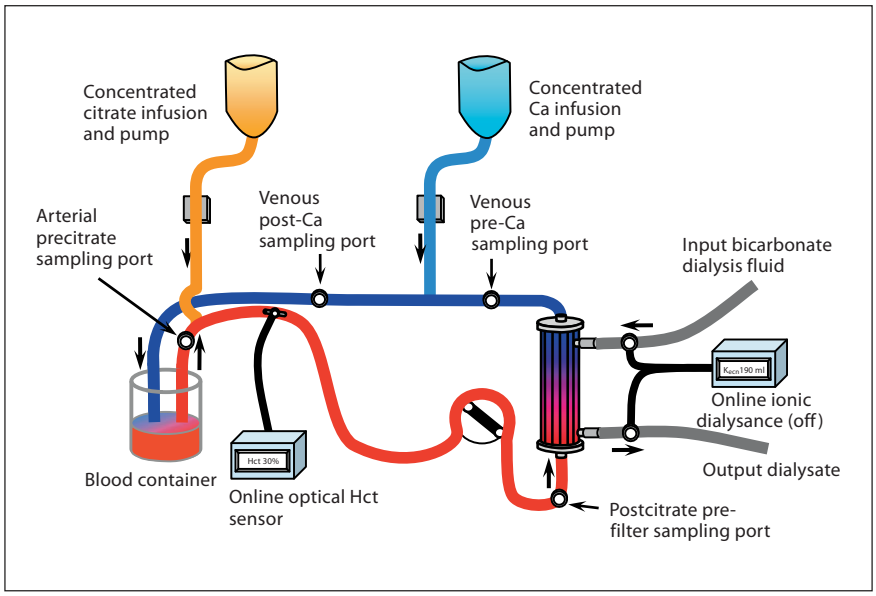

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Fig. 1. Ex vivo citrate dialysis circuit diagram. We used this fully heparinized system to confirm kinetic predictions of solute fluxes during SLED with RCA and to refine the dialysate composition and the Ca infusion dosing table with variable circuit hemoglobin and albumin levels. The ionic dialysance monitor of the dialysis machine is turned off during ex vivo dialysis but is utilized during clinical treatments to monitor filter performance.

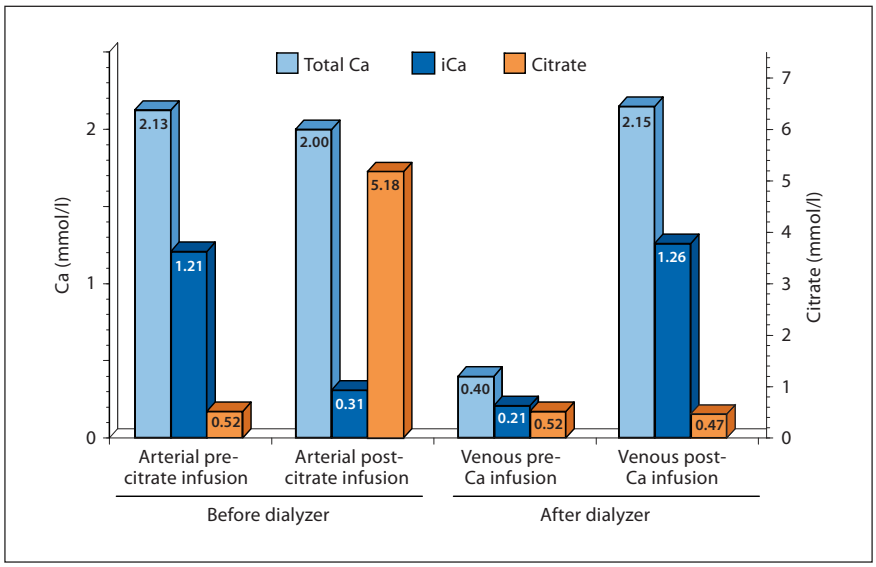

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Fig. 2. Representative dialysis circuit chemistry using the ex vivo model in figure 1 with blood flow 200 ml/min, an Hct of 30% and albumin 2.5 g/dl, ACDA anticoagulant at 400 ml/h, calcium-free dialysate flow at 400 ml/min (Na = 140 mmol/l and HCO_3 = 28), on an Optiflux 200NR filter with 136 mmol/l Ca infusion (10 g $CaCl_2$ in 0.5 liters of 0.9% saline) at 115 ml/h (citrate metabolism absent); ultrafiltration rate set at 510 ml/h (to remove the citrate and Ca infusion volume).

the continuous renal replacement therapy mode, in the IHD mode and with the above settings, the ionic dialysance and access recirculation monitors remain enabled and perform reliably, while the hourly uremic clearance is still limited by the low blood flow. Flexible selection of the online-generated dialysate sodium (Na^+), bicarbonate (HCO_3^-) and potassium (K^+) remains possible. We use a Ca-free dialysis fluid with a final [Na^+] = 140 mmol/l, [K^+] = 4 mmol/l, [Mg^{2+}] = 0.5 mmol/l, [HCO_3^-] = 32 mmol/l and glucose 100 mg/dl (5.5 mmol/l). We supplement the acid concentrate with 3 mol/l sodium phosphate to achieve a final dialysate phosphate level of 3.2 mg/dl (1 mmol/l) as needed clinically. The venous blood completely equilibrates with the fresh dialysate, and after the Ca infusion, always has a normal electrolyte composition. Clinically, systemic iCa levels have been normal (0.95–1.4 mmol/l) using the predictive Ca infusion dosing in all patients with normalization of all major systemic electrolytes and a 100% efficiency in eliminating circuit clotting [abstract submitted, ASN 2009, Szamosfalvi et al.]. Ca fluxes into and out of tissue stores do not seem to require modifications of the Ca infusion rate in

this protocol. Daily serum albumin measurements are sufficient for precise Ca dosing for most patients. We obtain 1–2 extra albumin values per SLED-RCA in patients on frequent albumin infusions.

Technological Barriers and Possible Solutions

The safe RCA-SLED protocol described could still lead to complications if a failure condition occurred during treatment. We performed a safety analysis in preparation for full automation of SLED-RCA. Some failure conditions and their mitigation are discussed below, loosely grouped into the human operator error category (risks 1–5) and the equipment malfunction category (risks 6–8).

Risk 1: Human Error in the RCA Prescription

Predictive dosing of the Ca infusion based on the serum albumin and the real-time Hct will only be accurate if all other parameters including the blood flow and filter selection are uniform. Calculation of the total net ultrafiltration goal is also difficult in the 10-hour IHD mode with the potential for severe volume management errors. Therefore we developed a secure, web-based, online prescription writing tool to generate all of our SLED-RCA treatment orders. This only allows flexibility in the selection of the hourly net ultrafiltration goal and automatically defines the initial Ca infusion rate once the most recent albumin and hemoglobin is provided. Orders are printed and are also available online, eliminating errors due to illegible handwriting. Obviously, in the future, integrating prescription writing and initial and subsequent Ca infusion rate calculations on the dialysis machine would allow us to use different blood flows, filters and therapy durations without compromising any of the principles of safe RCA delivery. This would be of particular importance if SLED-RCA were to be deployed in a 24-hour continuous fashion and if we wanted to adjust the delivered dose of small solute clearance based on body weight.

Risk 2: Wrong Connection of the Citrate or Ca Infusion

We use a 1-liter ACDA formulation with red legends on the plastic and a high-alert label. The Ca infusion is prepared in a 0.5-liter bag with black legends. We also label the dedicated citrate and Ca pumps and the citrate and Ca infusion tubing. The risk still persists that citrate could be wrongly infused into the return blood line and Ca into the arterial blood line prefilter. In Europe, blood circuit tubing integrated with a citrate and Ca infusion line already exists. Introduction of such tubing sets in the USA with larger citrate and Ca bags and unique plastic connectors would greatly increase the safety of RCA but would require regulatory approval from the Food and Drug Administration (FDA).

Risk 3: Loss of Manual Coordination of Machine Operation and the Citrate and/or Ca Infusion

By far the most common problem with the safe delivery of our RCA protocol is the current lack of dialysis machine-controlled citrate and Ca pumps in the USA. For instance, if the blood pump stopped for any reason, citrate and Ca would continue to infuse directly into the patient until the care provider restarted the machine or stopped the infusions. In Europe, such integrated pumps are now available from several manufacturers, and safe engineering approaches have been demonstrated in clinical practice. In our opinion, providing such integrated pumps and obtaining regulatory approval for their use in the USA should be a priority for dialysis equipment manufacturers for the sake of patient safety.

Risk 4: Use of a Ca-Containing Acid Concentrate with RCA

This error would lead to severe hypercalcemia. We use color-coded, dedicated jugs to deliver the Ca-free acid concentrate for RCA. All of our dialysis therapies (conventional and RCA) are delivered with a 45-fold proportioning system. Mix-up of these concentrates does not generate a conductivity alarm. In the future, integration of the Ca and citrate pumps may be accomplished in a way so that these pumps only become functional if the operator selects an RCA operational mode and Ca-free concentrate from the machine software menu. All RCA acid concentrates on the menu could be provided with sodium content for a 35-fold proportioning and all conventional Ca-containing acid concentrates with sodium content for a 45-fold proportioning. The same bicarbonate concentrate could be used with both types of acid concentrate with appropriate software control of bicarbonate proportioning. Mix-up of the acid concentrates would then result in a conductivity alarm due to the markedly different final sodium concentration of the dialysate. This simple method could be implemented with support from the dialysis equipment manufacturers today, but the implicit adaptation of the machine for RCA would again likely require regulatory approval from the FDA.

Risk 5: Variable Ca Losses on the Dialyzer with Fixed Blood Flow and Changing Hct

Ca losses on the dialyzer correlate positively with plasma flow and, hence, inversely with the Hct in this SLED-RCA protocol at a fixed blood flow. The Hct may change rapidly due to bleeding or blood transfusion. We monitor the Hct continuously with an optical Hct sensor, and the nurse adjusts the Ca infusion rate at least hourly based on the current Hct. In the future, precise optical Hct monitoring may be integrated with Ca infusion dosing on a custom RCA dialysis machine.

Risk 6: Declining Filter Performance and Citrate and Ca Removal

We have not observed this to date with the high, fixed citrate-flow-to-blood-flow ratio we use. In the SLED-RCA protocol, about 95% of the free $[citrate]^{3-}$ and 85% of the $[Ca\text{-}citrate]^{-}$ complex ions are cleared on the high-flux and high-efficiency Optiflux 200NR dialyzer. The filter performance in removing citrate is indirectly monitored by automated online measurements (OLC) of K_{ecn} by the dialysis machine and charted on the SLED-RCA flow sheet about every 1.5 h. The dialyzer would be replaced if the K_{ecn} value adjusted with the BTM measured access recirculation (R), i.e., $K_{ecn}' = 100 \cdot K_{ecn}/(100 - R)$, fell below 150 ml/min. To measure OLC, we must use the machine in the IHD operational mode with the longest treatment duration limited to 10 h. In the future, a continuous operational mode with RCA, OLC and BTM could be created in the machine software to increase user-friendliness. When RCA is used, the blood flow may be decreased to limit the hourly clearance while the dialysate flow may remain high in the range of 400–800 ml/min to allow reliable functioning of the OLC and BTM modules. When access recirculation occurs, the K_{ecn} appears reduced while citrate and Ca removal on the hemofilter remains as predicted without a need to change the Ca infusion rate. This can be detected by automatically measuring access recirculation and correcting the K_{ecn} as above.

Risk 7: Delivered Blood Flow below Set Value

This error may occur with high negative access pressures (deformation of the blood tubing segment) or due to a malfunctioning blood pump or the improper loading of the tubing segment into the blood pump. We use large-bore (12–13.5 F) catheter access and a fixed blood flow of 200 ml/min which typically results in a modest (50–100 mm Hg) arterial access pressure unlikely to result in a reduced delivered blood flow. We also emphasize good practices in maintaining and operating the blood pumps. In the future, very precise blood pumping technology may replace the current roller pump designs possibly with delivered blood flow monitors.

Risk 8: Puncture of the Ca Infusion Line

This error could lead to life-threatening hypocalcemia due to unreplaced Ca losses during therapy. Our current protocol requires that the nurses confirm the integrity of the citrate and Ca infusion systems hourly. In the future, a Ca and citrate sensor could be implemented in the filter effluent. This location avoids safety and biofouling concerns inherent in placing sensors directly into the circuit blood flow. Once the effluent fluid composition is measured, systemic plasma total Ca and citrate levels may be calculated in integrated RCA systems with clinically sufficient accuracy. Thus, developing systemic hypocalcemia could be detected before clinically significant sequelae occur. Finally, online Ca sensing would also allow minor modifications to the Ca infusion rate if needed in response to Ca fluxes into and out of tissue stores (for instance skeletal Ca release and hypercalcemia in a patient with multiple myeloma or tissue Ca sequestration and hypocalcemia in a patient with acute pancreatitis).

Conclusion

In the last few decades, a vast amount of clinical data was accumulated using RCA during renal replacement therapy. We now have sufficient knowledge to design safe RCA protocols ready for automated delivery. In turn, automated RCA will allow widespread use of the technique with all its well-established benefits. However, to implement automated RCA in the USA, where the commercial development of this technology has clearly lagged, a concerted effort will be needed by health care providers, equipment manufacturers and regulatory authorities alike. In particular, the responsibility of equipment manufacturers who have both the resources and expertise to bring automated RCA to the bedside cannot be overemphasized. Without their support, our patients will likely have to wait several more years to receive RCA in complete safety.

Conflict of Interest

The authors are inventors of patent pending Automated RCA Systems.

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Modeling Hepatitis C Virus Therapies Combining Drugs and Lectin Affinity Plasmapheresis

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Key Words
Hepatitis C virus · Lectin affinity · Hemodialysis · Plasmapheresis · Mathematical models · *Galanthus nivalis* agglutinin

Abstract
Hepatitis C virus (HCV) infection can be cured by standard pegylated interferon (IFN) + ribavirin drug therapy in 30–50% of treatment-naïve genotype 1 HCV patients. Cure rate is defined as a sustained viral response measured 6 months after the end of treatment. Recently, Fujiwara et al. [Hepatol Res 2007;37:701–710], using a double-filtration plasmapheresis (DFPP) technique, showed that simple physical reduction in circulating HCV using a 1-week pretreatment increased the cure rate for treatment-naïve type 1 HCV patients from 50 (controls) to 78% (treated). For previous nonresponders, the cure rate increased from 30 to 71%. This effect occurs even though the DFPP per treatment HCV viral load reduction averaged 26%. In clinical studies discussed here, a lectin affinity plasmapheresis (LAP) device caused an estimated 41% decrease in viral load as previously reported. A more detailed analysis using normalized data to correct for any variations in initial viral load gave an average 29% per treatment viral load reduction in 5 HCV-positive dialysis patients. The latter data indicate that continuous application of LAP could bring HCV viral load to undetectable levels in 4.1 days. Compared to DFPP, the LAP approach has the advantage that no plasma losses are incurred. In addition hemopurification can be carried out for extended periods of time analogous to continuous renal replacement therapy for the treatment of acute kidney failure, making the process much more effective. Calculations based on these data predict that continuous hemopurification would substantially increase the rate of viral load reduction (approx. 14-fold) and therefore increase the cure rate for HCV standard-of-care drug therapies without adding additional drugs and their associated side effects.

Introduction

Hepatitis C virus (HCV) infections currently affect more than 3.9 million people in the USA [1] and over 180 million worldwide. The virus is responsible for liver damage that drives much of the need for liver transplants in the USA and Europe.

The current standard of care for HCV infection uses pegylated interferon (IFN-α_{2b}) combined with ribavirin [2]. The therapy is curative in about 40% of patients [3]. According to the World Health Organization, only 30–50% of infected patients respond to pegylated IFN + ribavirin treatment after a 48-week course of therapy. Cure

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rates are related to both viral and human genetics [4]. There are 6 HCV genotypes and more than 50 subtypes. Genotype 1 accounts for 70–75% of all HCV infections in the USA and is associated with a 50% response rate to drug therapy. Genotypes 2 and 3 are more common in Asia and are more responsive to drug therapy [2], with genotype 2 having the best response rate at more than 80%.

The principal problem with the current standard of care is that a majority of patients suffer substantial adverse effects from IFN that can limit patient compliance or cause people to avoid therapy altogether. The side effects include influenza-like symptoms, hematological abnormalities and neuropsychiatric symptoms [2]. Present research in this area is aimed at the development of new, more powerful drugs that can inhibit the action of viral proteins such as the RNA polymerase. Other potential targets include the viral envelope (entry inhibitors) and viral proteases.

We have been developing an alternative strategy using a highly selective extracorporeal filtration therapy analogous to hemodialysis [5, 6]. This therapy combines plasmapheresis with affinity capture using lectins. Lectin affinity plasmapheresis (LAP) has been used in vitro and in clinical trials to rapidly and selectively clear viruses from blood and plasma [7].

In a recent clinical trial, hemodialysis patients infected with HCV were treated with a lectin affinity cartridge in combination with their kidney dialysis treatment [7]. Four patients received up to 3 four-hour treatments 3 times weekly. In a follow-up case study, 1 of these patients received extended treatment consisting of 12 four-hour treatment sessions on the same schedule. The LAP device caused an estimated 41% decrease in viral load in the initial studies. However, using the current data normalized to correct for variations in initial viral load gave an average 29% per treatment viral load reduction in 5 HCV-positive dialysis patients (table 1). As shown here, the predictions based on this result indicate that LAP in combination with drug therapy could reduce HCV viral load to undetectable levels in approximately 4 days, providing a substantial increase in cure rates relative to drugs alone.

Table 1. HCV clearance values in patients

	Average HCV, IU/ml		Change %
	before	after	
Fortis study 1 dialysis controls			
C1	7.90×10^7	1.49×10^7	–81
C2	8.96×10^7	4.60×10^7	–49
C3	4.00×10^7	3.70×10^7	–91
Fortis study 2 dialysis controls			
C1	1.64×10^6	5.82×10^6	256
C3	2.30×10^6	4.23×10^6	84
Mean			24
Fortis study 1 LAP treatment + dialysis			
T1	2.62×10^7	3.03×10^7	16
T2	3.72×10^7	1.86×10^7	–50
T3	4.41×10^7	1.43×10^7	–68
Fortis study 2 LAP treatment + dialysis			
T1	2.64×10^6	7.30×10^5	–72
T3	2.45×10^6	4.50×10^5	–61
T4	2.30×10^6	4.23×10^6	48
T6	2.03×10^6	1.10×10^6	–46
T7	2.48×10^6	3.64×10^6	46
T9	7.79×10^6	4.21×10^6	–46
T10	5.87×10^6	4.48×10^6	–24
T12	2.91×10^6	1.02×10^6	–65
Mean			–29

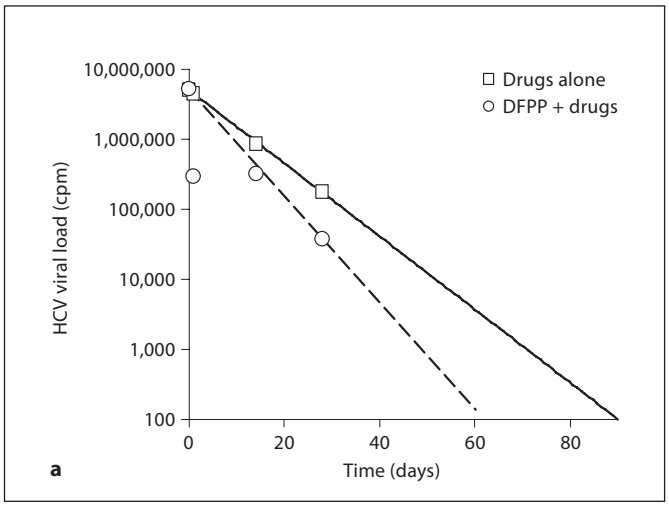
Fortis study 1, 2 = Study 1, entitled 'Controlled, Sequential, Phase I Safety Study to Evaluate the Use of the GNA Hemopurifier® during the Intermittent Dialysis of Subjects with End Stage Renal Disease', was a 3-treatment study involving 6 patients; study 2, entitled 'Single-Case Studies to Evaluate the Preliminary Efficacy of Prolonged Treatment of HCV with the Hemopurifier™ during Intermittent Dialysis', was a 12-treatment case study on 1 patient; both were ERB-approved studies conducted under the supervision of Dr. Vijay Kher at the Fortis Hospital in New Delhi; C = control; T = treatment.

Methods of Analysis

In vitro experiments have demonstrated that viral clearance using LAP is a first-order linear process in tissue culture media, plasma and human blood for a large number of different viral species [8]. Typical clearance half-times for most of these viruses range from 1 to 2 h in the absence of viral replication [6, 8]. Assuming a constant flow rate and total volume, virus clearance follows an exponential decay $C = C_o \cdot e^{-ct}$. For an apparent first-order process, the reaction half-time is related to the clearance rate by $t_{1/2} = \ln 2/c$.

Using this formulation, we can calculate a clearance rate constant (c) from the in vivo HCV clearance rate observed for HCV in our clinical trials (table 1).

In these two studies, we observed an average HCV viral load reduction of 29% per 4-hour treatment at a blood flow rate of 250 ml/min. This is equivalent to an 8.1-hour clearance half-time in the presence of in vivo viral replication. In contrast, there was a 24% increase in the average control viral loads over both studies. As is clear in the results presented, there was a large variation from sample to sample. Previous studies have shown that hemodialysis treatment can have some positive effect on HCV viral load which is quite variable from study to study [9, 10]. However, such changes tend to be transient. In one study, no significant reductions in viral load were observed when followed over the course of 13 months [11].



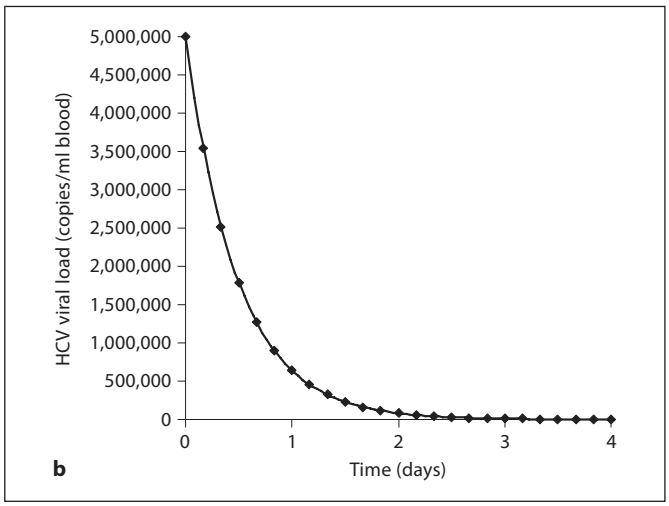


Fig. 1. a Effect of DFPP pretreatment on the response of HCV type 1 to standard-of-care drug therapy. Taken from the data of a clinical study by Fujiwara et al. [13]. The data were originally taken for 30 days and are extrapolated here for comparison with other calculations. The lines are a theoretical exponential decay. **b** Modeling HCV viral load clearance during hemopurification alone from clinical studies on HCV dialysis patients: log plot exponential theory reflecting an average clearance of 29% in 4 h that holds true during continuous affinity hemodialysis treatment and follows the same log linear process observed in vitro.

Inspection of the clearance equation makes clear that the most effective treatment regime would be one of continuous treatment (hemopurification) for as long a period of time as is safe. In continuous renal replacement therapy, commonly used to treat acute kidney failure, hemodialysis therapy is typically maintained for up to 24 h and has been used for up to 40 days on a single patient. By analogy, for continuous affinity plasmapheresis treatment, it is reasonable to suppose that a similar time frame for treatment would be safe.

In order to model the combination of the techniques, we needed an expression that combines exponential decay functions, one clearance rate for the drug treatment (k) and one for the device treatment (c). The combined expression is given by $C = C_o\, e^{-(k+c)t}$, where k = HCV virus daily clearance rate during treatment with pegylated IFN + ribavirin and c = HCV daily clearance rate during continuous hemodialysis of HCV dialysis patients and C_o = initial viral load in international units per milliliter. Using this formulation allows us to calculate the effect of combining continuous lectin affinity hemodialysis with standard-of-care HCV treatment in patients and compare it to the pattern for drug treatment alone. For the purposes of this discussion, the comparison applies primarily to a single continuous LAP treatment in combination with drug therapy where both mechanisms are operating.

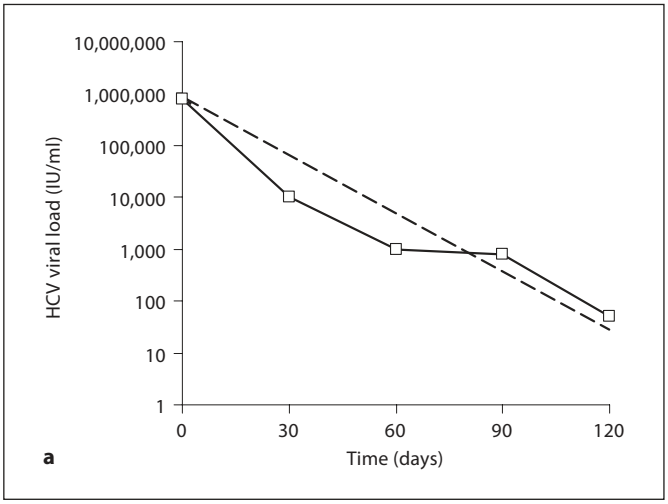
Results and Discussion

The regimen we envision proposes continuous hemopurification to rapidly reduce HCV viral load for the first week of standard-of-care treatment with pegylated IFN and ribavirin [2].

A similar approach developed by Asahi has recently been reported to improve sustained viral response outcomes from 50% without double-filtration plasmapheresis (DFPP) to 78% in genotype 1 HCV patients when DFPP is used in conjunction with ribavirin and pegylated IFN [12, 13]. In DFPP, plasma from venous blood is obtained from the patient and cleared of virus particles by ultrafiltration. In this study, an average of 3 treatments were given for 3.25 h during the first week of drug therapy. Typical results showed an approximate 100-fold drop in viral load in about 1 month (fig. 1a, replotted from data of Fujiwara et al. [13]). This simple 1-week pretreatment by physical virus removal increased the rate of virus clearance by the drug by approximately 33% and allowed drug therapy to be about 50% more successful in curing the infection.

These results are not without historical precedent. Table 2 shows a list of viral infections where viral load is correlated with the severity of viral disease and disease progression. For instance, it is well known that HCV is more responsive to drug therapy when the initial viral load is less than 800,000 copies/ml.

Thus, viral load reduction prior to or early in the treatment process should be expected to improve treatment outcomes. We have therefore looked to find more efficient methods to reduce viral load without the need for fluid replacement.



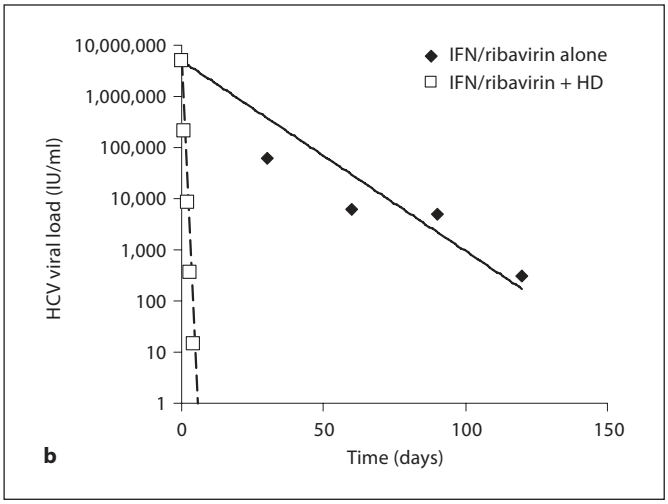


Fig. 2. a Modeling HCV viral load from patient data during IFN + ribavirin treatment (n = 34). The data for this figure were taken from Veillon et al. [33]. The log plot is fitted to a single exponential decay given by $y = 794{,}000^{-0.0857t}$ ($R^2 = 0.873$). **b** Modeling HCV viral load from patient data during IFN + ribavirin treatment in combination with affinity hemodialysis (HD). The data were recalculated and replotted against the predicted curve for the combination of continuous LAP + drug therapy. In both cases, the initial HCV viral load was set at 5×10^6 IU/ml.

Table 2. Correlation of viral load with disease severity and outcomes

Virus	Viral load correlation	Clinical benefit/ market clearance	Reference
Dengue	lethality correlates with high viral load	yes	14, 15
Ebola	lethality correlates with high viral load	yes	16, 17
Hepatitis C	pegylated IFN + ribavirin treatment	yes/yes	18
	disease severity and treatment response correlates with viral load	yes	19, 20
Herpes	antiherpetic drugs	yes/yes	21, 22
HIV	drug cocktails	yes/yes	23, 24
	long-term nonresponders (<1,000 cpm) and 'elite controllers'	yes	25–28
Influenza A	Tamiflu	yes/yes	29, 30
Marburg	plasmapheresis	yes (1 patient)	31
Sin Nombre	lethality correlates with high viral load	yes	32

LAP is a good candidate for such a process. Figure 1b shows the analysis of the HCV virological response predicted for continuous application of LAP in the absence of any other treatments. It shows a rapid reduction in HCV viral load based on a clearance rate of 29% in 4 h obtained in clinical studies. From this it may be calculated that starting from an initial viral load of 5 million IU/ml, continuous LAP should reduce HCV to undetectable levels in 2.28 days.

In order to predict the full effect of performing hemopurification in conjunction with the standard of care HCV treatment, we analyzed the kinetics of HCV drug treatment on a typical HCV patient population. Veillon et al. [33] have provided such treatment data in 34 patients using a combination of pegylated IFN-α and ribavirin. In this study, patients infected with genotype 2 and 3 HCV receiving weekly injections of pegylated IFN-α_{2a} and daily ribavirin were studied. In these patients, a more

than 100-fold decrease in viral load was predictive of a sustained virus response. Among sustained responders to combination therapy, 76 out of 96 (79.2%) had a viral load decrease of greater than 100-fold after 1 month of treatment.

A plot of the data averaged for the patients who showed a sustained virological response is shown in figure 2a. The rate of virus clearance during treatment was evaluated using a single exponential function. The clearance rate constant here was k = 0.0857 per day corresponding to an overall half-time of 8.09 days. While the data is clearly biphasic, a single exponential fit simplifies the picture and gives a reasonable correlation coefficient of 87%.

Figure 2b shows the results of combining drug treatment with 1 session of continuous LAP versus drug treatment alone. The striking feature of this comparison is that the combination treatment of continuous LAP with standard-of-care pegylated INF-α + ribavirin is predicted to proceed significantly faster than drug treatment alone. In this regard it is similar to DFPP, with the primary difference that continuous LAP-mediated virus clearance is at least 10 times faster and will also clear immunosuppressive free viral proteins and viral fragments which would be missed by DFPP.

Conclusions

Physical reduction of viral load has been demonstrated to substantially improve HCV cure rates. Based on the observed rates of virus clearance in clinical studies, we calculate that LAP applied continuously for 4.1 days would reduce HCV viral load to undetectable levels versus more than 30 days measured for DFPP in combination with drugs. This calculation suggests that 1 week of pretreatment with LAP used in combination with standard HCV drug therapy would probably lead to cure rates of more than 80%.

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Novel Insights into the Pathobiology of the Vascular Access – Do They Translate into Improved Care?

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Key Words
Thrombosis · Vascular access · Hemodialysis · Renal failure · Drug therapy

Abstract
While recent developments have allowed greater insight into the vascular pathobiology and intimal hyperplasia, very few of these advances have led to improved clinical care of hemodialysis vascular accesses. Indeed the most common procedure for the treatment of access stenosis and thrombosis is the same model for the creation and study of intimal hyperplasia. The evolution of our understanding of vascular thrombosis is reviewed with a current concept that includes a dynamic interplay of the biophysics, chemistry and biology of the blood vessel with the blood and its constituents. Implications for possible future interventions based on these novel concepts are offered, and the significance of improving our understanding of the pathobiology is emphasized.

Introduction

A set of rounds in a dialysis center or even a brief perusal of the latest clinical studies on vascular access (VA) should be enough for anyone to answer the title question. The most recent VA study in the most influential medical journal in the world is a randomized controlled trial (RCT) of 649 patients from 13 centers in the USA comparing the efficacy of 200 mg dipyridamole and 50 mg of aspirin to placebo for the unassisted patency of hemodialysis arteriovenous grafts. While the graft survival rate at 1 year was statistically better ($p = 0.03$) than that of controls, the treatment group achieved only a 28% 1-year survival compared to 23% for the controls [1]. The difference between the two groups appeared within the first month after which the survival curves became parallel. Differences in the first month are often considered too early for intimal hyperplasia to have developed and are more often considered to be the result of postsurgical thrombosis. Indeed, 44% of all thromboses occurred with a stenosis of less than 50%; however, an accompanying editorial concluded that this was an advance in our struggles against intimal hyperplasia [2]. Most would struggle to call 28% 1-year graft survival an advance, particularly since the drugs and result are quite similar to a study published in the same journal 30 years previously [3]. If most nephrologists thought at all about vascular pathobiology, they would undoubtedly conclude that current concepts are neither new nor are they of any major clinical impact; however, I doubt that the average nephrologist thinks much about vascular pathophysiology when making VA decisions. Indeed, they are most likely to deliberately ig-

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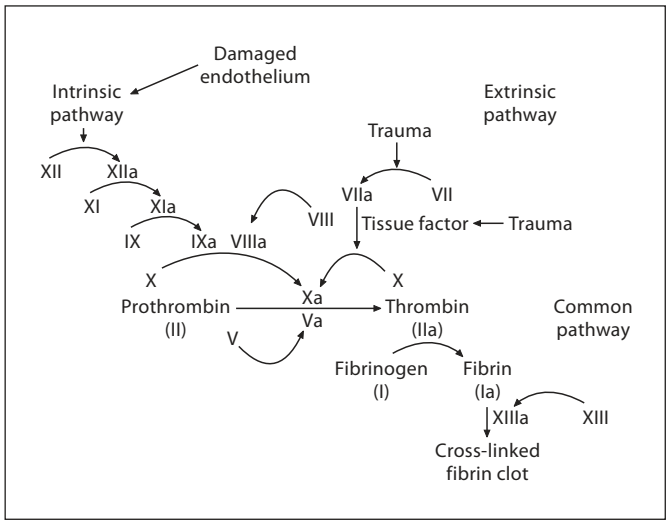

Fig. 1. Original factor: clotting cascade.

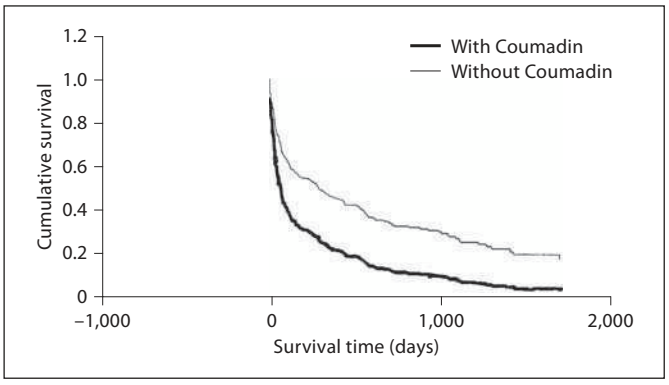

Fig. 2. The initial attempts at pharmacological intervention to prevent VA thrombosis were by the use of warfarin (Coumadin) which were unsuccessful. Some observational studies found even decreased access survival as noted above by our group but they may have been confounded by intention. Negative effect significant at $p < 0.05$.

nore them. Assisted patency by the use of angioplasty has been the major trend in access patency in recent years; however, it is the same angioplasty procedure on healthy blood vessels that is used as a model to produce the development of intimal hyperplasia and stenosis [4]. Therefore, the most common treatment tool creates the problem that we most wish to eliminate. Therefore, a more pertinent question would then be: 'Will the novel insights that we have discovered in vascular pathobiology translate into improved care?' For that to happen, we need to correct defects in both our knowledge and wisdom. To paraphrase Alexandre Dumas: 'The former requires information and memory while the latter requires philosophy' [5]. First we should review our knowledge of the pathobiology of access thrombosis as we have come to understand it, before we enter into the philosophical problems.

Historical Perspective

Theories of blood clotting have existed since antiquity. Aristotle, who felt that all disease resulted from an imbalance of humors, felt that blood was only kept fluid by heat from the heart [6]. Loss of that heat resulted in thrombosis. Variants of that theory existed even through the nineteenth century. John Lister attempted to prove that air was not the cause of thrombosis since he was able to produce thrombosis in a vacuum [7, 8]. Although Virchow discovered fibrinogen shortly thereafter, it was not until 1916 when Jay McLean, a second-year medical student at Johns Hopkins, discovered heparin that the modern-day theory of coagulation began with the clotting cascade. Thus, as the early years of chronic hemodialysis began, we understood coagulation as a series of plasma proteins cascading their way toward thrombosis (fig. 1). As a result, initial attempts at the pharmacological prevention of access thrombosis began with the interference of the production of fibrin polymers by the use of warfarin which interferes with the production of vitamin-K-dependent factors (II, VII, IX, X) in the days of Scribner shunts [9]. Bleeding complications and treatment failure were commonly chronicled [10–13], and success was often only anecdotal. Initially our group found that patients on warfarin had significantly decreased VA survival rates (fig. 2) in an observational study [14, 15]. That finding was echoed by the US Dialysis Outcomes and Practice Patterns Study (DOPPS) [16]; however, both of these studies could have been confounded by intention. More recently, an RCT also found no benefit to warfarin with significant complications of bleeding ($p = 0.03$) [17], but like the aspirin and dipyridamole study there was a slight early benefit that was lost after a few months. Eventually, most came to realize that intervention by inhibition of the clotting cascade alone appeared to be of little benefit.

With the discovery in the 1970s of prostacyclin (PGI_2) [18] and thromboxane A_2 (TxA_2), eicosanoid products of cyclooxygenase, our focus then shifted towards antiplatelet agents. Aspirin irreversibly inhibits cyclooxygenase by acetylation of a serine residue at its active site. Endothelial cells continuously produce cyclooxygenase and re-

cover the ability to produce PGI_2 within hours, but the nonnucleated platelets lose their ability to produce TxA_2 for the life of the platelet. Thus, low-dose aspirin prevents platelet aggregation and vasoconstriction while not interfering with the inhibition of clot propagation or vasodilatation. The use of low-dose aspirin to irreversibly inhibit platelet cyclooxygenase and TxA_2 production without affecting the synthesis of vascular PGI_2 became the standard to prevent coronary artery thrombosis. Subsequent trials to prevent hemodialysis VA thrombosis were initially thought to be successful in Scribner shunts [3]. Unfortunately, as endogenous arteriovenous fistulas or polyfluorotetraethylene (PTFE) grafts began to replace shunts as primary VA, we recognized that intimal hyperplasia was the most common cause of long-term access thrombosis [19]. Although intimal hyperplasia develops in Scribner shunts [20], the original results were particularly difficult to extrapolate, since histological evidence of intimal hyperplasia was not specifically examined. Initial investigations of aspirin use in arteriovenous fistulas and PTFE grafts were encouraging [21, 22], and the results correlated with our newly developed concepts of the roles of a platelet-endothelial balance to maintain vascular flow and hemostasis since experimental thrombocytopenia prevented intimal hyperplasia in rabbits [23]. Although there continue to be favorable reports with aspirin use [1, 24], many subsequent reports revealed a lack of efficacy of aspirin and dipyridamole on intimal hyperplasia [25] and patency rates of hemodialysis accesses [26]. Then thromboxane synthetase inhibitors were shown to have even less effect than aspirin [27], while PGI_2 production was demonstrated to be normal at the anastomotic site where intimal hyperplasia was produced. It appeared that platelet function, TxA_2 and PGI_2 had little to do with intimal hyperplasia. Subsequent studies from the USRDS suggested that aspirin, like warfarin before it, may be actually associated with decreased VA survival [28], and in 2008 all aspirin use to prevent vascular thrombosis in diabetics came under attack [29, 30] when two large RCTs found no benefit of aspirin over placebo to prevent vascular thrombosis or mortality [31, 32].

Similarly antiplatelet drugs that interfere with ADP production (dipyridamole [33–36]) and receptor attachment (cilostazol [37, 38], clopidogrel [39, 40]), along with competitive inhibition of cyclooxygenase (eicosapentaenoic acid [41]), followed a similar pattern to aspirin of early favorable reports [33, 35, 45–50] and subsequent failure of other groups to confirm benefits [15, 34, 36, 51–54]. Nevertheless, a Cochrane review of all RCTs comparing the use of antiplatelet agents to placebo to prevent hemodialysis VA thrombosis concluded that each agent studied did reveal at least statistical benefits of treatment [55]. Unfortunately, like the most recent RCT [1], while the clinical benefits are statistically significant, they are often less apparent with 1-year unassisted potencies for grafts often below 30%. Furthermore, since those benefits could only be purchased by an increased risk of bleeding with increased mortality [56], it has therefore become obvious that VA thrombosis involved more than platelets and fibrin polymers and that a better understanding of vascular physiology is needed [57, 58].

Current Pathobiology Concepts with Potential Novel Interventions

We have come to realize that there is more to hemostasis than platelet and fibrin. Endothelial cells arise from hemangioblasts just as hematopoietic cells [59] and intraaortic endothelial cells are themselves also thought to be a source of hematopoietic stem cells [60]. Endothelial cells can circulate in states of vascular damage and may be involved in repair and angiogenesis [61]. Under normal circumstances, a thin layer of glycocalyx lines endothelial cells, while endothelial protein C receptors, PGI_2 and thrombomodulin tissue factor pathway inhibitor (which is an anticoagulant protein that abrogates the activity of the tissue factor-factor VIIa catalytic complex) produce an anticoagulant effect. In order to maintain flow in the event of a thrombus, an endothelium-dependent relaxation factor, nitric oxide (NO), is produced along with interleukin (IL) 10 and PGI_2 for vasodilatation to maintain flow while providing for local production of tissue plasminogen activator, urokinase plasminogen activator and binding sites for plasminogen until thrombolysis occurs. However, when the endothelium is injured and the glycocalyx is disturbed, selectins known as endothelial linked adhesion molecules can be exposed as the endothelium becomes prothrombogenic. In order to understand the mechanisms under which the endothelium can be transformed from a surface preventing coagulation to that of a procoagulant, one must understand the glycocalyx. The glycocalyx is a 100-angstrom-thick layer of fibronectin, proteoglycans (such as heparan sulfate with similar anticoagulant properties to heparin sulfate), collagen and elastin that acts as a filter and regulatory barrier between the blood and the endothelial membrane [62]. Through this barrier there are many finger-like projections that allow the endothelial membrane direct ac-

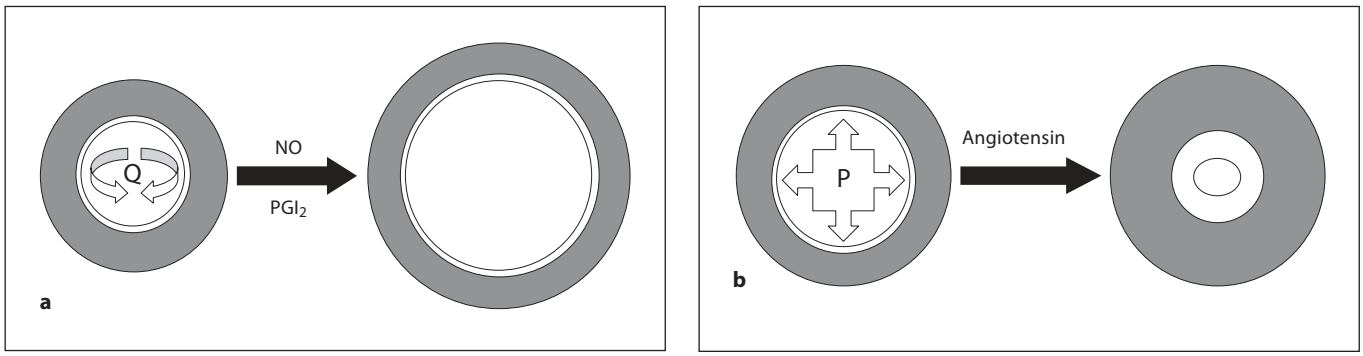

Fig. 3. a Increased flow (Q) results in vasodilatation and anticoagulant activity due to PGI_2 and NO. **b** Increased pressure (P) results in angiotensin-mediated smooth muscle migration, proliferation, intimal thickening and decreased luminal diameter. Intimal hyperplasia is the most common cause of access stenosis, and angioplasty has become the most common experimental model to induce this lesion.

cess to the blood and that respond to injury by unmasking latent C3b Fc receptors that activate complement and subsequently the coagulation cascade and neutrophil adhesion [63]. Release of platelet activating factor and endothelin 1 promotes vasoconstriction, while von Willebrand factor, tissue factor, plasminogen activator inhibitor 1 and factor V augment thrombosis [64]. While normally most of the vasodilators produced by the endothelium such as PGI_2, NO and ADP are platelet inhibitors, its vasoconstrictors are platelet activators.

After creation of an arteriovenous connection, there is an increase in shear stress, which is the frictional force exerted on the wall by the blood and defined as:

$$\text{shear stress} = 4\eta Q\pi r^3$$

where η is the blood viscosity, Q is blood flow and r is the vessel radius. The increased flow Q results in vasodilatation through upregulation of cyclooxygenase with increased PGI_2 production, as well as endothelial NO synthetase which produces higher levels of NO that relaxes vascular smooth muscle cells (VSMCs), inhibits VSMC proliferation and prevents adhesion of inflammatory cells and platelets (fig. 3a) [65]. It appears that increased Q increases GTP cyclohydrolase that increases cofactor tetrahydrobiopterin that regulates endothelial NO synthetase [66]. In contrast, the increase in transmural pressure (fig. 3b) that occurs results in increased VSMC proliferation and wall thickness [67] which prevents an increase in circumferential wall stress (σ) as defined as:

$$\sigma = P_iD_i/2T$$

where P_i is the internal pressure, D_i is the internal wall diameter and T is the thickness of the wall. Angiotensin II (Ang II) is thought to play a critical role in this proliferation (fig. 4). Very little endothelial surface ever comes into direct contact with blood. Only small finger-like projections protrude through the glycocalyx, which additionally creates large pits through which a molecular sieving occurs. In addition to the projections there are also nests of enzymes on the endothelial surface membrane sitting in the pits (caveolae) where only molecules of a particular size are allowed to enter and percolate. Here angiotensin and bradykinin come into contact with the converting enzyme. Since the angiotensin-converting enzyme is one of the most recognizable surface antigens for marking endothelial cells, it would theoretically be in an ideal position on the cell to sense and respond to injury, and recent studies have shown that VSMCs synthesize Ang II intracellularly. Once Ang I has been converted to Ang II by endothelial bound angiotensin-converting enzyme, Ang II can react with VSMC receptors, promoting the well-known vasoconstriction. However, Ang II also stimulates VSMC hypertrophy [68]. The mechanism of Ang-II-induced VSMC proliferation appears to occur at multiple steps, including promotion of synthesis of platelet-derived growth factor (PDGF) A chain, transforming growth factor β, thrombospondin and proto-oncogenes L-*myc* and c-*fos* [65]. All of those effects were inhibited by cilazapril in rats [69]. Similarly, in rats, a significant reduction of VSMC mitotic activity was found in ramapril-treated rats after balloon angioplasty injury [70] and captopril-treated New Zealand white rabbits [71]. Subsequent to the migration of VSMCs to the intima, they begin to proliferate and elaborate a glycoprotein. That is thought to be the traditional pathway of development of intimal hyperplasia, which is the

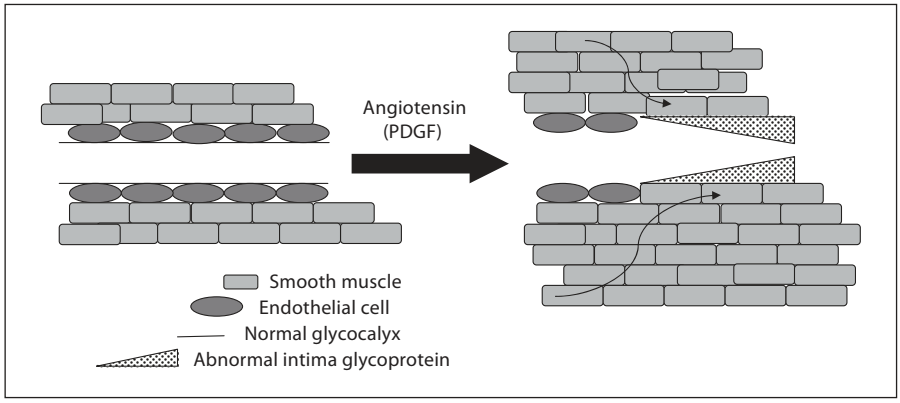

Fig. 4. Angiotensin is thought to mediate smooth muscle cell migration from the media to the intima with elaboration of an abnormal glycoprotein that thickens the intima and narrows luminal flow. PDGF = Platelet-derived growth factor.

most frequent cause of VA stenosis and failure, although there is evidence that adventitial cells also play a role [72]. Infusion of Ang II increases neointima formation and decreases endothelial function after stent placement [73] and is known to increase arterial thrombogenesis by activation of coagulation factors such as tissue factor [74] or by inhibition of fibrinolytic factors via activation of plasminogen activator inhibitor 1 or inhibition of tissue plasminogen activator [75, 76] via Ang II type 1 receptor stimulation. Those thrombogenic effects may be blocked by NO production [77].

Therefore, while increased flow results in increased NO production, vasodilatation and thrombus protection, increased transmural pressure results in increased Ang II, VSMC proliferation and thrombogenicity.

Implications for Intervention

Nonpharmacological Intervention

With an understanding of the physiology noted above, one can now understand how angioplasty would create intimal hyperplasia. The increased transmural pressure created on a normal vessel wall by angioplasty is now a common method of inducing intimal hyperplasia and stenosis in order to study its pathophysiology [78]. Nevertheless, the procedure has become the most common (at least at present) successful intervention for the treatment of stenosis [79]. As a result, drug-eluting stents have been developed to prevent the problems that angioplasty causes [80]. Although the antiproliferative agents paclitaxel [81] and sirolimus [82] have been successful in animal fistula models, meta-analysis reveals increased mortality with drug-eluting paclitaxel stents compared to placebo in patients with coronary artery stents [83]. Since the effect seems to result from a late thrombosis rather than stenosis that occurs usually after the patients have finished their course of anticoagulation, some have suggested that this problem may be improved. Similarly, sirolimus appears particularly ineffective in patients with renal failure who have vascular disease [84]. In fact, it is unclear whether any stent placement improves patency [85]. Despite the association with the significant complications of endothelial damage, stent fracture, migration and infection [86], the current role of stents in hemodialysis VA remains controversial. Similarly, radiation has been used for its antiproliferative effect after angioplasty in a pilot study of 25 dialysis patients, and although it was found safe there was no evidence of increased patency at 12 months [87]. Since angioplasty creates intimal hyperplasia which cannot be prevented by stents or radiation, one would hope that the improved understanding of vascular pathobiology will lead to better pharmacological solutions.

Pharmacological Intervention

One would have expected that nitrates would have a major beneficial effect on VA survival. Nitrates have been used as vasodilators since 1847 when Constantine Herring placed a small quantity of oily nitroglycerin onto his tongue and found that it could both relieve angina and produce very uncomfortable headaches. However, it was only in the last few decades that we have come to realize that the empirical use of organic nitrates for vasodilatation only mimicked the endothelium's natural production of endothelium-dependent relaxation factor which is now known to be NO. All NO-containing substances (including nitroprusside) can activate cyclic AMP and cyclic GMP in VSMCs. However, more recently it was shown [88] that by inhibition of nucleotide phosphodiesterase,

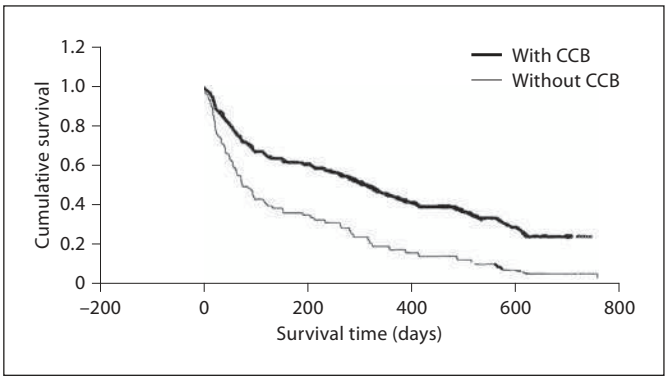

Fig. 5. In observational studies, calcium channel blockers (CCBs) have been shown to be associated with improved primary survival of PTFE grafts. In vitro they are known to inhibit intimal hyperplasia and preserve endothelial production of NO and PGI_2. Significant at $p < 0.006$.

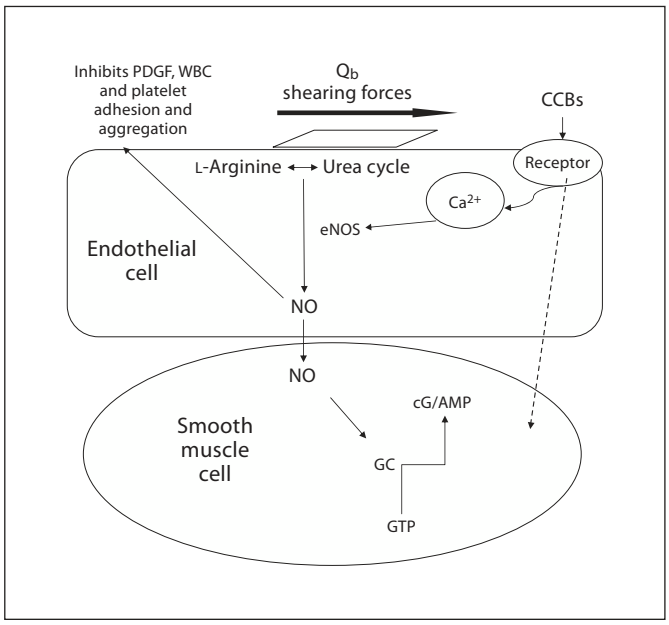

Fig. 6. CCB effects on VA survival may be due to the preservation of endothelial production of NO and PGI_2. Alternatively they also inhibit (dashed arrow) VSMC migration. WBC = White blood cell; Q_b = blood flow; eNOS = endothelial NO synthetase; GC = guanylate cyclase.

NO induces an increase in cyclic GMP, which inhibits both platelet aggregation and adhesion to endothelial cell membranes. Apparently, cyclic AMP controls platelet aggregation, but cyclic GMP controls both adhesion and aggregation. The impact of NO on the patency of vascular grafts was first demonstrated in 1988 [89], when it was shown that mammary artery/coronary artery bypass grafts had a higher patency rate and higher levels of NO than saphenous veins. Since that time, other investigators have confirmed the deficient venous production of NO that contributes to reduced graft patency [90]. In addition, NO also inhibits mitogenic substances such as PDGF, which may also contribute to intimal hyperplasia and improve endothelial dysfunction damaged by hypercholesterolemia. The normal endothelial cell maintains its own pool of L-arginine just to synthesize NO through its metabolism to citrulline [91]. Infusion of L-arginine into the atherosclerotic arteries of 15 patients undergoing cardiac catheterization restored normal endothelial function that had been lost [92].

Despite the theoretical promise, we found no statistical benefit to nitrates [15]. While there was a tendency towards improved access survival that did not reach significance in grafts, native fistulas displayed no such tendency. In contrast we found improved primary graft survival [15] associated with the use of calcium channel blockers (CCBs; fig. 5), which was confirmed by both the US and international DOPPS [16, 35], as well as improved graft [16, 35, 93–104] and fistula [16] survival for angiotensin-converting enzyme inhibitors (ACEIs) [95]. Given the pathophysiology, the fact that ACEIs were beneficial in humans and in animal models [96] is not surprising, but perhaps the effect of CCBs was the nitrate effect that we missed. CCBs are well known for inhibiting the entry of calcium into VSMCs and blocking calcium mobilization from intracellular stores and VSMC vasodilatation; however, interference with intracellular calcium stores by experimental CCBs may help preserve the function of NO and PGI_2 in endothelial cells damaged by atherosclerosis (fig. 6) [97–99]. The effect on preservation of NO function in hemodialysis patients by CCBs appears to come along with preventing oxidation stress. Patients on hemodialysis have evidence of extensive oxidation of lipids, thiols, proteins and nucleic acids and methylation of arginine that could prevent the local production of NO. Many of these changes can be reduced by short-term treatment with amlodipine [100]. Although some have suggested that cell permeability to calcium can modify endothelial cell structure reducing the biophysical wall shear [101] and thereby theoretically promote intimal hyperplasia, in practice such is not the case. Verapamil is known to inhibit intimal hyperplasia in experimental vein grafts despite a lack of effect on either the endothelium or platelets [102]. That effect may be due to the effect of CCBs to inhibit VSMC migration [103]. In cell culture

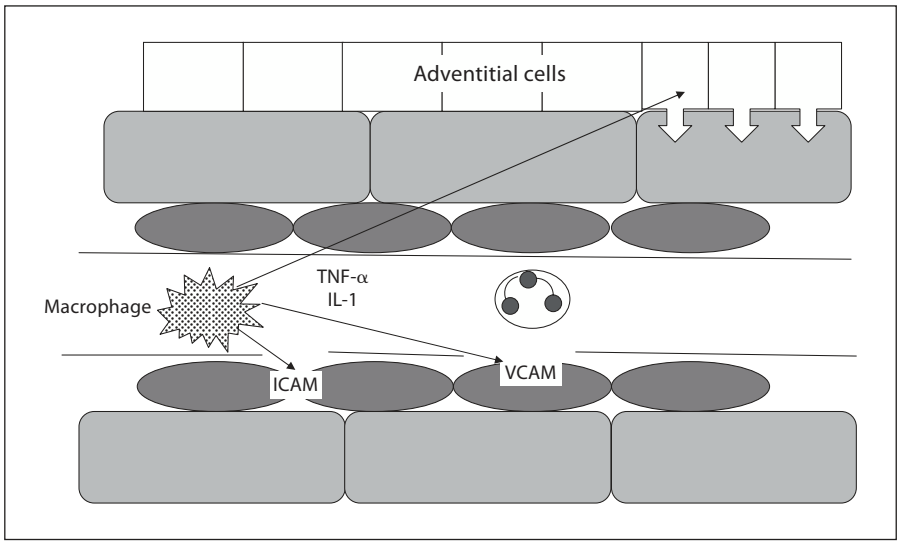

Fig. 7. TNF-α released from macrophages helps ablate intercellular adhesion molecule (ICAM-1) as well as expose vascular cellular adhesion molecule (VCAM) for leukocyte attachment and thrombosis. In addition, they can stimulate adventitial hyperplasia and migration to form intimal hyperplasia similar to smooth muscle cells.

experiments, VSMC migration is inhibited by both dihydropyridines (amlopidine, nifedipine or nicardipine [101]) and phenylalkylamines (like verapamil [102]). Most think that the effect is mediated by the ability to block VSMC L-type calcium channels (CaV1.2); however, some have argued that the effect is independent of CaV1.2 blockade [104]. One other possibility appears that CCBs also have the ability to inhibit the signaling of proinflammatory cytokines like granulocyte-macrophage colony-stimulating factor, tumor necrosis factor α (TNF-α), IL-1β, transforming growth factor β_1 or PDGF [105–107]. Since PDGF is well known to stimulate VSMC migration in intimal hyperplasia and is even used as an experimental model [103], this may possibly be the mechanism since it also appears that CCBs prevent activation of tyrosine kinase growth receptors that are well known to produce fibrosis due to inflammation in peritoneal dialysis patients. Perhaps anti-inflammatory agents may be of use.

Future Biotechnology Possibilities

While those drugs may prevent intimal hyperplasia, there is another approach to treat established disease by reducing wall mass through induction of cell death and extracellular matrix removal with antibodies to PDGF receptors that have induced intimal regression in baboon PTFE grafts [108]. Other possible molecular targets could include bone morphogenetic protein, nuclear factor κB [109] or the use of picropodophyllin to inhibit insulin-like growth factor 1 receptors [110].

Pathobiology

Sepsis is extremely common in patients on hemodialysis [111] and 23% of those infections occur even without central catheters [112], and a chronic inflammatory state unrelated to active infection is also common and is associated with malnutrition and poor survival [113]. Unfortunately, we now also know that inflammation increases thrombosis by increasing tissue factor, while decreasing thrombomodulin and plasminogen activator inhibitor 1. TNF-α released from macrophages helps ablate intercellular adhesion molecule 1 as well as expose vascular cell adhesion molecule for leukocyte attachment and thrombosis (fig. 7).

Implications for Pharmacological Intervention

Pentoxifylline is a methylxanthine with a structure similar to caffeine and theophylline. Drugs of this class are primarily known for their effect on nucleotide phosphodiesterase resulting in central nervous system stimulation and VSMC relaxation. However, pentoxifylline was originally marketed to improve the subnormal flexibility of erythrocytes in patients with claudication. While theophylline has been used for antiplatelet effects, presumably through phosphodiesterase inhibition of 5′-cAMP [114, 115], pentoxifylline has been shown to be an even more potent inhibitor of ADP-induced platelet aggregation [116]. Indeed, in one direct comparison with aspirin, pentoxifylline was felt to be even more effective as an antiaggregate [117, 118]. More interestingly, cyclooxygenase inhibition by aspirin and phosphodiesterase

inhibition by pentoxifylline were synergistic [119]. Additionally, pentoxifylline decreased viscosity and leukocyte and platelet adhesion to endothelial cell walls [120] and promoted PGI_2 release from endothelial cells [121, 122]. In human clinical studies it appeared more effective than aspirin in stroke prevention [123].

More importantly, pentoxifylline has been shown to inhibit inflammatory cytokines such as TNF-α released in sepsis (fig. 7) after bacterial endotoxins [124], and we have found that pentoxifylline might be a useful adjunct to ACEIs in reducing proteinuria in diabetic glomerular disease [125] by inhibiting monocyte chemoattractant protein 1 [126]. Recently investigators have found that markers of inflammation, such as C-reactive protein, sedimentation rate, total leukocyte count, IL-10 and TNF-α are reduced by pentoxifylline in diabetic and atherosclerotic patients and those with vascular disease [127–129]. Since uremic serum alone appears sufficient to upregulate expression of traditional inflammatory/thrombogenic mediators of monocyte chemoattractant protein 1, IL-8 and vascular cell adhesion molecule [130], inhibition of these mediators may have an important therapeutic benefit in promoting access survival. Similarly, while the exact mechanism may not be identified, we also found a significant benefit of pentoxifylline in the primary survival of hemodialysis grafts [15] (fig. 8) while another group found benefit in Scribner shunt survival [131]. Since we did not find an effect in fistulas, we speculated that the benefit may be less related to intimal hyperplasia and more related to inflammatory mediators; however, in experimental animals, pentoxifylline inhibition of cytokines resulted in less VSMC proliferation and neoadventitial hyperplasia within the vessel wall early after angioplasty and increased late lumen size [132].

Not all pharmacological interventions may be positive, however. There are many effects of recombinant human erythropoietin (rhEPO) that are unrelated to anemia [133]. Like Ang II, rhEPO appears to be a proliferative agent and while Ang II infusion increases EPO [134], ACEIs inhibit the effects of rhEPO [135]. Similarly both have receptors that are part of the tyrosine kinase family of growth receptors which are characterized by an extracellular N terminus and an intracellular C terminus that leads to phosphorylation. Analysis of amino acid sequences suggests that they both evolved, along with this family of growth receptors, possibly from primitive adhesion molecules (fig. 9) [135]. Therefore it is not surprising that since Ang II stimulates insulin-like growth factor by induction of tyrosine kinase receptors in VSMCs [136], rhEPO would likewise be shown to increase intimal hyperplasia [137] and that rhEPO is even more potent than vascular endothelial growth factor in predicting proliferative retinopathy in diabetics [138], and its use has been associated with an increased incidence of proliferative retinopathy [139, 140]. Therefore, the recent finding that rhEPO is associated with increased VA failure with subcutaneous administration ($p = 0.01$) [141] is not surprising due to the prolonged rhEPO exposure in the vascular system after subcutaneous administration.

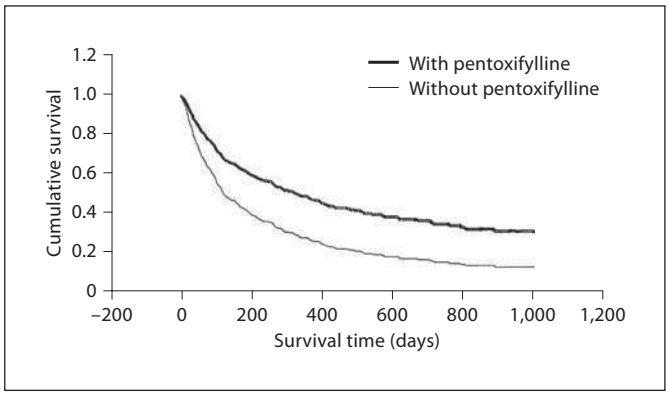

Fig. 8. In observational studies, pentoxifylline has been shown to be associated with improved primary survival of PTFE grafts. The effect may be the result of many pathways but it is known to be an important inhibitor of TNF-α, which is known to expose adhesion molecules and promote clotting. In animals it has been used to inhibit intimal hyperplasia due to cytokine-mediated adventitial hyperplasia. Significant at $p < 0.03$.

Other Future Possibilities

Inhibition of hydroxymethylglutaryl coenzyme A reductase is also an antiproliferative agent to reverse the effects of PDGF [142]. In tissue culture, it has been demonstrated to inhibit both VSMC and endothelial proliferation, as well as PGI_2 production, but the antiendothelial cell activity occurs above therapeutic dosing [143]. In rabbits with endothelial injury induced by balloon angioplasty, significant reduction of intimal hyperplasia was seen after treatment with lovastatin [144]. Simvastatin reduces TNF-α-induced invasion of human muscle cells by attenuating cell migration via ρ-kinase inhibition and subsequent cytoskeletal disruption, and by decreasing metalloproteinase 9 secretion via a posttranscriptional mechanism [145]. The effect was only observed for lipophilic statins when VSMC invasion was inhibited by an artificial basement membrane barrier (fluvastatin > atorvastatin > simvastatin >> lovastatin) [146]. Unfortunately, this effect has not been tested in dialysis patients. In

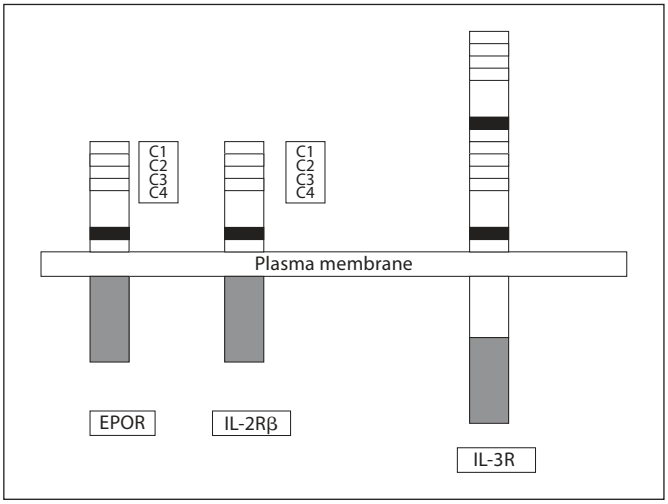

Fig. 9. The structural and functional homologies between erythropoietin and other growth factor receptors. They all may have evolved from a common adhesion molecule. The extracellular domains share a similar 20-amino-acid sequence including 4 cysteine residues (C1–4) and a Trp-Ser-X-Trp-Ser sequence (black box) which is repeated twice in the IL-3 receptor (IL-3R). The cytoplasmic domain has a region rich in proline, serine and acidic residues (cross-hatched lines). EPOR = Erythropoietin receptor; IL-2Rβ = IL-2β receptor.

addition since VSMCs have receptors for low-density lipoproteins, other groups have developed processes of direct photodynamic targeting to prevent neointimal hyperplasia in animal PTFE [147] and angioplasty models [148]. A liposomal formulation of a highly hydrophobic photosensitizing agent, Zn(II)-phthalocyanine, is readily taken up by cultured VSMCs, and subsequent red light irradiation induced cell death and neointimal hyperplasia in rabbits [144]. Another group has been successful with endovascular methylene blue followed by laser light during surgery in pigs with PTFE grafts [149].

Heparin has already been mentioned as an important constituent of the glycocalyx and binds an epidermal growth factor secreted from macrophages, heparin-binding epidermal-growth-like growth factor, that is activated by reduced endothelial shear stress, stimulates VSMC proliferation and appears to be the signal for proliferation in low shear stress states [150]. Heparin has long been a common drug used in the dialysis process and has been shown to reduce intimal hyperplasia after arterial injury VA implantation [151] and vascular injury [152–154]. In contrast to endothelial cells that produce heparan sulfate proteoglycan, VSMCs produce chondroitin sulfate and dermatan sulfate that promote cell migration and proliferation [155, 156]. As intimal hyperplasia proceeds, the new glycocalyx grows from a normal 100-angstrom size to lumen occlusion, and the proteoglycans are replaced by type I collagen and elastin [157] from VSMCs and fibroblasts. To bring control of the growth of the glycocalyx back under standard inhibitory factors could conceivably play a major role in preventing lumen occlusion. In one human study of 212 infragenicular vein grafts, direct infusion of heparin and nitroglycerin after thrombectomy contributed to the prolonged salvage of 80% of the thrombosed vein grafts [158]. Subcutaneous administration of low-molecular-weight heparin also significantly reduced the incidence of intimal hyperplasia [159] in another study. Unfortunately, some groups have found no benefit but only increased bleeding with the intraoperative use of intravenous heparin [160]. Since the antiproliferative effect appears disassociated from the anticoagulant effect, much promise appears in the study of heparin fragments with no anticoagulant hazards.

Also as biotechnology continues to advance, viral transfection with inhibitors of intimal hyperplasia may be available in the future. In animals, adenovirus transfection of tissue factor pathway inhibitor has been used to cause apoptosis of VSMCs [161] and to prevent intimal hyperplasia [162]. Another possibility is edifoligide, a double-stranded oligodeoxynucleotide that competitively inhibits the transcription factor E2F, a critical regulator of the cell cycle. Edifoligide has undergone extensive clinical testing for the treatment of intimal hyperplasia following vascular bypass procedures [163], although results so far have been disappointing [164, 165].

Finally there may still be a role for NO despite its lack of effect in observational studies when given locally. Perivascular delivery of the NO donor 1-[2-(carboxylato)-pyrrolidin-1-yl]diazen-1-ium-1,2-diolate on drug-eluting stents may significantly reduce the amount of neointimal hyperplasia and inflammation in rats [166].

Summary of Current Vascular Pathobiolgy and Implications

We have evolved from the humeral theories of the Ancients through chemical cascades and platelet plugs to an understanding of a dynamic interplay of physics, biology and chemistry of vascular thrombosis with the following conclusions:

– Increased flow appears to result in vascular dilatation and protection against thrombosis mediated through NO and PGI_2.

- Increased pressure results in VSMC hypertrophy and intimal hyperplasia mediated by angiotensin which also increases thrombogenicity.
- Inflammation may also stimulate adventitial migration and intimal hyperplasia, perhaps through TNF.
- Although TxA_2 and PGI_2 do not appear to be involved in intimal hyperplasia, antiplatelet agents appear to have a small but statistically significant effect particularly in the early course.
- Angioplasty results in creation of intimal hyperplasia and late stenosis.
- The beneficial effect of stents is controversial with conflicting and inconclusive studies; however, the complications clearly present.
- Drug-eluting stents used for coronary artery disease have poor results in renal failure with significant late thrombosis.
- ACEIs and CCBs appear to have benefit in observational studies, perhaps through their effect on angiotensin and NO, respectively.
- Pentoxifylline also has an effect, perhaps through its effect on inflammatory mediators.

Why We Should Care about Pathobiology: The Philosophy of Discovery

Several decades ago, a historian attempted to understand why China never discovered America, or at least Europe [167]. When Marco Polo visited in the 13th century, he was astounded by how far advanced beyond the technical capacities of Europe the Chinese were; yet, it was the Europeans that discovered both America and China. His conclusion was that China had already considered itself the only territory worth knowing. As a result, China has never been able to equal the success of the Western world. A similar problem led to the downfall of the mighty Babylonian empire and the rise of the Egyptian dominance that was to continue down to Cleopatra. The Babylonians never abandoned the lunar calendar which was uncomplicated and easy to follow in the evening sky. As a result they were unable to accurately predict the seasons and ensure proper planting time. A reliable solar calendar was more urgent to the Egyptians due to the seasonal loss of life with the flooding of the Nile. Both philosophical challenges face nephrologists. With the advent of interventional nephrology, most nephrologists feel that they already know everything worth knowing. We can intervene and reduce stenosis, perhaps quite a number of times, without ever needing to understand why the stenosis occurs. Unfortunately, our procedure for reversing stenosis is the model for creating stenosis. The newer concepts of pathobiology are not as simple as the concepts they replace and oftentimes appear arcane and irrelevant; nevertheless, in a transposition of our title question, it is clear that unless we come to improve our insight into the pathobiology of VAs, we will never be able to improve care.

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Blood Purification

Abstracts

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1

Comparison of Different Equations for Whole Body Bioimpedance Measurements to Estimate Body Fluid Volumes in Hemodialysis Patients

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Background: The aim of the study is to compare the estimation of ECV and intracellular volumes (ICV) in HD patients using the Xitron equation (XE) (Xitron Manual) and a recently modified equation (ME) developed by Moissl (ME, Moissl et al. Physiol Meas 2006;27:921). **Methods:** Three hundred and fifty-nine whole body bioimpedance measurements were performed pre- and post-HD in 23 HD patients as post-dialytic weights were gradually reduced to attain normal body hydration (Zhu et al. Physiol Meas 2008;29:S503). Whole body ECV (wECV) and ICV (wICV) were calculated by XE using Eq.1–3 and ME using Eq. 4–7, respectively:

$$wECV = \left(\frac{\rho_{ECV} \cdot K_B \cdot H^2 \cdot \sqrt{W}}{1025 \cdot R_E} \right)^{2/3} \quad (1)$$

$$wICV = wECV \cdot \left[\left(\frac{\rho_{MIX} \cdot (R_I + R_E)}{\rho_{ECV} \cdot R_I} \right)^{2/3} - 1 \right] \quad (2)$$

$$\rho_{MIX} = \rho_{ICV} - (\rho_{ICV} - \rho_{ECV}) \cdot \left(\frac{R_I}{R_I + R_E} \right)^{2/3} \quad (3)$$

where R_E and R_I are extra- and intracellular resistances in Ω, H is body height in cm and W is body weight in kg. The resistivity constants in ECV calculation (ρ_{EVC}) were 40.5 Ω·cm for males and 39.0 Ω·cm for females; and in ICV calculation (ρ_{IVC}) were 273.9 Ω·cm and 264.9 Ω·cm respectively. K_B is a constant factor (K_B = 4.3), correcting whole body measurements by relating the relative proportions of the leg, arm, trunk and height.

$$wECV = k_{ECV} \left(\frac{H^2 \cdot \sqrt{W}}{R_E} \right)^{2/3} \quad (4)$$

$$k_{ECV} = \frac{0.188}{BMI} + 0.2883 \quad (5)$$

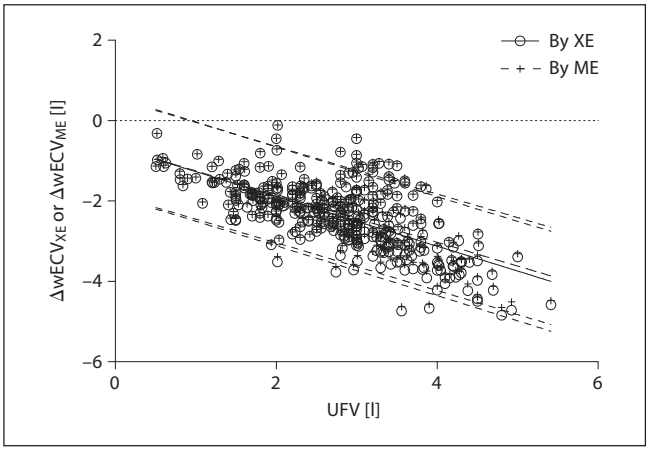

Fig. 1. Correlation between ΔwECV and UFV shown with regression line ±95%CI.

$$wICV = k_{ICV} \left(\frac{H^2 \cdot \sqrt{W}}{R_I} \right)^{2/3} \quad (6)$$

$$k_{ICV} = \frac{5.8758}{BMI} + 0.4194 \quad (7)$$

Total body water (TBW) is defined as the sum of wECV and wICV. ΔWeight (ΔWt), ΔwECV, ΔwICV and ΔTBW were calculated by the differences between pre- and post-HD values. UFV was considered as a reference value. Paired t-tests were applied to compare the same parameters calculated by two equations (XE vs. ME). **Results:** ΔwECVs calculated by XE and ME were not significantly different. ΔwICV was significantly less calculated by ME than by XE (table 1). ΔwECV, ΔwICV and ΔTBW calculated by both equations were significantly less than UFV or ΔWt. There was no significant difference between the degree of correlation of UFV and ΔwECV calculated by XE (R^2 = 0.45) or ME (R^2 = 0.44) (fig. 1). **Discussion:** ΔwICV calculated by ME is significantly lower than that of using XE, which results in less difference between ΔTBW and UFV or ΔWt. This reduced the error of the estimate of TBW removal. For examination of non-dialytic uses of this equation, confirmation by gold standard methods – isotopic dilution studies for ECV and TBW estimate are required. **Conclusion:** The Moissl equation improves accuracy of the measurement of ICV and therefore TBW in hemodialysis patients.

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Table 1. (for Abstract 1)

		By XE	By ME
Wt, kg	Pre-HD	76.2 ± 16.8	
	Post-HD	73.6 ± 16.6	
wECV, l	Pre-HD	16.3 ± 3.2	15.9 ± 3.1‡
	Post-HD	13.9 ± 3.0	13.6 ± 2.8‡
wICV, l	Pre-HD	16.9 ± 4.2	17.9 ± 3.3‡
	Post-HD	18.3 ± 4.5	18.5 ± 3.3†
TBW, l	Pre-HD	33.2 ± 6.7	33.9 ± 6.0‡
	Post-HD	32.2 ± 6.6	32.2 ± 5.6
ΔwECV, l		−2.4 ± 0.8§	−2.3 ± 0.8§, ‡
ΔwICV, l		1.4 ± 1.4§	0.6 ± 1.0§, ‡
ΔTBW, l		−1.0 ± 1.5§	−1.7 ± 1.4§, ‡
ΔWt, kg		−2.5 ± 0.9	
UFV, l		2.8 ± 0.9	

Compared to XE: † $p<0.05$; ‡ $p < 0.001$.
Compared to ΔWt or UFV: § $p < 0.001$.

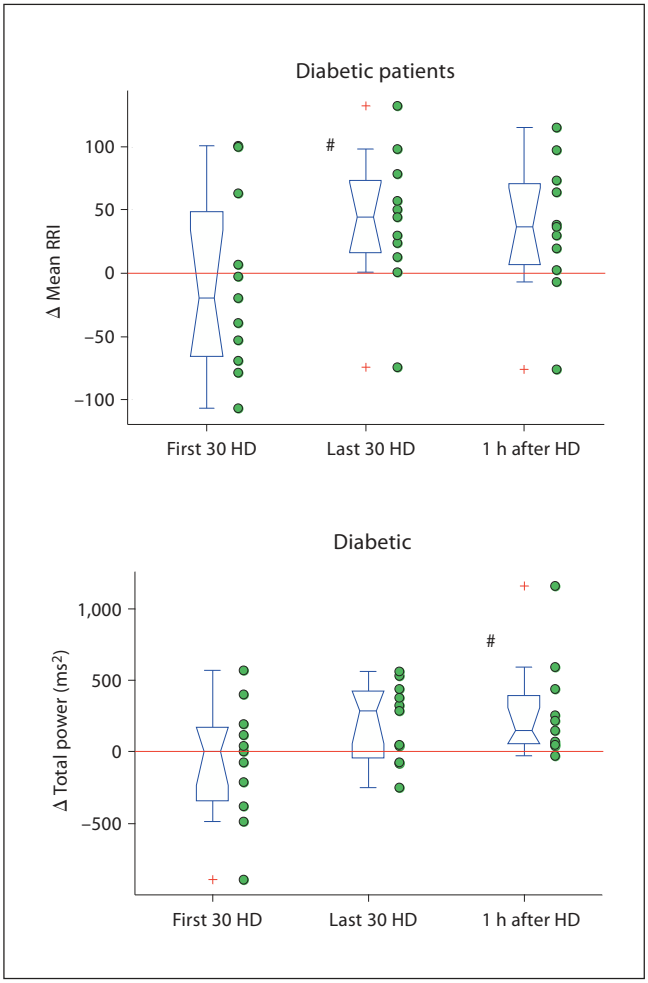

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Fig. 1. Boxplot showing the differences of mean NNI and total power (Δ = index value during High Dex dialysis minus index value during Norm Dex) in diabetics patients (# $p < 0.05$).

2

Heart Rate Variability and Autonomic Responses in Diabetic and Non-Diabetic Subjects during Dialyses with Different Dialysate Dextrose Concentrations

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Background: Power spectral analysis of heart rate variability (HRV) is a well established tool to assess activity of the autonomic nervous system (ANS). Differences during hyperglycemia (Kanaley, Metabolism 2007) and hyperinsulinemia are well known (Paolisso – Clin Sci 2000). This study aims to investigate the effects of different dextrose concentrations on the HRV and ANS in diabetic pts (D) and non-diabetic pts (ND) undergoing hemodialysis (HD) treatment using dialysate dextrose concentrations of 100 mg/dL (Norm Dex) and 200 mg/dl (High Dex). **Methods:** 24-Hour Holter-recording (clickholter, et medical devices Spa, Italy) of 11 diabetic (D) and 12 non-diabetic (ND) HD patients (pts) were analyzed during dialyses after a long non-dialytic interval, using Norm- and High-Dex respectively. Time domain and frequency domain parameters were computed: mean NNI, SDNN, VLF, LF, HF, LF/HF spectral component of the HRV signal and total power (Task Force, Circulation, 1996). The indices were assessed during the first and the last 30 min of HD, and 1 hour after HD treatment. Wilcoxon signed rank test was employed for statistical analyses. **Results:** Significant differences in the HRV indices were found in the diabetic population. In Ds HD using High Dex resulted in significantly higher values of SDNN, VLF, HF and total power in the first hour after HD and significantly higher values of VLF and mean NNI in the last 30 min (fig. 1). NDs do not show significant differences. **Conclusion:** Low Dex and High Dex seem to have impacts on HRV, which may be explained by higher glucose and insulin levels over the whole HD treatment. According to a recent study (Kanaley-Metabolim 2007), which reported an increase of total power and HF during hyperglycemia, an increased HF power and total power in Ds after hemodialysis can be thus explained by an increase of glucose in the blood. In contrast no significant differences were found in NDs, which may be explained a) by adequate insulin secretion (known to be a sympathetic stimulus) following glucose elevation and/or b) by HD related sympathetic stimulation masking the influences of increase in glucose concentrations.

3

Laparoscopic Peritoneal Dialysis Catheter Insertion and Outcomes in Pediatric ESRD Patients.

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Introduction: Peritoneal dialysis (PD) is the preferred method of renal replacement therapy in children. Complications are still common despite advances in surgical technique, products and postoperative catheter care. Peritonitis, catheter migration, exit site and tunnel infections, and outflow obstruction all contribute to inferior clearance and reduced quality of life. Laparoscopic approach to PD catheter placement has become more conventional in the last decade. We hope to add to the growing body of literature available to improve patient outcomes in this population. **Aim:** To determine the clinical outcomes of laparoscopically-placed PD catheters compared to open surgical technique among children and adolescents with ESRD. **Methods:** Two independent investigators conducted a retrospective study in all 0 to 21 year old patients who underwent peritoneal catheter placement or revision at the University of North Carolina Chapel Hill from October 1998 until August 2009. Patients enrolled in the home PD program at the UNC Carolina Dialysis Unit as well as inpatients requiring acute dialysis were included. Specific outcomes studied were grouped into surgical complications (adhesions, catheter malfunction or malposition, exit site leak, incisional hernia, exposed cuff) or infectious complications (exit site infections and peritonitis). Time on dialysis was calculated. Infection rates were calculated and compared using Poisson regression, and surgical complications were compared using logistic regression. Confounding by weight of the child was investigated, as laparoscopy is less likely to be performed in our institution in children smaller than 8 kg. **Results:** We identified 92 catheters in 58 patients, mean age 9.7 ± SD 5.7 (range 0.06–20.8 years) with the following characteristics at the time of first PD catheter placement: 31 (53%) males, 19 (33%) White, 31 (53%) Black, 8 (14%) other race; 17 (29%) with Public Insurance, 78% had a parent perform their home PD. The clinical and surgical characteristics included: 20 (35%) had a glomerular disease as a cause of ESRD, 78 (85%) had double cuff catheters, 44 (48%) of the catheters were laparoscopically placed, and catheter dysfunction was noted in 6 (24%) laparoscopic catheters vs. 7 (23%) in open catheters. No statistically significant difference was found when comparing laparoscopic placement to open placement for peritonitis, RR 0.91 (95% CI: 0.58, 1.44), exit site infections, RR 2.20 (95% CI: 0.20, 24.26), or surgical complications, OR 1.04 (95% CI: 0.30, 3.62). Adjustment for weight ≤8 kg did not alter the estimates, and thus only crude values were reported. **Conclusion:** In this pilot study, surgical approach to peritoneal catheter placement does not appear to impact on clinical outcome with regards to surgical or infectious complications. Further analysis is currently underway to determine if differences exist in time to first revision, initial failure of catheter function, and if omentectomy or costs independently influence catheter life.

4

Safety and Efficacy of a Liquid Formulation of PhosLo Gelcaps

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Objective: To assess the safety and efficacy of a liquid formulation of PhosLo (calcium acetate), a phosphate binder, by measuring urinary calcium and phophorus excretion and serum calcium and phosphorus levels in healthy subjects. **Methods:** A randomized, controlled, 3-arm, open label cross-over study was conducted in 46 healthy volunteers. Equivalent doses of either liquid PhosLo, PhosLo Gelcaps, or a calcium citrate positive control were administered for 3 days, TID during each meal, with a 5–10 day washout period between arms. All subjects were on a phosphate-, sodium- and calcium-controlled study diet prior to and during drug administration. Baseline samples of serum calcium, phosphorus, glucose and insulin and 24-hour urines were collected. PK samples were drawn on the third day following the last dose of test or control drugs. **Results:** Serum phosphorus and calcium from the liquid were comparable to that of the solid formulation based on the 90% CI of ratios for C_{max} and AUC_{0-6} using ratio adjustment to baseline. Urinary calcium from the liquid was not more than that of the solid formulation. The lower values of the 90% CI for R_{max} and Ae_{0-6} of the liquid formulation fell below the pre-established lower bound, indicating that the liquid formulation cohort excreted less calcium than did the solid formulation cohort. For urinary phosphorus, the lower values of the 90% CI for R_{max} and Ae_{0-6} for the liquid formulation fell below

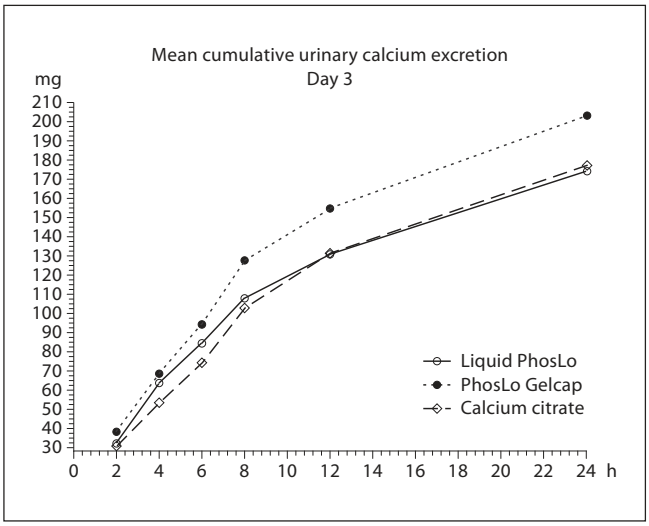

Fig. 1. Comparison of the mean cumulative urinary calcium excretion from liquid and solid calcium acetate and calcium citrate positive control after the last dose of 3 days of TID dosing.

the lower bound, indicating that the liquid formulation resulted in lower excretion of phosphorus than the solid formulation. No subjects exhibited hyperglycemia. Several subjects exhibited mild hypoglycemia (glucose <65 mg/dL) at repeated time points. Insulin levels demonstrated troughs between meals and postprandial peaks, indicating typical insulinemic responses, although ranges varied widely. There were no deaths or SAEs. The most common AE was mild, transient and self-limited diarrhea. **Conclusions:** Liquid PhosLo was found to be well tolerated. Liquid PhosLo and PhosLo Gelcaps were shown to be equivalent with respect to serum phosphorus and calcium using a 0–6 h assessment with ratio adjustment to baseline. Less calcium and phosphorus were excreted in the urine in the liquid compared to the solid PhosLo cohort, emphasizing not only that the liquid formulation excreted less calcium than that of the solid but also underscoring the ability of liquid PhosLo to bind phosphorus in the GI tract.

Disclosure of Financial Relationships: Fresenius Medical Care NA owns, distributes, and sells PhosLo Gelcaps, is developing liquid Phoslo, and sponsored this trial.

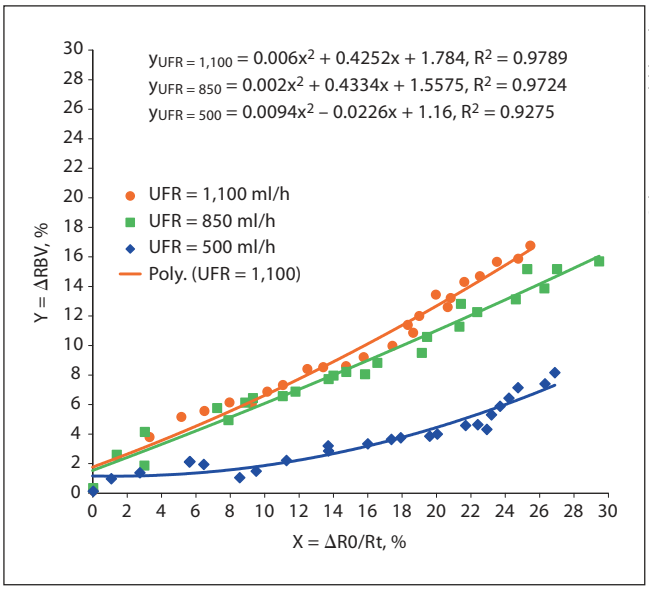

Fig. 1. Correlation between △RBV% and △R0/Rt% in one patient.

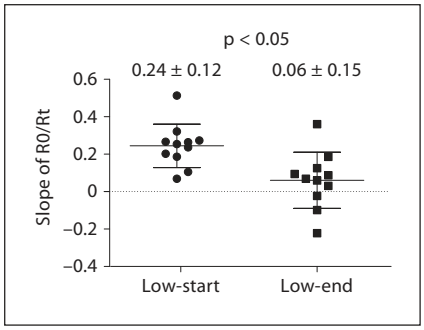

Fig. 2. Comparison of slope of R0/Rt in 10 min period at the start and end of the treatment in patients with lower pre-HD weight.

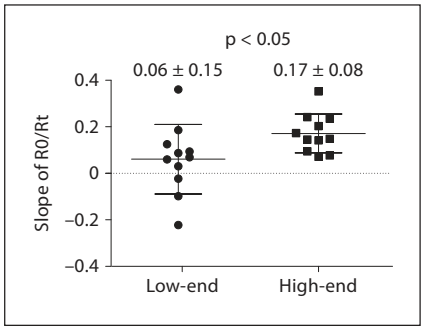

Fig. 3. Comparison of R0/Rt slope at the end in groups with low and high pre-weight.

5
Relationship between Relative Blood Volume and Calf Resistance by Bioimpedance Spectroscopy during Hemodialysis

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Hemodialysis with ultrafiltration (UF) is hemodinamically active process that extracellular fluid volume (ECV) shifts from interstitial compartment to intravascular compartment during the UF. Change in intravascular fluid can be monitored by relative blood volume (RBV) monitor and change in interstitial fluid in the calf can be measured with change in calf resistance (R0/Rt) with bioimpedance technique. The aim of the study was to investigate 1) what is the relationship between RBV and calf R0/Rt during HD with UF; 2) whether calf R0/Rt is influenced by hydration status. **Methods:** Thirty-three stable patients (age: 56 ± 12, 20 males) were studied in 219 HD sessions. Hydration was decreased by planned reduction of post-HD weights over a course of several HD treatments. RBV (Fresenius BVM) and R_0/R_t (Modified Hydra 4200) data were collected continuously during HD and examined at 10 minutes intervals. The relationship of ratio of resistance (R_0/R_t) and RBV were analyzed by linear regression in each treatment. Comparison of the slope of R0/Rt during 10 minutes period was made between different pre-HD weight (high and low) in the same patient (n = 11) at the beginning and end of treatment. **Results:** Mean correlation coefficient ($r_{RBV\text{-}R0/Rt}$) between RBV and R0/Rt was 0.953 ± 0.048 (0.500–0.996) for 219 treatments. Values of $r_{RBV\text{-}R0/Rt}$ were associated with the ultrafiltration rate (UFR) ($r = 0.29$, $p < 0.001$) Figure 1 shows the correlation between △RBV,% (100-RBV) and $\triangle R_0/R_t$,% (100-R_0/R_t) at different UFRs in one patient. Greater change in RBV was observed when hydration was reduced with higher UFR. However, R0/Rt did not

show much difference from this patient. The mean slope of R_0/R_t was significantly higher at the start than at the end in low weight group (0.24 ± 0.12 vs 0.06 ± 0.15, $p < 0.05$) (fig. 2). The slope was significantly higher in high group than in low group at the end of treatment. (fig. 3). **Conclusion:** Change in the blood volume with constant UF was closely correlated with change in the calf extracellular volume by R0/Rt during HD treatment. It can be assumed that change in the calf R0/Rt represents change in the refilling rate. Although UFR affected the relationship of RBV and R0/Rt, hydration is the major factor to change the slope of R0/Rt during the treatment.

6

Permeability of High-Flux Dialyzers to Low-MW DNA Fragments

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Short DNA fragments from bacteria, containing the non-methylated-CpG motif, are able to initiate inflammation via the Toll-like-receptor 9 on lymphocytes. These fragments have been detected by PCR in patient bloodstreams, indicating that they can transfer from the dialysate circuit, and might contribute to the chronic inflammation characteristic of many ESRD patients. We examined permeability in a Fresenius F180 high-flux dialyzer, of 5, 10, 20, 35, 50, and 100 base-length single stranded DNA fragments (oligos), with dialysate solution in both circuits, at T = 25°C. The transfer coefficient is the %DNA that crosses to the blood circuit from the dialysis circuit. DNA oligos were quantified by HPLC with a Waters X-Bridge C18 column. The results are shown below, reporting molecular weight on the X-axis. Both diffusion and convection were characterized. In control experiments, the transfer coefficient for diffusion of the protein lysozyme (MW 14,700) was 25%, and for Urea was 73%. DNA fragments of similar size to lysozyme showed lower permeability by diffusion than lysozyme. The transfer coefficients for convection were higher for the small DNA fragments than for diffusion. No significant DNA sequence effects on transfer coefficient for diffusion or convection were observed. 1% Bovine serum albumin in the blood circuit didn't interfere with the permeability of 20 base-length single stranded DNA fragment from dialysate circuit into blood circuit. **Conclusion:** Short DNA sequences (5–20 bases in length) have been shown to activate inflammation, and a portion of these short DNA sequences was able to permeate to the blood side of the dialyzer. Since these fragments may contribute to inflammation often seen in hemodialysis patients, studies of the amount of this type of DNA in dialysate used for patient treatment are warranted.

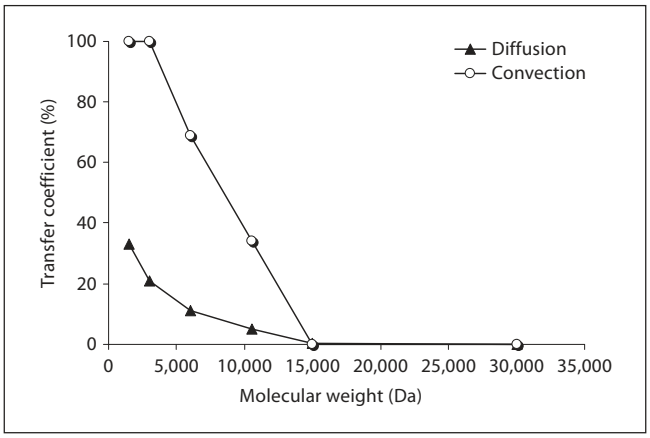

Fig. 1. Transfer characteristics for oligonucleotides, in both diffusion and convection.

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Quantified Management of Ca and P in Hemodialysis: A Clinical Trial

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Background: Chronic hemodialysis (HD) patients face multiple pathological consequences of renal failure, with disorders of bone and mineral metabolism being highly prevalent. Less than half of the patients achieve K/DOQI targets for phosphorus (P) and calcium (Ca). High serum levels of P and and accumulation of Ca throughout the body are associated with bone disease, cardiac disease, and mortality. The current approach to Ca and P metabolism lacks a comprehensive understanding of the quantitative aspects involved. We believe that in the style of urea kinetic modeling (UKM), it should be possible to calculate intake and removal of these species so that dialysis treatment parameters and medication prescriptions could be tailored to the individual's needs. We have developed a mathematical model of Ca and P kinetics (PKM) applicable to chronic HD. The goal of PKM is to generate a report which can help physicians with clinical decision-making regarding patient mineral metabolism. **Methods:** Initially, a set of formulae were developed that could be used to quantify the amount of P and Ca absorbed by the patient from their diet as well as the amount removed by dialysis treatment and P binders. A new parameter, the Phosphorus Protein Ratio (PPR), was also developed in order to evaluate patient compliance with diet and P binder regime. If a patient's PPR falls outside the acceptable range, the dietician and physician can be alerted to the possibility of non-compliance. The equations developed in this model quantify Ca and P absorbed and removed, generating mass balances of these species which can be used to suggest appropriate binder prescriptions that will bring a patient into target guide-

lines. In addition, the model suggests appropriate dialysate Ca concentrations to avoid Ca accumulation. The resulting report includes a graphical snapshot of relevant mineral metabolism data, including recent historical trends in individual patient's data as well as recommended changes in binder prescription and dialysate calcium concentration calculated from mass balance details. This information can aid the physician in making clinical decisions regarding prescription of dialysis treatment and drugs, including P binders, calcimimetics, and vitamin D. In spring 2009 a prospective single arm interventional trial on the efficiency of PKM was initiated. This trial tests the hypothesis that application of PKM over a period of 6 months results in improved control of serum P levels when compared to a 6 months baseline period. The enrollment target is 88 patients; only consistently hyperphosphatemic patients are enrolled. The trial has a power of 80% with a type I error of 0.05 to detect a reduction of serum P levels of greater 10%. Daily doses of phosphate binders (calcium acetate; PhosLo®) and dialysate calcium concentrations are calculated by PKM and communicated to the nephrologist in charge. At his/her discretion the model recommendations are then implemented. **Results:** As of September 1st, 2009 a total of 78 patients were enrolled. Mean age ± SD was 57.7 ± 15.4 years, 55.1% were males, dialysis vintage was 49.1 ± 38.7 months, African Americans 56.4%, Whites 33.3%, Asians 6.4%, other races 3.8%; 78.8% had fistulae, 14.1% grafts, and 7.7% catheters. In the 6 months prior to enrollment average eKt/V was 1.4 ± 0.2; nPCR 1.0 ± 0.2 g/kg/day; total serum Ca 9.0 ± 0.6 mg/dl; iPTH 469 ± 470 pg/ml; and alkaline phosphatase 100 ± 61 U/l. Serum phosphorus 1, 3 and 6 months before enrollment were 6.8 ± 1.0, 6.7 ± 0.9, and 6.5 ± 0.9 mg/dl, respectively. **Discussion:** Here we report the baseline characteristics of a population enrolled in an interventional prospective trial of P and Ca metabolism in chronic HD patients. The inclusion criteria guarantee the enrollment of patients with longstanding hyerphosphatemia.

8

Twenty-Five Years of Long-Term Reuse of Dialyzers in One Single Center

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In order to help low-income patients with renal failure and saving costs, 25 years ago our unit adopted and adapted the process of multiple manual reuse of hemodialyzers and its quality control from the National Nephrology Foundation. The aim of this paper is to assess patients´ safety and dialyzer efficiency in 25 years of follow up. **Methods:** We divided patients into chronic renal failure (CRF) and acute renal failure (ARF). The CRF group was subdivided in renal transplant program (RT-CRF) or temporally dialyzed patients (TD-CRF). Patients in ARF group were subdivided in hemodynamically unstable (HU-ARF) according to mechanical ventilation, amines and/or septicemia, and hemodynamically stable (HS-ARF). Patients' safety in RT-CRF group was defined as number of pyrogen reactions of any cause per 1,000 dialysis sessions. Dialyzer efficiency in ARF group was defined as rate of renal function recovery and patient survival. Dialyzer efficiency in RT-CRF subgroup was defined as the probability of getting a renal transplant and patient's survival. Dialyzer efficiency in TD-CRF subgroup was defined as patient's survival. **Results:** In these first 25 years, we had treated 2,556 renal failure patients and performed 110,773 consecutive dialysis treatments with 6,581 hemodialyzer with multiple manual reuses, with a mean reuse number of 16.8 ± 8 treatments per hemodialyzer. Patients´ characteristics according to subgroups are shown in table 1. Outcomes of dialyzed patients according to subgroups are shown in table 2. The HU-ARF subgroup had the highest mortality rate but similar to the reported in the literature in this patients. The CRF patients´ overall mortality was similar in both subgroups RT-CRF and TD-CRF. Renal function recovery occurred in 43% of HU-ARF patients and reached 72% in HS-ARF subgroup. In RT-CRF subgroup, 71% achieved renal transplantation. The main reason for not getting a renal transplant was lack of living donors; in that case patients were changed to peritoneal dialysis program where they could get help from the Government. There were 1.12 events of pyrogen reactions per 1000 dialysis sessions between 2005 and 2009, and 92.3% were associated to nontunneled catheter colonized by staphylococcus. **Conclusion:** Our one single center experience with manual long-term dialyzer reuse during 25 years has been favorable. Reuse allows us to maintain safe and efficient support for low-income patients.

Table 1.

	Number	Female %	age years	DM %	Number HD sessions, n
HU-ARF	606	47	57 ± 18	17	7.4 ± 9
HS-ARF	112	46	45 ± 18	21	7.7 ± 14
RT-CRF	866	48	32 ± 12	16	26.5 ± 124
TD-CRF	418	43	51 ± 20	49	11.8 ± 19

Table 2.

	Number	Death %	Recovery, %	Renal transplant, %	Dialysis, %
HU-ARF	606	49	43	–	8
HS-ARF	112	15	72	–	13
RT-CRF	866	7	–	71	22
TD-CRF	418	7	–	–	93

9

Predictive Factors for Phosphorous Removal during Hemodiafiltration

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Introduction: Patients under hemodialysis (HD) have a mortality risk 100 times higher than general population due to cardiovascular disease, and hyperphosphatemia is one of the main factors. Hemodiafiltration (HDF) provides greater phosphorous (P) clearance compared to HD. Recognition of variables involved in P clearance during HDF would help to understand its kinetics and to optimize prescription. **Objectives:** To obtain a predictive model for P removal during HDF and to compare it between two filter types and exercise. **Methods:** Fifteen patients without dietary restrictions or P binders were included. Each patient was assigned to both F-80 and Helixone (Fx60 or Fx80), and assessed with and without intradialyitic exercise, for a total of 4 sessions per patient. Total dialysis effluent volume was collected to determine the total P removal. Effluent dialyzate spot samples were taken at 30, 90 and 150 min. Blood samples were taken at the beginning, at the end, and 30 minutes post-dialysis. **Statistical analysis:** Data are shown as mean ± SD. A significant difference was considered if $p < 0.05$. **Results:** Fifteen patients were included for a total of 60 sessions, 10 women and 5 men. Ten patients had catheter and 5 had fistula. Thirty sessions were obtained for each group according to filter type and exercise. Total P removal was 797.5 ± 292.8 mg for women and 1,135.9 ± 436.2 mg for men ($p < 0.05$). The removal by F-80 filter was 1,073.6 ± 396.6 vs 746.9 ± 282.9 by Helixone filters ($p < 0.05$). There was no difference between Fx60 and Fx80, neither with or without exercise, or between catheter and fistula. Significant correlations with total P removal were weight, total ultrafiltration volume, serum P pre-dialysis, and effluent dialyzate spot samples at 30, 90 and 150 min. The predictive formula to estimate total P removal obtained by multiple linear regression was. Total P removal mg = e ^ [6.7 – ('1' if Helixone or '0' if F-80) × 0.3 + (serum P pre-dialysis mg/dl) × 0.08 + ln (Effluent dialyzate spot sample of P at 150 minutes mg/dl) × 0.31]]. $R = 0.75$, $R^2 = 0.56$, adjusted $R^2 = 0.54$, $F(3,56) = 24.26$, $p < 0.05$. **Conclusions:** Main factors associated with total P removal were filter type, serum P pre-dialysis and effluent dialyzate spot sample of P at 150 min. Although it is possible to predict P removal, there might be other factors that could contribute to a more precise estimation, like inter-compartmental movement of solutes and water. These data point out the importance of the second part of the dialysis session. **Limitations:** Patients with and without exercise had different serum P pre-dialysis as a result of different interdialytic time.

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Dynamics of the Electrocardiographic Cycle during Hemodialysis in Patients with End-Stage Renal Disease

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Several factors that depend of hemodialisis may induce arrhythmias: electrolytic and acid-base disturbances, hypoxia, and an elevated ultrafiltration rate. The traditional methods to evaluate the effects of hemodialysis in the electrocardiographic cycle consider slow beats, without showing relevant transitory events like extrasystoles, isquemia or tachycardia. The tridimensional electrocardiogram (TE) is an original technique that helps to visualize transitory changes in electric cardiac activity. **Methods:** Fifteen patients were studied, with an age of 44 ± 20 year old, in hemodialysis with 3 sessions per week for more than 6 months. A Holter device was used during the whole session. The register was analyzed and the beats were classified with Premier 11® program. The images were obtained with a program designed Matlab®. **Results:** There were no significant changes in arterial pressure before and after hemodialysis (systolic pressure 140 ± 33 vs. 132 ± 21 mm Hg; diastolic pressure 72 ± 18 vs. 64 ± 16 mm Hg). In 6 patients, sharp changes in arterial pressure with MAP lower than 70mmHg were related to a variation in cardiac frequency, with tachycardia followed by development of arrhythmias demonstrated in the TE with changes in RR interval. Serum potassium levels

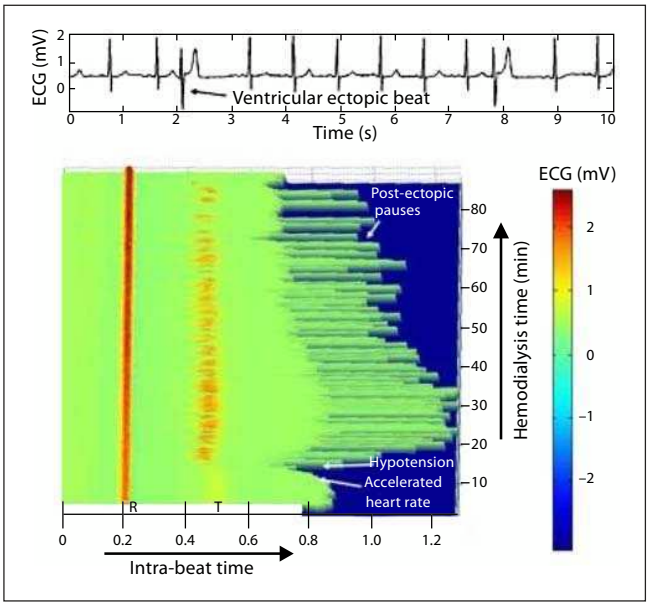

Fig. 1. Recording from a 32-year-old female patient during a hemodialysis session that had an episode of hypotension followed by the onset of isolated ventricular ectopic beats.

were 5.0 ± 0.8 mEq predialysis and 3.1 ± 0.4 postdialysis, which correlate in the tridimensional image with a progressive lowering in the T wave amplitude in all patients. **Conclusions:** The TE helps to identify different types of responses to hemodialysis: 1) arrhythmias associated with changes in cardiac frequency and hypotension, and 2) lowering of T wave amplitude and a rise in R wave. This study demonstrates the potential advantage of the TE.

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Effect of Edaravone on Endothelial Dysfunction and Contrast-Induced Nephropathy in a Model of Acute Renal Injury

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Background: Radiological procedures that require intravascular administration of iodinated contrast media is becoming the main source of contrast-induced nephropathy (CIN). CIN is associated to prolonged hospitalization, the need of renal replacement therapy and increased mortality of patients. There is still no universally accepted method for preventing CIN, except for extracellular volume expansion. The proposed pathophysiologic mechanisms of CIN are outer-medullary hypoxia due to decreased renal blood flow secondary to renal artery vasoconstriction, tubular obstruction and direct tubular toxicity. Also, decreased production of nitric oxide (NO) and increased oxidative stress play important roles in the pathogenesis of CIN. The aim of the present study was to assess the effect of edaravone, a strong novel free radical scavenger, on prevention of CIN. We hypothesized that edaravone may prevent CIN in a rat model due to its renal vasodilatation and antioxidant effects. **Methods:** Fourty-five Sprague-Dawley rats (300–350 g) rats were divided into five groups (n = 9 each): control (C), contrast media (CM), acute renal injury (ARI), contrast-induced nephropathy (CIN) and edaravone + contrast media (ECIN). ARI was induced by administration of intravenous L-NAME (10 mg·kg^{-1}) and iv indomethacin (10 mg·kg^{-1}) 15 min apart and prior to injection of normal saline or high osmolar contrast media diatrizoate (6 ml/kg). Edaravone (5 mg/kg) was administrated 1 hour before induced CIN. Kidney function was evaluated through serum creatinine, and endothelial dysfunction was evaluated in an 'in vitro' preparation of aortic rings subjected dose-response curves with phenylephrine (10^{-8} to 10^{-6}) to induce contraction, followed by dose-response curves (10^{-8} to 10^{-6}). With acetylcholine to induced relaxation. **Results:** The basal creatinine and the vasoconstrictor response were similar in the five groups. No significant changes were observed in serum creatinine or endothelial function in C group compared with the CM group. In contrast the basal creatinine value increased 27% and the vasodilatation response decrease 30% in the group with ARI ($p < 0.001$); the baseline creatinine increased 100% and the vasodilatation response decreased 54% in the CIN Group ($p < 0.001$). Furtheremore, edaravone attenuated CIN (38%) and improve endothelial dysfunction by 21% ($p < 0.001$). **Conclusion:** This study demonstrated the protective role of edaravone in contrast medium nephropaty, as well as in the endothelial dysfunction found in this model.

12

Calf Bioelectrical Impedance Spectroscopy Changes in Patients on Hemodiafiltration and after Renal Transplant

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Calf bioelectrical impedance spectroscopy (cBIS) is a reproducible method to estimated dry weight in hemodialysis (HD) patients. Renal transplant (RT) almost recovers normal kidney function. Hydric composition in early stages after RT from living donor has not been evaluated; neither comparison in hydric composition has been done in dry weight in HD patients and after a RT. These conditions were evaluated in this work. **Methods:** Fourteen patients in dry weight on hemodiafiltration (HDF) that received a RT from living donor were included. cBIS was performed with a multifrequency device (Xitron 4200) in seated position during HDF, 1 week, 1 and 3 months after RT. A healthy control group (HCG) was included. Resistivity, normalized resistivity, extracellular and in-

Table 1. Bioelectrical impedance spectroscopy measurements

	HDF	1 week	1 month	3 months	Control
Rho, Ω/cm	446 (400–556)	352 (316–386)*,†	393 (310–417)*	413 (397–461)	447 (416–472)
Rhon, Ω/cm /kg/m^2	21.3 (19.8–22.6)	16.9 (16.1–18.3)*	15.8 (14.0–18.9)*	17.6 (16.9–18.2)*	18.8 (17.2–20.0)*
Re, Ω	83.4 (70.5–90.3)	64.4 (58.8–69.7)*	60.3 (55.3–76.0)*	68.2 (59.1–73.1)*	69.1 (62.0–72.3)*
Ri, Ω	56.7 (47.7–64.8)	61.2 (47.0–67.7)	54.5 (49.4–63.2)	55.3 (42.7–60.5)	53.1 (43.4–63.3)
VEC, ml	118 (109–128)	135 (124–144)*	136 (119–153)*	132 (122–150)*	136 (128–148)*
VIC, ml	385 (361–422)	332 (305–431)	354 (320–405)	367 (340–431)	403 (342–487)

Data are expressed as median and intequartil range (25–75%). HDF = Hemodiafiltración, Rho = Resistivity, Rhon = Normalized resistivity, Re = Extracellular resistance, Ri = Intracellular resistance, VEC = Extracellular volume, VIC = Intracellular volume.

* p = <0.05 vs. HDF. † p= 0.03 vs. control.

tracellular resistance, extracellular and intracellular volume were obtained from cBIS. Continues variables were expressed as median and interquartile range. Friedman test, Wilcoxon test and Mann-Whitney U test were used, p = <0.05 was considered significant. **Results:** Fourteen (9 woman) patients with a median age of 22 (18–29) years old in CKD group and 14 (9 woman) with a median age of 28 (27–30) years old in HCG were included. No anthropometric differences were observed and allograft function was stable during evaluation period. The normalized resistivity and intracellular resistance were higher and extracellular volume was lower during HDF comparing with all moments post-RT and HCG. Other cBIS measurements are show in table 1.

Conclusions: cBIS shows that patients with a RT from living donor shows hydrated stage equal than healthy people since first week post-transplant. Dry weight in HDF is a lower hydrated stage from extracellular volume comparing with self after received a RT from living donor and with healthy people.

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Low Resistance Intradialytic Exercise Increases Phosphorous Removal in Hemodiafiltration Patients

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Background: Hyperphosphatemia is a risk factor for mortality in dialysis population. Compared with hemodialysis, Hemodiafiltration (HDF) improves phosphorous (P) removal, however still is necessary to prescribe dietary restrictions and phosphate binders to achieve P control. Intradialytic exercise could increase P removal related to better tissue perfusion and exchange between the different compartments. **Objective:** To compare phosphorous removal in chronic hemodiafiltration patients with and without low resistance isometric intradialytic exercise. **Methods:** In a cross-over study we included chronic and stable HDF patients who received 3 sessions per week and who regularly develop intradialytic exercise. We evaluated P removal by quantification in a whole dialysate collection. Every patient was recorded in 2 sessions, with and without intradialytic exercise. According to our program we did not prescribe phosphate binders nor dietary phosphorus restriction; HDF time was adjusted to P levels. Isometric low resistance intradialytic exercise was performed in a stationary bicycle equivalent to 3watts of work load. HDF was developed in 4008H machine with high flux membrane (polysulfone F80, FMC). Data are expressed as mean ± SD with exception of phosphorous removal (median, Interquartil range). Differences between groups were evaluated by t Student or Wilcoxon analyzes according to data distribution. **Results:** We included 11 patients; Male Sex 6/11 (54.5%); Age (years) 35.6 ± 15.9; Hemoglobin (g/dl) 11.4 ± 2.13; Predialysis BUN (mg/dl) 60.5 ± 13.7; Postdialysis BUN (mg/dl) 17.8 ± 4.9; URR (%)70.3 ± 6.66; Protein Catabolic Rate 1.53 ± 0.48; Albumin (g/dl) 4.47 ± 0.23; Calcium (mg/dl) 4.47 ± 0.23; Calcium (mg/dl) 10.0 ± 1.25; Alcaline Phosphatase (mg/dl) 315.8 ± 394.18; Phosphorus Intake (mg/d) 1,566 ± 491. **Conclusions:** In chronic hemodiafiltration patients, low resistance intradialytic exercise increase phosphorous removal by 18%.

Table 1.

Variable	Group 1, intradialytic exercise	Group 2 without exercise	p value
Predialysis P, mg/dl	5.44 ± 1.13	5.48 ± 1.08	NS
Postdialysis P, mg/dl	3.2 ± 0.67	3.06 ± 0.55	NS
P Removal, mg	887 (707, 1055)	751 (532, 871)	0.008
Dialysis time, min	208 ± 24	215 ± 22	NS
UF volume, l	1.7 ± 0.6	1.7 ± 0.4	NS
Qs, ml/min	437 ± 43	434 ± 61	NS
Qd, ml/min	631 ± 43	625 ± 41	NS
HDF substitution, l	16.5 ± 2.9	16.8 ± 2.8	NS
Kt/V	1.43 ± 0.3	1.39 ± 0.2	NS

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Better Uric Acid Control with Hemodiafiltration On-Line versus Conventional Hemodialysis and Peritoneal Dialysis

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Hyperuricemia (HU) has been associated with increased cardiovascular mortality, and related to endothelial disfunction which may contribute to hypertension and cardiovascular morbidity. HU is frequent in peritoneal dialysis (PD) patients (32.8%), and it has been associated with decline residual renal function. The mean uric acid (UA) removal is ~1 g per conventional hemodialysis session (HD), even with high-flux filters. Nevertheless, HU is still common in HD patients following HD therapy. HU, is a common complication among renal transplant (RT) recipients, and it has been associated with a decrease in renal allograft function; HU may be an independent cardiovascular risk factor in these patients. **Objective:** The aim of this cross-sectional study was to evaluate the relationship of uric acid levels and its association with PD, HD, HDF and RT. **Methods:** Between 2005 and 2008, 100 patients were evaluated, they were treated with dialysis and were included in the Renal Trasnsplant program, 54% were males and 46% females, age: 31 ± 12 years old. HDF was performed in 4008H FMC device, three times per week, with a high flux filter (polysulphone F80, FMC), dialysis time 198 ± 31 min. Average time on dialysis prior to transplantation was 14 ± 8 weeks, patients were under free intake and no anti-hyperuricemic therapy. **Results:** Mean AU levels were 9.1 ± 1.16 (pre-kidney transplant), 7.3 ± 1.79 (CAPD), 7.0 ± 1.72 (HD), 6.0 ± 1.47 (HDF) and 6.4 ± 1.57 (RT). No significant meaning was observed among data maybe due to a power sample minor of 0.8. Eighty one% of the patients in the group of RT with HU have a mean normal value of UA when they were under HDF. **Conclusions:** The HDF maintained lower levels of uric acid compared with conventional HD and CAPD. We need a long-term controlled study to confirm cardiovascular outcomes in high risk patients.

Table 1.

	RT	HDF	HD	CAPD
CKD	6.23 ± 1.65 vs. 9.12 ± 1.2 p < 0.01	6.33 ± 1.64 vs. 9.15 ± 1.29 p < 0.01	8.16 ± 1.64 vs. 9.42 ± 1.0 p = 0.11	7.05 ± 1.18 vs. 8.92 ± 0.61 p < 0.01
CAPD	6.65 ± 1.59 vs. 7.23 ± 1.8 p = 0.12	6.43 ± 1.46 vs. 7.43 ± 2.13 p = 0.02	7.5 ± 1.52 vs. 7.07 ± 1.57 p = 0.29	
HD	6.99 ± 1.87 vs. 7.0 ± 1.72 p = 0.98	5.88 ± 1.42 vs. 6.85 ± 1.62 p = 0.01		
HDF	6.5 ± 1.62 vs. 6.11 ± 1.55 p = 0.14			

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Infective Endocarditis: A Rising Challenge in Chronic Hemodialysis Patients with Av Graft

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End Stage renal disease patients are more prone for cardiovascular disease.They are at increased risk of infective endocarditis (IE) thereby posing greater threat of morbidity and mortality compared with the general population.Traditonally the incidence of IE is higher with dual lumen catheters than with PTFE grafts or AV fistula.We report two unusual presentations of IE in patients receiving chronic hemodialysis. Cases of IE at our hospital hemodialysis unit were identified using the modified Duke criteria. Both patients were diabetic with abnormal valve with calcification, mean age 61 years, on long term hemodialysis for 12 months and 59 months respectively before they had an episode of IE. During the time of diagnosis of IE one patient presented with mild dizziness and the other had an episode of fever. Both patients didn't show visible signs of infection in the AV graft with no obvious foci or portal of entry of infection. Primary hemodialysis access was polytetrafluoroethylene graft in both patients.In one patient staphylococcus aureus (methicillin sensitive staph aureus) was isolated in the blood with involvement of mitral valve treated with antibiotics. The other patient grew coagulase negative staphylococcus considered to be a relatively rare (<5%) causative organism for IE with involvement of aortic valve requiring both valve replacement and antibiotic. The probable sources of bacteremia in both patients were skin commensals with possible entry from the site of cannulation of the AV graft respectively. Generally coagulase negative staphylococcus is ignored in the fear of development of antibiotic resistance with the assumption that it is a contaminant. Infection with coagulase negative staphylococcus could be as severe requiring valve replacement. No definitive guidelines are available for categorization and indication for antibiotic prophylaxis for hemodialysis patients with abnormal valve calcification. The prognosis of IE in HD patients remains poor and a high index of suspicion is required for prompt diagnosis and intervention. Large scale studies are required to assess whether a subset of hemodialysis patients require long term prophylaxis for prevention of IE.

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Relationship of Serum Magnesium to Body Composition and Inflammation in Peritoneal Dialysis (PD) Patients (Pts)

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Magnesium is the fourth most abundant cation in the body and is involved in many cell functions. Serum magnesium concentration is maintained within a narrow range by the kidney and digestive tract. It has been recently reported that lower serum magnesium level is a significant predictor for mortality in hemodialysis pts (Ishimura et al., Mag Res, 2007). Body composition and inflammation are important predictors of mortality in PD pts. The objective of the present study was to examine the relationship of serum magnesium to body composition and inflammation in PD pts. Sixty two PD pts treated at the Long Island College Hospital were enrolled in this study between November 2000 and July 2008. Demographic, clinical and biochemical data were recorded. Body composition parameters including fluid status were determined by Bioimpedance analysis (BIA). High sensitivity C-reactive protein (hs-CRP), a marker of inflammation was measured by immunoturbidimetric method. The mean age was 55 years. The majority were African-American (63%), and 55% were women. Twenty-five percent were diabetic. Mean serum magnesium was 1.597 ± 0.28 mEq/l. Mean high sensitivity C-reactive protein (hs-CRP) was 13.53 ± 20.8 mg/l. Serum magnesium was directly correlated with serum nutritional markers, albumin (r = 0.42, p = 0.001), creatinine (r = 0.43, p = 0.0001), and total protein (r = 0.44, p < 0.0001). Serum magnesium was also

directly correlated with BIA parameter and marker of cellular health, phase angle (PA) (correlation coefficient, r = 0.35, p = 0.006) and inversely correlated with extracellular mass (ECM)/body cell mass (BCM) ratio (r = –0.34, p = 0.008), a highly sensitive marker of nutrition. There was an inverse correlation between serum magnesium and hs-CRP (r = –0.33, p = 0.037) in PD pts. In conclusion, lower serum magnesium is associated with poorer nutritional status, deteriorating cellular health and increased inflammation which may contribute to the increased risk of mortality in PD pts.

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Black Specks in the Effluent of a Pediatric Peritoneal Dialysis Patient

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We report the second case of peritonitis caused by Curvularia in a pediatric peritoneal dialysis patient. Our 13 yo male resumed peritoneal dialysis after a failed transplant and was maintained on cellcept and monthly infusions of IVIG to reduce his panel reactive antibody level. He developed a low grade fever, cloudy fluid and abdominal pain without peritoneal signs. His mother reported seeing some black 'flakes' in his effluent. Peritoneal fluid showed a WBC count of 5,600 per mcL with no growth when cultured. He received empiric antibiotics. His fluid remained somewhat cloudy and he developed intermittent drainage problems. A second sample of fluid contained 243 WBCs per mcL. Empiric antibiotics were started, but again there was no growth on bacterial or fungal cultures. Our patient remained asymptomatic with little abdominal pain, but had progressive difficulties with drainage. Eventually a black 'speck' was obtained and analyzed. The fungal culture grew Curvularia. His peritoneal dialysis catheter was removed on hospital day 2 and was maintained on hemodialysis. Amphotericin B was started intravenously. Fungal peritonitis is a serious complication of peritoneal dialysis [1]. Filamentous species such as Curvularia are uncommon [2] with a reported 8 cases in the adult population and now 2 cases in pediatrics [2, 3]. Although it has been reported as a disseminated infection, it is rarely pathogenic in humans [1]. Curvularia is ubiquitous, but is more prevalent in warm, humid environments. Risk factors for fungal peritonitis include recent exposure to antibiotics, immunosuppressive therapy, recent bacterial peritonitis, and presence of a bowel perforation [2]. Curvularia species can present with catheter obstruction, peritonitis, or both [3]. Of the ten reported cases, 7 reported seeing black proteinaceous material in the lumen of the Tenckhoff catheter [1–3]. The most important feature in treatment of peritonitis caused by Curvularia is removal of the peritoneal dialysis catheter [2]. Curvularia species have been found to be susceptible in vitro to many antifungals [2]. Currently the International Society of Peritoneal Dialysis recommends antifungal chemotherapy for 4–6 weeks with catheter removal if no improvement in 4–7 days [2].

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Diasafe plus Filter Performance in the Removal of Bacterial DNA Contamination

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Water designated for hemodialysis (HD) treatment is maintained by complex treatment systems to conform to strict standards set to ensure patient safety. A typical concern of water sterility is bacterial contamination. Currently, regulatory standards for water for dialysis and dialysis fluid include bacterial counts and endotoxin. These parameters have long been used to assess the biological component of water that can negatively affect patient health. However, recent focus has been placed on bacterial DNA fragments (bDNA) as a component of contaminated water that may elicit a patient reaction. Some have theorized that bDNA may have a causative effect of micro-inflammation in patients. This study focused on the ability of the Diasafe plus filter to sequester bDNA from solution. Additionally, the effect of cleaning with bleach was tested to determine how this process would alter the removal of bDNA by the filter. Finally, the presence of endotoxin in the filter was also tested to determine any effect on the passage of bDNA through the filter. All experiments were designed to show differentiation between bleach treating or endotoxin pre-loading, and the control. In vitro simulations evaluated the ability of the filter to remove bDNA from a challenge solution of water for injection (WFI) spiked with 10 mg/l of bDNA from E coli. bDNA was used in high concentration to exhaust the capacities of the filter. Bleach-treated filters were treated using the bleach rinse cycle on the Fresenius 2008K machine; filters were tested either 2, 4, 8 or 12 times, rinsed and then stored until testing. Endotoxin pre-loaded filters were challenged for 24 hours with endotoxin-spiked saline, with concentrations of 500, 1000, 1500 and 2000 EU/ml. The 1-liter challenge solution was re-circulated for 24 h at 37°C; afterwards, all filters exhibited <10 EU/ml in the challenge solution. Filters were then rinsed and ready to test. A filter was tested by re-circulating 1 liter of WFI at 300 ml/min with a spike of 10 mg bDNA. Samples of the return line and reservoir were analyzed for bDNA concentration. **Results:** The majority (>97%) of bDNA re-circulating through the control Diasafe plus is removed within 30 min. The removal of bDNA by the bleach-treated and endotoxin pre-loaded filters was shown to be >96% and >93%, respectively. Results show that the bleach-treat

cycle has no significant effect on bDNA removal. However, the removal rates for pre-loaded filters suggest that endotoxin within the filter may effect the way bDNA is trapped by the Diasafe plus membrane.

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Establishing a Sustainable Peritoneal Dialysis (PD) Treatment Program for Acute Kidney Injury in Kilimanjaro Christian Medical Centre, Moshi, Tanzania

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United Republic of Tanzania is a country of 38 million people in East Africa, with a stable democratic government since 1961. The treatment program for acute kidney injury (AKI) using peritoneal dialysis (PD) has been founded at the Kilimanjaro Christian Medical Centre (KCMC) located in Moshi, with the focus on treatment of children and women of child bearing age, goals consistent with the United Nations Millennium Development Goals 2015, to reduce child mortality, improve maternal health and reduce mortality from HIV/AIDS, malaria and other diseases. Diarrheal diseases are a leading cause of morbidity and mortality among young children in developing countries. The diarrhea specific mortality in children less than 5 years of age in Africa has been estimated at about 10.6/1,000. When kidneys fail acutely, the result of timely dialysis treatment is usually complete restoration of kidney function and return to daily life in many patients. Prevention of gastroenteritis and oral repletion is the basis of management of AKI and may not be prevented in all instances. PD is the modality of choice because of its simplicity and low cost. The pilot program includes the following elements: (1) training of doctors and nurses on principles of PD and techniques of catheter insertion and care, (2) securing a reliable, high quality supply of dialysate solutions, (3) expansion of the model to Regional Hospitals, (4) establishment of a database to track outcomes of patients treated with PD for AKI, (5) collection of epidemiological data to establish incidence and prevalence of AKI, (6) distribution of saliva strips, a simple diagnostic test to be used as a surrogate for blood urea nitrogen at the villages, (7) involvement of the local laboratory facilities, (8) development of a financial model sustainable at the local level, (9) engagement of the hospital administration and involvement of the local and central governments. Working with KCMC, industry, universities, International Society of Nephrology and a private donor, Sustainable Kidney Care Foundation (SKCF), secured initial funding for training, acquisition of reliable high quality PD solutions and supplies. In order to achieve sustainability, payments from patients will be used for the ongoing replenishment of related supplies and solutions. Without treatment children with reversible AKI requiring dialysis will die in most cases. This approach is the beginning of a substantial long term effort to be used as a model in other countries in need of renal services.

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Pediatric Renal Transplantation and the Cylex ImmuKnow: A Single Center Experience

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Introduction: The Cylex® ImmuKnow™ assesses cell-mediated immunity by measuring ATP generation in lectin (PHA)-stimulated CD4 cells. We present our experience with Cylex® in pediatric renal transplant patients. **Methods:** All patients aged <19 years at time of transplant and now less than 25 years with Cylex levels measured between 11/2006 and 4/2009 were included. Patients with very low Cylex levels (<100 ng/ml ATP) or very high levels (>600 ng/ml ATP) were retrospectively evaluated for infection or rejection within the 3 months following the result respectively. We divided the cause of ESRD by glomerular vs. not (all other conditions). Acute rejection was biopsy verified. Viral infection was defined as either a clinical syndrome or a positive PCR in blood or urine. The cost of the cylex test was provided by the UNC Hospital lab. **Results:** 366 Cylex tests were obtained in 50 patients with the following characteristics: 24 (48%) males, 23 (46%) White, 22 (44%), Black and 5 (10%) other race. 33 (46%) had non-glomerular disease, current mean age of 14.2 ± 4.9, and mean age at transplant 9.9 ± 5.2. The mean Cylex level was 359.7 ± 186.4. There were no bacterial infections. Of the 12 patients with low levels (<100ng/ml) 5 had viral infections (42%). None of the 8 patients with very high Cylex levels had acute rejection, but one developed parvovirus and another developed BK virus infections. In this cohort, 3 (6%) patients had acute rejections despite therapeutic Cylex levels within the previous two months. The total cost of the Cylex tests in this cohort was between USD109,068 and USD133,224 based upon a test cost of USD298 and test charge of USD364. **Conclusions:** Cylex levels in most of these patients were in therapeutic range. 40% of patients with very low levels developed viral infections as did 25% of patient with very high Cylex levels. Episodes of acute rejection were not associated with abnormal levels of this test. Cost-benefit analysis of this test is currently underway.

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The Role of Implantable Cardioverter Defibrillators in Patients with End-Stage Renal Disease: Results from a Multi-Center Registry

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Background: Clinical trials of implantable cardioverter defibrillators (ICD) have excluded patients with end-stage renal disease (ESRD) receiving dialysis. Small single center retrospective analyses suggest that dialysis patients do not obtain as much of a survival benefit from ICDs as non-dialysis patients. We wanted

to assess the survival benefit in ESRD patients with ICDs compared to 1) ESRD patients with poor LV function and 2) patients with normal renal function and ICDs. **Methods:** Data from two registries was used to select 50 ESRD patients with ICDs (referent group), 65 randomly selected controls with normal renal function (estimated glomerular filtration rate >60 ml/min/1.73 m^2) (ICD only group) and 50 patients with ESRD and poor LV function (defined as ejection fraction <0.35) (ESRD only group). Crude event rate for all-cause mortality was calculated. Multivariable analysis using Cox proportional-hazards regression was performed, including age, beta-blocker and amiodarone use, prior history of coronary artery disease and ejection fraction in the model. **Results:** Over the follow-up period, there were 21, 29 and 26 deaths in the referent group, the ESRD only group and the ICD only group respectively. Overall survival in the full cohort was a mean of 8.0 years (95% CI: 5.6–9.1), without loss of follow-up. The median survival in the referent group, ESRD only, and ICD only groups were 8.0, 3.1 and 10.4 years, respectively. The unadjusted Cox proportional hazards regression model showed that there was a non-significant trend towards better survival in the referent group as compared to the ESRD only group (p = 0.054). There was also a non-significant trend towards better survival in the group with ICD only as compared to the referent group (p = 0.11). In the multivariable analysis, the ESRD only group showed a significantly lower survival than the referent group (HR 2.15, 95% CI 1.05–4.40, p = 0.037). **Conclusion:** In this retrospective cohort analysis, use of an ICD in patients with ESRD and left ventricular dysfunction was associated with a significantly higher survival as compared with ESRD patients with left ventricular dysfunction without an ICD. This result merits further study in a larger cohort of patient.

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Blood Purification

(A) = Abstract

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