Pharmaceutical Aspects of Cancer Chemotherapy

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Series preface

There is, in our view, an important need to draw the attention of both students and practitioners concerned with drug therapy to recent and continuing developments in the pharmaceutical sciences. The purpose of the *Topics in Pharmacy* series is to fulfil this need by providing up-to-date, concise, readable accounts of current aspects of pharmacy—with particular emphasis on those aspects of the pharmaceutical sciences related to clinical practice. In this endeavour we have been fortunate in securing the collaboration of academics and practitioners recognised as experts in the subjects of their contributions.

Each volume in the series has a theme, and each constituent chapter provides a concise account of a particular area; the accounts are intended to be introductions to a topic rather than comprehensive and complete reviews, and hence, wherever possible, each chapter is appended with a short bibliography for further study.

The series is aimed at a readership comprised of senior undergraduate and postgraduate students in pharmacy, medicine, nursing and allied health sciences, and practitioners in these fields. However, it is our hope that the series will also be generally useful to other professionals concerned with the preparation and administration of medicines and the monitoring of drug therapy.

Alexander T. Florence, London
Eugene G. Salole, Glasgow

Dedicated to the memory of Brenda Salole, who fought hard against but finally succumbed to cancer in December 1992

Preface

Nowadays the treatment of cancer results in cure in 40 per cent of patients [1]. The Cancer Research Campaign points out that the major challenge is to improve the prospects for cure in the remaining 60 per cent, and to improve the quality of survival and lessen the side-effects of treatment in the entire group of patients [1]. Drug formulation and delivery play an important part in anticancer chemotherapy, by providing appropriate concentrations at sites of activity and also ensuring that local and systemic side-effects are minimised. This topic is dealt with in Chapter 1. A proper understanding of the absorption and distribution of anticancer drugs facilitates their more efficient use. The utilisation of carriers such as microspheres and liposomes allows the absorption and distribution of anticancer agents to be modified, so that some active moieties may be targeted directly to tumour sites, or by controlling release, the achievement of maximal drug concentration at the tumour may be facilitated. This area, which continues to require much development, is reviewed in Chapter 2.

Monotherapy is rare in cancer treatment protocols, hence the opportunity for complex interactions, both between cytotoxic drugs or between them and other drugs administered concomitantly, is considerable. This often problematical aspect of cancer chemotherapy is dealt with in Chapter 3.

Because few anticancer drugs exhibit completely selective toxicity, they can adversely affect healthy tissues and healthy subjects. This is an important consideration as an occupational hazard for staff in situations where cytotoxic drugs are routinely handled, e.g. pharmacy laboratories and hospital wards. In Chapter 4 some of the issues involved in minimising the risks are highlighted.

The physicochemical properties of anticancer drugs must also be given proper attention, particularly in the early stages of clinical assessment, when inappropriate formulation might compromise trials of efficacy. In Chapter 5 aspects of the stability of anticancer drug solutions are discussed, and in Chapter 6 some of the problems faced in the development and production of formulations for Phase I trials are described.

The contributors to this third volume in the *Topics in Pharmacy* series have considerable experience in the scientific, pharmaceutical and

clinical evaluation of anticancer drugs and therapy. It is our hope that the information assembled here will be of value to those working in the many areas of cancer chemotherapy, whether in industry, in the hospital pharmacy, biochemistry laboratory or ward. We are indebted to Dr Andrew Bosanquet, Mr Jonathan Cooke, Dr Jeff Cummings, Mr Ian Goss, Drs Martin Graham and John Green, Professors David Kerr and John Smyth, Drs Ivan Stockley and Roger Vezin and Professor Paul Workman for their sustained interest and contributions to this book. We also thank Alison Duncan and the staff at Butterworth-Heinemann for their professional interest and attention.

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1. *Cancer Research Campaign. Scientific Yearbook.* Cancer Research Campaign, London, 1992.

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Chapter 1

Absorption and distribution of anticancer drugs

M. A. Graham, D. J. Kerr and P. Workman

Introduction

Chemotherapy is generally administered with the aim of selectively eradicating the tumour cell population in an attempt to improve the length and quality of life of the cancer patient. In practice these aims can be compromised by a number of factors associated with the disease state, such as drug resistance or aspects of drug disposition, which may have a serious effect on the efficacy of treatment.

Unfortunately, for the vast majority of conventional antineoplastic agents the margin between an effective dose and a toxic dose, i.e. the therapeutic index, is extremely narrow. Consequently, a fundamental problem in cancer chemotherapy is the inherent lack of selectivity of established antitumour agents. Although the processes and mechanisms which determine drug selectivity are complex, in general terms they can be explained by pharmacodynamic or pharmacokinetic considerations.

Pharmacodynamics describes the therapeutic activity and toxicity of a drug treatment. For example, one important pharmacodynamic consideration can be the emergence of a multidrug-resistant cell population which is refractory to treatment. The causes of drug resistance are likely to be multifactorial and may include: (i) impaired drug uptake into the cell or its rapid efflux by the P-glycoprotein pump; (ii) the utilisation of DNA repair mechanisms; (iii) the elevation or reduction of target enzyme levels; and (iv) the induction of protective mechanisms such as glutathione S-transferase [1].

In addition to these pharmacodynamic considerations, drug pharmacokinetics may also have a major influence on treatment outcome. Pharmacokinetics describes the absorption, distribution, metabolism and excretion of a drug. Examples of pharmacokinetic processes which determine the whole body outcome of drug action include: (i) bioavailability from the site of administration; (ii) extent of plasma protein binding; (iii) distribution into poorly vascularised areas or across the blood–brain barrier; (iv) extent and nature of drug

biotransformation; and (v) rate and extent of clearance from the systemic circulation.

This chapter reviews the pharmacokinetics of some commonly used anticancer drugs with particular reference to how variability in drug absorption and tissue distribution may affect drug efficacy.

Route of administration

Commonly, cytotoxic chemotherapeutic agents (e.g. the anthracyclines, epipodophyllotoxins, vinca alkaloids) are administered intravenously as a bolus injection in order to achieve high systemic levels. By definition, bioavailability following this route of administration is 100% and patient compliance is generally assured.

Alternatively, drugs may be administered by continuous infusion into either a peripheral vein or through a central line. This mode of administration may have the effect of decreasing peak plasma levels (thus reducing toxicity associated with high plasma levels) but in some cases may increase total drug exposure as measured by the area under the plasma drug concentration versus time curve (AUC). Toxicities such as the acute neurotoxicity caused by the experimental antifolate MZPES and doxorubicin-induced cardiac dysrhythmias may be attributable to peak plasma levels, and may be partially alleviated by intravenous infusion. Also, for drugs with a relatively short plasma half-life or for cell cycle-specific agents, prolonged drug exposure by infusion should theoretically result in a greater degree of cell killing.

Cytotoxic agents have also been administered by a variety of other routes (e.g. intraperitoneally and intrathecally) to try and achieve a regional pharmacokinetic advantage. However, intravenous and oral dosing still represent the most common routes of administration. Oral dosing is an important route of administration for a number of anticancer drugs including the topoisomerase II inhibitor etoposide, the DNA-crosslinking bifunctional alkylating agents melphalan, cyclophosphamide and ifosfamide, and the antifolate methotrexate. The pharmacokinetics of these and other anticancer agents will be examined in detail later in this chapter.

Oral administration is a convenient route, as medication can be administered on an outpatient basis and may therefore be more acceptable for patients undergoing protracted and/or multiple courses of treatment. It also eliminates the hazards associated with drug extravasation and the problems in maintaining a central line catheter. However, there are a number of drawbacks with orally administered chemotherapy which require particular attention. One of the most serious problems is incomplete and variable absorption of

the drug from the gastrointestinal tract, which may give rise to unpredictable plasma levels. The consequences of this may be either failure to achieve a therapeutic plasma concentration and the likelihood of treatment failure, or, more seriously, the danger of absorbing a highly toxic dose. Not all drugs will be suitable for oral administration, particularly agents which may be rapidly degraded at acidic gastric pH. Absorption through the cell membrane of the gastrointestinal mucosa is governed by the molecular size of the drug, its lipid solubility (i.e. its partition coefficient) and the presence of certain highly specific transport systems. The latter include the folate transport systems for antifolates and the amino acid carriers utilised by melphalan. In the absence of such systems, very water-soluble or hydrophilic anticancer drugs will not be absorbed well after oral dosing and systemic administration may be more appropriate.

Another advantage of oral administration is that some agents can be substantially more emetogenic when given orally, or produce high localised concentrations of drug which may aggravate gastrointestinal toxicities. In addition, vomiting may lead to loss of a large proportion of an oral dose. Moreover, highly emetogenic anticancer drugs such as cisplatin cannot be given in simultaneous combination with orally dosed agents. Extensive liver uptake and/or metabolism and biliary excretion may also limit systemic drug levels of parent drug after oral administration. In some cases food can affect oral absorption of anticancer drugs. Finally, the absorptive capacity of the gastrointestinal tract may be affected by the disease state or by prior chemotherapy.

The remainder of this chapter reviews the pharmacokinetics of anticancer drugs which can be administered orally, and discusses the merits and problems associated with their use. Firstly, some general pharmacokinetic models relating to oral absorption are discussed.

Pharmacokinetic models for absorptive processes

It is possible to formulate relatively simple pharmacokinetic models which describe a drug's plasma concentration–time profile following absorption from distant sites, e.g. the gastrointestinal tract, muscle, subdermis and body cavities. These models are important when we consider concepts such as bioavailability, first-pass metabolism, pharmacokinetic interactions with other drugs and the effects of disease states.

The majority of plasma concentration–times curves following oral or intramuscular administration can be described as a one-

compartment model with first-order input and output:

$$C = \frac{FDk_a}{V(k_a - k_{el})} \cdot (e^{-k_{el}t} - e^{-k_a t})$$

where

C = plasma concentration
D = drug dose
F = fraction of administered dose D that is absorbed
V = volume of distribution
k_a = apparent first-order absorption rate constant
k_{el} = elimination rate constant
t = time

This formula can be modified so that the maximum plasma concentration (C_{max}) and the time taken to achieve C_{max} can be derived.

The model also helps define the degree of first-pass metabolism which occurs after oral administration. Cytotoxic drugs administered subcutaneously (e.g. methotrexate and actinomycin D) or intramuscularly (e.g. bleomycin) enter the peripheral venous circulation directly, and less than 30% of the administered dose passes through the liver on first-pass through the body. This differs from oral (and intraperitoneal) administration, in which cases the drug gains access to the systemic circulation via the hepatic portal venous system. Drug uptake and clearance by the liver during the passage of drug through the portal veins may effectively prevent a significant proportion of the drug reaching the systemic circulation and, ultimately, the site of action within the tumour.

Another important parameter which can be described mathematically for orally administered drugs is bioavailability. This may be defined as the measurement of both the relative amount of an administered dose that reaches the general circulation intact and the rate at which this occurs. The usual approach to measure bioavailability is to compare plasma concentration or urinary excretion data following oral and intravenous administration. For example:

$$\text{bioavailability} = \frac{\text{plasma AUC after oral administration}}{\text{plasma AUC after intravenous administration}}$$

where AUC is the area under the plasma drug concentration–time curve. There are various subtleties which can be used to modify this formula. However, if the test drug is administered by both routes to

the same patient, in a crossover study, this method represents one of the best approaches to assessing bioavailability.

There is increasing interest in developing concentration–effect models which relate pharmacokinetic parameters to toxicity and efficacy. If there are large interindividual differences in drug handling, absorption and distribution, this could lead to some patients being overdosed and some underdosed. Evans and Relling [2] have shown that there is a relationship between steady-state plasma concentrations of methotrexate and survival in childhood leukaemia. Rather than administer the drug in a fixed dose, these investigators plan to administer methotrexate according to its pharmacokinetics, based on drug clearance using a Bayesian forecasting technique. A similar approach has been described for suramin using an 'adaptive control with feedback technique to individualise and optimise drug dosing [3].

Effect of cancer on cytotoxic drug absorption and distribution

It is important to consider the effect that the cancer burden may have on the various factors governing drug absorption from the gastrointestinal tract. A number of cancers, arising from, or metastasising to, the gastrointestinal tract can reduce gastrointestinal motility by interfering with autonomic innervation. Gastrointestinal motility can also be affected by co-administration of antiemetics like metoclopramide, which can increase the rate of gastric emptying. This interaction has been shown to influence the pharmacokinetics of the nitrosourea TCNU. The gastric emptying rate can also be decreased by concomitant medication, including morphine, vincristine, vinblastine and amitriptyline, which in turn may affect the lag time and maximum achievable plasma concentration (C_{max}). If metastatic spread involves the peritoneal cavity with subsequent blockage of peritoneal lymphatics, significant mucosal oedema can occur which may impair gastrointestinal absorption.

Some cancers, e.g. carcinoid and neuroendocrine pancreatic tumours, are invariably associated with diarrhoea as a result of increased gastric emptying and enhanced permeability of the gut wall. Steatorrhoea, which is associated with decreased enterohepatic cycling of bile acids, causes reduced absorption of fat-soluble drugs.

If the cytotoxic agent undergoes significant first-pass metabolism on oral administration, hepatic metastases may also perturb drug clearance as a result of hepatic dysfunction. Protein-binding is another area which can be altered by cancer, which is often associated with hypo-albuminaemia and elevated serum concentration of the acute phase protein α_1-acid glycoprotein [4]. Competitive drug inter-

actions, e.g. salicylate displacing methotrexate from plasma protein binding sites, can also lead to alterations in the fraction of free drug in plasma water and therefore alter drug clearance and volume of distribution. These and other factors which may perturb drug absorption and distribution will be examined more closely in the following section in relation to specific classes of anticancer drugs.

Pharmacokinetics of orally administered anticancer drugs

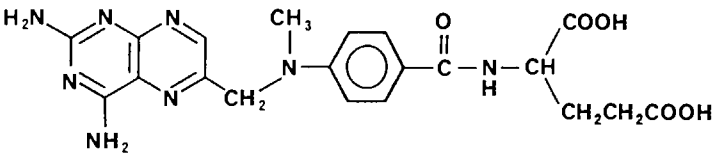
Antimetabolites

Methotrexate

Methotrexate (MTX; Fig. 1.1a) is an inhibitor of the enzyme dihydrofolate reductase, which is responsible for converting folic acid to reduced folate cofactors. Reduced folates are necessary for the metabolic transfer of 1-carbon units during the biosynthesis of thymidylic acid and, in turn, DNA. It is an important cytotoxic drug and is used clinically in the treatment of leukaemia, lymphoma, sarcoma, breast cancer, head and neck tumours and bladder cancer.

Absorption of MTX following oral administration is rather variable, and hence this route is not often used in cancer therapy, although it is employed in treatment of psoriasis and rheumatoid arthritis. Doses less than 30 mg/m^2 are virtually completely absorbed, whereas doses of 80 mg/m^2 are poorly absorbed and lead to plasma levels which are only 10% of those achieved following intravenous administration [5]. This is consistent with a saturable mechanism of intestinal absorption. A carrier-mediated, energy-coupled, cellular transport system exists for reduced folates and it is likely that MTX competes for this carrier [6]. Intravenous infusion is generally therefore the preferred method of dosing. However, the drug has also been administered intramuscularly and subcutaneously.

Pearson *et al.* [7] compared the pharmacokinetics of oral and intramuscular methotrexate in children with acute lymphoblastic leukaemia. Although intramuscular administration resulted in higher serum concentrations, there was substantial interpatient variability and this route offered no real advantage over oral administration. However, pharmacokinetic studies following subcutaneous administration in experimental animals and in patients have proved more successful. Balis *et al.* [8] examined the pharmacokinetics of methotrexate in the rhesus monkey after subcutaneous administration. The



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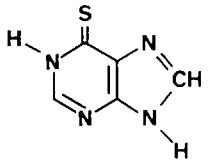

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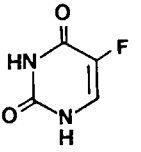


(c)

Figure 1.1 Antimetabolite drugs: (a) methotrexate; (b) 6-mercaptopurine; (c) 5-fluorouracil.

dose was completely absorbed and there was no localised toxicity at the site of administration. Subcutaneous administration has also been examined in children with acute lymphoblastic leukaemia [8]; again, the dose was completely absorbed, in contrast to the relatively poor oral absorption (approximately one third of the dose) at 40 mg/m^2. Overall, subcutaneous dosing is well tolerated and overcomes the problem of incomplete and variable absorption observed with oral and intramuscular administration.

MTX distributes rapidly into a volume approximated by total body water and diffuses slowly into interstitial fluid spaces, e.g. cerebrospinal fluid. Furthermore, 50–70% of the drug is protein-bound, predominantly to albumin, and MTX can be displaced from its binding sides by salicylate and nonsteroidal anti-inflammatory drugs. However, penetration into the central nervous system generally is low, which may have implications for the treatment of brain metastases. Clearance is primarily renal and persistent drug levels are associated with increased toxicity, which may require extra folate rescue, underlying the need for careful pharmacokinetic monitoring [9].

6-Mercaptopurine

6-Mercaptopurine (6MP; Fig. 1.1b) is an analogue of the purine base hypoxanthine, which inhibits *de novo* purine biosynthesis. This drug is used in the treatment of leukaemia as a component of maintenance therapy. In common with the majority of cytotoxic drugs, 6MP is prescribed in a standard dose regimen. This implies that the drug has consistent bioavailability and that interindividual differences in drug absorption and distribution are relatively small. However, a number of recent studies have challenged this supposition. For example, the bioavailability of 6MP is very poor (16% in one study) and extremely variable [10]. The pharmacokinetics of oral 6MP have been studied in children receiving therapy for acute leukaemia and non-Hodgkin's lymphoma, and large interindividual variations in peak plasma and the fraction of dose excreted in urine were found. Although food has been implicated in the variable absorption of 6MP, a study by Lafolie *et al.* [11] does not fully support this view. The effect of food intake was examined in children receiving oral 6MP following a standardised meal. Although food intake reduced the time taken to achieve C_{max}, pronounced inter- and intraindividual variations in pharmacokinetics were still observed in both groups [11]. In addition, the variability could not be clearly attributed to genetic polymorphisms in cytochrome P450 expression [11]. In view of the low bioavailability and the variability in pharmacokinetics, it is perhaps puzzling that the drug is given orally. Further work is required to investigate the correlation between the pharmacokinetics of 6MP and its clinical activity.

5-Fluorouracil

5-Fluorouracil (5FU; Fig. 1.1c) is metabolised within cells to 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) which binds to and inhibits the enzyme thymidylate synthetase. The drug is widely used in the treatment of breast, colorectal, gastric and pancreatic cancers and is usually given in combination with other agents rather than as a single agent. 5FU has been used orally, but it has extremely variable absorption [12–15] due to saturable intestinal transport and extensive saturable first-pass metabolism [16, 17]; for example, oral bioavailability may be as low as 25%, but is generally increased at higher doses [18]. In addition, interindividual variation in pharmacokinetics can lead to variability in efficacy and toxicity, and therefore use of oral 5FU cannot be recommended. Designing a controlled-release formulation could avoid the problem of saturable intestinal transport, but would potentially increase the problems of first-pass metabolism, as discussed in the previous section.

Platinum coordination complexes

Cisplatin and carboplatin

The cytotoxicity of platinum coordination complexes is thought to be due primarily to the formation of platinum–DNA adducts, including both inter- and intrastrand crosslinks.

A number of analogues of cisplatin (Fig. 1.2a) have been synthesised which possess different physicochemical properties and patterns of toxicity. Carboplatin (Fig. 1.2b) has a less reactive chemical structure compared to cisplatin, which probably accounts for its more favourable toxicity profile [19], i.e. myelosuppression with reduced nephro- and neurotoxicity. van Hennick *et al.* [20] performed a pharmacokinetic study following oral administration of carboplatin. The drug caused nausea and vomiting for 3–9 hours after administration and the bioavailability, relative to a standard intravenous dose of carboplatin, was very low ($< 10\%$). This trial highlighted a problem that can be associated with oral administration of cytotoxic agents, namely the induction of nausea and vomiting. This is another contributory factor to variable bioavailability, as a substantial and variable proportion of the dose could be lost during emesis, which may seriously compromise the efficacy of the treatment.

Experimental studies have demonstrated that the antidiarrhoeal agent loperamide can enhance the oral bioavailability of carboplatin in mice [21]. However, it is unlikely that carboplatin will be pursued further as an oral preparation as other platinum complexes with better bioavailability are now under development. Clearance of carboplatin can be predicted by the glomerular filtration rate, and dosage formulae based on this parameter have been developed to individualise therapy [22, 23].

Tissue-distribution studies in mice and rats have shown that tumour uptake of platinum was generally good, with equivalent

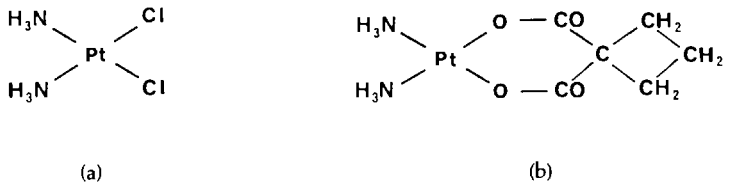


Figure 1.2 Platinum coordination complexes: (a) cisplatin; (b) carboplatin.

levels to those seen in the plasma [24, 25]. High levels were seen in kidney and low levels in brain. Whole-body scanning studies using ^{195m}Pt cisplatin in patients showed that the drug was rapidly concentrated in the kidneys and head region (within 3 hours), with the highest levels eventually accumulating in kidney, liver and intestine 40 hours post-treatment [26, 27].

Alkylating agents

The alkylating agents are a diverse group of compounds which are thought to exert their antitumour activity by forming alkyl adducts with DNA bases, either directly or after spontaneous or metabolic breakdown, thus inhibiting DNA replication and transcription. The majority of agents contain two alkylating centres and are described as bifunctional, forming crosslinking DNA adducts at the N–7 position of adjacent guanines. Melphalan and chlorambucil are directly-acting nitrogen mustards (containing an $-N(CH_2CH_2Cl)_2$ moiety), while cyclophosphamide and ifosfamide require metabolic activation. CCNU (lomustine) is a chloroethylnitrosourea which also forms DNA crosslinks; however, this drug differs from the nitrogen mustards in that the initial adduct formation involves chloroethylation at the O–6 position on guanine.

Chlorambucil

Chlorambucil is the phenylbutyric acid derivative of nitrogen mustard (Fig. 1.3a). It is used in the treatment of chronic lymphocytic leukaemia, Hodgkin's disease, ovarian cancer and certain inflammatory disorders. In clinical use it is normally given orally in low doses for 2–3 weeks; higher doses cause nausea and vomiting, neurotoxicity and myelosuppression. It is rapidly absorbed orally, with peak blood levels occurring within 2 hours. Although comparative studies of oral versus intravenous routes are limited, the results suggest that the oral bioavailability is 70–100% [28, 29]. An unusual feature of the drug is the extensive metabolism of the side-chain to form phenylacetic mustard by mitochondrial β-oxidation. Drug distribution studies with chlorambucil in rats demonstrate that only low concentrations of the parent drug and metabolite phenylacetic mustard enter the brain (with brain/plasma ratios of 0·021 and 0·013 respectively), which may account for the relatively poor antitumour activity of this agent against implanted brain tumours [30].

In an attempt to overcome the difficulties of drug penetration, a number of lipophilic chlorambucil prodrugs have been synthesised. Greig *et al.* [31] have evaluated a number of aliphatic esters including

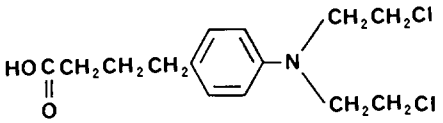
chlorambucil–methyl, –propyl, –hexyl, –octyl and aromatic esters. Although these compounds have desirable lipophilicity (log partition coefficients (octanol/water) 4·05–> 8·0), they were rapidly broken down in plasma, and yielded only low drug concentrations in the brain [31]. The approach was more successful, however, with a chlorambucil–tertiary butyl ester. Equimolar doses of chlorambucil (10 mg/kg) and the tertiary butyl ester (13 mg/kg) were given i.v. to rats. Brain/plasma drug concentration ratios of total active compounds were found to be 0·018 and 0·68 for chlorambucil and the ester respectively, indicating excellent brain penetration for the lipophilic analogue [32]. This was accompanied with a 5-fold lower plasma concentration for the ester. Together, these results suggest that the tertiary butyl ester may have pharmacokinetic advantages in the treatment of brain tumours [32], although care may have to be taken with respect to possible neurotoxicity.

Melphalan

Melphalan (Fig. 1.3b) is the phenylalanine derivative of nitrogen mustard and is used in the treatment of melanoma, breast cancer, ovarian cancer and multiple myeloma. It can be given both orally and intravenously. Comparative studies have indicated poor and extremely variable oral absorption (in some patients the drug could not be detected in plasma after oral administration) [33–37]. Food appears to be a factor in the efficiency of oral absorption [33, 35, 36]. In one study, the bioavailability ranged from 32% to 100% with a mean of 71% and absorption was found to be more efficient when melphalan was taken with food [33]. In contrast, another study showed that oral bioavailability was 85% (26–96%) after fasting compared to 58% (7–99%) with food [35]. Therefore the effect of food on melphalan absorption remains unclear.

Oral melphalan has also been used at high doses (140–157 mg/m^2) with autologous bone marrow transplantation [38, 39]. The systemic bioavailability with these very high oral doses of melphalan was extremely variable, and this cannot therefore be recommended as a regimen for patients undergoing autologous bone marrow transplantation [39].

Unlike chlorambucil, melphalan is not extensively metabolised. Clearance mechanisms are not clearly understood, but hydrolysis of the mustard group (OH replacing Cl) may be a significant feature and renal clearance may also be important [40]. A study by Loos *et al.* [41] in multiple myeloma showed that the plasma AUC rose by approximately 45% in patients after three courses given at 6-week intervals. This was not attributable to alterations in drug metabolism, elimi-



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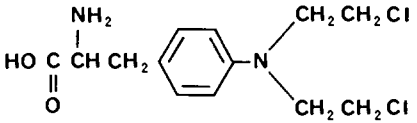

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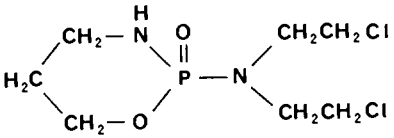

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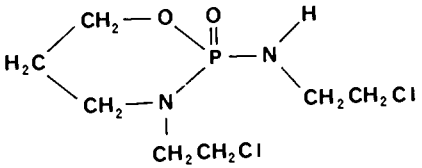

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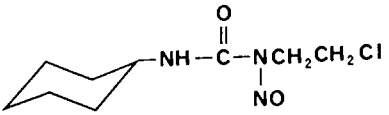


(e)

Figure 1.3 Alkylating agents: (a) chlorambucil; (b) melphalan; (c) cyclophosphamide; (d) ifosfamide; (e) CCNU.

nation or protein binding, and the authors postulated that covalent binding sites became progressively saturated during the courses of treatment, which was reflected in the elevated plasma AUC values [41].

The volume of distribution of melphalan approximates to the total body water in man and studies with radiolabelled drug in animals showed that tissue levels can exceed plasma concentrations in kidney and liver. The uptake of melphalan into tumours has been investigated in mice with adenocarcinoma 16/C. Concentrations of drug in well perfused areas of the tumour were similar to plasma levels at 30 minutes. However, the results indicated that tumour-to-tumour variations in perfusion and drug distribution were major determinants of drug efficacy [42]. The uptake of melphalan into cells is thought to occur via two separate high-affinity amino acid transport systems, the so-called L and A systems [43]. Similar uptake systems have been found to operate at the blood–brain barrier [44] (although uptake here is low) and are probably also involved in gastrointestinal absorption. Evidence from an *in situ* rat intestine model suggests that melphalan uptake does involve an active transport mechanism. Hence, dietary amino acids as well as food mass may affect absorption [45]. Interestingly, the L system may be absent or deficient in bone marrow cells and some human tumour cells, which may have therapeutic implications [46].

Given the variability of oral absorption, it is clear that the intravenous administration of melphalan is preferable. If the latter is not practicable it has been suggested that the dose should be escalated if myelosuppression or antitumour effects are not seen. Since some evidence suggests that bioavailability decreases with repeat dosing, it has been proposed that the drug might be given for fewer days than the 4-day regimen studied [36].

Cyclophosphamide

Cyclophosphamide and its relative, ifosfamide, are very commonly used alkylating agents. A wide range of schedules and doses are used and both drugs are often given in combination with other agents. The oxazaphosphorine ring of cyclophosphamide (Fig. 1.3c) deactivates the nitrogen mustard moiety. Hence, DNA alkylation requires metabolic activation [47]. A cascade of reactions is set in train by a cytochrome P450-mediated oxidation to 4-hydroxycyclophosphamide which is in equilibrium with aldophosphamide (Fig. 1.4). Oxidation takes place mainly in the liver and the 4-hydroxy metabolite then circulates as the major transport form. A detoxification pathway catalysed by aldehyde dehydrogenase forming 4-keto and

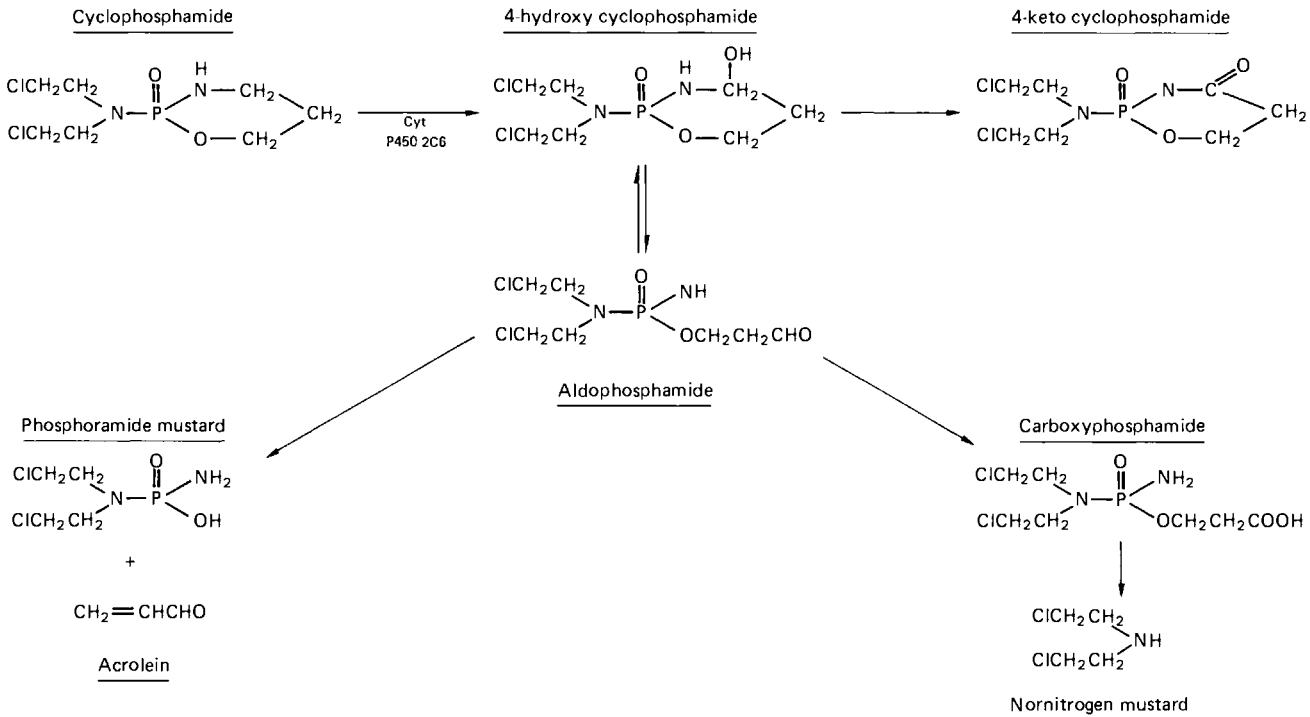


Figure 1.4 Metabolic activation of cyclophosphamide.

carboxyphosphamide seems to predominate in normal tissues such as liver, kidney and gastrointestinal mucosa. In the absence of the detoxification pathway, which is deficient in some tumours, aldophosphamide breaks down to yield the toxic metabolites acrolein and phosphoramide mustard. The latter is a potent DNA crosslinking agent and is believed to play the major role in the antitumour activity of cyclophosphamide. In contrast, acrolein probably does not have therapeutic activity but causes the characteristic haemorrhagic cystitis associated with cyclophosphamide therapy. When high doses are used, this problem can be alleviated by co-administration of the protective thiol agent mesna (sodium 2-mercaptoethanesulphonate).

The oral absorption of cyclophosphamide is generally high, but rather variable [48]. In one study with doses of 1–2 mg/kg, bioavailability was 97% and the range was 35–130%; at 3–6 mg/kg, bioavailability was 74% (34–90%) [49]. A more recent crossover study showed very similar systemic bioavailabilities for the important metabolites 4-hydroxycyclophosphamide and phosphoramide mustard [50].

Cyclophosphamide clearance has been reported to increase with repeat dosing [49] and its elimination rate is also increased in patients receiving the hepatic enzyme-inducer phenobarbitone. The reverse is likely to be the case with co-administration of the cytochrome P450 inhibitor cimetidine. Differences in the handling of cyclophosphamide are likely to be due to such induction/inhibition effects, together with genetic differences in cytochrome P450 expression, particularly the isoform 2C6 [51]. However, the therapeutic and toxic effects of such differences remain uncharacterised, as are the consequences of poor renal function.

Ifosfamide

Ifosfamide (Fig. 1.3d) is structurally related to cyclophosphamide but has one of the chloroethyl side-chains attached directly to the oxazaphosphorine ring. Ifosfamide is similar to cyclophosphamide in that it requires metabolic activation to 4-hydroxyifosfamide and ultimately ifosphoramide mustard, which is a potent alkylating agent. In contrast to cyclophosphamide, N-dealkylation can occur to form dechloroethyl metabolites and chloroacetaldehyde [52], and the latter has been implicated in ifosfamide-induced neurotoxicity. Another difference is that a smaller proportion of ifosfamide is converted to biologically active metabolites.

Ifosfamide can be administered by both oral and intravenous routes [53]. Oral bioavailability is generally accepted to be complete. However, there is evidence of higher levels of the metabolites

carboxyifosfamide, dechloroethyl ifosfamide and isophosphoramide following oral administration, indicative of first-pass hepatic metabolism [54]. Interestingly, neurotoxicity appears to be enhanced following oral dosing, which may be related to the formation of chloroacetaldehyde, implicated in the neurotoxic syndrome [55]. Although ifosfamide has essentially 100% bioavailability, differences in metabolism due to first-pass effects preclude oral administration at conventional i.v. doses.

CCNU

CCNU (lomustine) (Fig. 1.3e) is an orally administered chloroethylnitrosourea. Therapeutic indications include melanomas and brain tumours. The latter activity may be related to the excellent penetration of this highly lipophilic drug across the blood–brain barrier, a property also shared with the related agents BCNU (carmustine, given intravenously) and methyl CCNU (semustine, given orally). The dose-limiting toxicity is myelosuppression.

Until recently, there was very little information on CCNU pharmacokinetics. However, a recent small study [56] showed that CCNU could not be detected in the plasma of patients receiving the standard oral dose of 130 mg/m^2; instead, the metabolites *cis*- and *trans*-4-hydroxy CCNU were present. Apparently, the first-pass metabolism of CCNU is complete and hydroxylation is catalysed by cytochrome P450. Some typical plasma profiles of the hydroxylated metabolites are shown in Fig. 1.5. The two hydroxylated metabolites show similar activity to the parent drug, but their combined peak levels do not exceed 1 μg/ml. This concentration would not cause marked cytotoxicity in many tumour cell lines, especially those with high levels of the O–6 alkyl transferase repair protein, which removes the damaging O–6 adduct [56]. The same study reported concentrations of the hydroxylated metabolites in the tumour tissue of a patient with melanoma. Both metabolites were readily detectable in the tumour, and the levels were found to be 48% and 60% of the corresponding plasma levels 4 hours after oral dosing.

Anthracyclines

Idarubicin

The anthracyclines have a spectrum of antitumour activity second only to the alkylating agents and are the most valuable of the natural product anticancer drugs. Although it was once considered that they owed their cytotoxic activity to DNA intercalation, it is now known

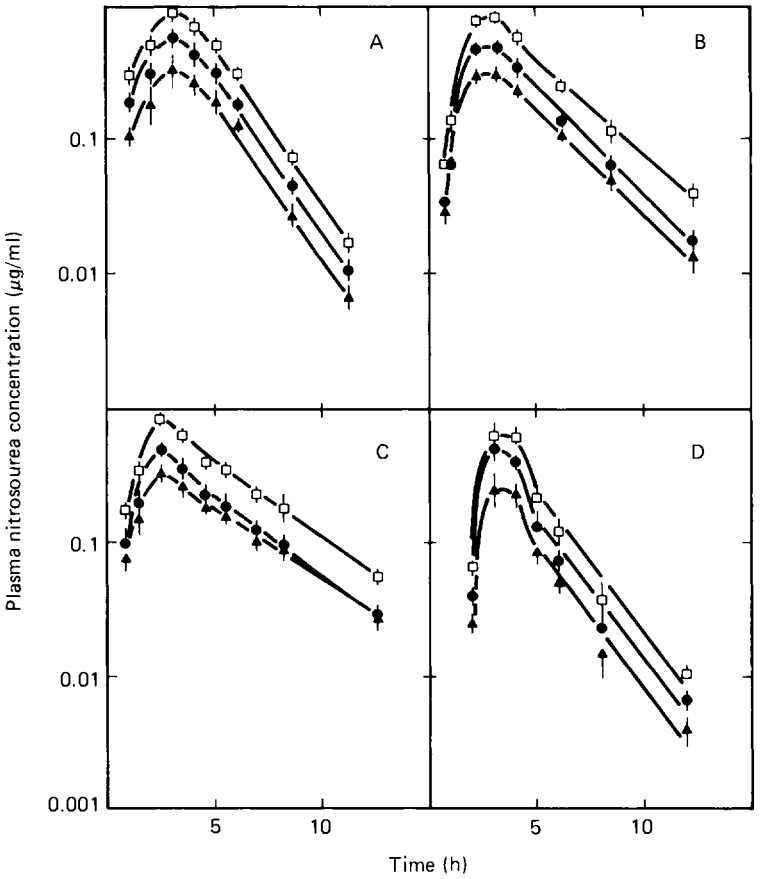


Figure 1.5 Mean plasma concentrations (error bar = 1 SD) of (□) total *cis*- and *trans*-4-hydroxy CCNU, (●) *trans*-4-hydroxy CCNU and (▲) *cis*-4-hydroxy CCNU in four patients receiving 130 mg/m^2 CCNU orally. (Modified from reference 56.)

that the formation of topoisomerase II-dependent DNA damage is one of the most important mechanisms of action. Effects on cell membranes and free radical formation may also contribute to cytotoxicity. Side-effects include myelosuppression and cardiac toxicity, the latter possibly involving free radical formation.

Anthracyclines are generally not active when given by the oral route. The best known and most widely used anthracyclines, doxorubicin and daunorubicin, are always given intravenously.

However, a daunorubicin analogue, idarubicin (Fig. 1.6a), does exhibit adequate oral bioavailability [57], and demonstrates activity in acute lymphocytic leukaemia, acute non-lymphocytic leukaemia and breast cancer.

Idarubicin differs from daunorubicin only in lacking the 4-demethoxy group at the C–4 position on the chromophore. After oral administration, peak plasma levels were achieved 2–4 hours post-treatment and the bioavailability was in the range 9–39% [58]. A more detailed analysis of the bioavailability of the drug in elderly leukaemic patients showed that the bioavailability of idarubicin alone was about 21%, whilst the summed bioavailability of idarubicin and the major metabolite 13-dihydro-4-demethoxydaunorubicin) was about 41% [59]. The drug was rapidly and extensively metabolised to 4-demethoxy-13-hydroxydaunorubicin, the levels of which exceeded those of the parent drug, and probably contribute significantly to its pharmacological effects [58, 59]. Urinary recovery was 16% for the intravenous route compared to 5% for oral dosing, which is in accordance with the plasma bioavailability estimates.

The sum of the plasma AUC for idarubicin and idarubicinol was lower in breast cancer patients with rapid disease progression than in those who responded, and a correlation between AUC and relative leucocyte decrease was found [60].

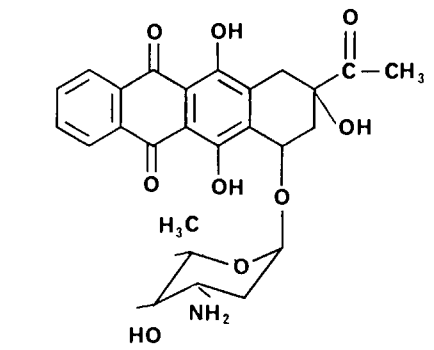
Uptake of anthracyclines into cells is generally good and a very high proportion is usually located within the nucleus. Recent studies have shown that resistance to anthracyclines may develop as a result of increased expression of the P-glycoprotein membrane efflux pump [1]. Clinical trials are currently underway with anthracycline analogues which retain activity in resistant cells, apparently by failing to act as substrate for the efflux pump [61]. Inhibition of the pump by agents as diverse as verapamil and cyclosporin is also under evaluation.

Diffusion of anthracyclines into tissues is often limited to 2–3 cell layers from the nearest blood vessel, apparently because of extensive binding to DNA in these cells.

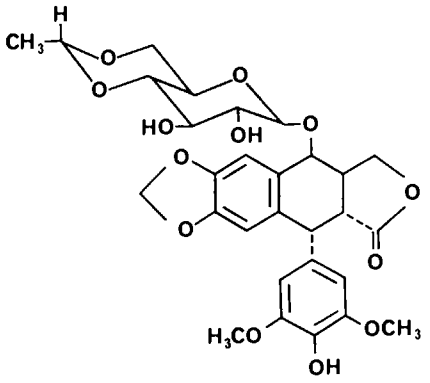
Podophyllotoxins

Etoposide

Etoposide (VP-16; Fig. 1.6b) is a podophyllotoxin derivative extracted from the roots and rhizomes of *Podophyllum peltatum* and *P. emodi*. It is an important anticancer agent widely used in combination with drugs such as cisplatin in the treatment of testicular teratoma, lymphoma, small-cell lung cancer, leukaemia and Kaposi's sarcoma. Mechanistically, the drug differs from podophyllotoxin itself in its



(a)



(b)

Figure 1.6 (a) Idarubicin; (b) etoposide.

mechanism of action, as it is a poor inhibitor of microtubule assembly. This may be due at least in part to the bulky glucoside constituent on the molecule. A number of alternative mechanisms of action have been proposed, including the formation of reactive metabolites and inhibition of DNA topoisomerase II.

In vitro metabolism studies have shown that etoposide can undergo O-demethylation to form the orthodihydroxy (catechol) derivative which may be involved in covalent binding to cellular macromolecules. The formation of a catechol and subsequent con-

version to the orthoquinone probably involves the formation of a semiquinone free radical which may cause DNA strand breaks.

Originally, etoposide was given by the intravenous route, but oral administration is now gaining ground because of the improved therapeutic index with fractionated dose schedules. Various preparations of etoposide have been looked at for oral administration, including solutions and soft gelatin capsules. The bioavailability of oral preparations is around 50%, but there is considerable interpatient variability (17–137%) [62]. The physicochemical characteristics of etoposide have been examined to determine if pharmaceutical factors contribute to the low and variable oral bioavailability of the drug. The intrinsic dissolution rate was low (0·0094 mg/min/cm^2) whilst the octanol/water partition coefficient was relatively high (9·94). These studies indicate that absorption of the drug is limited by the rate of dissolution rather than the rate of permeation. It was also found that etoposide was chemically unstable at pH 1·3, so degradation at gastric pH may also contribute to its low oral bioavailability [63].

Oral etoposide is convenient for outpatient use, particularly when long periods of treatment are desirable. During a Phase I study of oral etoposide at 50 mg/m^2/day, 5 of 16 patients had a partial response [64]. The success with oral etoposide given at low doses over a protracted period indicates that the sustained inhibition of topoisomerase II, particularly the cell cycle-dependent form, may be therapeutically advantageous [65].

A more soluble derivative, etoposide phosphate (BMY-40481-30) is currently undergoing early clinical trials and seems to give much improved and more consistent bioavailability. Preclinical studies demonstrated that the drug was more active orally than etoposide against the P388 leukaemia in mice [66], and that the etoposide phosphate acted as a prodrug, releasing etoposide following the action of phosphatases *in vivo*. This prodrug approach has been developed further by covalently linking monoclonal antibodies (directed towards antigen-positive tumour cells) with alkaline phosphatase. The intent was to modify the distribution of the drug and liberate etoposide at the site of the tumour [67, 68]. Unfortunately, this approach has been largely unsuccessful *in vivo*, presumably due to hydrolysis by non-specific plasma phosphatases. However, preliminary clinical studies with i.v. etoposide phosphate are encouraging. The enhanced water solubility of etoposide phosphate has led to significant improvements in formulation, facilitating administration by infusion. Initial pharmacokinetic studies in patients indicate that the drug is rapidly converted to etoposide [69]. Tolerance and efficacy studies with this compound are underway presently, and it

will be of interest to see if the drug has useful antitumour activity following oral administration.

Concluding remarks

Pharmacokinetic studies are now playing an increasingly prominent role in the development of new anticancer agents. Detailed preclinical and clinical studies are carried out to help us understand the therapeutic and toxic effects of both new and established anticancer drugs. Similarly, efforts are being made to develop pharmacokinetic–pharmacodynamic models so that these can be used to individualise doses for particular patients. As illustrated in this chapter, preclinical and clinical studies of oral bioavailability are essential when this convenient, but potentially problematic, route of administration is

Table 1.1 Tumour and plasma concentrations of flavone acetic acid in human melanoma

Patient no.	*Time after infusion*	*Tumour conc.*		*Plasma conc.*	*Tumour/plasma ratio*	
		(μg/g)	*(Mean)*	*(μg/ml)*	*(%)*	*(Mean)*
1*	0	196·8	183·4	390	50·5	47·1
		157·3			40·4	
		196·6			50·3	
2	0	114·1	131·8	283	40·2	46·6
		123·0			43·4	
		122·3			43·1	
		165·4			58·4	
		134·3			47·4	
3	10 min	183·3	178·7	429	42·7	41·6
		174·1			40·6	
4	22 min	125·1	120·5	290	43·1	41·5
		116·5			40·1	
		119·9			41·3	
1*	65 min	169·0	176·3	290	58·3	60·8
		183·6			63·4	
1*	12 h	14·5	20·1	78	18·5	25·7
		20·1			25·7	
		23·6			30·1	
		22·4			28·7	

*Three samples taken from one patient after three different infusions.
Data from reference 70.

being considered. Detailed studies of tumour penetration by anti-cancer agents are often lacking, particularly in patients. However, the development of non-invasive techniques for studying tissue pharmacokinetics, such as magnetic resonance spectroscopy and positron emission tomography for example, are likely to play an increasingly important role in early clinical trials. The value of measuring tumour penetration has recently been illustrated with the experimental drug flavone acetic acid (FAA) [70]. The uptake of FAA into the tumours of patients with malignant melanoma was reasonably good, at around 40–50% of the corresponding plasma levels (Table 1.1), and hence it was concluded that poor tumour penetration did not account for the disappointing clinical results with this agent. In conclusion, a detailed knowledge of the absorption and distribution of anticancer agents will facilitate the optimisation of treatment regimens, which in turn should improve both the duration and quality of life of the cancer patient.

Acknowledgement

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Further reading

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Chapter 2

Cytotoxic drug delivery

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Rationale for cytotoxic drug delivery

With limited exceptions, cytotoxics (also known as antineoplastic agents or antiproliferatives) represent the most effective form of drug therapy currently available to treat human cancer. They are unusual as a class of pharmacological agents in that: (i) their action is irreversible, i.e. they cause cell death; and (ii) their action is directed against the normal replicative machinery (usually DNA itself) present in all cells. Thus, they will attack any cell that has entered into a cycle of replication. Originally, it was believed that tumour cells divided faster than normal cells and, because of this, cytotoxic drugs would have inherent tumour cell specificity. However, it is now clear that many types of cancer (e.g. colorectal, adenocarcinoma of the lung) contain only a small fraction of proliferating cells (1–5% of the total tumour population) whereas, in contrast, normal tissues such as the bone marrow can contain up to 75% dividing cells [1]. Hence, in some cases it is probably more accurate to say that cytotoxic drugs have an inherent lack of tumour cell selectivity. This lack of specificity limits the strength and frequency at which doses can be administered, seriously compromising their therapeutic efficacy. As a consequence, many of the major forms of human cancer, e.g. lung, gastric, colon and breast carcinoma, cannot be controlled by chemotherapy.

Therefore the rationale for cytotoxic drug delivery is clear: in the absence of agents that interact with unique biochemical pathways in cancer cells and could, theoretically, be administered safely systemically, a mechanism is required to alter the disposition of currently available compounds so that they are directed more towards the tumour and less towards host organs. The pharmaceutical approach to this problem is to reformulate the cytotoxic agent in a drug delivery system (DDS); this approach is the subject matter of this chapter, where the drug delivery systems that have been tried are discussed along with the results they have achieved with specific agents.

Characteristics of an ideal drug delivery system

There are many properties that one would wish to incorporate into an ideal DDS. However, in reality, it is questionable whether this could be achieved with any one particular system. A set of ideal parameters has been defined, which are as follows [2]:

Selectivity. The DDS must be able to transport the drug agent intact to its site(s) of action without leakage during transit, drug metabolism or distribution into non-diseased host tissues.
Load factor. The DDS should be capable of delivering a therapeutically effective dose to the target site(s). From a pharmaceutical viewpoint, the dose incorporated in or associated with the DDS has to be controllable and predictable.
Immunology. The DDS should be suitable for repeated administration without causing immune responses and hypersensitivity.
Toxicity. The DDS has to be non-toxic in its own right.
Scope of disease. It would be advantageous if many disease types could be treated and many different therapeutic agents used.
Pharmaceutical feasibility. The formulation has to make practical sense in terms of both production and administration.

Classification of drug delivery systems

Due to the obvious need to improve the outcome of cancer chemotherapy, a large number of different drug delivery approaches have been investigated, often out of sheer frustration, with little scientific rationale. For the purposes of clarity, we have separated the systems currently available into three major classes and within these have identified several subdivisions. The major classes are vesicular, microparticulate and soluble macromolecular carriers.

Strategies for targeting cytotoxic drug delivery systems

Different levels of targeting are possible within the whole organism and these are defined by degrees: 1st order targeting requires delivery of the drug-carrier to a particular organ or tissue; 2nd order targeting refers to selective uptake into specific cell type(s) within an organ or tissue (i.e. the tumour); and 3rd order targeting involves delivery to particular intracellular compartments in the target cells. It does not necessarily follow that one is always striving to achieve the highest

order of targeting: in the treatment of cancer, 2nd order may be sufficient with certain drugs.

Many factors dictate the eventual destination of a DDS upon administration and hence the level of targeting achieved. The physicochemical and pharmacological properties of the drug and the carrier are both important. If the DDS is administered intravenously then the permeability of the vasculature at the target site becomes a key determinant. Macromolecules, microparticles and vesicles can be taken up into cells by endocytosis or can escape from the circulation through capillary endothelia, which are sinusoidal or fenestrated, as in the liver and spleen. Therefore, a variety of physiological variables are also important, such as the phagocytic and pinocytic activity of the tumour, its degree of vascularisation and the maturity of the vessels. Two broadly based strategies have been applied: passive targeting and active targeting.

Passive targeting

Passive targeting relies on the inherent properties of the DDS without intervention from another agent or approach. Perhaps the most important parameter here is size and this alone will have a profound affect on eventual destination. The effects of size on passive targeting of DDS have been well researched [3] and are listed for microspheres in Table 2.1 below.

Passive targeting is unlikely to achieve good tumour selectivity for a variety of reasons. One of the major barriers that has to be overcome is the reticuloendothelial system (RES). The RES is the collective name for a group of highly phagocytic cells derived from the bone marrow. Although these cells are found throughout the body, large concentrations reside within the liver (fixed macrophages

Table 2.1 Relation between drug delivery system size and anatomical destination

Size (μm)	*Destination*
0·05	Spleen and bone marrow
0·1–2·0	Cleared from the blood stream by macrophages of the reticuloendothelial system
2·0–12	Entrapped in lung, liver and spleen following intravenous administration
> 12	Lodge in the first capillary bed encountered (chemoembolisation)

Adapted from reference 3.

termed Kupffer cells), spleen and bone marrow. Smaller particles ($< 2\ \mu m$) tend to be preferentially sequestered by the RES and will target naturally to the liver, spleen and bone marrow, the latter being the major organ of toxicity for cytotoxic drugs. Larger particles unable to escape from the circulation or be endocytosed can avoid the RES and achieve good 1st order targeting by becoming physically trapped in the first bed of small blood vessels they encounter after administration (chemoembolisation), but will not produce 2nd or 3rd order delivery. Upon injection into the blood stream all colloidal particles will normally become coated with various blood components and this process (opsonisation) enhances uptake into the RES or by circulating macrophages.

Active targeting

Several approaches have been adopted to target more actively to tumours and these are dictated by the properties of individual DDS. For instance, ultrafine particles of magnetite (Fe_3O_4) can be incorporated into microspheres so that their movement after intra-arterial administration can be controlled by an externally applied magnetic field [4]. This approach has been successful in inhibiting the growth of subcutaneous tumours, but is of limited usefulness for disease sites located deep inside body compartments. Co-administration of the smooth muscle vasoconstrictor angiotensin II with intrahepatic arterial doxorubicin (DOX)-loaded protein microspheres improves dramatically 2nd order targeting to colorectal liver metastases [5]. To avoid liposomes being taken up into the RES, modification of surface charge has resulted in a longer residence time in the circulation and greater uptake into tumours in experimental animals [6]. Liposomes which do not release their drug content unless elevated to a temperature above the liquid-crystal transition point of the lipids can be formulated. By causing hyperthermia at tumour sites in the body, more selective drug uptake is envisaged. Synthetic copolymer, soluble, macromolecular drug carriers can be produced where the cytotoxic agent is attached via a peptide spacer, which is selectively cleaved once uptake into cell lysosomes is achieved [7]. These techniques are discussed in more detail in the appropriate sections below.

Vesicular systems

Vesicular systems are distinguished from microparticulate systems by being in a more fluid state and, therefore, are not normally

chemoembolised. The corollary is that they also tend to be less stable, and leakage of drug into the circulation can occur before targeting is achieved.

Liposomes

Liposomes are microscopic structures which consist of one or more closed concentric lipid bilayers surrounding an equal number of aqueous spaces and are the DDS which has been studied most extensively. Water-soluble compounds will partition preferentially into the aqueous spaces and water insoluble compounds will partition into the closed bilayer, allowing for a versatile choice of cytotoxic drug. Weinstein and Leserman [6] have described the following 11 possible advantages of liposomes as a DDS: (1) prolonged drug effect due to longer residence time in the circulation; (2) the possibility that liposomes may be sequestered in the target location (the tumour); (3) reduction of toxicity in tissues (e.g. cardiac muscle) which do not accumulate liposomes; (4) protection of drug content from metabolism and immune attack until it reaches the tumour; (5) confinement of liposomes to a chosen anatomical compartment; (6) selective targeting by attaching an antibody or other ligand; (7) direction of liposomes to their natural target, i.e. the phagocytic cells of the liver, spleen and other organs; (8) amplification of therapeutic effect by incorporation of numerous drug molecules in each target-directed particle; (9) selective local release from liposomes as a function of physical factors such as the local temperature or pH; (10) circumvention of permeability barriers by endocytosis or fusion of liposomes with cells; and (11) delivery of drugs designed to be active after endocytic uptake.

Three major types of liposomes have been described. Multilamellar vesicles (MLV) are in the size range 0·1–10 μm and will form spontaneously when bilayer lipids are hydrated in aqueous solution. Their eventual size distribution will depend on the method of manufacture and subsequent processing through size exclusion filters. Small unilamellar vesicles (SUV) are produced from MLV by an input of energy, usually in the form of ultrasonication; they can be as small as 0·02 μm. SUV are the most commonly used form for cytotoxics drug targeting. They are more uniform in size than MLV, are osmotically stable and remain in the circulation longer, but have a tendency to aggregate with time. Due to their high surface/interior volume ratio, they trap very little water-soluble material per unit of lipid, but because of their size they appear to be endocytosed by fibroblasts and lymphoid cells more readily than larger particles, and their contents may subsequently be released into the cytoplasm. The

third major type is the large unilamellar vesicle (LUV); their methods of production are more complex but they have the advantage of a large ratio of trapped volume to lipid.

A detailed account of the chemistry of the lipids used to produce vesicular structures and the physical properties of these particles is beyond the scope of this chapter. In brief, lipids which form bilayers generally have two acyl chains attached to a polar head group which can be either neutral (phosphatidylcholine), positively or negatively charged (e.g. stearylamine and phosphatidylglycerol, respectively). Cholesterol is frequently included to strengthen the liposome for *in vivo* application. Below the transition temperature of the acyl side-chains, liposomes are more rigid and will retain their drug content better. Degree of drug incorporation depends on the oil–water partition coefficient of the drug, the type of vesicle involved and its lipid content.

Considerable disruption of liposomes can occur in the blood stream. Originally albumin was thought to be responsible, but it has been shown that this effect is more likely to be due to apolipoproteins of the high density lipoprotein complex (HDL), which break the vesicles into micellar structures whose rims are wrapped with apoprotein. The largest openings in the cardiovascular system are in the liver, where blood flows through sinusoids with endothelial linings that contain 0·1 μm pores, large enough for SUV alone to pass through. The continuous endothelia of most tissues only have intracellular junctions, which allow particles of $< 0{\cdot}02$ μm to diffuse through and exclude most liposomal formulations. There is evidence, however, that some primary and metastatic tumours may have impaired endothelia which would enhance liposome uptake.

Liposomes can theoretically interact with cells in several different ways including endocytosis, adsorption on to the external surface of the cell, fusion with the plasma cell membrane and transfer of lipid from the vesicle to the cell membrane, with resultant particle disruption and drug release. Of these, the major route into the cell appears to be the endocytic pathway, and cell fusion probably does not occur *in vivo*. Recently, prions have been incorporated into liposomes in an attempt to improve their uptake directly into cells through the plasma membrane by non-endocytic mechanisms [8].

Four categories of targeting have been pursued with liposomes [9]:

(1) Natural (or passive) targeting, where particle destination would be predominately to phagocytic cells; this is least appealing for cytotoxics.
(2) Compartmental targeting, where the liposomes are administered directly into an anatomical region poorly perfused by blood, such as

joints, peritoneal cavity, lymphatics, etc.
(3) Physical targeting, using temperature- or pH-sensitive, magnetic, etc. liposomes.
(4) Ligand-mediated targeting, by incorporating an antibody, hormone, lectin, carbohydrate, prion, etc. into the structure of the liposome to act as recognition molecules for complementary molecules on the surface of the target tumour cells.

Option (4) has been the one most studied. Although experimental studies have shown that by judicious use of antibodies the uptake into tumour cells growing *in vitro* can increase 4-fold [10], the major problem for ligand-mediated targeting to solid tumours in patients is the lack of consistently expressed tumour-specific determinants to target against. This is compounded by the fact that tumours are normally made up from heterogeneous subpopulations of cells, each with their own characteristic biochemical and immunological properties. Considerably more progress is required before liposomes can be expected to make any significant inroads into improving the outcome of the major forms of human cancer.

Table 2.2 shows some of the cytotoxic drugs that have been incorporated into liposomes, along with typical entrapment efficiencies and the type of liposome employed. This list is by no means exhaustive however, and at present only a limited number of drug formulations have progressed to the clinic, including adriamycin, the lipophilic analogue of cisplatin codenamed L-NDDP, and the new analogue of mitomycin C codenamed NSC-251636.

Doxorubicin (adriamycin, DOX)

DOX is one of the most effective anticancer cytotoxic drugs in clinical use, having a broad spectrum of activity against both leukaemias and the major solid malignancies such as breast cancer. Its mechanism of action is most probably mediated via non-covalent intercalative binding to DNA and stabilisation of a drug/DNA/topoisomerase II complex termed the cleavable complex [11]. Formation of the cleavable complex results in an inhibition of DNA and RNA synthesis, leading to a block in cell replication and eventually cell death. The drug also undergoes biotransformation by quinone reduction to a semiquinone free radical which can redox cycle with molecular oxygen and generate a cascade of potentially toxic reactive oxygen species, including the superoxide radical, hydrogen peroxide and the hydroxyl radical. Free radicals have been shown to be uninvolved in antitumour activity but are strongly implicated in the severe cardiotoxicity of DOX. Cardiotoxicity limits

Table 2.2 Liposomal cytotoxic drug preparations

Drug	*Type of liposome*	*Drug entrapment (%)*
Hydrophilic compounds		
Actinomycin D	SUV	15
Cytosine arabinoside	SUV	20–35
Cisplatin	SUV	1–7
Doxorubicin	SUV	50–60
	SUV	65–68
	SUV	50–60
	LUV	>95
Melphalan	SUV	8
Methotrexate	SUV	4–9
Vincristine	SUV	10
Lipophilic drugs		
Cytosine arabinoside–phospholipid complex	SUV	85–95
Methotrexate–phospholipid complex	SUV	>95

SUV, small unilamellar vesicles; LUV, large unilamellar vesicles.
Data modified from reference 18.

the cumulative dose of DOX that can be administered safely to 550 mg/m^2, where a typical therapeutic dose would be 40–50 mg/m^2 every 3–4 weeks. Apart from cardiotoxicity and the usual spectrum of drug-induced side effects (e.g. bone marrow toxicity, nausea and vomiting, gastrointestinal disturbances), the therapeutic efficacy of DOX is hindered by the presence of *de novo* and acquired drug resistance, including the multidrug resistance phenotype (MDR). MDR is mediated via the expression of a plasma membrane glycoprotein, P-170, which has a wide substrate specificity and is believed to function as a transport pump, actively effluxing the cytotoxic drug from the cell [12]. Levels of expression of P-170 correlate inversely with drug levels and with therapeutic efficacy in human tumours, as would be expected from its proposed mechanism of action.

The rationale for incorporating DOX into liposomes is two fold: (i) reduction of toxicity, especially cardiotoxicity; and (ii) overcoming drug resistance. Strom and coworkers [13] determined the key factors for liposome encapsulation of DOX. They found that negatively charged phospholipids (phosphatidylserine and phosphatidylglycerol) were better than neutral lipids for increased loading capacity and increased stability against particle aggregation, and the inclusion of cholesterol reduced leakiness. These findings correlate with the known physicochemical properties of DOX which, at physiological

pH, carries a positive charge on its daunosamine sugar group that can interact with the negative charges on lipids. DOX is also known to bind to cardiolipin, a major lipid component of heart mitochondrial membranes, and this interaction is believed to be important in its cardiotoxicity. Inclusion of cardiolipin in positively charged liposomes slows down drug egress from these particles.

Several preclinical animal pharmacokinetic and antitumour activity studies with different liposome preparations have yielded consistent results [14, 15]. DOX-liposomes tend to accumulate preferentially in the spleen, although repeated administration does not result in enhanced immunotoxicity [16]. High uptake also occurs in the liver, which may be beneficial in the treatment of liver metastases. Reduced uptake is observed into the heart, due to this organ's poor phagocytic activity, resulting in an attenuation of the toxic histopathological lesions normally seen with DOX, but no reduction in its antitumour activity. Limited studies are available on DOX-liposomes in drug resistance, although when actinomycin D (another cytotoxic in the MDR class of drugs) was encapsulated in liposomes, the formulation was found to be ineffective against a drug-resistant murine tumour [17].

The promise of reduced cardiotoxicity has prompted the entry of DOX-liposomes into clinical trials at two different centres (Georgetown University, USA and Hadassah University, Israel) using two different formulations [18]. The Georgetown preparation consists of SUV liposomes (0·05–0·1 μm) made from egg phosphatidylcholine, cholesterol, cardiolipin and stearylamine at a molar ratio of 5 : 3·4 : 1·9 : 1 with a drug entrapment efficiency of 50–60%. The Hadassah preparation also consists of SUV liposomes (0·05–0·2 μm) composed of egg phosphatidylcholine, phosphatidylglycerol and cholesterol at a molar ratio of 7 : 3 : 4 which have an entrapment efficiency of 65–85%. Another formulation is available for clinical trials from a commercial source (the Liposome Company, USA); this comes as a kit ready for reconstitution in the hospital pharmacy and consists of LUV liposomes (0·2 μm mean size) composed of egg phosphatidylcholine and cholesterol, and offers the advantage of $>95\%$ drug loading. Preliminary results from the clinical trials show that higher doses of DOX can be administered and high response rates can be achieved in tumour types which are chemosensitive to non-encapsulated DOX. Data are still lacking on cardiotoxicity. Encouraging results have also been achieved by the Georgetown group, using their formulation for intracavity peritoneal administration of DOX-liposomes to patients with advanced ovarian cancer [19]. These clinical trials will ensure that liposomes have a future in the treatment of human cancer.

Other anticancer drugs

In general, liposomes tend to alter the disposition of other cytotoxic drugs in a manner similar to DOX, where raised levels in the circulation are observed along with elevations in the spleen and liver and reduced or normal uptake into the majority of tissues, although this is not necessarily associated with increased antitumour activity.

An example of the potential usefulness of liposomes is with new cytotoxic substances which are difficult to formulate due to poor water solubility, e.g. the lipophilic analogue of the anticancer drug cisplatin codenamed L-NDDP. Cisplatin is one of the most successful anticancer agents available, having curative potential in testicular cancer and good activity in ovarian and lung cancers, but it suffers from severe nephrotoxicity as well as the problem of pleiotropic drug resistance. However, L-NDDP is a water insoluble analogue and is almost completely inactive when administered as non-entrapped drug in a micellar suspension. Liposomal-L-NDDP is as active as cisplatin against animal tumour models, is non-cross resistant, and in dogs produces no significant renal dysfunction. L-NDDP is formulated in MLV liposomes of mean size 2 μm, consisting of phosphatidylcholine and phosphatidylglycerol at a 7 : 3 molar ratio, and entrapment efficiency is close to 100%; clinical trials are planned with this formulation at the MD Anderson Cancer Centre, USA. A similar philosophy has been applied to lipophilic analogues of mitomycin C.

Nonionic surfactant vesicles (NSV)

NIV should be considered an alternative to liposomes. It has long been recognised that, from a pharmaceutical viewpoint, liposomes exhibit certain disadvantages. Due to the predisposition of phospholipids to undergo oxidative degradation (lipid peroxidation), they have to be stored and handled in an inert (usually nitrogen) atmosphere. Problems can also be encountered with the variable purity and high cost of naturally-occurring phospholipids. To a certain extent, these drawbacks have been overcome with the advent of commercially available kits which allow reconstitution immediately prior to use. Nevertheless, an alternative to the liposome with different pharmacological properties would be useful. NSV were first shown to form when a mixture of a single-alkyl chain, non-ionic surfactant and cholesterol were hydrated [20]. The resultant vesicles were stable multilamellar species of 0·3–1·0 μm diameter, depending on the method of preparation and the cholesterol content. These vesicles entrap solute molecules with an efficiency that again depends on

composition and method of formation. Sonication generates a mixture of unilamellar and small multilamellar vesicles (0·1 μm average size). Incorporation of cholesterol, which has been shown inessential for vesicle formation, reduces rate of release of water-soluble drugs [21]. Entrapment efficiencies have been reported to be lower than for liposomes, e.g. only 14% for DOX-NSV compared to 60–95% for DOX-liposomes. The critical test for the NSV is whether it can favourably alter the disposition and activity of clinically active cytotoxic drugs.

Methotrexate

Methotrexate has been formulated into two similar preparations of small vesicles (0·1 μm average diameter) consisting of 'surfactant 1', cholesterol and diacetyl phosphate at a ratio of 47·5 : 47·5 : 5 in type A NSV and 60 : 30 : 5 in type B NSV [22]. Entrapment efficiency of drug was 20–30%. NSV were administered to mice both orally and intravenously and levels of the drug and its 7-hydroxy metabolite were measured in plasma and several tissues. Like liposomes, levels of circulating drug increased with NSV administration and remained elevated for longer. NSV targeted very high levels to the liver, indicative of tissue uptake being mediated via endocytic mechanisms. Interestingly, the NSV improved penetration into the brain, suggesting that they may be able to perturb the blood–brain barrier, and they protected the drug from rapid metabolism. No antitumour studies were performed during these investigations.

Doxorubicin

Incorporation of DOX into NSV made with 'surfactant 1' have yielded hopeful results in preclinical animal tests. In these studies, DOX was encapsulated in two different formulations of large multilamellar NSV (0·8–0·9 μm mean diameter): 100% 'surfactant 1' and 50 : 50 'surfactant 1' : cholesterol. Drug entrapment efficiency was 14% for both [23]. In preformulation studies, cholesterol was shown to retard the release of DOX significantly. However, *in vivo* the two different formulations behaved similarly [23]. As anticipated, NSV produced elevated blood levels, but surprisingly did not cause an accumulation of drug in the liver (coating liposomes with neutral surfactant has been reported to circumvent the RES). Additionally, drug levels appeared not to be reduced in the heart, although closer examination using fluorescence microscopy revealed that the distribution was different; it was observed that whereas most free drug was located intracellularly, with the NSV most drug was located

extracellularly in association with intact NSV which were trapped in the vasculature. Particle aggregation and entrapment in the vasculature was also implied in the lung. Perhaps the most unusual finding was that the cholesterol-containing system was able to achieve higher drug levels in a model tumour (a subcutaneous sarcoma, S180) and this correlated with superior antitumour activity. Much more work is required with this promising drug delivery system.

Multiple emulsions

Oil-in-water (ow) emulsions can be given by the intravenous route as carriers of lipid-soluble anticancer drugs. More complex systems can be produced where the dispersed droplets themselves contain smaller dispersed droplets and these are known as multiple emulsions. They are prepared by the re-emulsification of a primary o/w or w/o system to yield o/w/o and w/o/w systems respectively [24].

The w/o/w systems have been the most extensively employed biomedically. They can be envisaged as slow-release formulations for water-soluble drugs, which are trapped in small particles surrounded by lipid. Table 2.3 contains a list of the cytotoxic drugs that have been administered as multiple emulsions together with their specific application. Emulsions tend to be inherently unstable, and choice of components and method of preparation are critical parameters for their effective use in anticancer drug delivery. Their potential can be illustrated with methotrexate, where a dose of 3 mg/kg administered in a w/o/w achieved a better survival rate in a leukaemic mouse model than a single dose of 80 mg/kg as an aqueous solution [24].

Targeting studies with anticancer drugs have been limited to the lymphatics after regional treatment. In these experiments 5-fluorouracil was the drug of choice and the emulsion systems were

Table 2.3 Application of multiple emulsions to cytotoxic drug delivery

Application	*Drug*
Drug targeting	Bleomycin 5-Fluorouracil
Slow-release for parenteral use	Methotrexate Cytosine Vinblastine

Adapted from reference 24.

o/w, w/o and w/o/w. Following intratesticular injection, emulsion droplets reached the regional lymph nodes after 15 min and were still present 7 days later. The w/o/w system gave the best regional targeting [24].

Microparticulate systems

Microparticulate carriers are distinguished from the more fluid vesicular carriers by their solid nature. This property has major implications for targeting strategies where chemoembolisation becomes a more important determinant and particles of greater dimensions (up to 200 μm) are considered. The major classes are defined broadly on the basis of size: nanoparticles (< 1 μm), microspheres ($1-100$ μm) and microcapsules (> 100 μm).

Microspheres

Microspheres are monolithic structures, solid throughout. They can be prepared by a variety of techniques: coacervation (or phase separation); interfacial polymerisation; and emulsification by mechanical methods (such as vortex mixing or sonication). The latter has been most widely employed, as it has the flexibility to incorporate drugs that are either hydrophilic or lipophilic. In practice, the particular method chosen is dictated by the properties of the matrix material, drug and additives to be microencapsulated, characteristics of the final product, and economics. A variety of materials can be used, including protein, polylactic acid, starch, gelatin and polyacryl starch. Eventual selection of matrix material will depend on several factors: (i) size of particles required; (ii) properties of the drug and its incorporation efficiency (i.e. drug 'payload'); (iii) surface properties of the particles (such as shape and charge); (iv) biodegradability of the microspherical system, its toxicity and antigenicity; (v) drug release rate profile desired; and (vi) stability of both the drug during formulation and particles during use and storage.

The use of microspheres as DDS is based on two major considerations: targeting and slow release of drug.

Targeting

Microspheres provide 1st order targeting due to chemoembolisation and the target organ can be specified by appropriate administration, e.g. the lung by systemic intravenous injection, the liver by intrahepatic arterial injection and the kidney by intrarenal arterial injection. More selective 2nd order targeting can be achieved by modulation of

the blood vessels supplying the target organ with vasoactive agents such as angiotensin II. It is important to note that microspheres do not readily (if at all) leave the circulation nor are they liable to internalisation into cells by transport mechanisms. Therefore, 3rd order targeting is unlikely to occur—but because of the close proximity of particles to the tumour, a high degree of uptake of locally-released drug seems a distinct possibility. Nevertheless, there has been an unconfirmed report that microspheres can migrate from the vasculature as a result of an endocytic response secondary to mechanical pressure against endothelial cells [4]. Additionally, it has been shown that in some experimental models tumour cells actually make up part of the vascular endothelial lining, therefore microspheres trapped in such vessels will be in direct contact with the tumour [25].

Slow-release of drug payload

Microspheres can be manufactured such that matrix biodegradation and the rate of drug release can be controlled. Slow-release will prevent or diminish drug leakage during transit and hence reduce systemic toxicity; sustained release at the target site will improve therapeutic efficacy. Drug release will also be fixed to a large extent by the duration the microspheres remain chemoembolised. As they probably do not leave the circulation, their clearance from the body relies on their rate of biodegradation to smaller particles which can then be handled through the RES. Biodegradation is dictated by the amount of glutaraldehyde used as a protein crosslinker to stabilise the particles, as well as type of protein, and can vary from a half-life of 24–48 hours with 1–2% glutaraldehyde to non-biodegradable systems at 3% crosslinker. Chemoembolised microspheres are eliminated by circulating macrophages and other phagocytic cells in discrete stages, so their gradual reduction in size can be monitored with time. Figure 2.1 is an electron micrograph of two partially degraded doxorubicin-loaded albumin microspheres chemoembolised in the liver of a rabbit.

For successful chemoembolisation, the all-important determinant is the blood supply to the target organ harbouring the tumour. The key questions are: (i) is there a greater density of functional blood vessels supplying tumour tissue compared to non-diseased tissue which could be exploited for more selective 2nd order targeting, and (ii) are there any qualitative differences in blood supplies, such as innervation or smooth muscle content, which could be manipulated pharmacologically? Using whole body scanning (scintigraphic) techniques, patients with primary or metastatic hepatic tumours have been studied following injection of a radiolabel into the hepatic artery in

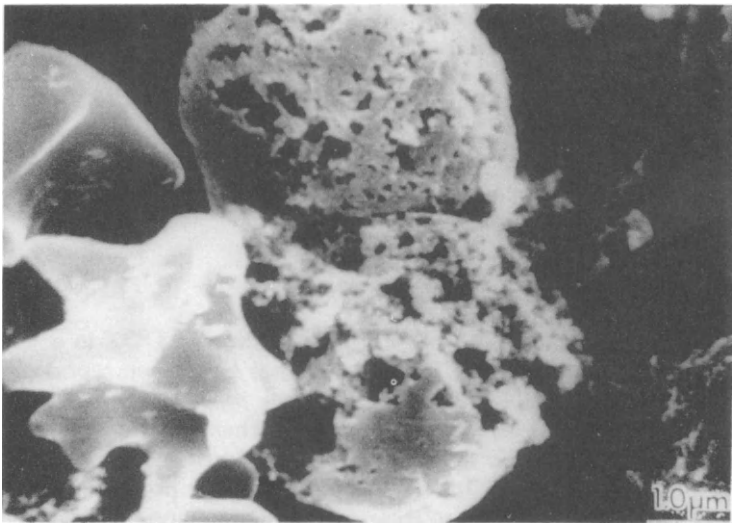

Figure 2.1 Electron micrograph of two partially degraded doxorubicin-loaded albumin microspheres chemoembolised in the liver of a rabbit. Sample was taken 5 days after intra-arterial injection. Size bar = 1·0 μm.

order to examine uptake into normal tissue versus tumour. Injection of ^{99m}Tc-labelled macroaggregated albumin together with single photon emission computerised tomography showed that in 18 patients the ratio of uptake of radioactivity into the tumour compared to uninvolved regions of the liver varied from 1·2 to 8 (median 2·7) in favour of the tumour [5]. In a separate study with ^{81m}Kr, 9 patients (14 lesions) were investigated [26]: here, tumour to normal ratios were 0·38 to 3·36 (median 1·4). Therefore, there is good clinical evidence that a high percentage of liver tumour lesions are hypervascular, and should preferentially sequester microspheres. There is now also evidence that certain animal and human tumours have vasculature which is either poorly innervated or deficient in smooth muscle; theoretically, administration of a vasoconstrictor in this situation could constrict normal blood vessels and exclude the microspheres but leave the blood vessels supplying the tumour unaltered and sufficiently patent to allow the microspheres to hyperconcentrate. Several pharmacological agents, including catecholamines, have been examined and angiotensin II has proven effective in patients with hepatic tumours.

On a cautionary note: the effectiveness of microspheres in advanced malignant disease, where the primary tumour has metastasised to many different sites in the body (which can quite often include the bone marrow, brain and lymphatics) is likely to be limited. Only certain highly defined disease types are liable to be effectively controlled by this DDS approach, such as: primary hepatoma and renal carcinoma; liver metastases of colorectal origin; and, possibly, advanced localised breast cancer.

Doxorubicin

The pharmacological rationale for incorporation of DOX in a DDS has been discussed above. Extensive studies have been reported in the literature with DOX and microspheres prepared from albumin [27]. Albumin has several advantages over other proteins as a matrix material: (i) it is readily available in purified form and is chemically and physically stable; (ii) it has excellent storage properties and is biodegradable; (iii) it has low antigenicity and immunogenicity; (iv) it is easy to conjugate with a number of drugs, and (v) it will undergo controlled denaturation by heat or chemical cross-linking to form microspheres. A typical procedure for making DOX-loaded albumin microspheres involves chemically cross-linking protein molecules in the presence of the drug using glutaraldehyde. Cross-linking occurs in the aqueous component of a water-in-oil emulsion produced by vortex mixing. The appearance of these microspheres under dark-field light microscopy is shown in Fig. 2.2: their spherical nature is obvious (as would be their red-coloured content of entrapped DOX); typical mean size distribution is 15–40 μm [32]. Drug loading is nominally 1%, but closer investigations have shown that a fraction of incorporated drug associates covalently to the protein matrix via a molecule of glutaraldehyde, and is not amenable to analysis by conventional techniques but probably remains pharmacologically active [28]. Taking this fraction into account, loading is more like 3–5% and clinical trials have shown that this factor is sufficient to be able to deliver therapeutically active drug levels to human tumours.

DOX-albumin microspheres can be lyophilised, stored for several weeks and reconstituted without alteration in pharmaceutical characteristics (size, drug content and slow-release properties) or loss of pharmacological efficacy (antitumour activity and pharmacokinetic profile) [29]. Half-life *in vivo* in the rat is 2 days for particles chemoembolised in the lung and 3·6 days for particles lodged in the liver. *In vitro*, the release of DOX from microspheres is significantly slow. All the above parameters are likely to vary when different proteins and methods of preparation are implemented [30].

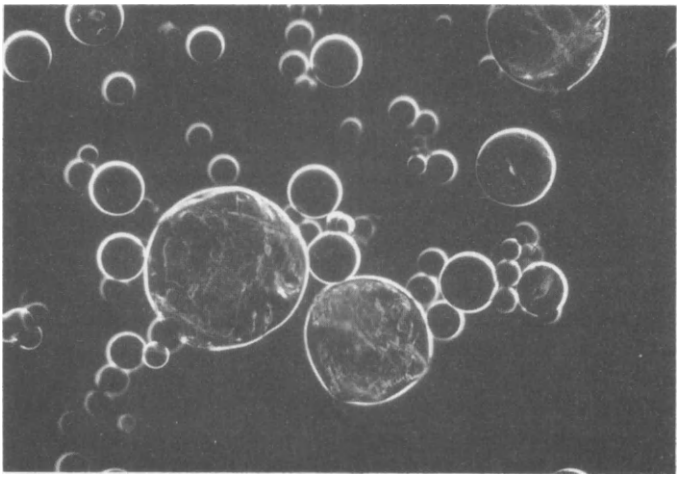

Figure 2.2 The appearance of doxorubicin-loaded albumin microspheres viewed under dark field light microscopy. Typical mean size distribution is 15–40 μm.

Targeting

Results of targeting ^{99m}Tc-labelled albumin microspheres to specific organs in the rabbit are shown in Table 2.4 [31, 32]. For the liver and kidney, the degree of 1st order targeting was greater than 90%, with few particles reaching the circulation, indicating that loaded microspheres would produce very low plasma drug levels compared to an equivalent dose of free DOX. In addition, increased drug uptake into the kidney occurred and the net result was a drug delivery advantage (therapeutic advantage) 106 times better than arterial free DOX, which in its own right is 1·5–2 times better than conventional intravenous treatment.

In human studies, labelled microspheres have accomplished 1st order targeting to the liver (Fig. 2.3, scan B) [33]. Additionally, with co-administration of a short infusion of angiotensin II (4 min, 10 μg/min) starting 100 seconds before injection of microspheres, 2nd order localisation was realised in tumour deposits (hepatic metastases of colorectal origin, scan C). The neoplastic regions were originally visualised in a separate diagnostic test where labelled tin colloid was administered intravenously to the patient (Fig. 2.3, scan A). Tin colloid is only taken up into normal tissue, and tumour cell deposits (identified by the two arrows in scan A) appear as areas of darkness. The pattern in Fig. 2.3 has been repeated in a large cohort of patients [5] including

in some cases excellent 2nd order targeting without the need to infuse angiotensin II. These outstanding results have prompted further clinical investigations.

A phase I trial (i.e. testing for safety rather than therapeutic efficacy) of drug-free albumin microspheres (15–40 μm) has now

Table 2.4 Targeting efficiency (% administered dose) of radiolabelled albumin microspheres in the rabbit after arterial injection

	Liver	*Kidney*		*Lung*	*Blood**
Right renal arterial injection	0·6	97 (right)	0·3 (left)	0·2	1·9
Hepatic arterial injection	93	1·2		5·2	0·3

Kidney data modified from reference 31. Liver data from reference 32.
* per litre.

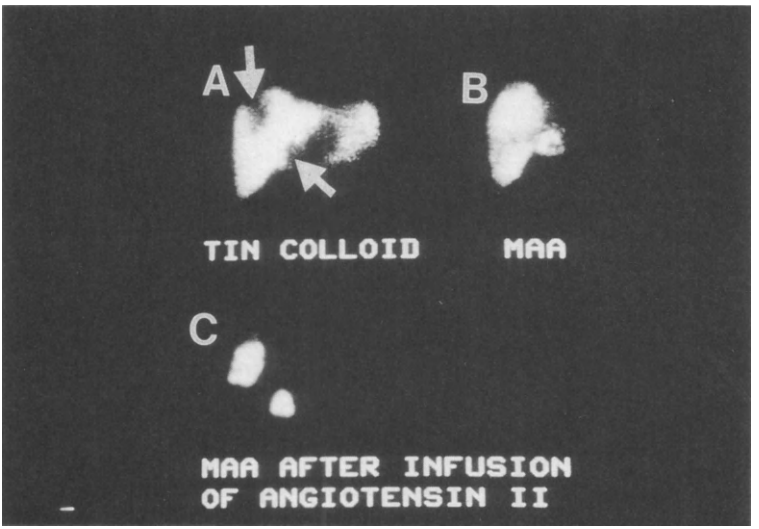

Figure 2.3 Administration of radiolabelled microspheres (MAA) to a cancer patient with colorectal cancer liver metastases. Degree of targeting to the liver was determined by whole body scintigraphy. Scan A: radiolabelled tin colloid (i.v.) scan showing the contours of the liver (left) and the spleen (right). Areas of darkness indicated by the arrows are diagnostic of tumour deposits. Scan B: radiolabelled microspheres. Chemoembolisation has occurred in both the normal liver and tumour deposits. Note that the microspheres do not reach the spleen after intrahepatic arterial injection. Scan C: microspheres plus angiotensin II. Embolised microspheres are located predominately in tumour deposits. Compare with areas of darkness in scan A.

been completed for intrahepatic arterial administration [33]. Doses were escalated from 100 mg to 350 mg in 50 mg increments. The maximum tolerated dose was 350 mg, which caused right upper quadrant pain in 4 out of 5 patients, therefore 300 mg is recommended for phase II investigations. Clinical trials of drug-loaded microspheres in localised breast cancer have been carried out (J. Cummings *et al.*, unpublished results). Here, the starting dose was 5–10 mg DOX and administration was via a major artery supplying the breast. In one patient, a dramatic tumour regression was recorded. However, severe skin necrosis was also manifest, which was nevertheless reversible. Limited pharmacokinetic assessment confirmed that high drug levels were being delivered to the neoplastic tissue, but also demonstrated that 10-fold higher levels were directed towards the normal breast. Clearly, further pharmacological manipulation is required before this treatment can be considered as standard for breast cancer. Follow-up trials in this disease were terminated due to anatomical difficulties in perfusing the breast via arterial access. However, the early work validated the view that albumin microspheres have clinical potential.

Slow-release

It is axiomatic that the higher the levels of DOX targeted to a tumour, the greater the antitumour effect; it is also now known that the longer the duration of exposure, the greater the effect. Intratumoural injection of DOX-albumin microspheres proved 5 times more active against the Sp 107 tumour in rats than an equivalent dose of free DOX administered the same way [34]. The increased drug potency was associated with two alterations in tumour drug disposition. The first was expected and was in the form of elevated and sustained drug levels. The second was less expected and was a 155-fold increase in drug biotransformation of DOX to its inactive metabolite, the 7-deoxyaglycone. These studies disclosed that the microspheres were behaving not just as passive instruments of drug delivery, but could actively modulate drug disposition. It has subsequently been shown that this property of initiating drug metabolism via specific pathways (anaerobic quinone reduction) is detrimental to the overall antitumour activity of DOX. Whereas the microspheres increased efficacy 5-fold, this value could have been much higher without the unwanted quinone reduction. However, the potential for exploiting this attribute in a positive manner remains.

Doxorubicin has been encapsulated in many other forms of microspherical system, two notable examples being: (i) poly (L-lactic acid) microspheres displaying slow-release characteristics (6·3 days for 50% drug recovery) have been administered locally to patients in the

treatment of malignant pleural effusions with some success [35]; (ii) immobilisation of DOX covalently attached to agarose beads or polyglutaraldehyde microspheres appears to confer new properties on the drug [36]. When cancer cells were exposed to these particles, which are far too large to be internalised, cytotoxicity still occurred, indicating that DOX does not need to enter the cell to be active. This finding has implications for *in vivo* delivery, suggesting that 3rd order targeting of microspheres may not even be necessary.

Mitomycin C

Initial clinical experience with mitomycin C revealed a drug with a broad spectrum of anticancer activity but which was profoundly toxic to the bone marrow. This finding discouraged further trials until it was shown that intermittent high dose therapy with a 4–6 week interval reduced toxicity without affecting activity. Due to its low therapeutic index, mitomycin C is an ideal candidate for selective drug delivery approaches and indeed a large number of systems have been investigated.

Mitomycin C, whilst being comparatively stable chemically in the solid state and at neutral pH, does contain three potentially labile chemical groups: a quinone moiety, a fused aziridine ring and a carbamate function, and these are readily activated, resulting in the drug forming covalent adducts with a variety of nucleophilic species as well as undergoing hydrolysis. Although these properties are important for the biological activity of the drug, DNA-covalent adducts being implicated strongly in its mechanism of action, production of microspherical systems can be problematic due to significant drug loss. In a study of methods of microsphere production, matrix material and drug degradation [37], albumin microspheres prepared by heat denaturation resulted in 37% drug loss whereas microspheres prepared by chemical cross-linking with 0·5 M biacetyl resulted in complete degradation of encapsulated drug. Preparation of polylactic acid microspheres by emulsification at 55°C resulted in no apparent breakdown of mitomycin C.

Preclinical studies with mitomycin C-loaded albumin microspheres have been reported [38]. Here, average particle size was 45 μm and drug entrapment efficiency was 5%, although over 3 days only 20% of drug was released, suggesting degradation and covalent binding. Particles were infused into the femoral artery of rabbits with a VX-2 tumour implanted into the flank of the hindleg, or into the hepatic artery of rats. In the case of the rabbit, high drug levels were maintained in the tumour for several hours and the microspheres produced a much enhanced antitumour activity. Sustained release of

high drug levels into the rat liver were recorded. Microspheres of mitomycin C are, however, still at an early stage of development.

Microcapsules

One of the first vehicles used for chemoembolisation was microcapsules prepared from materials such as ethylcellulose. Microcapsules are essentially vesicular structures in which a membrane encloses drug, and the physical process underlying their formation (coacervation) produces particles over 100 μm in diameter. As a consequence of their consistency (which is distinct from the solid nature of microspheres), high drug entrapment efficiencies have been reported (over 80%) but drug release can be complete after only 1 or 2 hours [39]. Together with drug targeting and slow drug release, microcapsules are believed to exert their influence by infarction also. Although ethylcellulose is the wall material most widely studied, microcapsules have also been made from albumin and polylactic acid.

Mitomycin C

Several centres have now studied mitomycin C in ethylcellulose microcapsules, essentially as formulated originally by Kato and colleagues [40]. These studies include clinical investigations and pharmacokinetic trials. The microcapsules were prepared by coacervation and the resultant product had irregular morphology, an average particle size of 224 μm, and contained 80% mitomycin C : 20% drug carrier by weight. *In vitro* release was delayed compared to free drug but still must be considered as relatively fast, where with slight mechanical agitation almost 100% recovery of drug occurred after only 2 hours.

Favourable results have been observed with microcapsules containing 0·5 mg/kg mitomycin C when they were infused into the hepatic artery of patients with unresectable (incurable) hepatocellular carcinoma in cirrhosis [41]; both an increase in quality of life and patient survival rate occurred. Clinical pharmacokinetic studies, again with liver perfusion of a total dose of 20 mg mitomycin C, showed that peak plasma levels were approximately 10-fold lower after the microcapsules [39]. However, in a more detailed clinical study it came to light that clumping occurred in the saline injection vehicle prior to administration, despite vigorous shaking, and that considerable force was frequently required to deliver them through the catheter [42]. Additionally, serious problems with local toxicity were encountered in the form of pancreatitis and gastroduodenal ulceration. It was

concluded that microcapsules of a smaller size (or a new formulation) were required for further progress.

Cisplatin

A similar pharmacokinetic modulation to that observed above with mitomycin C (approximately 10-fold lowering of systemic blood levels) has been noted in patients when microencapsulated cisplatin was infused into the maxillary artery [43]. A reduction of over 50% in the diameters of measurable tumours (carcinoma of the maxillary sinus or oral cavity) was seen in 9 out of 14 subjects treated with the microcapsules. Significantly, no renal toxicity was reported but there was again an indication of high local toxicity in the form of pain. The future of cisplatin microcapsules remains unclear.

Nanoparticles

Although submicron diameters can be achieved with particles made from phospholipid and protein, the term 'nanoparticle' is normally applied to systems produced from poly(alkyl 2-cyanoacrylate). Nanoparticles are formed by anionic polymerisation of an alkyl (methyl, ethyl, propyl and butyl) 2-cyanoacrylate monomer which is added to a rapidly stirred aqueous solution (at pH 2–4) containing a stabiliser such as dextran or a non-ionic surfactant. The resultant particles can vary greatly in size throughout a normal range of 30–500 nm, but the eventual diameter will depend on many factors including stirring rate, monomer concentration, pH, temperature, electrolyte and preferred stabiliser and can be as large as 3000 nm [44]. Nanoparticles have been shown to be discrete spheres with a smooth surface and a highly porous interior; they carry a net negative surface charge but are more or less hydrophobic depending on the alkyl moiety chosen. Drug can be loaded either by incorporation during formation or by adsorption once the particles are isolated, although the latter tends to be preferred since addition of drug may interfere with the polymerisation process. Nanoparticles will have a preference to adsorb hydrophilic un-ionised molecules and this will increase with increasing length of the alkyl chain. However, enhanced adsorption of ionised compounds can be achieved by covalent attachment of charged dextrans to particle matrix. Typical loading capacities for two different anticancer drugs on a weight basis are 0·1% for actinomycin D and 1–10% for daunorubicin. Although drug release has been proposed to mirror particle degradation and is therefore controllable, recent *in vivo* data incorporating radionuclides suggest that their highly porous nature leads to significant leakage of drug.

Biodegradation of nanoparticles is complex and may occur by chemical hydrolysis and by enzyme catalysed pathways; it can result in the release into the body of toxic species such as formaldehyde and alkyl 2-cyanoacetates. A reduction in toxicity may be achieved by changing the alkyl group from methyl to isobutyl.

In vivo, nanoparticles tend to be highly sequestered by the reticuloendothelial system, especially Kupffer cells in the liver, but this can be attenuated by the choice of alkyl moiety. Incorporation of actinomycin D in methyl cyanoacrylate nanospheres reduced uptake into the liver and spleen but resulted in greater localisation in the lung and small intestine. These particles also caused a considerable decrease in the growth of a rat soft tissue sarcoma [45]. Doxorubicin nanoparticles produced by adsorption have reduced cardiotoxicity. Overall, nanoparticles seem to increase antitumour activity and toxic side effects.

Soluble macromolecular carriers

The soluble macromolecule as a drug carrier is based on two principles: (i) alteration of pharmacokinetics due to the complex avoiding filtration by the kidney; and (ii) uptake into cells predominately by pinocytosis. In the absence of a homing molecule (such as an antibody or lectin), selective targeting to a tumour is based solely on rate of endocytosis. Drugs usually have to be covalently attached to the macromolecular species, and thus require a mechanism for selective decoupling which is robust enough to survive transport through the cardiovascular system, but is sufficiently labile to allow release into the cytoplasm of target cells. Since macromolecules are normally taken up by endocytosis, this means the link will have to be cleaved enzymatically (or chemically) in lysosomes. Such complexes are referred to as lysosomotropic agents. A large number of different macromolecules have been investigated but perhaps the most versatile are the synthetic polymers as, with these, a number of desirable properties can be engineered into the system. Low drug capacity of soluble macromolecules compared to vesicular or particulate DDS militates against this system.

Synthetic copolymers

The use of synthetic copolymers has been extensively reviewed [7], including chemical aspects (which will not be covered here). The review reports on N-(2-hydroxypropyl) methacrylamide copolymers (HPMA) with the anthracyclines DOX and daunorubicin. Drugs were

conjugated to the HPMA copolymer via a small peptide spacer molecule, and particular attention was paid to the amino acid sequence. The tetrapeptide link of Gyl–Phe–Leu–Gly (a preferred substrate for lysosomal cathepsins) permitted cleavage of the anthracycline from the carrier following internalisation, promoting effective antitumour activity, whereas the non-lysosomally-degradable spacer Gly–Gly produced a conjugate that was inactive *in vivo*. Such anthracycline conjugates were shown to retain antitumour activity against a panel of animal tumours *in vivo* including L1210 murine leukaemia, the rat Walker sarcoma and murine B16 melanoma, and exhibited reduced toxicity [46]. Approximately 10-fold more DOX could be administered to animals using HPMA without overt signs of toxicity [47].

Miscellaneous

Examples of other macromolecular molecules that have been tested as cytotoxic drug carriers include: mitomycin C covalently attached to dextran with different surface charge and mitomycin C adsorbed onto activated carbon particles; the anthracyclines DOX and daunorubicin with DNA, poly(carboxylic acid) polymers, polyvinyl alcohol and low density lipoprotein (LDL); and methotrexate with LDL. These systems have met with variable results.

Summary

The 1980s saw an intensification of the search for the elusive 'magic bullet' (a term first coined by Paul Ehrlich in 1906) and a burgeoning of different drug delivery systems to target cytotoxics to tumour cells. Although a vast literature has been produced evaluating these new systems, little if any significant impact has been made on the clinical outcome of the disease they were meant to cure. In the 1990s selective delivery of anticancer drugs will probably find itself at an impasse. Microparticulate carriers of a size suitable for chemoembolisation (i.e. $> 10\ \mu m$ diameter) are capable of a high degree of tumour-specific targeting but are also toxic to local normal tissue and are unsuitable for disseminated disease. Vesicular systems, nanoparticles and soluble carriers avoid the problem of local toxicity but still tend to be sequestered by phagocytic cells of the reticuloendothelial system, predominately located in the liver, spleen and bone marrow. Whether this impasse will be overcome by further optimisation of existing systems or will await the development of new

systems is a matter of conjecture. What is clear is that resolution is bound to require the identification of recognition determinants which reside only in the transformed cancer cell.

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Chapter 3

Drug interactions with cytotoxic agents

J. A. Green and I. H. Stockley

Cytotoxic drugs are now widely used in the management of many types of cancer. In many cases, further medication is given to alleviate side-effects, or to reduce toxicity, and may include anti-emetics, antibiotics or laxatives administered either therapeutically or prophylactically. In tumour types including leukaemias, high-grade non-Hodgkin's lymphoma, testicular cancer, small-cell lung cancer and ovarian cancer, these drugs are frequently given in combination with the intention of achieving cure or significant gain in survival. As many as six drugs may be employed in alternating sequences in intensive treatment protocols in an attempt to minimise the emergence of drug resistance. As a result, the possibilities for interactions between the cytotoxic drugs used in the protocol are considerable, and over the last 10 years there has been a greater understanding of the underlying mechanisms, in some cases allowing these effects to be used to therapeutic advantage. Interactions between cytotoxic agents and other types of drugs are also possible, particularly in the palliative context, in patients who may be receiving a range of medications prescribed for symptom control or other medical conditions.

Irregular compliance with cytotoxic agents or other medications used in the management of cancer is no less common than in other therapeutic areas, and may complicate the clinical picture and impair precise diagnosis of a suspected interaction. Detailed study of the pharmacokinetics and pharmacodynamics of interactions is precluded by the toxicities of the cytotoxics, and as a result much of the data that are available come from animal studies. Definite predictions are therefore rare, but prescribers should be made aware of the possibilities of these interactions.

Cytotoxic drugs can be arranged into broad categories according to their mechanisms of action (such as alkylating agents, anti-metabolites and biological response modifiers), but the precise way in which these drugs exert their cytotoxic action is far from clear. For example, mitomycin C has been shown to have both alkylating

activity and the potential to cause direct free radical damage to the DNA backbone. Similarly, the nitrosoureas have both alkylating and carbamoylating activities. In the case of the interferons, it is still not clear whether the immune modulation or antiproliferation properties account for the tumour responses observed. Alkylating agents may form adducts with both DNA and protein: the former give rise to their anticancer effect, while the latter may contribute to their toxicity profile. Furthermore, the precise route of metabolism or the activity of the metabolites produced is not well established for the majority of the 40 or so cytotoxic drugs currently licensed in the UK. It is therefore difficult to predict with accuracy what effect a potential interaction will have: there may be enhancement of the effect of one or other drug, the development of totally new effects, the inhibition of the effect of one drug by another, or even the absence of a demonstrable effect in spite of gross changes in kinetics or metabolism.

The clinical importance of many reported cytotoxic interactions is hard to define; it does, however, make sense to avoid a potentially hazardous interaction. In this chapter an attempt is made to illustrate certain potentially serious interactions and to highlight those few interactions which have potential utility, before describing the majority of those known according to their estimated clinical importance. The use of cytotoxic agents as a class generally involves a long interval between administration and a therapeutic effect; the consequence of an interaction may be seen in relation to acute side-effects, or in therapeutic activity, or both. However, in the latter case an interaction may either be wrongly associated with therapeutic failure, or attributed to a tumour-related effect. Interactions between cytotoxics as well as those with other non-antineoplastics are considered in this chapter.

Drug interaction mechanisms

A drug interaction exists when the effects of one drug are altered by prior or simultaneous use of another drug; therefore, the effect may be beneficial or adverse. In clinical oncology both additive and antagonistic effects are described, while synergy is more frequently an *in vitro* observation than of proven clinical relevance.

Five different types of interactions are considered: (i) gastrointestinal absorption; (ii) drug distribution; (iii) drug metabolism; (iv) renal excretion; and (v) target site interaction. It may be possible to generalise between drugs of a given class for some interactions, but this principle cannot be universally applied to heterogeneous groups such as antimetabolites and antibiotics.

Gastrointestinal factors

Absorption of drugs from the gastrointestinal tract can be affected by altered binding or chelation as well as by changes in gastrointestinal motility. Metoclopramide is widely used as an antiemetic for both orally and parenterally administered cytotoxics, but for the commonly administered oral drugs (which include melphalan, busulphan, cyclophosphamide, chlorambucil, and the antimetabolites methotrexate and 5-fluorouracil), this effect on gut motility is unlikely to be of significance, as most are given continuously for up to 14 days. Unless there is a significant reduction in the extent of absorption, as opposed merely to the rate, the clinical effect will be minimal. A recent study has reported that metoclopramide *increased* the bioavailability of TCNU (tauromustine), a new orally administered nitrosourea [1]. The effects of gastric mucosal damage on the absorption of oral drugs should be borne in mind, one example of which is the reduction in serum digoxin levels, which has been shown to occur with the concurrent use of combination chemotherapy [2]. It is frequently important to maintain contraception in young female patients receiving chemotherapy: although the effects on absorption of the oral contraceptives are not known, it makes sense to avoid 'low dose' steroidal preparations.

Distribution

Many cytotoxic drugs, including cisplatinum, methotrexate and etoposide, are highly protein-bound, and distribution effects may be seen where there is competition for plasma protein binding sites, as the active drug is generally the free form. Oral anticoagulants, hypoglycaemics, salicylates, phenytoin and tetracyclines are all protein-bound, and may displace cytotoxics from albumin. In many cases of advanced malignancy, the serum albumin concentration may be low, and may also change during successive cycles of chemotherapy.

As the doses of drugs are initially empirically based on surface area (or weight in children), but subsequently modified in the light of acute toxicity, distribution effects giving rise to increased toxicity should become 'titrated out' with dose reductions in successive cycles in most patients. In the case of neurotoxicity attributed to ifosfamide, the serum albumin level has been shown to be one of the main risk factors along with renal function in predicting this serious effect [3], which has in some cases been associated with WHO grade III or IV toxicity in the first cycle. One study has suggested that the surfactant polysorbate 80 (Tween 80), which is included in the formulation of etoposide, may reduce the area under the concentration/time curve

(AUC) of doxorubicin [4]. While displacement from tissue binding sites could, in theory, alter effective drug levels, most cytotoxics are fairly tightly or irreversibly bound, and this effect should be slight.

Drug metabolism

Induction of hepatic microsomal drug metabolising enzymes is now a well recognised phenomenon [5], and may be an important factor with anticonvulsants given for cerebral metastases, or steroids given concurrently for a number of indications. Both phenytoin and rifampicin have been shown to lower the AUC of etoposide. The full effect of induction may take 7–10 days to become apparent and the decline following cessation of the inducing agent may be similarly protracted. In the same class of interactions are the effects of allopurinol, which competes for the catabolic site of xanthine oxidase with 6-mercaptopurine, which is detoxified to 6-thiouric acid. Concurrent therapy with allopurinol will have the effect of increasing plasma levels of 6-mercaptopurine while delaying excretion, giving rise to increased toxicity [6]. A reduction of 30–50% in the usual dose of mercaptopurine is required when both drugs are given simultaneously, although in practice because the cytotoxic agent is introduced after the tumour induction phase and used as a maintenance drug, the allopurinol may often be discontinued at this stage. Azathioprine is chemically related to 6-mercaptopurine and similar effects may be observed.

Cimetidine, which may be given for steroid-induced dyspepsia, is an enzyme inhibitor which has a potential for clinical interactions with cytotoxics, and has been shown to increase the bone marrow toxicity of cyclophosphamide and reduce the AUC of 5-fluorouracil [7]. On account of its monoamine oxidase inhibitory activity, procarbazine should be used cautiously with alcohol or high amine foods [8]. The drug may also interfere with alcohol detoxifying enzymes and give rise to headache and flushing [9]. The clinical significance of the enzyme-inhibition effects of sulphonamide is less clear.

Renal excretion

Alterations in urinary pH may affect the excretion of drugs that are weak acids or bases, as a result of which their lipid solubility and consequent tubular reabsorption is reduced. Some non-steroidal anti-inflammatory drugs (NSAID) may reduce the renal excretion rate of methotrexate as a result of inhibition of renal prostaglandin synthesis [10]. This is an increasingly important effect, as NSAIDs are widely

used for pain control in cancer patients, as well as in patients with coexistent rheumatoid arthritis. High dose cisplatin ($> 120\ mg/m^2$) may be associated with acute nephrotoxicity, and this complication may be minimised by the concomitant infusion of hypertonic saline [11].

Target-site interactions

As the mechanisms of action of all the cytotoxic drugs are far from clear, and the selection of combination therapy may be in part empirical, pharmacodynamic interactions (where two similar drugs compete for activity at the target site such as a membrane receptor or DNA nucleotide) are likely to be common. The sensitivity of normal tissue, for example bone marrow, may be the limiting factor in these cases, and is so common that it is often not considered an interaction in the strict sense. With higher doses made possible by bone marrow transplantation or haematopoietic colony stimulating factors, kidney, gut or nerve tissue sensitivity may become the limiting factor. In these cases it is the 'receptor site' in the normal tissue that is being targeted by the competing pharmacologic attack. In the last 5 years, several attempts have been made to turn these interactions into therapeutic benefit, the basis of the modulation strategy being a differential relative concentration between normal and tumour tissue.

Examples of interactions with specific cytotoxics

The three drugs whose pharmacology has been most widely studied are cyclophosphamide, methotrexate and 5-fluorouracil, and they will be used as examples to illustrate common mechanisms. Compared with β-blockers or antibiotics, cytotoxic drugs are much less widely prescribed, and the duration of exposure is generally shorter, but the possibility of a previously unrecognised interaction should always be considered, particularly in the case of the newer agents, and may be enhanced by the coexistence of renal or hepatic dysfunction.

Cyclophosphamide

Cyclophosphamide is an alkylating agent which requires activation by the liver microsomes to 4-OH cyclophosphamide and aldophosphamide. The alkylating moiety is phosphoramide mustard, which is a breakdown product of 4-OH cyclophosphamide. There is great variation in microsomal metabolising capability between animal species,

and as a result there is conflicting evidence about the net effect of the interaction between cyclophosphamide and steroids or phenobarbitone, for example. Pretreatment with phenobarbitone accelerates the production of the alkylating metabolites and, as a result, there is rapid depletion of the parent compound and metabolites. Brain tumour patients with chronic phenobarbitone exposure show short plasma half-lives of the order of 2 hours [12]. Peak plasma levels for the active metabolites may be increased, but in man the *total concentration* × *time* product of alkylating levels for a given cyclophosphamide dose may not be altered sufficiently to change the toxicity or therapeutic effect [13]. Table 3.1 lists those compounds which have been shown to alter the plasma levels of cyclophosphamide [14].

The predicted effects of prednisolone on cyclophosphamide activation could be an immediate inhibitory effect on the $NADPH_2$-dependent activation system as a result of competition for prednisolone hydroxylation and cyclophosphamide activation, and a more delayed effect of increased activation due to microsomal enzyme induction [15]. Therefore, the dose and timing of corticosteroid administration may affect the resultant cyclophosphamide toxicity. Steroids and cyclophosphamide are widely used in combination chemotherapy protocols (e.g. with vincristine in lymphoma or methotrexate and 5-fluorouracil in breast cancer). In addition to any possible lympholytic effect, they may improve the patients' sense of well-being as well as appetite. More recently dexamethasone has been found to be an effective antiemetic, but it is not known whether this drug may have an effect on the anticancer activity of the cytotoxic agents.

Haemorrhagic cystitis is a rare complication of cyclophosphamide therapy with an incidence around 1%, and is attributed to the acrolein

Table 3.1 Drugs which may interact with cyclophosphamide to alter its blood plasma levels

Increased plasma levels	*Decreased plasma levels*
Phenobarbitone	Phenobarbitone
Prednisolone	Chloramphenicol
Triiodothyronine	Apomorphine
Chlorpromazine	Corticosteroids
Chloroquine	Nicotine
	Atropine
	Ephedrine
	Cocaine
	Dapsone

Modified from reference 14.

metabolite. With the analogue ifosfamide, this toxicity occurs in about 50% of cases, but can be almost entirely prevented with the use of the thiol 2-mercaptoethane sulphonate (mesna), which is excreted by the kidney and irreversibly binds the acrolein without interfering with the cytotoxic activity of the drug [16]. This form of selective detoxification is an example of antagonism being used for therapeutic effect, but does not apply to the majority of alkylating agents, which do not all require hepatic activation. Bone marrow depression remains the product of the dose-limiting toxicity of cyclophosphamide, but cumulative damage is rarely seen owing to high levels of the enzyme aldehyde dehydrogenase in the haematopoietic stem cells. As a result, cyclophosphamide may prove a useful drug to be used in combination with the haematopoietic colony stimulating factors (CSFs) such as G-CSF (granulocyte) or GM-CSF (granulocyte macrophage). Thiol compounds including glutathione may form covalent interactions with cyclophosphamide (and melphalan) in enzyme-catalysed reactions, and this is one area where modulation strategies are currently being explored. Furthermore, the alkylating agents may themselves inhibit hepatic microsomal enzymes and affect the activation or degradation of other drugs by this mechanism [17].

Methotrexate and 5-fluorouracil

Methotrexate is an antimetabolite which blocks purine and pyrimidine synthesis, primarily by inhibition of the enzyme dihydrofolate reductase. With the use of the drug antagonist folinic acid, the enzyme block can be bypassed, and this has been employed therapeutically to allow administration of a higher dose of methotrexate followed by folinic acid 'rescue' 24 hours later. However, in practice, the areas where this therapeutic manoeuvre has been found clinically beneficial have proven limited [18], and intermediate dose methotrexate with rescue is now routinely employed only in the leukaemias and lymphomas. This observation may be explained by the fact that methotrexate polyglutamates, the active intracellular form of the drug, are increased by a factor of only two, even with a several hundred-fold increase in dose [19].

In conventional doses, methotrexate may be given orally or intravenously. Antibiotics may destroy bacterial flora which metabolise methotrexate in the gastrointestinal tract, but the clinical effect of concomitant parenteral administration is unclear. Impairment of glomerular filtration will increase the toxicity and antitumour effect of methotrexate, as will organic acids such as salicylates and some antibiotics which affect tubular reabsorption. The use of non-steroidal

anti-inflammatory drugs has recently been associated with severe methotrexate toxicity [20]. There was marked prolongation of methotrexate half-life, ascribed to decreased renal elimination secondary to inhibition of renal prostaglandin synthesis. Where high dose methotrexate is being administered, it is now common practice to alkalinise the urine, which should minimise this potential interaction, as well as prevent drug crystallisation in the tubules.

Methotrexate is protein-bound, and altered therapeutic effect and changes in toxicity might be expected to occur when other protein-bound drugs are administered. Barbiturates, tranquillisers, phenytoin and sulphonamides may be expected to potentiate methotrexate in this way. As methotrexate is taken into cells by a carrier-mediated process, uptake may be reduced by other folates, or organic anions such as antibiotics, probenecid or hydrocortisone [21]. Allopurinol inhibits xanthine oxidase and as a result may reverse the antitumour effect of methotrexate by reducing catabolism and thus increasing metabolite accumulation within the cell [22]. Elevated plasma concentrations of purines and thymidine, possibly produced from tumour lysis, may serve to ameliorate toxicity to tumour or host tissues.

The potential interaction between methotrexate and the antifolate antibiotics has long been suspected, but there have been recent reports of severe bone marrow depression [23]. This may be particularly important in the haematological malignancies, where intensive treatment protocols with high or intermediate dose methotrexate are given with co-trimoxazole prophylaxis. An additional factor may be displacement of methotrexate from protein binding sites by the sulphonamide [24]. Asparaginase may antagonise the cytotoxic effect of methotrexate by preventing progression into the S-phase of the cell cycle.

The interaction of methotrexate with 5-fluorouracil has also been explored, based on the rationale that methotrexate pretreatment may deplete reduced folate pools while 5-fluorouracil inhibits the enzyme thymidylate synthetase, and synergy has been clearly demonstrated *in vitro* [25]. Clinical trials have, however, failed to prove any more than additive effects from alterations in sequence or schedule of these two compounds. The use of high dose folinic acid to enhance the therapeutic efficacy of 5-fluorouracil has also recently been evaluated, based on the rationale that prolonged inhibition of thymidylate synthetase could be achieved by increasing the levels of the co-factor 5,10-methylenetetrahydrofolate, a metabolite of folinic acid. Unfortunately, these attempts at biochemical modulation by manipulation of intermediary metabolism have been largely unsuccessful in the clinic [26]. However, one recent study has suggested improved survival in colorectal carcinoma with the combination of low dose folinic acid

and 5-fluorouracil [27].

Table 3.2 summarises potential drug interactions with methotrexate.

Summary and conclusions

As our knowledge of the mechanism of action of drugs has increased over the last few years, an awareness has developed that the effects of drugs given concurrently may not be predictable from their known effects when given alone. Conversely, theoretical pharmacokinetic and metabolic changes may not translate to a clinically demonstrable effect. Cell line and animal models have severe limitations in the case of the anticancer drugs, and most of the clinical reports are based on single cases or small series. However, interactions with the anticancer drugs are common (Tables 3.3 and 3.4), and the purpose of this review has been to provide a framework for their analysis.

There are multiple types of interaction and, given the age, toxicity and variety of drug prescription in these patients, they are frequent

Table 3.2. Potential interactions with methotrexate

Drug	*Effect on methotrexate*	*Mechanism*
Folinic acid	Reversal	Bypasses enzyme block
Neomycin	Decreases absorption	Destroys gut bacteria
		Direct mucosal damage
Co-trimoxazole	Bone marrow depression	Folate depletion
Sulphonamides	Alters plasma level	Displaces from proteins
Salicylates	Alters plasma level	Decreases renal clearance
		Displaces from proteins
NSAID	Prolongs half-life	Diminished renal excretion
Barbiturates	Alters plasma level	Displaces from proteins
Tranquillisers	Alters plasma level	Displaces from proteins
Phenytoin	Alters plasma level	Displaces from proteins
	Enhanced toxicity	Folate depletion
Corticosteroids	Decreases intracellular level	Inhibits cellular uptake
Penicillin	Decreases intracellular level	Inhibits cellular uptake
	Increases intracellular level	Reduced tubular secretion
Triamterene	'Pseudo-resistance'	Increases DHFR level
Allopurinol	Decreases antitumour effect	Increases intracellular purine level
Probenecid	Increases plasma level	Reduced renal excretion

DHFR, dihydrofolate reductase.
Modified from reference 14.
Note: few of these interactions have been demonstrated in man.

Table 3.3 Potentially hazardous interactions between cytotoxic drugs and other agents

Cytotoxic	*Other agent*	*Effect*
All agents	Vaccines	Reduced immune response
	Live vaccines	Generalised infection
Aminoglutethimide	Anticoagulant	Increased anticoagulant requirement
Bleomycin	Oxygen above 30%	Pulmonary fibrosis
Cisplatin	Aminoglycosides	Enhanced ototoxicity and nephrotoxicity
Hexamethylmelamine	Antidepressants	Hypotension
Methotrexate	Salicylates	Protein-binding displacement
	Co-trimoxazole	Myelosuppression
	NSAID	Enhanced toxicity
Procarbazine	Alcohol	Flushing headache
	High-tyramine foods	Monoamine oxidase inhibitory effect

NSAID, non-steroidal anti-inflammatory drugs.

but rarely significant. They may be both detrimental and beneficial; in the former case, this could be by massive increase in the effective dose leading to severe or life-threatening toxicity, or severe underdosing which may be initially welcomed by the patient as relative freedom from the expected side-effects such as emesis or alopecia, yet later lead to sub-optimal control of the cancer. One major problem in the optimisation of cytotoxic chemotherapy is the long time interval between administration and anticancer effect, yet the dose is limited by acute toxicity, principally myelosuppression. With intermittent administration, whether every 7, 14, 21 or 28 days, doses may be adjusted progressively downwards based on acute toxicity, but rarely upwards to compensate for drug antagonism.

Beneficial interactions, such as folinic acid rescue with methotrexate, or mesna uroprotection for ifosfamide, form the basis of many modulation strategies currently being developed. Increasing the selectivity of these protection approaches to concentrate on bone marrow, liver or gastrointestinal tract is currently the subject of much research. A related problem is that certain tissues, including tumours, possess or acquire a drug efflux mechanism to remove doxorubicin from cells, and agents such as verapamil or nifedipine are under investigation as possible poisons of this pump [28]. Of the interactions with other non-cytotoxic drugs, many of these are common to other areas of clinical practice (such as the protein-binding effects of warfarin or the non-steroidal anti-inflammatory agents, and the

Table 3.4. Other significant interactions between cytotoxic drugs and other agents

Cytotoxic	*Other agent*	*Effect*	*Source*		*Strength of association*
Actinomycin	Doxorubicin	Cardiotoxicity		M	2
Aminoglutethimide	Diuretic	Hyponatraemia		M	1
	Digoxin	Increased clearance		M	2
Asparaginase	Hypoglycaemic agents	Glucose tolerance impaired		M	2
Azathioprine (see mercaptopurine)					
Bleomycin	Cisplatin	Enhanced pulmonary toxicity		M	1
	All cytotoxics	Pulmonary toxicity		M	1
Carmustine (BCNU)	Cimetidine	Myelosuppression		M	1
	Metronidazole	Myelosuppression		M	1
	Barbiturates	Reduced efficacy	A		1
Chlorambucil	Phenylbutazone	Myelosuppression	A		1
Cisplatin	Methotrexate	Nephrotoxicity		M	2
Cyclophosphamide	Allopurinol	Myelosuppression		M	2
	Barbiturates	Enhanced toxicity	A	M	2
		Minimal effect		M	1
	Benzodiazepines	Enhanced toxicity	A		1
	Chloramphenicol	Reduced toxicity	A	M	1
	Chloroquine	Enhanced toxicity	A		
	Cimetidine	Enhanced toxicity	A		1
	Corticosteroids	Inhibit activation	A		1
	Dapsone	Reduced cytotoxicity		M	1
	Hypoglycaemic agents	Increased hypoglycaemia		M	1
	Metronidazole	Enhanced toxicity		M	1
	Morphine	Enhanced toxicity		M	1
	Suxamethonium	Pseudocholinesterase	A	M	1
	Warfarin	Lowered prothrombin time		M	1
Cytosine arabinoside	Methotrexate	Antagonism	A		1
	Alkylating agents	Repair inhibition	A		1

	Cisplatinum	Repair inhibition	A		1
	Etoposide	Increased DNA stromal breaks	A		1
Doxorubicin	Aminoglycosides	Inhibition	A		1
	Barbiturates	Increased clearance		M	1
	Mercaptopurine	Hepatotoxicity		M	2
	Propranolol	Cardiotoxicity	A		1
Etoposide	Warfarin	Bleeding		M	1
	Phenytoin	Reduced etoposide activity		M	1
	Verapamil	Increased accumulation	A		1
	Methotrexate	Slower polyglutamate efflux	A		1
Fluorouracil	Allopurinol	Inhibition		M	1
	Aminoglycosides	Delayed absorption		M	1
	Cimetidine	Increased levels		M	1
	Methotrexate	Synergism	A		2
				M	2
Hydroxyurea	CNS depressants	Drowsiness		M	1
	Cytosine arabinoside	Increased DNA incorporation	A		1
Ifosfamide	Cisplatin	Nephrotoxicity		M	2
Lomustine (CCNU)	Theophylline	Myelosuppression		M	1
Mercaptopurine	Allopurinol	Enhanced toxicity		M	2
	Co-trimoxazole	Enhanced toxicity		M	1
Methotrexate	Alcohol	Hepatotoxicity		M	1
	Retinoids	Hepatotoxicity		M	1
	Aminoglycosides	Reduced GI absorption		M	1
	Cholestyramine	Reduced GI absorption		M	1
	Barbiturates	Alopecia	A		1
	Cephalothin	Leucopenia		M	1
	Milk	Reduced GI absorption		M	1
	NSAID	Renal clearance		M	2
	Penicillin	Mucositis		M	1
	Probenecid	Elevated serum levels		M	1
	Tetracycline	Mucositis		M	1
	Fluorouracil	Synergism			

Table 3.4. (*continued*)

Cytotoxic	*Other agent*	*Effect*	*Source*		*Strength of association*
Mitomycin C	Doxorubicin	Cardiotoxicity		M	1
	Chlorozotocin	Pulmonary fibrosis		M	1
Mustine	Morphine	Increased cytoxicity	A		1
	Procarbazine	CNS toxicity		M	1
Procarbazine	Antihypertensives	Hypotension		M	1
	Sedatives	Drowsiness		M	1
Streptozotocin	Phenytoin	Reduced cytotoxicity		M	1
Tamoxifen	Allopurinol	Hepatotoxicity		M	1
Thiotepa	Muscle relaxants	Enhanced effect		M	1
Vinca alkaloids	Isoniazid	Neurotoxicity		M	1
	Mitomycin C	Bronchospasm		M	1

NSAID, non-steroidal anti-inflammatory drugs; GI, gastrointestinal; A, animal or *in vitro* experiment; M, data from man.
Strength of association: 1, slight or anecdotal; 2, multiple reports.

enzyme-inducing agents, including phenytoin) but an understandable focus on the tumour and the anticancer drugs with their anticipated severe effects may lead many interactions to go unrecognised. The precise effects of steroids are not yet clear, nor are the effects of cytotoxic drugs on the general drug detoxification mechanisms such as p450 cytochromes, glutathione transferase or UDP glucuronyl-transferase. Scheduling of drugs with the development of infusion pumps and integration with other modalities such as radiation therapy are areas of much active interest. The interaction of doxorubicin with radiation therapy is well recognised [29], but similar problems may be recognised as other agents are incorporated into integrated regimens.

Many of the new anticancer drugs introduced over the last few years have been analogues of existing agents such as carboplatin or epirubicin, and their interaction profiles could be expected to be similar, but by no means identical. However, we are now entering an era of new antineoplastic agents, either synthesised or derived from natural product screens. The biological response modifiers (such as the haematopoietic colony stimulating factors, interferons and lymphokines) represent a whole new class of molecules, which are not always chemically defined and which frequently have complex structures. The optimum dose of these, alone or in conjunction with conventional agents, will be hard to define and the possibilities for interactions will be considerable. Together with newer analgesics, antiemetics (e.g. HT_3-receptor antagonists) and antibiotics (e.g. the quinazolines), previously unrecognised effects will be encountered. Constant awareness of the mechanisms of interactions constitutes the principal defence against this form of iatrogenic disease, which in addition to effects on patient morbidity and mortality, utilises increasingly scarce Health Service resources.

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Chapter 4

Handling cytotoxic drugs

J. Cooke and I. J. Goss

Environmental exposure to the harmful effects of antineoplastic chemotherapeutic agents is a cause for concern to staff involved in their manufacture, dispensing and administration. Evidence from both animal work and observation of patients treated for malignant disease suggests that many antineoplastic drugs are carcinogenic, mutagenic and teratogenic [1–7]. However, direct evidence of harmful effects from environmental exposure is conflicting and with current developments in operator protection and protected environments such as vertical laminar flow cabinets and isolators, it becomes even more difficult to be able to assess any danger to the operator.

The first report of the potential for adverse effects of these drugs on the personnel who handled them was a controlled study which reported an increase in mutagenic activity in the urine of nurses working in an oncology clinic [8]. Mutagenic activity in this respect involves incubating test samples of urine with reference strains of tryptophandependent *Escherichia coli* or histidine auxotroph strains of *Salmonella typhimurium* and measuring the relative frequencies of spontaneous mutations. These nurses were involved with the reconstitution of cytotoxic drugs and their administration to patients, but operated without the benefit of masks, rubber gloves or a protected environment. However, the value of these urinary mutagenic assays as predictors of exposure is somewhat equivocal. A similar study failed to show any difference in urinary mutagenic activity between exposed and control groups [9]. The lack of sensitivity and the potential effects of environmental and dietary factors on the results led another group of workers to conclude that urinary mutagenicity tests could not be used routinely for the detection of accidental absorption of cytotoxic drugs [10]. A later study suggested that urinary mutagenicity could be decreased by dietary modification and could be used subsequently to monitor the mutagenic potential of administering cytotoxic drugs to patients [11]. Thus, this issue remains somewhat inconclusive and further work is required to provide us with a definitive answer.

An epidemiological study among nurses who worked in hospital units which prepared cytotoxic agents prior to administration to

patients found an increased incidence of foetal losses [12]. Again, none of the currently recommended operator protection techniques was employed during the study period [13–16].

Methods of absorption

The potential for systemic absorption of cytotoxic drugs was shown in a study which detected cyclophosphamide and its metabolites in the urine of nurses handling the drug, and of volunteers who had it applied to their skin [17]. Any cytotoxic drug which possesses lipophilic properties and has a relatively small molecular configuration might be expected to be able to be absorbed through the skin. Increased absorption might be expected when the skin is damaged or broken. Furthermore, many of these drugs are strong vesicants and possess corrosive properties against the skin. Sometimes the corrosiveness is a property of the vehicle in which the drug is formulated. Thus, there may be a localised toxicity as a result of inadvertent topical administration which then causes a greater systemic absorption.

Other potential portals of systemic entry of cytotoxic drugs are ingestion, either directly or as a contaminant of food or drink. Thus, eating or drinking in the environment where these drugs are being manipulated should be prohibited. For the same reasons, no food or drink should be stored in the same environment as cytotoxic drugs. The same arguments apply to inhalation, and smoking should not be permitted in these areas. Furthermore, careless manipulation of these formulations can produce aerosols which can then be passively inhaled. Protection from entry through all these portals can be enhanced by such things as protected environments, masks, goggles, gloves, garments and by assiduous attention to handling procedures.

Are there reliable tests to monitor environmental exposure?

Cytogenetic analysis of peripheral blood lymphocytes has been employed to demonstrate dose-related increases in the number of chromosomal aberrations after exposure to ionising radiation in industrial workers [18]. Similar effects have been seen on examination of sister chromatid exchanges (SCE) in workers handling organic chemicals [19]. SCE damage was observed in one study of nurses handling antineoplastic agents [20], but another study gave equivocal results [21]. Chromosomal damage in peripheral lymphocytes was

examined in a group of nurses who had been handling antineoplastic drugs. There was a higher incidence of chromosome-type breaks in the exposed group compared with a control group. The nurses had handled the drugs over a number of years without appropriate protection [22].

In many institutions in the United Kingdom, reconstitution of antineoplastic drugs has been centralised in designated areas in pharmacy departments in accordance with current professional and health and safety recommendations [23]. However, there is still concern from workers in these units, who are often females of child-bearing age, concerning the possible deleterious effects of handling these drugs and the lack of any reliable monitoring tests for demonstrating exposure to them.

Work conducted in Leeds used cytogenetic methods to determine whether staff who handled cytotoxic drugs were at risk from potential environmental exposure [24]. Blood samples were taken from pharmacy staff from a number of UK hospitals and were then examined for chromosomal damage and compared with a control group of office staff, a group of nurses working where patients were being treated for malignant diseases and a group of patients who had recently received cytotoxic drugs therapeutically. The results for the pharmacy and nursing staff showed no difference in mutagenic toxicity compared with the control group. The patient group showed significantly greater toxicity than the other groups, as might be expected, due to the specific toxic effects of these agents towards both malignant cells and normal host cells. These results are very reassuring for pharmacy staff who handle a large amount of cytotoxic compounds, as they validate the current recommendations for protective equipment and environmental control. This study was performed on pharmacy staff who were using vertical laminar flow aseptic cabinets. It is likely that the newer isolator units will offer equal, if not greater, protection to these individuals. The study results also offer reassurance to nursing staff who administer these agents to patients and attend to them in wards and clinics.

The measurement of cytogenetic changes in peripheral blood lymphocytes can thus offer a relatively non-invasive method for monitoring individuals who handle mutagenic compounds in their day-to-day occupations. The method has been shown to validate the various operative procedures which have become standard practice within the UK. On balance, it is probably sufficient to accept these validated results without recommending that all staff need to be subjected to regular blood checks. Health and Safety legislation, procedures and training should offer the greatest degree of operator protection.

Equipment

Increased awareness of the potential for chemotherapeutic agents to exhibit both acute and chronic toxicity in the context of occupational exposure has been the stimulus for wide-ranging developments in equipment and facilities for cytotoxic drug handling. The responsibility for the safe provision of chemotherapy in a ready-to-administer form now rests, quite properly, with the pharmaceutical service.

Equipment design is concerned with three factors: (i) protection of the environment; (ii) protection of the product; and (iii) protection of the operator. In contrast, the traditional aseptic dispensing/manufacturing facility is concerned only with product protection. Although the basic manipulative techniques are common to both areas, the additional criteria of operator and environmental protection necessitate adaptations to standard facilities. Any suitable system must provide a controlled environment for aseptic manipulation, as well as enable complete containment of hazardous material produced or released during the process. Drug material may be liberated as microaerosols when diluent is added or withdrawn from vials. Liquid droplets on the outside of needles, vials, etc., or even gross spillage due to breakage, all pose an exposure hazard.

The standard pharmacy aseptic dispensing cabinet relies on horizontal laminar airflow through a HEPA (high-efficiency particle arrestor) filter prior to the workspace. Such airflow potentially puts the operative at greater risk than with no cabinet at all! Existing technology was borrowed from other disciplines handling dangerous materials, notably microbiology, and thus Class II Microbiological Safety cabinets were pressed into service for the purpose of cytotoxic drug dispensing. Subsequent designs have been specifically for cytotoxic drug handling, with greater emphasis on product sterility. They must conform to the requirements of BS5726, and be sited in a clean room, ideally with a Class I (manufacturing standard) environment, although a Class II room may be considered adequate. The key design features are: (i) a balanced airflow which produces an air 'curtain' across the front of the workspace aperture (see below); (ii) vertical laminar flow of HEPA-filtered air to provide optimal conditions for aseptic manipulations; and (iii) postworkspace filtration to retain any drug material released. Figure 4.1 illustrates the principles of operation. Air passes through a HEPA filter and flows vertically down over the workspace. Laminar flow conditions are maintained within the working area. Vents at the front and rear of the work surface allow air passage across a second filter, which retains any drug material, as the air is recirculated. On each cycle, approximately 30% of the airflow is exhausted from the system. An equiva-

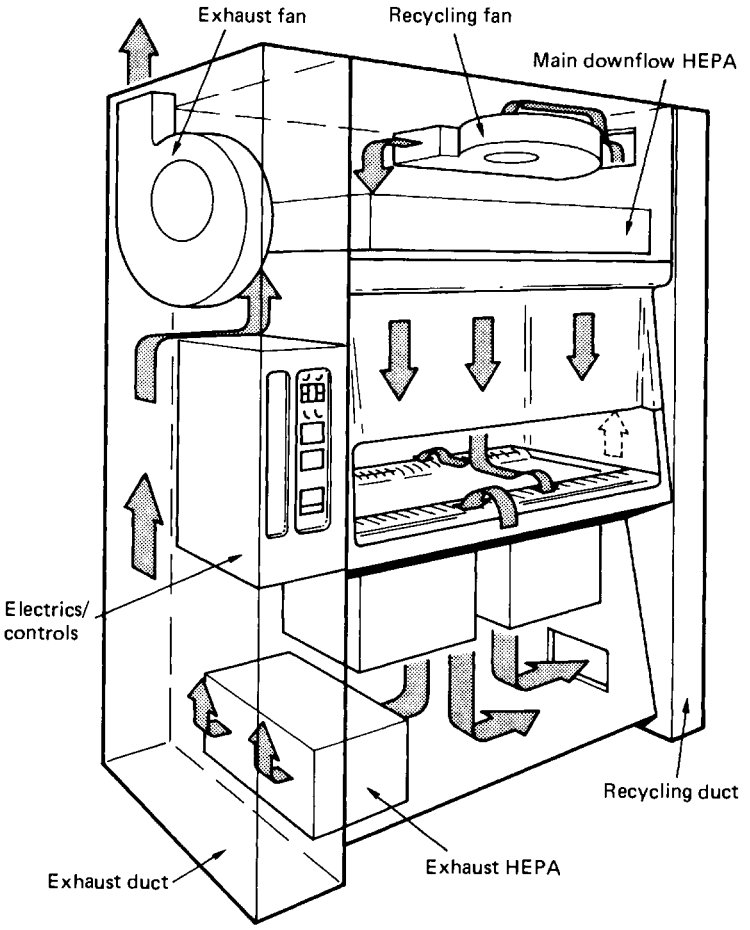

Figure 4.1 Airflow diagram of a typical vertical laminar flow cytotoxic cabinet. (Courtesy of Envair (UK) Ltd.)

lent amount is drawn in through the front opening. The basis of the operator protection afforded by this system is the production of a 'curtain' of air across the front opening of the cabinet. The downflow and inflow of air must be balanced to ensure that this is achieved. Exhaust air is 'clean', and may be blown back into the room, or ducted to atmosphere. Cabinet monitoring involves regular filter-integrity testing, airflow balancing, plus a challenge test of the 'curtain'. Small particles (usually of potassium iodide) generated

within the workspace should not pass through to a detector device placed where the operator would stand.

Isolators

Pharmaceutical 'clean rooms' are expensive to build and maintain, and occupy vast areas of space. Recent developments have been to isolate the working cabinet from the room to such an extent that it is independent of the surrounding conditions. Such containment facilities have acquired the generic name 'isolators'. Starting with a simple glove-box design, a host of options are now available. An isolator (Figs. 4.2 and 4.3) is a totally enclosed work station, supplied with filtered air which should meet BS5295 Class I standards. Operators have access to the working area via glove ports with flexible sleeves, or, in larger models, by means of a 'half-suit' arrangement. Air-locked access ports allow the introduction and removal of materials. Throughout the operation, the workspace is totally sealed from the

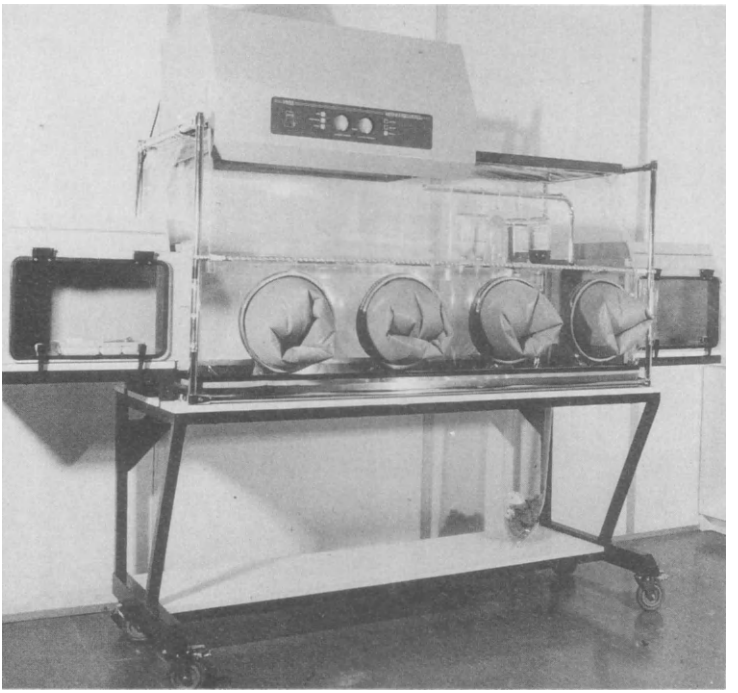

Figure 4.2 Flexible film isolator. (Courtesy of Envair (UK) Ltd.)

external environment. The design may be based on a rigid or flexible structure.

Flexible film isolators

These comprise a chrome or stainless steel frame supporting a PVC 'tent' (Fig. 4.2). The air supply may provide negative or positive pressure within the working area. Cytotoxic materials must be handled under negative pressure. Any leakage of seals or puncture of the film will lead to air ingress, rather than the escape of hazardous material. Additional support frames are necessary if operating under negative pressure. Air flow is usually turbulent, although more sophisticated designs may accommodate a laminar-flow screen within the structure. Flexible film isolators can be made very much larger than the rigid variety. Disinfection is by gaseous means, peracetic acid or formaldehyde being the usual agents used. This process is more applicable to larger scale manufacturing facilities.

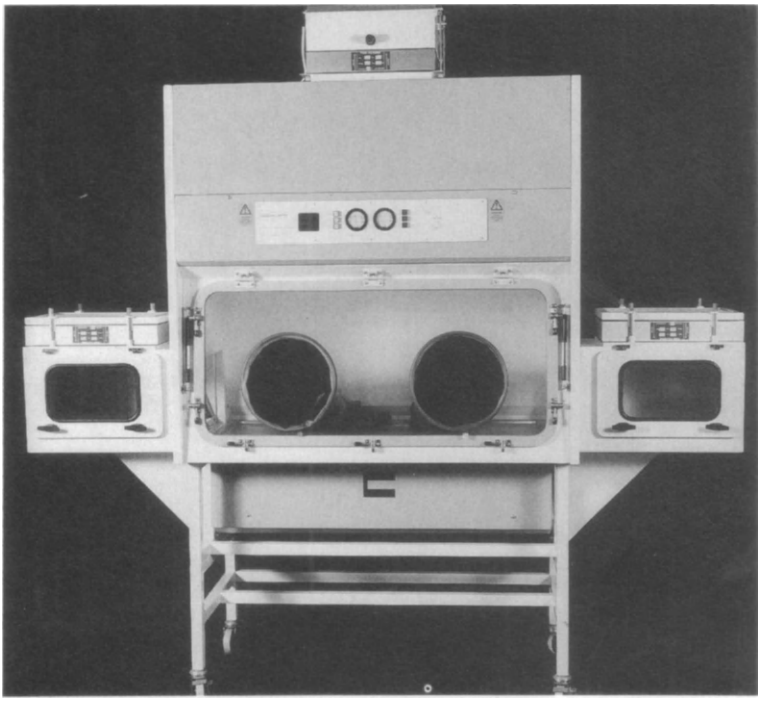

Figure 4.3 Rigid isolator. (Courtesy of Envair (UK) Ltd.)

Rigid isolators

These have a construction very similar to a traditional HEPA cabinet (Fig. 4.3). The workspace is fronted by a clear panel, with up to four glove ports. Newer models have laminar airflow, both in the workspace and also at the entry and exit hatches. The rigid walls allow positive or negative air pressure, often at the flick of a switch. As mentioned above, this should always be negative if handling cytotoxic materials. Disinfection is much easier, as hard-surface swabbing is adequate. The relative lack of working space renders these cabinets more suitable to smaller scale dispensing operations. Rigid isolators have a much longer working lifespan than the flexible versions. The choice of facility will depend on such things as cost, workload type and volume, number of operatives, extent of existing facilities, projected service developments, resources, etc.

Disposable elements of operator protection

Traditional pharmaceutical clean room clothing will be worn in the majority of situations. Its close-fitting, low-linting design ensures a high level of product protection. Cytotoxic drug handling imposes the additional need for an impermeable layer to provide a physical barrier to prevent drug penetration through clothing and, ultimately, the operative's skin. The usual way of satisfying both requirements is to wear a disposable impermeable garment over the normal cleanroom suit. An apron plus armbands will generally suffice for this purpose. The cleanroom suit is protected from contamination, hence it can be of a non-disposable type, which is more comfortable and acceptable for the operatives. For isolator-working, armbands alone are required, as damage to the sleeves may allow drug to escape from the cabinet. If faced with a major spillage, masks and goggles should also be worn. Provision must be made to deal with such an eventuality at locations remote from the dispensing area, e.g. stores, goods receipt bay, ward/clinic, corridors, etc.

The final area of equipment/clothing to consider is arguably the most critical, in that the point of contact with any hazardous material is by touch. Thus, which gloves should be worn? In recent years, the literature has been complicated and confusing. Some advice has appeared to be contradictory, and the testing techniques bear no relation to the way gloves are used 'in action'. In summary:

(1) No glove material offers total protection against penetration by cytotoxic drugs. Glove material, glove thickness and glove integrity are the major factors affecting drug penetration.

(2) Generally speaking, the thicker the glove, the better the protection provided. For cleaning spillages, the thickest possible gloves should be used.
(3) Latex gloves are the gloves of choice, despite earlier recommendations for PVC. Good quality latex (surgeons') gloves provide a good tight 'fit' (unlike PVC), good 'touch' and a reasonable barrier to penetration.
(4) 'Double-gloving', a traditional technique in some units, confers no advantages. Given that all materials will be penetrated if sufficient time is allowed, it is sensible to ensure regular changes of gloves and, particularly, immediate removal of a glove where direct contact with a cytotoxic agent has occurred.

For a further discussion of the various equipment options, plus details of the original investigations of glove/clothing materials, references 25–30 may be useful.

Techniques and procedures

It must be stressed that the most sophisticated and specialised equipment is intended to complement good manipulative technique, and can never be a substitute for the appropriate technical skills. By the same token, in those situations where the availability or provision of facilities falls well short of the standards outlined above, good technique (allied to common sense) will reduce to a minimum the degree of hazard involved. All operatives handling cytotoxic drugs must be fully trained in aseptic dispensing, and have their technique validated at regular intervals. The following represents a highlighting of the main procedural considerations in providing a cytotoxic reconstitution service.

In addition to the standard procedures for aseptic dispensing, consideration should be given to the following:

(1) Containment of all materials at all stages of the dispensing process. Spillage may contaminate the work station for future usage. Working within a tray, preferably lined with absorbent material, will reduce the risk of future contamination.
(2) Drug reconstitution must employ 'pressure equalisation' techniques. This is the key area of working practice if aerosol formation is to be avoided. Liquid transfers under pressure pose a serious potential hazard. Vials must be vented at all times during the addition and withdrawal of solution; there are now many proprietary devices to facilitate this. The operative's technique must be validated in this area (see below).

(3) Proper closure of the drug administration system. Infusions prepared in PVC bags should have a tamper-evident seal fitted to the additive port. To eliminate the risk of leakage, all syringe/needle/giving-set connections should be of luer-lock type. Syringes should be sealed with luer-lock hubs. Where the dose is to be given some distance from the dispensing unit (e.g. community-based treatment), consideration should be given to specifically-designed 'transportation syringes'.
(4) Safe systems of transport of dispensed products. Drugs are invariably administered at sites remote from the pharmacy. In addition to the points made above, packaging must be selected to contain any material inadvertently spilled. Clear labelling as to the hazardous nature of these materials is essential.
(5) Procedures for spillage must extend not only to those staff involved in dose preparation, but to all individuals handling the product between its arrival in the hospital (via stores/porters) to its final disposal (as clinical waste). Written procedures should be available, with the provision of suitable 'spill packs' as appropriate. Cytotoxic drugs are specifically covered by the Control of Substances Hazardous to Health Regulations (COSHH) [31].
(6) Safe disposal of waste. 'Sharps' present the possibility of needle-stick injury. Procedures to reduce this risk to an absolute minimum should be adopted. Re-sheathing of needles is the usual cause, and a strict 'no re-sheathing' rule is advisable. Rigid, plastic 'sharps' containers are to be preferred to the traditional (and cheaper) cardboard variety. In addition to preventing needle puncture, they will retain small volumes of liquid which may leak from 'empty' vials. All empty and part-used vials, syringes, needles, disposable equipment, etc., should be clearly labelled as hazardous waste, and incinerated at 1000°C.
(7) Staff training and validation is essentially that for a standard aseptic dispensing service. Exercises such as broth transfer tests should be employed routinely. Few specific techniques exist for the assessment of competence in eliminating aerosol formation during fluid transfers. A useful exercise is to reconstitute and transfer a dye such as carmine or methylene blue. Any deficiency in preventing liquid escape will be all too evident. A solution of quinine is colourless but will fluoresce under UV light and can be used to monitor the efficiency of spillage-cleaning procedures.

Acknowledgement

Figures 4.1–4.3 have been reproduced by kind permission of Envair (UK) Ltd.

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Chapter 5

Stability of solutions of anticancer drugs

A. G. Bosanquet

Introduction

The chemical stability of solutions of drugs used for treating cancer is of prime importance when considering how effective they will be. Although this aspect of cancer chemotherapy is unlikely to result in any more patients cured of their disease, the subject can only be ignored at peril.

Alongside considerations of chemical stability is the problem of potential microbiological contamination, as few anticancer drugs are formulated with antimicrobial preservatives in them. This is often the reason for the sometimes surprisingly short shelf life of drugs after reconstitution. However, this chapter will deal only with the stability and interactions of the drugs themselves. Listed in the Appendix are the names, synonyms, acronyms and US National Cancer Institute National Service Center numbers of some experimental anticancer agents and drugs in common clinical use.

The points covered in this chapter fall broadly into four areas: the stability of (i) drugs made up for i.v. bolus injection, (ii) drugs diluted for i.v. infusion, (iii) drugs in experimental systems; and (iv) the development of new drugs.

Intravenous injection

How long can a drug prepared for injection be left before it must be discarded? Many anticancer drugs are available for i.v. bolus injection, and drug stability information should be given on the package insert. However, it may not be clear whether this information relates to the chemical or microbiological stability of the drug, which makes it difficult to extrapolate to other conditions and situations. Often a drug will be tested for *chemical stability* by an undisclosed method, yet the time limit stated is usually constrained by *microbiological* considerations.

Stability problems with injections are usually minor since handling of drugs can be controlled both during manufacture and at the point

of use by simple instruction (e.g. 'use within 8 hours'). The problems that do occur are often associated with high drug concentrations, which give rise to potential crystallisation or precipitation reactions which, for instance, could be caused by storage of the solution at too low a temperature.

Intravenous infusion

Is it possible to infuse drugs over a long time scale without compromising drug stability? Infusions of anticancer drugs of medium term (hours) and long term (up to 14 days or more) are increasingly being used in efforts to overcome lack of efficacy, toxicity to patients and tumour resistance. Conditions of use are less easy to control in these situations. For instance, the pH of diluents such as 150 mM sodium chloride solution (normal saline) can vary widely (see below). Thus, stability information crucially required in practice is not so readily available from manufacturers; relevant information is more often found in the scientific literature. Particular problems can be encountered which arise because the drug is diluted; for instance, absorption and adsorption are more likely to occur.

Experimental systems

How can anticancer agents be handled and stored for use in experiments *in vitro*? This will include much work undertaken with anticancer drugs in *in vitro* chemosensitivity assays. Here, the problems can be legion in that drugs may be handled and experiments performed under a variety of conditions which may affect drug stability.

New drugs

How can a potential new anticancer drug be best formulated to maximise stability and ease of use? As discussed in Chapter 6, the development of potential new anticancer drugs also requires drug stability information. Is the drug compatible with solvents, diluents and containers? Can it be formulated to be stable in concentrated solution so that it does not have to be dissolved immediately before administration to the patient?

In all these areas, except possibly for i.v. bolus injections, there is no one definitive value for the stability of a drug. Any figure that is quoted would ideally have certain riders attached to it, such as a statement of pH, diluent composition, container material, etc., unless it has been shown that these have no effect. It is also important to

consider the quality of the data on drug stability: by what method has stability been measured?

Stability-indicating assays

The chemical stability of a drug can be measured by a variety of different means, some more accurate than others. This problem is illustrated by the work of two groups who separately studied six drugs in common and arrived at different conclusions about the stability of five of them. One group suggested that doxorubicin, carmustine, cisplatin, 5-fluorouracil and melphalan were stable for between 10 and 61 weeks at −60°C [1], whilst the other group concluded that these same drugs were unstable after only 3 weeks at either −20°C or −70°C [2]. These conflicting data may be the result of the use of different drug concentrations by the two groups. However, it is more likely, as the results are so divergent, that the discrepancy came about because both groups used biological assays in their attempts to assess drug stability. It seems clear, as was stated by Trissel in 1983 [3], that stability-indicating assays (i.e. assays which have been shown to detect parent drug unequivocally and are, almost by definition, not biological) are the only way to obtain reliable data and clarify discrepant results. However, 5 years later, problems were still being observed with stability testing programmes [4].

Obviously, reports of the stability of the stated drug (without contamination by degradation products, which in themselves could also be toxic) and an accurate assessment of a value such as $t_{0.90}$ (the time required for 10% degradation) are required. This information can be accurately gained only by the use of a stability-indicating assay. Thus, all results obtained using a system which is not stability-indicating will be open to criticism and should always be treated with caution.

Factors affecting drug stability

Many factors affect drug 'stability'. In this context 'stability' refers to both chemical and physical stability, and some factors are obvious, while some are more obscure.

Drug adsorption

If 'stability' is measured by determining the concentration of drug in

solution as a function of time, apparent degradation may actually be due to a reduction in concentration due to adsorption. Adsorption is unlikely to be a problem with drug concentrations above 1 mg/ml, but at greater dilutions can have a very significant effect on drug 'stability'. Drugs can adsorb to filters (some filter sets may adsorb up to 5 mg drug substance) or to containers (e.g. the adsorption of doxorubicin, see below), and can dissolve into the plasticisers used in the formulation and manufacture of flexible polyvinylchloride (PVC) tubing and packaging. This latter problem may be part of the cause of the reduced 'stability' of some drugs when stored in PVC containers and is likely to be most serious for lipophilic drugs.

Twelve of thirty antineoplastic drugs tested in dilute solution by Pavlik *et al.* [5] showed $>5\%$ adsorption on to sterile filtration units made from cellulose nitrate/cellulose acetate esters, and seven of these drugs also showed binding of $>10\%$ to polytetrafluoroethylene (PTFE) filters. Greater than 95% of the two drugs most affected, dactinomycin and doxorubicin, were adsorbed by the cellulose filters, and they were also adsorbed to a considerable extent by the PTFE units. It is of interest to note that whilst vincristine showed significant adsorption, vinblastine, with a very similar structure, showed negligible binding. Similarly, doxorubicin was adsorbed far more strongly than daunorubicin.

Dactinomycin and doxorubicin have also been shown to adsorb to a number of other materials, but not to siliconised glass or polypropylene. However, the use of siliconised glass is not a panacea for this problem, as some drugs bind more strongly to it than to glass. It is becoming generally recognised that polypropylene is probably the best material in which to store dilute aqueous drug solutions.

Light

Many anticancer drugs are sensitive to light and therefore need to be protected from light if they are to be stored for any length of time. This phenomenon sometimes becomes more acute as the drug is diluted and is particularly noticeable amongst the antitumour antibiotics (see Chapter 6).

pH

The stability of a number of drugs is pH-dependent, and caution is required when making up, storing or investigating the stability of these drugs in unbuffered solutions such as water or normal saline. Glass-distilled water and saline from PVC containers have a pH of

4–5, whereas water for injection and saline from glass containers have a pH closer to 7; this will make a considerable difference to the stability of drugs such as the nitrosoureas (which are 5–10 times more stable at pH 5 than at pH 7).

Freezing

Recently, pharmacists have become more interested in the stability of anticancer drugs frozen in solution, as this would enable them to provide a faster and more efficient service. Researchers also require a number of drugs in solution at short notice, and so store them frozen.

Although storage of a solution at below-zero temperatures would be expected to increase its shelf life, it can sometimes produce very surprising results. For instance, the new anticancer agent, diaziquone, has been found to have a *minimum* stability at −35°C in phosphate buffered saline, and have far *greater* stability at 4°, −7° and ≤ −70°C [6].

The results presented in the literature about the stability of frozen solutions of carmustine, 5-fluorouracil and melphalan are discrepant. Possibly, this is due in part to the problem of not using stability-indicating assays, as mentioned above. Most anticancer drugs can probably be frozen without problems (the lower the temperature, the better, as it is quite possible for a solution in a domestic freezer at about −15°C not to be completely frozen), but care must be taken with concentrated solutions, which are more likely to crystallise or precipitate.

Solubility

Many anticancer drugs are not very soluble in water or normal saline. Some require an alkaline pH (e.g. 6-mercaptopurine and 6-thioguanine), whilst others require acid–alcohol (e.g. melphalan) or more complex solvents (e.g. etoposide) to dissolve them (see Chapter 6). Care then needs to be exercised when diluting these drug formulations with intravenous infusion solutions to ensure that precipitation does not occur. For instance, etoposide shows greater stability at lower concentrations (100 as opposed to 1000 μg/ml) because it takes longer to crystallise out [7]. A number of other drugs are packaged in solution and, according to the manufacturer's instructions, should not be refrigerated and/or frozen [8]. The opposite can also occur: carmustine and doxorubicin show reduced stability at lower concentrations.

Table 5.1 Compatibilities of anticancer drugs

Drug	*Compatible with*	*Incompatible with*
Amsacrine		Methylprednisolone
Bleomycin	Cisplatin	Methotrexate
	Cytarabine	Mitomycin
	Dacarbazine	
	(Dexamethasone)	
	(Fluorouracil)	
	(Vinblastine)	
	(Vincristine)	
Carboplatin	Etoposide	
Carmustine	Cisplatin	
	Dacarbazine	
Cisplatin	Bleomycin	Etoposide?
	Carmustine	Fluorouracil?
	Cytarabine	
	Dacarbazine	
Cyclophosphamide	Dacarbazine	
	Doxorubicin	
	Fluorouracil	
Cytarabine	Bleomycin	Fluorouracil?
	Cisplatin	Methotrexate?
	Dacarbazine	Methylprednisolone
	Daunorubicin	
	Etoposide	
	Prednisolone	
	Vincristine	
Dacarbazine	Bleomycin	
	Carmustine	
	Cyclophosphamide	
	Cytarabine	
	Dactinomycin	
	Doxorubicin	
	Fluorouracil	
	Mercaptopurine	
	Methotrexate	
	Vinblastine	
Dactinomycin	Dacarbazine	
Daunorubicin	Cytarabine	Dexamethasone
	Etoposide	
Dexamethasone		Daunorubicin
		Doxorubicin
Doxorubicin	Cyclophosphamide	Dexamethasone
	Dacarbazine	Fluorouracil
	Vinblastine	
	Vincristine	
Epirubicin	Ifosfamide	Fluorouracil
Etoposide	Carboplatin	Cisplatin?
	Cytarabine	
	Daunorubicin	

Table 5.1 (*continued*)

Drug	*Compatible with*	*Incompatible with*
Fluorouracil	Cyclophosphamide	Cisplatin?
	Dacarbazine	Cytarabine?
	Prednisolone	Doxorubicin
	Vincristine	Epirubicin
		Methotrexate?
Ifosfamide	Epirubicin	
Mercaptopurine	Methotrexate	Prednisolone
	Dacarbazine	
Methotrexate	Dacarbazine	Bleomycin
	Mercaptopurine	Cytarabine?
	Vincristine	Fluorouracil?
		Prednisolone
Methylprednisolone		Amsacrine
		Cytarabine
Mitomycin		Bleomycin
Prednisolone	Cytarabine	Methotrexate
	Fluorouracil	Mercaptopurine
Vinblastine	Dacarbazine	
	Doxorubicin	
Vincristine	Cytarabine	
	Doxorubicin	
	Fluorouracil	
	Methotrexate	

Drugs in parentheses were not tested for stability.
Question marks indicate that under some conditions the drugs were found to be compatible [16, 17].
Adapted from references 7, 9–14, 16, 17.

Additives

The presence or absence of a preservative has also been found to have a considerable effect on the stability of aqueous solutions of cyclophosphamide, dactinomycin and a number of other drugs. This is a reminder that small details which may seem insignificant can be quite important for drug stability. Many anticancer drugs also show incompatibilities with other substances (see Chapter 6).

Some work has been undertaken on the compatibility of anticancer drugs. The results are summarised in Table 5.1.

The anticancer drugs

Table 5.2 summarises a large amount of work that has been undertaken on the stability of anticancer drugs. Where it is known, information is given on the most stable pH, whether drugs adsorb to

membrane filters or are sensitive to light, and an indication of their chemical stability in solution at room temperature when diluted with saline or a similar solution. Below are summarised some of the findings about drug stability for different classes of anticancer agents, and of some individual drugs.

Alkylating agents

The alkylating agents are a rather diverse group of drugs including some of the most unstable anticancer agents (e.g. chlorambucil, nitrogen mustard, procarbazine and treosulphan). Hexamethylmelamine and ifosfamide, on the other hand, are very stable in solution. Chlorambucil (although not administered i.v.) and melphalan were found to be more stable when bound to albumin (chlorambucil by a factor of 100 times).

Nitrosoureas

Much work has been undertaken on the nitrosoureas in attempts to determine the alkylating species of these drugs. This work has shown that they are very labile in aqueous media. In general, the stability of these compounds is better in acid medium (pH 3–5), or in ethanolic solution.

The degradation of three of the nitrosoureas has been shown to be catalysed by protein, and other nitrosoureas can be expected to be similarly affected.

Anthracyclines and other antitumour antibiotics

Much work has been done on the stability of these drugs, particularly doxorubicin. Its stability is affected by many different factors, and probably because not all of these have been controlled whilst others were under investigation, many contradictory results have been published [15]. There have also been problems with measuring the drug, as it adsorbs to many materials, even chelating to the stainless steel of high performance liquid chromatography (HPLC) columns. Table 5.3 summarises some of the drug stability results that have been published. Note that very different results have been recorded at virtually every temperature, and these are now discussed further.

As reconstituted for i.v. administration, doxorubicin is reasonably stable. At 2 mg/ml, only 10.5% of doxorubicin degraded when the solution was stored in the dark at 4°C for 180 days, whereas at 37°C, 10% degraded in approximately 14 days. The deep colour of this photolabile drug at this concentration may well protect it against

Table 5.2 Stability of the common anticancer drugs

Drug	*Stability of the i.v. formulation*[1]	*Stability diluted at room temperature*[2]	*Most stable pH*	*Effect of light*[3]	*Adsorption to filters*[4]
Aclarubicin	...[5]	52 h	4–4·5	+	0
Actinomycin D	24 h	21 wk	(5·5–7)[6]	+ +	+ +
Amsacrine	> 2 d at 5 mg/ml	...	..	0	..
Asparaginase	2 d/20 d (37°)	2 d	5–9 (7·4)	..	..
Bleomycin	28 d/36 mo (4°)	14 d/5 wk	(4·5–6)	+	0
Busulphan	...	∽ 3 h	..	..	..
Carboplatin	> 24 h/91 d at 15/10 mg/ml	24 h	(5–7)	..	..
Carmustine	8 h/24 h (4°)	40 min	4–5 (5·6–6)	+	..
Chlorambucil	...	15 min	< 3	..	+ / –
Chlorozotocin	...	20 min	4–5 (3·8–4·2)	..	+
Cisplatin	14 d/21 d at 1 mg/ml	> 24 h at 0·3 mg/ml	(3·5–6)	+ / –	0
Cyclophosphamide	6 d/48 d at 8/20 mg/ml	...	(3–7·5)	..	..
Cytarabine	> 2 yr/3 yr (4°)	12 d	7 (5–7)	0	+ / –
Dacarbazine	8 h/4 wk	24 h	3·5–4 (3–4)	+	..
Daunorubicin	2 d	40 h	4–6 (4·5–6·5)	+ +	+
Dexamethasone	3 yr	...	(7–8·5)	+	0
Dianhydrogalacticol	...	> 8 h	..	..	..
Diaziquone	...	21 h	6·3 (6·5)	0	..
Dibromodulcitol	...	∽ 30 min	..	..	..
Dibromomannitol	...	∽ 30 min	< 7	+	..
Doxorubicin	14 d/96 d at 1/2 mg/ml	24 h	3·5–5 (3·8–6.5)	+ +	+ +
Epirubicin	14/50 d at 2 mg/ml	24 h/28 d	..	+	..
Esorubicin	...	24 h	..	..	..
Etoposide	5 yr	6 h/7 d/28 d ($\leq$ 400 μg/ml)	5 (3–4)	+	0
Fludarabine	> 16 d	2 d	7·6 (6·5–8·5)	0	..
Fluorouracil	4 yr	7 d/28 d	9 (9·2)	+ / –	0
Hexamethylmelamine	...	? > 7 days	..	..	..

Ifosfamide	24 h/21 d/90 d	> 7 days	(6)	+	..
Interferons α and β	2 wk/1 mo (4°)	< 3 h (no carrier protein)	..	..	..
Iproplatin	24 h	> 24 h at 0·75 mg/ml	..	+/−	..
Lomustine	...	50 min	4–5	..	..
Maytanzine	...	4 d	..	0	+ +
Melphalan	...	50 min	< 3	+/−	0
Mercaptopurine	> 3 wk at 10 mg/ml	7 d	(10–11)	+	..
Methotrexate	2 yr	10 d/4 y (dark)	7 (7–8·8)	+	0
Mithramycin	...	24 h	≥ 7	+	+ +
Mitoguazone	> 2 d at 100 mg/ml	7 d	..	0	..
Mitomycin C	12 h/14 d at 0·5 mg/ml	24 h	> 6 (6–8)	+	0
Mitoxantrone	2 yr	2 d/28 d	< 7 (3–4·5)	0	+ +
Neocarzinostatin	...	4 mo	5	+ +	..
Nimustine	...	10/30 min	4–5	..	..
Nitrogen mustard	4 h at 1 mg/ml	1 h	(3–5)	..	..
Pentostatin	3 d	2 d	(6·7–8·7)	..	..
Prednisolone	...	92 d	(7–8)	+/−	..
Prednisone	...	Crystallises	..	+	..
Procarbazine	...	1·5 min	..	+	..
Rubidazone	...	1·3 h	..	+/−	..
Semustine	...	50 min	4–5	..	..
Steptozotocin	2 d/3 mo	50 min	4–5	..	0
Teniposide	...	24 h at ≤ 0·1 mg/ml	..	..	..
Tetraplatin	...	> 6 h at 0·1 mg/ml	..	..	..
Thio-TEPA	5 d (4°)/7 d at 0·25 mg/ml	> 12 h	..	+	0
Thioguanine	< 24 h at 15 mg/ml	24 h	(11–12)	..	..
Treosulphan	6 h	5 min (pH 8·5)	..	..	..
Vinblastine	2 yr	24 h/3 wk/39 d	2–4 (3·5–5)	+/−	+/−
Vincristine	58 d/6 mo	24 h/3 wk	4–5 (3·5–5·5)	+/−	+
Vindesine	30 d (4°)	3 wk	2 (4·2–4·5)	..	..

[1] Where two experiments have been conducted for different times, or produced different results, both values are given.
[2] Diluted with intravenous infusion solutions.
[3] 0, no effect; +/−, uncertain effect; +, slight effect; + +, considerable degradation caused by light.
[4] Adsorption of the drug from dilute solutions onto cellulose acetate and/or PTFE filtration units. 0, no adsorption; +/−, uncertain adsorption; +, some adsorption; + +, considerable adsorption.
[5] No data available.
[6] Figures in parentheses are i.v. solution pH values [7].

photodegradation: its light sensitivity is very dependent on drug concentration, with a 10-fold reduction in half-life on dilution of a solution from 100 μg/ml to 10 μg/ml.

Dilute solutions of doxorubicin have been found to adsorb to PTFE, glass, polyethylene, steel and 'plastic' (most likely polystyrene) containers, but not to siliconised glass or polypropylene. In dilute solution and with small volumes (a few ml), the problem of drug adsorption becomes very acute, with >95% doxorubicin being adsorbed to cellulose ester membranes and about 40% being bound to PTFE units.

Doxorubicin reacts with aluminium, flocculates with Fe^{3+} ions and is possibly catalytically degraded by steel. This latter observation may be part of the cause of problems in the HPLC determination of the drug by some authors, since steel columns are most commonly used for HPLC. It has also been shown that Al^{3+}, Cu^{2+}, Fe^{2+}, Mg^{2+} and Ni^{2+} are all chelated by anthracyclines.

As well as the observed incompatibilities with other anticancer drugs (Table 5.1), doxorubicin is incompatible with heparin at concentrations above 1·3 units/ml. Three amino acids and sodium bicarbonate ($NaHCO_3$) have also been implicated in causing the degradation of the drug.

Much work has been undertaken investigating the stability of mitomycin C. Again, there are discrepancies in the literature about the facts. It is more stable between pH 7 and 8, but its stability at −20°C or in cell culture medium is a matter for debate. As with other similar drugs, it degrades in light and chelates iron.

Platinum drugs

The stability of cisplatin and its analogues is rather different from that of the other anticancer drugs, due to the ionic nature of the bonding between platinum and the other groups. For this reason, the degradation of these compounds does not usually follow first-order or pseudo-first-order kinetics. The components of the solution tend to form mixtures of different drug species, the proportions of which can often be relatively stable as long as the solution composition remains unchanged. In general, it seems that solutions containing the leaving groups (from the platinum) confer the best stability on the drug; thus chloride is best for cisplatin and sulphate for spiroplatin, and water and chloride are best for both iproplatin and carboplatin.

For cisplatin, the main stability consideration is the chloride concentration of the solution, so that whether the drug is in water, dextrose, plasma or cell culture medium, an increase in the chloride concentration to 0·1 M or above confers the best stability. It is

Table 5.3 Stability of solutions of doxorubicin

Temperature (°C)	*Concentration (μg/ml)*	*Solution**	*Length of experiment (days)*	*Condition/% drug remaining*	*Approximate $t_{0.90}$ (hours)*
−70	1·5	NS	21	Stable	–
−70	0·6	NS	21	Unstable	–
−60	100	NS	70	Stable	–
−20	1·5	NS	21	Stable	–
−20	0·6	NS	21	Unstable	–
−20	1400	NS	30	Stable	–
−20	2000	Water	30	Stable	–
−20	–	Plasma	180	60%	–
−20	–	Plasma	14	Stable	–
4	2000	Water	180	89.5%	–
4	1·5	NS	21	Stable	–
4	0·6	NS	21	Unstable	–
RT	180	5% dextrose in PVC	2	Stable	–
RT	180	5% dextrose in glass	1·8	90%	–
RT	100	NS	–	–	40
RT	10	NS	–	–	40
RT	10	NS	–	–	3
RT	10	Water	–	–	4
RT	10	Ringer's injection	–	–	1
21	10, 20	Dextrose	–	–	100
21	10, 20	Ringer's injection	–	–	28
21	10, 20	NS	–	–	62
37	1·2	Dilute acid	–	–	9
37	0·4	CEM + 15% fetal calf serum	–	–	4
37	1·0	Enriched CRML 1066	10	∽90%	–
37	1000	Various media (no serum)	–	–	1
37	10	Various media (plus serum)	–	–	2

*Solution pH unknown.
NS, normal saline; RT, room temperature.
Data modified from reference 15.

suggested that cisplatin is not refrigerated when the solution concentration exceeds 0·6 mg/ml due to the possibility of precipitation. Cisplatin has been successfully stored frozen at high concentrations showing minimal decomposition (measured by non-stability-indicating biological assays) over a number of weeks, but it is suggested that it is not so stable when stored at more dilute concentrations.

Cisplatin is said to slowly isomerise to the *trans* form, an inactive but toxic form, the reaction possibly being catalysed by UV light. Most authors agree that normal laboratory lighting will not affect solutions. The drug is incompatible with aluminium and binds to proteins.

Antimetabolites

Methotrexate has been used for many years, but only relatively recently has it been found to be photolabile, this tendency being increased by drug dilution. Its degradation in the light showed a lag phase followed by zero-order kinetics, which suggested a free radical mechanism.

Methotrexate (at 0·5 mg/ml) is found to be stable during freezing and thawing. It binds strongly (94%) to serum albumin. A decade ago, methotrexate was only $>85\%$ pure 4-amino-10-methyl-folic acid, but although now it is 94% pure it still contains significant amounts of other substances.

Fluorouracil is a reasonably stable drug. It degrades at high pH (hydrolyses) and under UV light, but at high concentration (50 mg/ml) in amber bottles it is stable for 8 weeks. The drug is compatible with solutions of amino acids, total parenteral nutrients, and dextrose. It shows no change in UV spectrum after repackaging, and can be brought back into solution after precipitation by careful heating in a microwave oven.

Cytarabine is stable *in vitro* but is metabolised rapidly *in vivo* to the deaminated and inactive uracil arabinoside. A number of prodrugs have been suggested in an attempt to limit this metabolism *in vivo*.

Tubulin-binding agents

The tubulin-binding agents include the vinca alkaloids, vincristine, vinblastine and vindesine, the podophyllotoxins, etoposide (VP 16) and teniposide and maytansine.

Originally it was suggested that the vinca alkaloids were used 'as soon as possible' after reconstitution and were protected from light, but it is now known that they are reasonably stable drugs. Vincristine

and vinblastine both bind strongly to α-and β-globulins. Despite the similarity of the structures, vincristine binds significantly (5–15%) to cellulosse ester membranes (and the problem is increased by more dilute solutions), whereas vinblastine does not. The use of PTFE membranes does not reduce this binding of vincristine. Concentration-dependent crystallisation is a limiting factor in the stability of VP-16 at room temperature, and the drug has been observed to precipitate in dextrose solution.

Proteins

L-asparaginase is a protein acting as a cytotoxic drug, it is thought, by reducing asparagine levels and thereby inhibiting protein synthesis and cell proliferation. It has been isolated from a number of different sources including *Erwinia carotovora* and from *Escherichia coli*, the molecule from the latter having a molecular weight of 255 000 daltons. At high concentrations (> 2 mg/ml) it is stable in phosphate buffered saline for 2 days at 20°C but dilution to < 1 μg/ml causes rapid deactivation, probably due to adsorption onto container surfaces, PVC being found to be worse than other materials. The drug's stability is also reduced by preservatives and shaking.

The interferons are proteins of around 20 000 daltons. Their stability is difficult to assess because of the large number of different species known and because accurate determination of their activity is difficult. There is a distinct need for a good assay.

Interferons α and β are more stable than interferon γ, the latter being heat and acid sensitive. Good stability has been reported for interferon α at < -10°C. The most important aspect of the stability of the molecules seems to be the presence of 5 mg/ml human serum albumin as a carrier protein; without this, a solution of interferon β in normal saline was only stable for 2 hours at room temperature.

Steroids

Prednisone, prednisolone and dexamethasone are derivatives of cortisone usually used in chemotherapy in combination with other cytotoxic agents. Prednisolone sodium succinate and prednisolone sodium phosphate are used for i.v. injection and quite often *in vitro* due to their increased solubility, but the formulations contain a number of other additives. Prednisolone binds strongly to proteins even at low protein concentrations. Surprisingly, this binding is not inhibited by prednisone but is inhibited by hydrocortisone. The

succinate and phosphate esters are more stable in solution than is prednisolone.

Dexamethasone (as the sodium phosphate salt) stability is probably similar to that of the other cytotoxic steroids; the sodium succinate ester also shows good stability in a number of dosage forms.

Conclusion

At high concentrations for injection, the stability of the anticancer drugs presents few problems, as instructions are available to follow. However, when these drugs are diluted, many problems can occur, and their stability is often not known precisely. As the use of long-term infusions of anticancer drugs increases, care needs to be exercised in defining under what conditions the drug will be stable. It is also surprising how much is *not* known (or at least unpublished) about the stability of many anticancer drugs!

Appendix

The names, synonyms, acronyms and United States National Cancer Institute National Service Center (NSC) numbers for some experimental anticancer agents and drugs in clinical use are shown in Tables 5.4 and 5.5 below.

Table 5.4 National Service Center (NSC) numbers

NSC no.	*Drug name*
740	Methotrexate
750	Busulphan
752	6-Thioguanine
755	6-Mercaptopurine
762	Mustine (hydrochloride)
3051	N-Methylformamide
3053	Dactinomycin
3088	Chlorambucil
6396	Thiotepa
8806	Melphalan
9120	Prednisolone
10023	Prednisolone
13875	Hexamethylmelamine
19893	5-Fluorouracil
24559	Mithramycin
26271	Cyclophosphamide
26980	Mitomycin C

Table 5.4 (*continued*)

NSC no.	*Drug name*
27640	Floxuridine
32065	Hydroxyurea
32946	Mitoguazone (dihydrochloride)
34521	Dexamethasone
39069	Treosulphan
39084	Azathioprine
45388	Dacarbazine
49842	Vinblastine (sulphate)
63878	Cytarabine
67574	Vincristine (sulphate)
77213	Procarbazine (hydrochloride)
79037	Lomustine
82151	Daunorubicin (hydrochloride)
85998	Streptozotocin
89199	Estramustine (sodium phosphate)
94100	Mitobronitol
95441	Semustine
102816	5-Azacytidine
104800	Mitolactol
109229	L-Asparaginase
109724	Ifosfamide
122758	Isotretinoin
119875	Cisplatin
122819	Teniposide
123127	Doxorubicin
125066	Bleomycin (sulphate)
126849	3-Deazauridine
129943	Razoxane
132313	Dianhydrogalactitol
134087	Prednimustine
141540	Etoposide
148958	Ftorafur
153858	Maytansine
157365	Zinostatin
164011	Rubidazone
169780	ICRF 187
178248	Chlorozotocin
180024	Carubicin (hydrochloride)
180923	Tamoxifen (citrate)
181815	4-Hydroperoxycyclophosphamide
182986	Diaziquone
208734	Aclarubicin
218321	Pentostatin
227114	4-Hydroperoxyisofosfamide
241240	Carboplatin
245382	Nimustine (hydrochloride)
245467	Vindesine (sulphate)
249992	Amsacrine
256439	Idarubicin
256927	Iproplatin

Table 5.4 (*continued*)

NSC no.	*Drug name*
256942	Epirubicin (hydrochloride)
267469	Esorubicin (hydrochloride)
287513	Ametantrone (acetate)
301739	Mitoxantrone
311056	Spiroplatin
312887	Fludarabine (phosphate)
337766	Bisantrene
345842	Mafosfamide
352112	Trimetrexate
363812	Tetraplatin
404421	Vidarabine
409962	Carmustine

Table 5.5 Alternative drug names

*NSC no.**	*Drug name*
	Aclacinomycin—see aclarubicin
208734	Aclarubicin
	ACNU—see nimustine
	4′-[(9′-acridinyl)amino]methanesulfon-*m*-aniside—see amsacrine
	ActD—see dactinomycin
	Actinomycin D—see dactinomycin
	Adenine arabinoside—see vidarabine
	Adriamycin—see doxorubicin
	Altretamine—see hexamethylmelamine
287513	Ametantrone (acetate)
	3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea—see nimustine
	m-AMSA—see amsacrine
249992	Amsacrine
	Ara-A—see vidarabine
	Ara-C—see cytarabine
109229	L-asparaginase
	ASTA-Z-7557—see mafosfamide
	Azacitidine—see 5-azacytidine
102816	5-azacytidine
39084	Azathioprine
	3′-azido-3′deoxythymidine—see azathioprine
	Aziridinylbenzoquinone—see diaziquone
	AZQ—see diaziquone
	BCNU—see carmustine
125066	Bleomycin (sulphate)
750	Busulphan
	2,5-*bis*-(Carboethoxyamino)-3,6-diaziridinyl-1,4-benzoquinone—see diaziquone

Table 5.5 (*continued*)

*NSC no.**	*Drug name*
241240	Carboplatin
	Carminomycin—see carubicin
409962	Carmustine
180024	Carubicin (hydrochloride)
	CBDCA—see carboplatin
	CCNU—see lomustine
	CHIP—see iproplatin
3088	Chlorambucil
	Chlormethine—see mustine
	1,3-*bis*-(2-Chloroethyl)-1-nitrosourea—see carmustine
	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea—see lomustine
178248	Chlorozotocin
119875	Cisplatin
	CTX—see cyclophosphamide
26271	Cyclophosphamide
63878	Cytarabine
	Cytosine arabinoside—see cytarabine
	Cytoxan—see cyclophosphamide
	Czt—see chlorozotocin
45388	Dacarbazine
3053	Dactinomycin
	Dag—see dianhydrogalactitol
	Daunomycin—see daunorubicin
82151	Daunorubicin (hydrochloride)
	DBD—see mitolactol
	2DC—see pentostatin
	Dcf—see pentostatin
126849	3-Deazauridine
	4-Demethoxydaunorubicin—see idarubicin
	4′-Deoxyadriamycin—see esorubicin
	2-Deoxycoformycin—see pentostatin
	4′-Deoxydoxorubicin—see esorubicin
34521	Dexamethasone
	DHAD—see mitoxantrone
	cis-Diamine-1,1-cyclobutane dicarboxylate platinum(II)—see carboplatin
	cis-Diaminedichloroplatinum—see cisplatin
132313	Dianhydrogalactitol
182986	Diaziquone
	Dibromodulcitol—see mitolactol
	Dibromomannitol—see mitobronitol
	1,3-di(2-chloroethyl)-1-nitrosourea—see carmustine
	cis-Dichloro,*trans*-dihydroxybis-isopropylamine platinum(IV)—see iproplatin
	Dihydroxyanthracenedione (HCl)—see mitoxantrone
	Dihydroxyanthraquinone (HCl)—see mitoxantrone
123127	Doxorubicin
	DTIC—see dacarbazine
	4′-Epiadriamycin—see epirubicin
	4′-Epidoxorubicin—see epirubicin

Table 5.5 (*continued*)

*NSC no.**	*Drug name*
256942	Epirubicin (hydrochloride)
	Epodyl—see ethoglucid
267469	Esorubicin (hydrochloride)
89199	Estramustine (sodium phosphate)
	Ethoglucid
	Etoglucid—see ethoglucid
141540	Etoposide
27640	Floxuridine
312887	Fludarabine (phosphate)
19893	5-Fluorouracil
148958	Ftorafur
	FudR—see floxuridine
	4-HC—see 4-hydroperoxycyclophosphamide
13875	Hexamethylmelamine
	HN2—see mustine
181815	4-Hydroperoxycyclophosphamide
227114	4-Hydroperoxyisofosfamide
	Hydroxycarbamide—see hydroxyurea
32065	Hydroxyurea
	ICRF 159—see razoxane
169780	ICRF 187
256439	Idarubicin
109724	Ifosfamide
	IL2—see interleukin 2
	IMI-30—see idarubicin
	Interferon α
	Interferon β
	Interferon γ
	Interleukin 2
256927	Iproplatin
122758	Isotretinoin
	JM8—see carboplatin
	JM9—see iproplatin
79037	Lomustine
	LPAM—see melphalan
345842	Mafosfamide
153858	Maytansine
	MeCCNU—see semustine
	Mechlorethamine—see mustine
	MeGAG—see mitoguazone
806	Melphalan
755	6-Mercaptopurine
740	Methotrexate
	Methyl-CCNU—see semustine
3051	N-Methylformamide
	Methyl-GAG—see mitoguazone
	Methylprednisolone
	MGBG—see mitoguazone
24559	Mithramycin
94100	Mitobronitol

Table 5.5 (*continued*)

*NSC no.**	*Drug name*
32946	Mitoguazone (dihydrochloride)
104800	Mitolactol
26980	Mitomycin C
301739	Mitoxantrone
	Mitozantrone—see mitoxantrone
	MMC—see mitomycin C
	6-MP—see 6-mercaptopurine
	MTX—see methotrexate
762	Mustine (hydrochloride)
	MZN—see mitoxantrone
	Navelbine—see vinorelbine
	NCS—see zinostatin
	Neocarzinostatin—see zinostatin
245382	Nimustine (hydrochloride)
	NMF—see N-methylformamide
	Novantrone—see mitoxantrone
	Oncovin—see vincristine
	L-PAM—see melphalan
218321	Pentostatin
	Pharmarubicin—see epirubicin
	cis-Platinum—see cisplatin
	Plicamycin—see mithramycin
134087	Prednimustine
9120	Prednisolone
10023	Prednisone
77213	Procarbazine (hydrochloride)
129943	Razoxane
	13-*cis*-Retinoic acid—see isotretinoin
164011	Rubidazone
	Rubidomycin—see daunorubicin
95441	Semustine
311056	Spiroplatin
	Streptozocin—see streptozotocin
85998	Streptozotocin
180923	Tamoxifen (citrate)
	Taxol
	Taxotere
	Tegafur—see ftorafur
122819	Teniposide
363812	Tetraplatin
752	6-Thioguanine
	6-Thiopurine
6396	Thiotepa
	Tioguanine—see 6-thioguanine
	TNO-6—see spiroplatin
39069	Treosulphan
404421	Vidarabine
49842	Vinblastine (sulphate)
67574	Vincristine (sulphate)

Table 5.5 (*continued*)

*NSC no.**	*Drug name*
245467	Vindesine (sulphate)
	Vinorelbine
	VM-26—see teniposide
	VP-16—see etoposide
157365	Zinostatin
	Zorubicin—see rubidazone

NSC, National Service Center.
* Drugs without NSC numbers usually have an alternative name.
For some agents an NSC number could not be identified.

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Chapter 6

Development and production of cytotoxic drug formulations for Phase I trials

W. R. Vezin and E. G. Salole

Uniquely among all therapeutically active substances, Phase I trials of anticancer drugs are carried out on cancer patients and not on healthy individuals. Projected formulations of the compound for human use are first administered to mice (a total of 5 kg body weight of animals) to establish an LD_{10} level on which the starting dose of the escalating Phase I regimen is based.

Phase I studies are concerned with determining the maximum tolerated dose of an experimental compound. A number of individual patients, having provided informed consent to the authorised clinicians, are given a Fibonacci-type series of doses starting at a fraction of LD_{10}, increasing to a maximum tolerated dose on which the subsequent Phase II study is based [1]. The Phase II study that follows is designed for initial evaluation of the compound's efficacy against specific tumours [2].

This chapter deals with the pharmaceutical formulation and manufacture of material for clinical use. The terms 'formulation' and 'manufacture' are sometimes confused; 'formulation' refers to the conversion of a pure compound to a dosage form suitable for administration, while 'manufacture' is the batchwise replication of the same dosage form (subject to systems of quality assurance) for supply to clinical centres. This chapter describes the experiences of formulation and manufacture of a number of compounds at the Cancer Research Campaign (CRC) Formulation Unit at the University of Strathclyde, and illustrates some of the methods used to overcome insolubility, instability and production difficulties.

Formulation strategy from receipt of raw material

Subject to inevitable pressure from clinicians for an early completion of the formulation process and start of manufacturing and clinical

trial, there is some conflict for the formulator between seeing the exercise as merely providing a product which is adequate for valid Phase I and II input on the one hand, and providing an optimised formulation approximating (in terms of aesthetics and stability at least) to the final marketed form, a development process which may take a much longer period. If the compound is successful, there will be later (Phase III and IV) studies, with tighter regulatory requirements, which must be carried out on formulations which are not different (or, if at all, differ only 'insignificantly') if repetition of the Phase I and II studies is to be avoided (it should be noted that a small change in an apparently insignificant parameter can have a marked outcome, e.g. the effect of hypotonicity on the haemolytic characteristics of a surfactant drug).

Although total optimisation is often unnecessary, a formulation too hastily developed may be either unstable or too inconvenient to use and can, unfortunately, become 'locked' into an unlicensed 'special' or even a marketed product requiring, ideally, reformulation (e.g. the freeze-dried cyclophosphamide formulation, once the only product available in the UK, that sometimes took more than 10 min to reconstitute [3]). However, even if a better (more soluble, say) formulation subsequently becomes available, even if a significant improvement, it may have limited applicability because of the expense and inconvenience of repeating the regulatory process virtually *ab initio*.

The most common formulations of anticancer compounds are generally intravenous (i.v.) injections, especially in Phase I (about 90%), although, particularly in Phase II, solid oral dosage forms are indicated for compounds with good oral bioavailability and often overlap with injectable formulations. Oral liquid formulations, although rare, may be used in cases of poorly soluble compounds of high dose and good oral bioavailability. In the total absence of reliable toxicological data, the choice of a formulation for Phase I studies has to involve some assumptions about toxicity—resulting in a certain degree of overformulation, and running the risk of having to reformulate in the event of these assumptions being proved wrong later. However, this is unavoidable, demonstrating the virtual impossibility of assuring that a formulation is the final one before the end of Phase I studies. As the objective of preclinical toxicology and Phase I is to establish an upper tolerable dose limit, initial assumptions must be made about drug concentration and other formulation details (e.g. the use of cosolvents or surfactants, each with their own distinct toxicities, in order to solubilise sufficient drug) which may subsequently prove to have been unnecessary because of the toxicity of the test compound. For instance, the solubilisation of amsacrine by

the surfactant Cremophor EL (BASF) was found to be unnecessary in toxicological testing and it was therefore deleted from subsequent formulations. Occasionally, reformulation may also be necessitated by the prior appearance of excipient toxicity, or synergistic toxicity, during the escalation of dosing. Very often, the new compound is supplied with crude animal LD_{50} data based on intraperitoneal administration of a suspension of the solid compound, occasionally in an oil; in the case of an insoluble drug this preparation would act as a very crude sustained-release formulation, potentially giving rise to an exaggerated toxic dose or an exaggerated estimate of the drug loading required in the trial formulation, and also tending to encourage overformulation at the start.

Typical progress of a new compound from receipt to Phase II would be as follows: receipt of pure compound from the originator (an industrial or academic source); preformulation studies; pilot formulations; physicochemical studies of pilot formulations' stability; choice of formulation; small scale manufacture; preclinical toxicology; pilot scale manufacture; quality control; release to clinical centres for Phase I studies; further manufacture (and quality control) of supplies; release to clinical centres for Phase II studies. The compound's progress is either terminated at this point or, if results are significantly promising, advanced to Phase III studies.

Table 6.1 outlines some of the factors involved in preformulation studies. Although it is reasonable with non-cytotoxics to expect an average of about 1000 man-hours work in preformulation and formulation development prior to first appearance in Phase I (it has been said that one cannot learn too much about a compound!), time is often short and safety requirements often result in the handling of cytotoxics being more labour intensive. However, the programme may sometimes be accelerated; for instance, a full study along the lines in Table 6.1 would be unnecessary in the case of a potent drug for i.v. administration which is clearly stable and extremely soluble—but this is not usually the situation with many cytotoxic agents. The preformulation agenda in Table 6.1 has been placed in the order of routine practice at the CRC Formulation Unit: items in the first group are invariably carried out, those in the second possibly less frequently and those in the last group not necessarily (although some of these can occasionally be very important, e.g. compatibility with excipients for an injection when a choice is to be made on the basis of stability assessments of only a few pilot formulations). The formulator must either receive the more fundamental data (from the first group of assessments) from the originator of the test compound, or the data must be generated in full elsewhere. Full documentation of each raw material batch must include assay, and identification and quantification

Table 6.1 New drug characteristics assessed in preformulation studies

Chemical structure: key characteristics noted
Synthesis and purification: identification of likely recrystallisation solvents
Purity: identification of impurities
Characterisation: by spectroscopy (NMR, IR, UV, MS), melting point, X-ray diffraction
Assay: methods and validation
Solubility and rate of solution
Raw material stability
Hygroscopicity
Precipitation rate from supersaturated solutions
Dissociation constant
Complexation characteristics
Partition coefficient
Light sensitivity
Identification of chemical instability, specific reaction mechanisms and methods of stabilisation
Compatibility with excipients
Polymorphism and solvation characteristics
Powder properties: particle size and shape, compactability, flow properties
Organoleptic properties

NMR, nuclear magnetic resonance; IR, infrared; UV, ultraviolet; MS, mass spectroscopy.

of every identifiable impurity. This is not always available, therefore, to avoid regulatory problems and the necessity of repeating studies later, samples of every raw material batch are stored at low temperature ($-85°C$) for future reference and analysis. Some of the preformulation procedures are now discussed in more detail.

Characterisation

Characterisation of a new compound is obviously vital. Each raw material batch received should be numbered at source as well as fully documented (it has been known for the CRC Unit to receive unsolicited material with no analytical data, batch number or documentation of any kind). It should be noted that the assignment of an arbitrary reference number by the receiving formulator may make it difficult to correlate the batch later with any of the originator's manufacturing runs. Standard characterisations are required to assure that the compound is received as described, with descriptions of any departures from the Standard Operating Procedures (SOPs) during manufacture. On one occasion it was found that a satisfactory formulation of an injection of a soluble drug could not be replicated;

it transpired that, as a result of a change of solvent of recrystallisation, later supplies of raw material consisted of a polymorph with about only one-sixth the solubility of the original batch. Early in the study a raw material reference standard (or primary sample) must be placed on storage at low temperature for comparison with all subsequent batches.

Assay method and validation

Development of a variation of the assay may be necessary if, for example, an analytical method is required in studies of the stability, compatibility or pharmacokinetics of the test compound, or where the compound is to be assayed in the presence of formulation components or breakdown products which are familiar and where only resolution from synthetic impurities is required. The demonstration that the assay is quantitative, robust, easily transferable between laboratories (even if the validation procedure itself is quite complicated) and specific to the compound is essential (e.g. in a high performance liquid chromatography (HPLC) method, the probability of a derivative peak being unresolved and hidden in the main compound peak should be negligible). This can be checked for an HPLC assay by scanning the peak with a diode array (if the compound is UV absorbing) or by using other detectors (e.g. nuclear magnetic resonance, mass spectroscopy), or elemental analysis in comparison with the raw material. Some compounds do not absorb in the UV and some (e.g. pyridoglutethimide, below) exist in two optically active forms, supplied to the CRC Unit as 50 : 50 racemic mixtures and resolved in the non-chiral HPLC methods developed for them. It should be remembered that only one isomeric form of a drug may be the pharmacologically active agent [4].

It may be that separate assays are necessary for assessment of stability, compatibility and pharmacokinetics, involving the resolution of differing compounds; this situation requires overinvestment in validation routines and, fortunately, is relatively rare. The identification and quantification of impurities should approximate to the requirements of the Pharmaceutical Expert Report accompanying a Product Licence Application. However, it has been suggested that since the majority of new compounds tested have only low probabilities of success, there might be a compromise on time-consuming initial characterisations and that the Phase I and II studies of successful compounds be repeated after they have been more thoroughly characterised.

Solubility and rate of solution

Solubility studies should be carried out in a range of aqueous buffers, particularly at pH equal to the pKa of the compound, in a range of organic solvents acceptable to regulatory authorities (e.g. ethanol, methanol, propylene glycol, polyethylene glycol 400, dimethylacetamide (DMA) and dimethylsulphoxide (DMSO), and a small range of admixtures of these solvents with water or buffers, in order to examine departures from ideal log–linear solubility [5] and also to get a 'feel' for the compound. Solubility determinations carried out in the usual way can be wasteful on experimental compounds (usually available only in very small quantities), hence specially designed miniaturised apparatus is advantageous. If aqueous solubility is insufficient, it may be possible to increase it using the following range of options: an increase in the degree of ionisation; formation of soluble salts; the use of cosolvents or complexing agents; a combination of these in the case of very insoluble compounds [6]. Failing these, solubilisation in surfactant systems (surfactant–water and surfactant–alcohol systems, which frequently do not precipitate on dilution) should be studied, limiting attention to surfactants acceptable to regulatory authorities, e.g. polysorbates (Tweens, ICI), Cremophor EL and Solutol HS15 (BASF). Each of these approaches can, as rule of thumb, increase solubility by a factor of about 10, and in combination by up to 10^4. It should be noted that although rates of solution correlate well with solubility and powder specific surface area, large clumps of a poorly soluble compound may take longer to dissolve than smaller masses of particles of the same size and this may have implications for compounds that are unstable in solution (e.g. methylene dimethanesulphate, below).

Raw material stability

Protracted stability studies over desiccant and in atmospheres at a range of relative humidity (RH) values (e.g. 11–93% RH) should be undertaken at 25°C and, if possible, at higher temperatures. Occasionally, a compound proves extremely unstable even at temperatures as low as −20°C, due to, for instance, the presence of small amounts of impurities such as water or acid, but in a pure form proves stable at higher temperature (e.g. 37°C) under nitrogen, thus necessitating its formulation as a pure compound, without excipients, to be reconstituted and diluted immediately before administration (e.g. Compound No. 9, below). Aspects of the stability of anticancer compounds are discussed in more detail in Chapter 5.

Hygroscopicity

Compounds absorbing water rapidly from the air should be identified early. Hygroscopicity makes weighing, manufacture and standardisation difficult, occasionally requiring special methods of handling (in glove boxes and under dry nitrogen, for instance, e.g. SDZ 62 434, below). Water absorption isotherms should be determined for each compound at 20°C over a full RH range.

Precipitation rate

Formulations utilising cosolvency for enhancing the solubility of an insoluble drug are often prone to precipitation on dilution (e.g. on injection), perhaps even requiring instructions for a maximum permissable i.v. injection rate (e.g. diazepam injection [7]). However, within certain limits depending on the compound, solutions can exceed solubility and briefly supersaturate on dilution and injection without causing adverse effects. Model systems (effectively artificial blood vessels [7, 8]), which afford an assessment of solubility limits and rates of injection, should be used if such formulations are unavoidable.

Dissociation constant, complexation and partition coefficient

The dissociation constant of a compound is a vital parameter and should be determined by the best means available [9].

Complex formation is occasionally used to supplement other methods of increasing the solubility of an insoluble drug or of an insoluble degradation product [6]. For instance, nicotinamide complexes with a wide range of compounds and, although intrinsically toxic, can be used to solubilise the more toxic antineoplastics [10]. However, when used to formulate Compound No. 807 (see below), nicotinamide proved unsatisfactory due to synergistic toxicity.

The partition coefficient should be studied with a small range of organic phases (e.g. hexane, *n*-octanol, soya bean oil) against an aqueous phase composed of buffers (particularly at pH values around the pKA if possible).

Light sensitivity

It is important not to spend too much time on this characteristic. Although many industrial formulation units study light sensitivity routinely (sometimes by simple exposure to south daylight, sometimes by exposure to a range of discrete wavelengths), the results may be difficult to interpret and translate to a practical situation [11].

It is only necessary to establish sufficient stability under standard handling and manufacturing conditions (say fluorescent light or daylight for 24 hours), since both light-sensitive and light-stable materials are generally stored in darkness (it is worth noting that the use of amber glass and other dark packaging for ampoules and vials may contribute additional problems by hampering visual inspection for particulate contamination).

Methods of stabilisation, identification of specific reaction mechanisms and compatibility with excipients

Methods of stabilisation, identification of specific reaction mechanisms and compatibility with excipients can be very important in specific cases, as described under particular compounds below (e.g. BZQ and didox). Hydrolytically and alcoholytically unstable compounds may have to be formulated with no excipients at all (e.g. Compound No. 9 below).

Formulation excipients chosen should be unreactive with the drug. This screening overlaps with the assessment of drug stability, and includes assessment of compatibility in the final formulation by shelf-life studies and stability assay validation. Studies of the compatibility between new compounds and the excipients likely to be used in the preparation of solid dosage form are always carried out (perhaps initially using thermoanalytical techniques as rapid screening methods [12]); however, 95% of all Phase I anticancer formulations are injectable solutions of simpler design.

Polymorphism and solvation

Some industry-based formulation groups have described routine searches for soluble polymorphs and solvates ('pseudopolymorphs') of insoluble drugs. This is generally a last resort, being usually less fruitful than the other means of increasing solubility described above. However, a brief screen to assess a novel compound's capacity for adopting different crystalline forms (which may exhibit widely differing stabilities, dissolution rates, powder properties, etc. [13]) is advisable.

Powder properties: particle size and shape, compactability, flow properties

Particle size and shape, compactability and flow properties are important when encapsulation or tableting by direct compression are contemplated [14].

Organoleptic properties

Taste and colour may be important in the case of oral liquid formulations (suspensions, emulsions, solutions) in terms of patient compliance and placebo design (e.g. amphethinile, below). However, some basic drugs with surface-active properties have a qualitative bitterness that is difficult to mimic in a placebo.

Pilot formulation studies

Once a feel for the physical chemistry of a compound has been obtained through preformulation studies, a choice from a small selection of formulations is then made. The most common problems with antineoplastics are chemical reactivity (particularly with the alkylating agents) and solubility (which can be improved by a number of approaches, as outlined previously). When a stabilising or solubilising excipient is required, instead of optimising the formulation, a compromise may be necessary between the excipient concentration required to produce a particular drug loading, stability, etc., and its toxicity. For example, the use of a higher cosolvent concentration to produce a greater solubilising effect on an insoluble compound will almost always lead to greater local irritancy and a higher risk of local drug precipitation on injection, leading to the development of phlebitis [15]. Parenteral products may have pH values between 2 and 12, but these outer limits are only suitable for single-dose i.v. injectables and must be reduced to between pH 3 and 9 for compounds, such as the antineoplastics, which are administered repeatedly. The risk of local tissue intolerance to parenteral formulation excipients is further increased in the case of intramuscular and, further still, subcutaneous injections; fortunately, both these routes are rarely used in anticancer therapy. For a review of typical concentrations of formulation excipients in marketed parenterals see reference 16.

A reconstitutable or freeze-dried, bulk or liquid-fill, formulation may have a maximum stability well within acceptable pH ranges but be sufficiently unstable to require an extensive formulation exercise. Another compound may have optimum stability outside the safe pH range (e.g. BZQ below) and require compromise and some extra formulation to provide reasonable stability at an acceptable pH. Such compromises of physicochemical non-ideality and physiological acceptability may also be required with solubilised formulations [17].

In the search for a sufficiently elegant product, total optimisation is not always necessary. Optimisation is required, however, in the pilot

formulation process where a number of closely related formulations are prepared for simultaneous stability testing by making slight variations in a single parameter, e.g. SDZ 62 434, below. With an apparently stable, soluble material such as SDZ 62 434, whose desirable properties may be obvious after the most perfunctory preformulation study, it is sufficient to produce only three injectable formulations, of pH approximately equal to, slightly above and slightly below that projected for the product, for simultaneous stability testing (typically in triplicate, at 4, 25, 37, 55 and possibly 70°C, and sampling at 1, 2, 4 and 12 weeks). If a freeze-dried product is required, at least three lyophilisates should be submitted to a similar stability program after drying at a single temperature below the determined eutectic temperature [18]; the three lyophilisates should be prepared at three different secondary-drying temperatures around the value projected as suitable for the drug. Stability studies of freeze-dried products should be limited to a narrower range of temperatures (usually 25°C and 37°C are sufficient) because of the greater difficulty of extrapolation of solid-state degradation rates from higher to lower temperatures [19]. The development of lyophilisates is complicated by the possibility of varying the particle size and crystallinity of the final product (and therefore dissolution rate, reconstitution time and stability on storage) by varying the freezing rate [18, 20].

Although non-isothermal stability studies have been cited as sometimes furnishing shelf-life values more quickly than isothermal methods [21], the wide range of activation energies of drug degradation reactions (which commonly range from 50 to 250 kJ/mol, though are usually around 80 kJ/mol) effectively precludes a simple method of predicting shelf-life of a product in less than about 3 months after making the first pilot formulations. For instance, assuming a 'worst case' scenario of degradation following first-order kinetics with a low activation energy of 50 kJ/mol, to provide data indicating stability for a year at room temperature, a formulation would require to be autoclaved at 115°C for about two and a half days.

Stability studies

This work is particularly important and takes up most of the time of the CRC Formulation Unit. Stability studies are initiated as soon as possible after the final choice of formulation, and usually last for at least 3 months before the formulation is released for preclinical toxicology. Stability studies are best carried out according to the

guidelines suggested by the United States Food and Drug Administration (FDA), which are sufficient to validate clinical trial input and are subsequently usable for regulatory batch documentation [22]. At least 3 months' stability data should be available before the start of Phase I studies, and 9 months' data should accompany the Phase II data.

Minimum FDA guidelines for liquid-fill and freeze-dried parenterals are at least three batches, manufactured according to Quality Assurance procedures, on store in the dark at 4, 25, 37 and 55°C, with a similar sub-batch stored at a south-facing window (or undergoing similar light sensitivity studies); unit samples from each batch at each temperature are taken at 3, 6, 9, 12, 24, 36, 48 and 60 month time points and assayed (in duplicate at least) against two separate standards. The FDA guidelines state an assay window of 15 days per time point, although late assays are sufficient to validate clinical input (and in any case stability studies can be repeated if necessary). If the product requires storage at a lower temperature, say in a freezer at −20°C, the stability storage conditions can be confined to three separate batches at temperatures slightly above and slightly below the recommended value, say −15°C and −25°C. In the case of a liquid fill formulation, two extra batches, at pH slightly above and below that of the standard formulation, should be placed on store for simultaneous sampling; in addition, the truncated stability studies carried out during the formulation exercise should have extra sampling points at 1 and 2 weeks. For tablets and capsules, three separate batches should each be placed on store as follows: at room temperature and ambient RH in a clear container on a south-facing window; at 20°C and 100% RH; at 20°C and 60% RH; at 31°C and 70% RH; and at 55°C and ambient RH. Tests at each time point usually include an assessment of appearance and physical characteristics (i.e. dissolution, disintegration and friability) as well as chemical assay for content. These stability studies often require larger numbers of tablets or capsules (compared with units of parenteral dosage forms), at least 20 per time point, to be sufficient for all the tests; however, Product Licence submissions may not require all the data described.

Preclinical toxicology: animal studies

These studies are carried out with the final formulation intended for human use in order to determine the starting dose of the escalating regimen to be used in the Phase I study. They involve giving increasing doses of the formulation (along with a placebo of diluent to exclude formulation-related toxicity [23]) in order to establish an

LD_{10} dose level, a multiplier of which is used as a starting dose in the Phase I programme [1]. On average, about 5 kg total weight of mice are used per compound in a typical preclinical toxicity study.

Manufacture of dosage forms for clinical studies

In terms of current good manufacturing practice (GMP), the manufacturing requirements for cytotoxics in early clinical trial are no different from other compounds [24]. However, there are a number of additional safety considerations arising from their toxicity which tend toward greater labour intensiveness (see Chapter 4). Ampoule and vial filling of solutions in small quantities, with minimal risk of aerosols generation or entrainment of particulates, may be carried out in a cytotoxic safety cabinet conforming to Australian Standard 2567 [25], with attendant use of protective clothing and immediate access to effective means of decontamination in case of accidents. A similar set-up may also be used for some less toxic (e.g. non-alkylating) compounds when filling into capsules. On the other hand, encapsulating alkylating and other dangerous agents, or the tableting of any anticancer compound generally requires a totally washable isolated room under negative pressure, with hermetically sealed suits and individual air supplies for operators; large glove boxes may also be used for filling the more toxic compounds in less rigidly controlled areas (see Chapter 4).

There is a certain conflict between safety and GMP requirements in the manufacture of sterile anticancer and other biologically hazardous products. Good sterile practice generally requires an environment under slightly positive pressure with a sufficient rate of filtered air changeover, while a safe environment for hazardous materials requires a negative pressure with a filtered or scrubbed air outflow. Both configurations must be combined in a sterile suite dedicated to cytotoxic sterile manufacture, as a negative pressure area enclosed within a positive or vice versa. At the CRC Formulation Unit, small-scale filling of ampoules or vials is carried out in effectively negative-pressure cabinets in a positive-pressure room. Here, the contamination of the positive-pressure area in an accident is a theoretical possibility, in which case the entire sterile area can be put under negative pressure by means of a manually operated override.

Liquid-fill and freeze-dried ampoules and vials are often the main Phase I and II formulations, and a manufacturing unit should be able to produce 1000–3000 dosage units (with accompanying quality control) of a compound for studies over a 3-month period. Require-

ments for solid dose forms are similar. A final decision on dose and pack size of a formulation is made at the end of Phase I and the approximate requirements for Phase II manufactured in, ideally, only a few batches.

A concensus has been reached within the United States National Cancer Institute (NCI), a view somewhat shared by the Joint Formulation Working Party (a formal collaborating group drawn from the NCI, the European Organization for Research on Treatment of Cancer and the CRC) that, as far as possible, anticancer formulations should be freeze-dried. This has a number of aesthetic, safety, and stability advantages, as well as tending to accelerate the formulation development process [20]. However, not all compounds can be freeze-dried (e.g. amphethinile and Compound No. 9, below), and there is a body of opinion in favour of a wider range of products. Nevertheless, despite freeze drying being a labour intensive, resource expensive and 'environmentally unfriendly' process [18], it is the commonest operation in the manufacture of anticancer formulations and is carried out routinely at the CRC Formulation Unit.

A tablet or capsule formulation may be more convenient with a compound showing evidence of good oral bioavailability because it may be soluble at physiological pH or at low pH or in bile salts. It may be advantageous to design, *ab initio*, a capsule formulation for a fully automatic hard shell capsule filling machine (cf. a semi-manual plate-filling type), in order to have a final formulation suitable for large volume manufacturing; often such formulations require only little extra work, and a formulation designed with only plate-filling in mind is unlikely to work on subsequent transfer to a fully automatic machine without reformulation and the attendant risks of altered bioavailability, etc. Stability considerations may indicate tableting by direct compression; in this case, with sparingly soluble compounds the quality control specifications for raw materials must include particle size, which could be a critical parameter for reproducible dissolution and bioavailability. Occasionally, a very high dose of insoluble material may be suitable for administration only as an oral suspension (e.g. amphethinile, below).

Quality control and manufactured batches

Certificates of analysis of all raw materials, actives and formulation excipients alike, are required whether they are the subjects of pharmaceutical compendial monographs or not. With experimental new compounds, the documentation received with the raw material is often inadequate, in which case samples of all batches must be placed on store at $-80°C$ in sufficient quantity for quality control assess-

ments later. It should be remembered that the failure of (or lack of data on) even one batch of raw material could compromise a clinical trial, in the event of the data being required later for regulatory purposes. Quality criteria applied to finished batches generally follow compendial specifications and requirements, e.g. for vials and ampoules their fill and extractable volumes, a contents assay and assessment of particulates (using an Allen viewer or more sophisticated particle sizing instruments like the Coulter Counter). For capsules, the CRC Formulation Unit generally follows somewhat tighter limits than in the compendia (usually 5–7.5% margins on content, say), supplemented by assays of samples taken from the bulk throughout filling runs to confirm the homogeneity of the powder mixture, as well as end-process compendial tests for dissolution and disintegration.

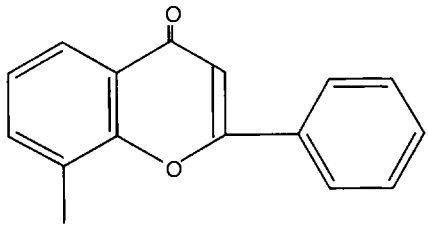
Specific problems encountered with some compounds formulated at the CRC Formulation Unit*

LM 985 (flavone-8-acetic acid, N,N-diethylaminoethyl ester; Lipha)

The compound LM 985 (Fig. 6.1a) hydrolyses *in vitro* and *in vivo* to the pharmacologically active, and *in vivo* longer lived, flavone acetic acid, LM 975. LM 985 is thus a prodrug of LM 975, which has itself been tested clinically. The *in vivo* half-life of the conversion of LM 985 to LM 975 is about 70 min, very nearly equal to the *in vitro* hydrolysis rate in aqueous buffers at 37°C and pH 7·4 (there is a slight ionic strength effect), suggesting negligible contribution by non-specific esterases, lipases, etc., to hydrolysis *in vivo* and to initial clearance.

Figure 6.2 shows the pH-solubility profiles of LM 985 and LM 975. Hydrolysis of LM 985 is pH dependent (i.e. it is stable in acid and the reaction is $[OH^-]$ catalysed), the rate constant being nearly inversely proportional to $[H^+]$ at a given temperature. Because of the different solubility of LM 975, the time taken for the appearance of this degradation product on dissolution of LM 985 (theoretically about 4 hours at room temperature but in practice longer than this due to supersaturation) is nearly invariant between pH 2 and 5. Frozen solutions of LM 985 were formulated at pH 4·0 and stored at −80°C; LM 975 was formulated as a lyophilisate, although it is stable as a sodium salt in solution at pH 9·0 (Fig. 6.2).

* Compounds that are confidential at time of writing are ascribed a Unit code only, without chemical or structural description.



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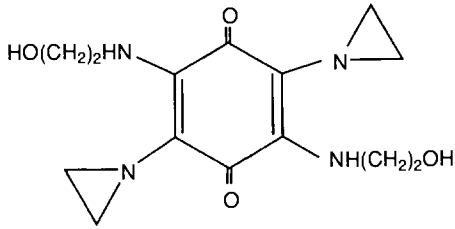


(b)

Figure 6.1 (a) LM 985; (b) BZQ.

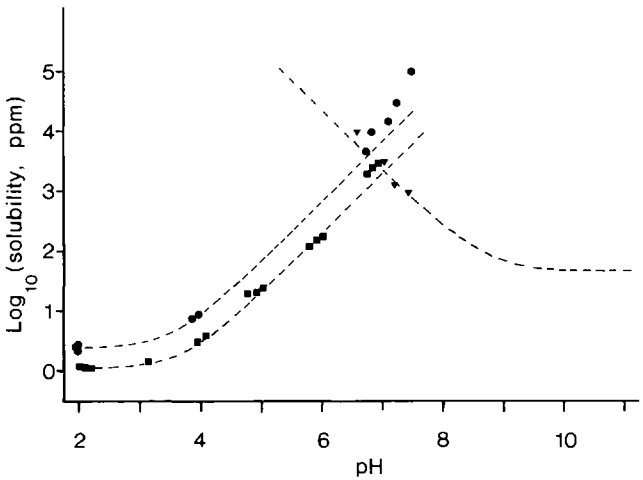


Figure 6.2 pH-solubility curves for: LM 985 at 37°C (▼); LM 975 at 20°C (■) and 37°C (●).

BZQ (2,5-di-N-aziridinyl-3,6-di-(N-2-hydroxyethyl) benzoquinone)

BZQ (Fig. 6.1b) degrades, especially in acid but also in alkali, by aziridine ring-opening to the tetra (N-2 hydroxyethyl) benzoquinone; maximum stability in solution occurs at pH 9·2 (Fig. 6.3), but the degradation rate remains high and precludes aqueous formulation. It is also unstable in all biocompatible mixed-solvent systems, and polymerises on freezing of aqueous solutions (at rates dependent on the additives present and on pH and rate of cooling, but not apparently on rate of warming or thawing). A lyophilisate was developed by incorporating (in solution with BZQ) a series of biocompatible polymers which inhibited its polymerisation, the inhibition being measured by assay of the compound and particle counts after repeated freeze–thaw cycles at freezing rates similar to those encountered in a freeze-drier (Fig. 6.4). Dextrans were finally chosen, combining good biocompatibility with efficacy as polymerisation inhibitors and stabilisers. The lyophilisate incorporated bicarbonate–carbonate buffer to provide a biocompatible pH near the stability maximum on reconstitution. The *in vitro* degradation profile of the freeze-dried BZQ on storage (Fig. 6.5) was characteristic of closed systems in which a finite reservoir of reactants (i.e. secondary water) is depleted; the degradation of untreated BZQ raw material in sealed tubes showed the same type of profile, with degradation rates increasing with decrease in particle size, suggesting a solid-state surface reaction [26].

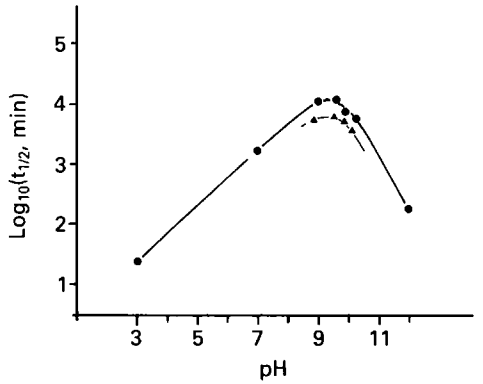


Figure 6.3 pH stability profile of BZQ in solution in: 1·26% $NaHCO_3$ (●); 0·16% $NaHCO_3$, 5·4% dextran 70 and 4·5% dextrose monohydrate (▲).

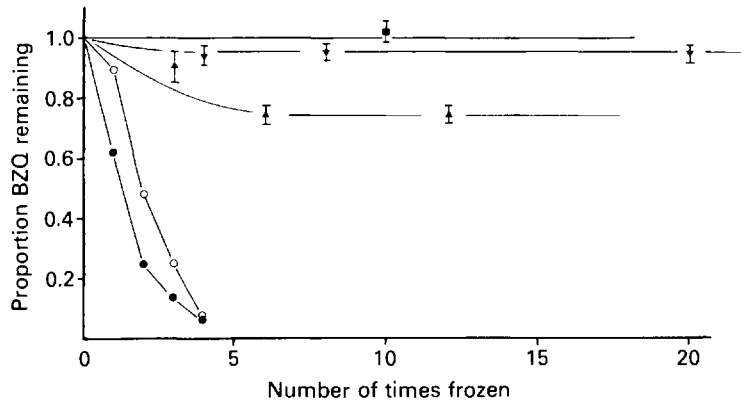


Figure 6.4 Stability of BZQ on repeated freezing (20 to −30°C in 30 min) and thawing (−30 to 20°C in 2 min) 0·1% w/v solutions in: 1·26% $NaHCO_3$ at pH 9.0 (●) and pH 10·0 (○); 1·26% $NaHCO_3$ and 0·5% PVP (polyvinylpyrrolidone, K 30–35 grade) at pH 10·0 (▲); 1·26% $NaHCO_3$ and 6% PVP at pH 10·0 (■); 0·13% $NaHCO_3$, 4·5% dextran 70 and 4·5% dextrose monohydrate at pH 9·5 (▼). The pH was adjusted with 0·1 M NaOH.

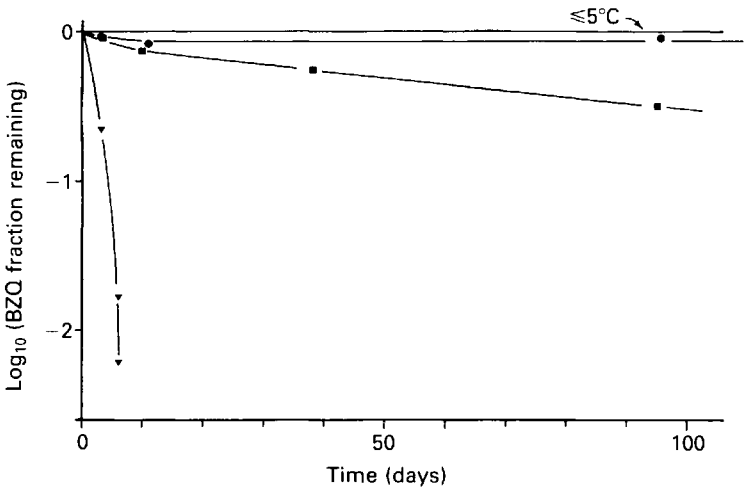
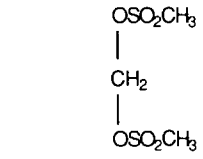


Figure 6.5 Stability of freeze-dried BZQ on storage at: ≤5°C (top horizontal line); 20°C (●); 37°C (■); 55°C (▼).

MDMS (methylene dimethanesulphate)

A busulphan analogue, MDMS (Fig. 6.6a) is unstable in aqueous solution (half-life 30 min at 37°C and about 70 min at 20°C, invariant with pH but varying slightly with ionic strength). It is also unstable in alcohols and in a wide variety of polar, biocompatible, organic solvents. It was formulated as a lyophilisate prepared from a solution in DMSO, in which it is sufficiently stable to permit production runs. The freeze-dried product (100–200 mg) is reconstituted in two stages, first with dimethylformamide and then made up to volume with water or saline. The acceptable solubility of MDMS in DMSO solved some scale-up problems, and the slower rate of evaporation of DMSO during lyophilisation was compensated for by the smaller volume of DMSO required. It is also possible to prepare vials of reactive compounds like MDMS by vacuum evaporation from solutions in ketones or esters, but although sometimes better aesthetically than DMSO formulations, these possibilities require a freeze-drier conveniently capable of coping with the evaporation of flammable solvents (Pirani vacuum gauges can ignite flammable solvent–air mixtures!). A formulation of MDMS prepared by vacuum evaporation from acetone solution had a better assay and superior stability to the DMSO lyophilisate, but the product proved unsuitable because it discoloured soon after manufacture, possibly due to reaction with adsorbed acetone. Vacuum evaporation of MDMS



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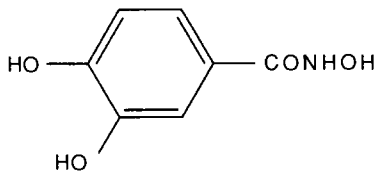


(b)

Figure 6.6 (a) MDMS; (b) Didox.

solutions in DMA produced a reasonably attractive and stable product, but the process took too long to be cost-effective; it was also difficult to see when the compound had fully dissolved during the first stage of the two-stage reconstitution procedure. Although MDMS reacted with dry DMSO (about 25% in 24 hours), no reaction products appeared (as detected by NMR) in the final freeze-dried product; the process was carried out on 2 ml aliquots of a 5% DMSO solution, all subject to a standard exposure of 25 min at 20°C before placing the trays of vials on pre-frozen shelves. The batch sizes (originally 120 vials in a small freeze-drier) were too small to allow the guidelines of the Joint Formulation Working Party on validation of the stability of individual batches to be followed; material supplied for clinical trial was checked by replicating the conditions of transport to, and storage (at −20°C) at, the clinical centres using only a small part of each batch produced (about 10 vials). The lyophilisate, which contained about 4% DMSO (detected by NMR), appeared stable over a period of at least 3 years at −20°C.

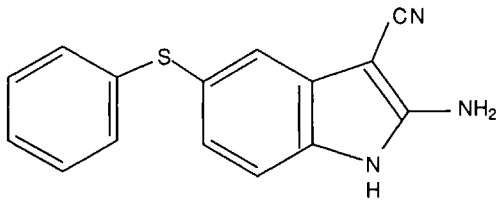
Didox (3,4-dihydroxybenzhydroxamic acid)

Didox (Fig. 6.6b) is a good example of a case where a minor reaction is stability-limiting rather than the main mode of degradation. A liquid-fill formulation (20 mg/ml) was prepared in saline, with ascorbic acid 0·02% w/v as stabiliser (antioxidant). Although purged with inert gas during manufacture, very slight irreproducibilities in residual oxygen content resulted in a slight within-batch variation in rate of discoloration of the product. On the basis of 'main peak' HPLC assay this formulation was apparently very stable (calculated to take a few years at 4°C to degrade by 5%). However, although the main mechanism of didox degradation is hydrolysis to protocatechuic acid and hydroxylamine, pharmaceutically-speaking the most important degradation mechanism is the relatively slow (by a factor of 100) formation of insoluble dark oxidation products of unknown structure. This precipitate amounts to only a few parts per million by weight of didox but results in compendial limits for particles in injections to be exceeded after only 3 months or so, after which time in-line filters are necessary when injections are to be used clinically. As is typical of catechol derivatives, the 'main peak' assay remains good even when oxidation results in a black mirror of a precipitate! This oxidation process can be much reduced by freeze-drying the product (25 mg/ml in water) without antioxidant or other excipients, and reconstituting it to isotonicity in hypotonic saline without antioxidant. Didox is administered i.v. as a 0·3% w/v solution over 12 hours. As with all compounds administered in this way, compatibility of the solution

with the giving set under the conditions of administration has to be demonstrated in advance [27]. Generally, it is sufficient to carry out a stability study on the injection solution stored in the giving-set bag over 24 or 48 hours, and also analyse samples from successive 50 ml aliquots taken throughout a complete simulated administration; up to 12 mg of didox from a 3 g/l saline infusion were lost either by adsorption to or diffusion into the plastic material of the giving set (but this proved too small a loss to affect the trial).

Amphethinile

Amphethinile (Fig. 6.7a) is a tubulin binder that combines high dose with very low aqueous and mixed-solvent solubility. It is very soluble in pure organic solvents (ethanol and, particularly, DMA and DMSO) but precipitates on dilution into or with aqueous systems, raising the potential for problems in use (precipitation on injection, for instance). The solubility of amphethinile in DMA or DMSO is greater than that in ethanol by an order of magnitude, but there is no advantage in the former solvents because of the much higher positive



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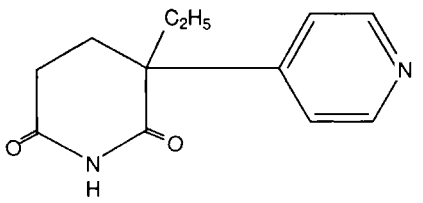
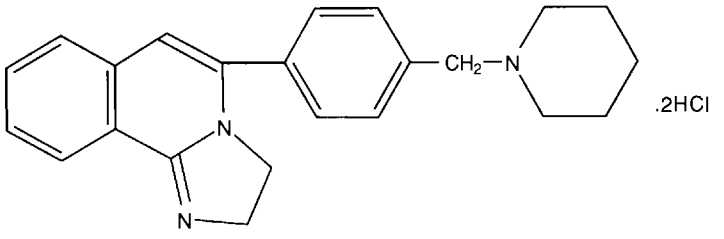


(b)

Figure 6.7 (a) Amphethinile; (b) Pyridoglutethimide.

deviations from ideal log–linear solubility with ethanol–water mixtures. The problem of precipitation and consequent phlebitis on injection was solved by formulating the compound in ethanol and Solutol HS15 (BASF); a 5% solution of amphethinile in a 50% alcoholic solution of this new surfactant (a hypoallergenic Cremophor EL analogue prepared from 12-hydroxystearic acid instead of castor oil, and used previously only in veterinary products), further diluted at least 50 : 1 prior to administration, was acceptable (precipitation of amphethinile on dilution is prevented by micellisation). It should be noted that strengthened ampoules may be required if alcoholic solutions are to be sterilised by autoclaving; a curious effect was noticed during accelerated stability studies of extremely stable compounds, such as amphethinile, in ethanolic solution—a significant loss of volatile solvent, sufficient to give a significantly higher assay, apparently occurred sometimes. The mechanism appeared to be molecular diffusion through the heat-sealed tips of the glass ampoules as a result of increased internal pressure, although the seals were apparently proof to conventional pressure ('dye diffusion') testing.



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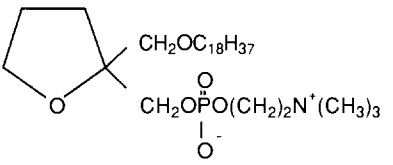

(b)

Figure 6.8 (a) SDZ 62 434; (b) SRI 62 834.

Thus in accelerated stability testing of formulations such as these the possibility of substandard ampoules should be eliminated by a system of individual ampoule weighing.

The required oral dose of amphethinile (> 1 g) was too high to administer as capsules or tablets, so an oral formulation was prepared in the form of an orange-flavoured suspension of the finely-divided compound (prepared by precipitation from solution in propylene glycol). Further development of this formulation is required, as bioavailability was apparently variable and highly particle size-dependent, due to the very low aqueous solubility of amphethinile (1·9 ppm at 20°C).

Pyridoglutethimide

The free base of pyridoglutethimide (Fig. 6.7b) is slightly soluble, but the compound is stable and soluble as the hydrochloride salt and has been formulated as such (40 mg free base/ml). The pKa of pyridoglutethimide is about 4·3, so the hydrochloride salt formulation which is adjusted to pH 2·7 contains a small quantity of free base. However, such a pH is not ideal and since the free base has good oral bioavailability, pyridoglutethimide has been formulated as such into both capsules and tablets for Phase I and II studies.

SDZ 62 434 (Sandoz)

SDZ 62 434 (Fig.6.8a) is a rare example of a very stable, highly soluble compound of high potency, the formulation of which, therefore, does not require a high loading, nor the application of most of the preformulation programme outlined earlier. It was more difficult to formulate this compound as a lyophilisate than as a liquid-fill preparation; the freeze-dried plug prepared from aqueous solution appeared to be a solid solution of water in amorphous compound, and development of a crystalline lyophilisate would have required extensive development with mixed-solvent systems. It was much easier to prepare a stable aqueous parenteral formulation, at a concentration of 50 mg/ml, in a very low-capacity buffer at pH 4·5. Stability was verified with simultaneous stability studies at pH 4·3 and 4·7; the compound appeared to associate in solution, giving a lower osmolarity than theoretically expected.

SRI 62 834 (Sandoz)

SRI 62 834 (Fig.6.8b) is a phospholipid analogue with interesting physical chemistry. As it lacks glyceride ester linkages it is stable in

solution. It has a very high temperature coefficient of solubility but, peculiarly, after the compound was precipitated from aqueous solution by cooling it was difficult to redissolve. It was formulated as an isotonic heat-sterilised solution in citrate buffered saline at pH 4·8. The compound is a surfactant, apparently micellising or associating in solution; at the formulation concentration of 25 mg/ml, its osmotic characteristics were of a compound of about five times its molecular weight.

Compound No. 9 (Uniroyal)

This compound was only very slightly soluble in aqueous mixed-solvent systems, highly reactive and unstable in most biocompatible organic solvents, including DMSO. There was a concentration-dependent reactivity with alcohols: at low concentration (0·1%) there was slow degradation (10% in 2 months at 20°C), but at high concentration (10%) a second, much more rapid, reaction occurred with the alcohol. This rapid reaction is catalysed by an acidic breakdown product of the first reaction, resulting in an apparent increase in the linear rate of degradation due to build-up of the catalytic acidic intermediate; the degradation appeared to be inhibited by small quantities of water (Fig. 6.9). As Compound No. 9 is a liquid, it was most easily formulated as the plain compound, without excipients, filtered through inert (PTFE) filters and packed in ampoules under an inert gas blanket. The compound degraded rapidly in the presence of small quantities of impurities and in the presence of air, even at −20°C, but if pure and stored under nitrogen or argon it appeared stable for years, even at 37°C. Although degradation in alcohol is rapid (30% degraded in 3 hours and 70% in 8 hours), the formulation was reconstituted from the pure material in ampoules by a two-stage process: first with ethanol to prepare a 44% w/v solution, then secondly by incorporation into Intralipid 20% (Kabi Pharmacia), a total parenteral nutrition emulsion, to produce a final concentration of 4% w/v in the emulsion [28, 29]. The latter process was carried out using an ultrasonic probe, applied for the 2 min during which alcoholic solution was added to the Intralipid 20%, and for a further 2 min after addition. The reaction in alcohol, although rapid, resulted in less than 1% degradation at 20°C if the addition to Intralipid 20% was carried out within 8 min of preparing the ethanolic solution. Stabilisation of the compound in the emulsion is by preferential partition of both the reactive alcohol and the water-soluble acid degradation product (catalyst) into the aqueous (continuous) emulsion phase, while the compound is protected by migration to the anhydrous

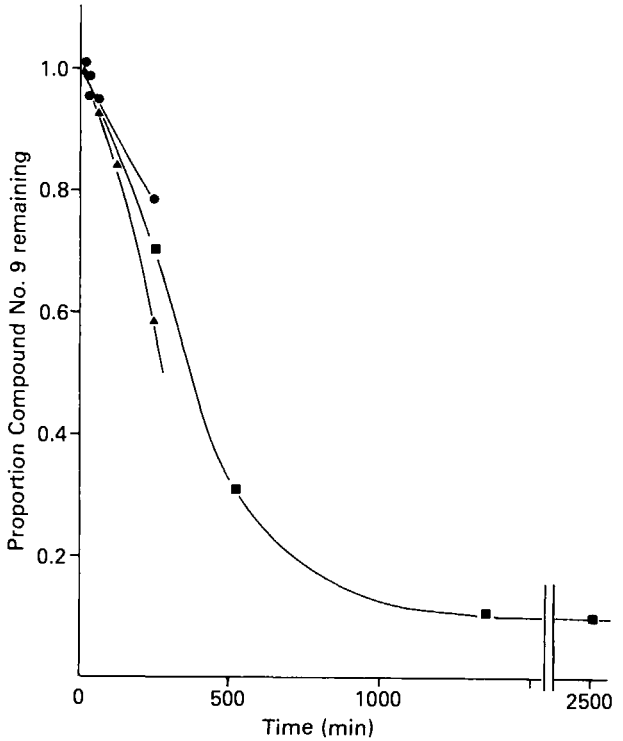

Figure 6.9 Degradation of Compound 9 in: ethanol (▲); ethanol containing 0·2% water (■) and 0·5% water (●).

disperse phase. Validation of this formulation required:

(1) Evolution of the SOP for reconstitution by examining the emulsion droplet size (e.g. by laser light diffraction) before and after incorporation of the alcoholic solution of compound. The measured increase was insignificant and consistent with the theoretical increase (of about 5%) in droplet diameter expected due to loading with drug (assuming negligible droplet coalescence).

(2) Examination of changes in droplet size and distribution over a period of time after reconstitution, to demonstrate a stability sufficient for parenteral administration. The emulsion appeared very stable at room temperature, with no measurable changes in droplet size or distribution being detected after about 3 days. However, after

this period of time the emulsion was observed to suddenly destabilise and crack over a period of only a few hours, large droplets (of about 1 mm, cf. 1 μm) rapidly forming two distinct phases; this may be a zeta potential effect caused by adsorption of breakdown products of the compound. The cracking was accelerated by increased temperatures and deferred by cooling, but the emulsion also cracked after freezing.

(3) Proof of chemical stability over a reasonable period: degradation at room temperature was less than 5% over 3 days—thus the stability of the reconstituted formulation was limited by physical rather than chemical factors.

(4) Experiments on scale-up: the effects of changes in batch size on droplet size were tested. The SOP which evolved was originally designed for 100 ml volumes made up immediately prior to injection, but during preclinical toxicology a potential 'scale-down' problem emerged in that it was impossible to use an ultrasonic probe on much smaller volumes of injection.

Compound No. 805

The dose of this compound is about 2–3 g, but it is only slightly soluble (4 mg/ml) and highly unstable in aqueous solution, presenting problems of reconstitution. The rapid aqueous degradation occurs partly by side-chain scission to formaldehyde and partly by polymerisation. An attempt was made to formulate Compound No. 805 by freeze-drying aqueous solutions with mannitol as a filler; however, on scale-up the process was severely limited by the rapid rate of degradation both during and after dissolution, and also by the slow dissolution rate. Further attempts (at another centre) to formulate the drug by freeze-drying with low molecular weight polyethylene glycols (PEGs) as solubility enhancers failed because of the very low eutectic temperatures and extremely long freeze-drying times of PEGs. Also, all the freeze-dried aqueous formulations exhibited very short shelf-lives, apparently due to solid-state polymerisation of the drug by secondary absorbed water (which though present at only about 1% w/w of the compound, was sufficient to catalyse the reaction), which could not be removed by even protracted secondary drying at high temperatures. Subsequent approaches involving freeze- drying solutions in DMSO, in which the compound is soluble and stable, were successful, producing a lyophilisate free of water and affording a good shelf-life. Unfortunately the DMSO lyophilisates were unsuitable for direct reconstitution with an aqueous vehicle due to their low solubility; therefore reconstitution required a two-stage process similar to MDMS above—first, dissolution with either DMA

or DMSO (3 ml/g of drug), followed by addition of aqueous diluent. In this case the drug dose was thus limited (fortunately to slightly above the clinical trial level) by excipient toxicity rather than by instability on reconstitution.

Compound No. 807

This phenolic compound was of low potency (high dose) and of very low solubility. A common characteristic of phenols is a pKa value of about 10, which precludes, on pH grounds, the use of salt formation to enhance solubility for parenteral purposes [6]. Compound No. 807 was more soluble in DMA and DMSO than in alcohols, but larger positive departures from ideal log–linear solubility afforded no advantages to these solvents. In fact, it proved impossible to load any mixed-solvent or surfactant system with a sufficient concentration of compound to allow a preclinical trial that was not limited by formulation (rather than drug) toxicity. A neutral solubilisate in nicotinamide solution produced an unexpected synergistic toxicity of the nicotinamide, and complexes in N-methyl glucamine and lysine had too high a pH and buffer capacity; cyclodextrins also proved ineffective [30]. However, the solubility profile of the compound made it suitable for processing through the Violante Medisperse System—a patented method of preparing precipitates of very fine, controlled, particle size by adding mixed solvent-surfactant solutions of the compound to aqueous diluents.

Springer prodrug

The concept of a freeze-dried formulation for this compound was developed at the United States National Cancer Institute. It was the first case of a compound which cannot be handled because of its instability, being processed for freeze-drying as a stable precursor, the ditertiary butyl ester, the active form being produced in the process.

The ditertiary butyl ester is dissolved in pure formic acid and incubated at 10°C for 48 hours, which hydrolyses the two ester groupings from the precursor to give the free acid. This acidic solution is then freeze-dried to give pure free acid. A certain amount of formulation development is required for this product: the secondary drying temperature must be sufficient to drive off residual water (present at about 1–2% in analytical grade formic acid), which will reduce shelf-life if present, but not so high as to degrade the product at the manufacturing stage. For Phase I the product was freeze-dried at a shelf temperature of 0°C from a fill-volume of 100 ml in a 125 ml

vial, with secondary drying at 30°C for 20 hours. The compound is very hygroscopic and rapidly degrades, physically and chemically, in the presence of even small quantities of water; thus the formulation development process will also involve a suitable choice of freeze-drying container and stopper, with, if possible, vial closure under dry inert gas.

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