THE CLINICAL AND PHARMACOLOGICAL EVALUATION OF NEW CHEMOTHERAPEUTIC AGENTS

DAVID BALFOUR SMITH

A THESIS SUBMITTED TO THE UNIVERSITY OF EDINBURGH FOR THE DEGREE OF M.D.

1987

Declaration

I declare that this thesis was composed by myself and that all the work described was my own with the exception of technical assistance with a proportion of the Idarubicin assays. Furthermore no part of this thesis has been submitted in canditature for any other diploma, degree or professional qualification.

Signed :

Contents

Acknowledgements

Professor Derek Crowther for help and encouragement and allowing me to undertake this work in his department.

Dr. Nick Thatcher, Dr. Howard Scarffe and Dr. Tony Howell who allowed their patients to be entered in the clinical trials.

Dr. Peter Wilkinson and Dr. Sam Lucas for assistance with pharmacokinetic analyses.

Jennifer Margison for help with Idarubicin assays.

Professor Brian Fox and all members of the Department of Experimental Chemotherapy, Patterson Institute for Cancer Research, for tolerating my presence in their laboratory.

Colin Ewan for help with treating mice with amphethinile.

The Christie Hospital library staff

The Cancer Research Campaign and Farmitalia Ltd. who provided financial support.

Abstract

Early clinical trials with new chemotherapeutic agents must be performed quickly and efficiently in a setting that allows a low probability of erroneously rejecting an active agent.

A phase I clinical trial was conducted with methylene dimethane sulphonate. The MTD was 225mg/sqm which was reached in 7 escalation steps using the modified Fibonacci series. The dose limiting toxicity was cumulative thrombocytopenia. Neutropenia was mild but 1/3 of patients at higher doses (100mg/sqm - 225mg/sqm) required blood transfusions during treatment with MDMS. Non-haematological toxicity consisted of grade 0-2 nausea and vomiting of short duration, and increasing degrees of alopecia at doses above 100mg/sqm. The doses recommended for phase II testing were 125mg/sqm in patients who had received previous chemotherapy and 150mg/sqm in those who had not.

During the MDMS study I became concerned at the numbers of patients treated at doses with little or no chance of therapeutic activity. Dose escalation schemes for phase I clinical trials of new chemotherapeutic agents have traditionally been rather conservative with the result that over 50% of patients receive doses below those subsequently recommended for phase II testing. A survey of phase I studies published between 1971 and 1986 suggested that the dose of drugs showing no toxicity at the starting dose (n) could immediately be increased to 5n with consequent saving of two dose steps. It was decided to combine this idea with those of Dr. Collins (Collins et al, 1986) who suggested the use of pre-clinical pharmacology data to guide dose escalation in phase I clinical trials.

A phase I clinical trial of Amphethinile using an intermittent iv

schedule was conducted. The dose escalation scheme incorporated both the ideas outlined above. An HPLC method was developed to measure amphethinile in serum and urine and the AUC at the LDl0 in the mouse was found to be 3l0ug/l.h-l. This was adopted as the target AUC for the trial. The MTD was 1200mg/sqm, thirty times the starting dose. This was reached in four escalation steps, a saving of five steps compared to the modified Fibonacci series. Methods such as these for increasing the speed of escalation therefore appear to be of practical value.

The dose limiting toxicity in this trial was neurotoxicity consisting of light headedness, lethargy and a sense of detachment. It is likely that some of the toxic effects seen at higher doses were due to the Solutol vehicle and it was felt that the drug could not be recommended for phase II testing using this schedule.

A phase II and pharmacokinetic study of 4-Demethoxydaunorubicin administered orally was conducted in patients with advanced breast cancer. These patients had had no prior chemotherapy but the protocol included an option for early cross-over to Adriamycin in the event of progression. Comparison of the survival of patients in this trial with those in previous trials of conventional chemotherapeutic regimens at this institute showed no disadvantage for the 4-DMDNR treated patients. The objective response rate in the trial was 15% and there was no evidence of alteration in the kinetics of 4-DMDNR with continuous weekly therapy.

In conclusion the efficiency of phase I clinical trials can be substantially improved using the methods outlined above. In addition the data from phase II studies may be improved by using new agents first-line with early cross-over to an active regimen on progression.

List of Tables

List of Figures

17 Alteration in kinetics of Amphethinile with 50% dose reduction in patient FL

18 Plasma decay curves of Amphethinile in patients receiving 1200mg/sqm

19 Plasma decay curves of Amphethinile adminstered orally and intravenously to patient JB

20 Ultraviolet absorbance scan of Amphethinile

21 Plasma decay curves of Amphethinile in mice

22 Amphethinile chromatogram

23 Plasma decay curves of Amphethinile in man

24 Plasma decay curves of Amphethinile metabolite in mice and man.

Introduction

The concept of curative drug therapy for human disease dates back to the use of cinchona bark (containing quinine) and ipecac (containing emetine) for the treatment of malaria. These remedies were discovered in the seventeenth century and were introduced into Europe from South America by the Jesuits. In a situation not dissimilar to that of cancer treatment today they were used without knowledge of the cause of the disease, nor of the identity or action of the active principle. Further progress in the treatment of infectious diseases was not made until the turn of the 19th centuary when Paul Ehrlich discovered the anti-parasitic properties of arsenicals. From 1903-1915 Ehrlich devoted much of his attention to the development of these agents. He synthesised a long series of organic arsenic compounds and developed animal models to test the effect of these drugs. The 606th of these arsenicals was highly active against trypanosome infections and rabbit syphilis. Ehrlich named this drug Salvarsan, "saviour of mankind". It was the first man made chemical shown to be active against human parasitic disease and he coined the term "chemotherapy" to describe such a compound.

The first indication that a systemic treatment might also be used for the treatment of cancer was the demonstration by Lissauer in 1865 of the anti-tumour effect of potassium arsenite (Fowler´s solution). Ehrlich was also employed to work on the problem of cancer and although he discovered the first alkylating agent, ethylenimine, in 1898 nearly half a century passed before similar compounds began to be used in the treatment of human cancer.

The modern age of cancer chemotherapy may be said to begin with the work of Huggins and Hodges in the late 1930s. Their observations on serum acid phosphatase levels in patients with carcinoma of the

prostate provided a rational basis for the use of endocrine manipulations in this disease (Huggins and Hodges 1941). This was the first example of the use of a "marker" to follow the effect of a systemic therapy in the treatment of cancer.

The gas warfare programmes of the two World Wars provided the next great stimulus to cancer research when it was noticed that members of the armed forces dying of mustard gas poisoning developed marked aplasia of the lymphoid organs. In the early 1940´s mustard gasses were submitted for study to Goodman and Gilman at Yale university. In a series of experiments on rodents they demonstrated that these chemicals had profound effects on the lymphatic and haemopoetic systems. In addition Dr Dougherty at the same institution showed that nitrogen mustards could produce remissions in spontaneously occurring leukaemias and lymphomas in rats. These observations suggested that nitrogen mustard might be of some value in the treatment of leukaemias and lymphomas in man. The first patient, a terminal case of lymphosarcoma, was treated at the New Haven Hospital in 1941 with a dramatic regression of involved glands. Subsequently nitrogen mustard was used in the treatment of a group of 67 patients suffering from a variety of neoplastic diseases including lymphosarcoma and Hodgkin´s disease. A number of encouraging responses were seen in this limited study but because of the secrecy surrounding the war gas programme the results were not published until 1946 (Goodman et al 1946). Additional studies by Alpert and Peterson (1947) and Dameshek et al (1949) confirmed that remissions could be induced by the nitrogen mustards particularly in the lymphoid malignancies. Unfortunately the duration of these early remissions was short but despite this there was hope that advanced

cancer might soon be curable with drug treatment.

Shortly afterwards in 1947 Sidney Farber noticed that the administration of folic acid conjugates to children with acute leukaemia appeared to induce an acceleration in the leukaemic process (Farber et al 1947). This observation led Farber to experiments with folic acid antagonists in the treatment of acute leukaemia firstly using pteroyldiglutamic acid and subsequently the more powerfull antagonist aminopterin (a forerunner of Methotrexate). Again these early studies produced encouraging results with 10/16 patients showing some response to aminopterin therapy (Farber et al 1948).

Following these early successes the search began in earnest to identify other chemicals with anti-tumour activity. The chemicals investigated were either natural products from plants or micro-organisms or synthetic compounds. Some compounds were synthesised and screened because they bore a structural similarity to agents of known activity ("rational" approach) others were merely included in a random screening programme ("empirical" approach). Regardless of source every anti-cancer agent currently in use was first tested clinically on the basis of activity in animals bearing malignant tumours (Venditti 1975, Gellhorn et al 1955, Goldin et al 1979, Goldin et al 1980). The development of transplantable animal tumours in syngeneic hosts had been pioneered by George Clowes at Roswell Park Memorial Institute, and four of the more widely used transplanted tumours, sarcoma 37, sarcoma 180, Walker 256 and Ehrlich ascites tumour were developed before 1930. One of the earliest screening programmes was set up by Shear of Harvard University in the 1930s (Shear et al 1947) to test and isolate bacterial polysaccharides capable of producing haemorrhage and necrosis in

sarcoma 37.

With the stimulus provided by the war research and the work of Shear and Huggins and Hodges, the end of hostilities ushered in a new era of intensified interest in cancer research. Drug development programmes were instituted at the Sloan-Kettering Institute for Cancer Research under Rhoads, at the Chester Beatty Institute in Great Britain under Haddow and at the University of Tokyo under Yoshida. The Sloan-Kettering Institute rapidly emerged as the dominating force in cancer chemotherapy. Sarcoma 180, a tumour which grows in out-bred mice and is therefore suitable for mass screening, was established as the standard screen. By 1955 approximately 20,000 chemicals had been tested and the Sloan-Kettering programme accounted for 75% of chemotherapy screening in the USA (Zubrod et al 1977).

In 1953 the US Congress first proposed the establishment of a National Integrated drug discovery programme and this led in 1955 to the setting up of the Cancer Chemotherapy National Service Centre (CCNSC) in Bethesda. (the NSC numbers of compounds tested in the NCI programme is a contraction of CCNSC).

One of the earliest tasks of the new National Cancer Institute in Washington was to decide which, if any, of the available biological systems could predict activity in the agents that had already found their way into clinical use. In 1953 the American Cancer Society commissioned a systematic study of the methods used in different laboratories for screening potential agents for anti-tumour activity and this led to a report in 1955 by Gellhorn and Hirschberg. After examining 74 different assay systems they concluded that no one system could identify all the agents then in current clinical use (nitrogen mustard, methotrexate, 6-MP, and actinomycin-D) and also

that in vivo systems could not be replaced by in vitro non-tumour models. As a result the NCI selected a panel of three murine tumours (L1210 leukaemia, sarcoma 180 and adenocarcinoma 755) for use in an extensive systematic screening programme. This programme continued for a decade until the mid 1960's during which time busulphan, chlorambucil, 5FU, the vinca alkaloids, cyclophosphamide, melphalan, hydroxyurea, mithramycin, Mitomycin-C, Procarbazine, Cytosine Arabinoside and Daunorubicin all entered clinical use.

In 1966 Goldin, Serpick and Mantel completed a retrospective analysis of the predictiveness of over 100 tumour models including those used in the NCI screen. They concluded that all but one of the clinically active drugs (mithramycin) would have been selected by the use of the mouse L1210 system and the rat Walker 256 carcinoma. As a result the NCI screen was reduced to these two tumours. However it soon became clear that the Walker model, being particularly sensitive, was identifying too many agents (ie had too low a threshold for activity). This tumour was therefore replaced by another murine tumour, the P388 leukaemia, which was less expensive than the rat Walker tumour and more sensitive than L1210. Over the next 5 years there were a number of changes and new models were examined until a new procedure was adopted in 1975. By this time BCNU, streptozotocin, DTIC, asparaginase, bleomycin, and Adriamycin had been added to the clinical armoury but this represented a small return for the annual screening of 30-40,000 compounds.

The new system involved a "pre-screen" using P388. Any compound achieving a certain level of activity in this screen (greater than 20% increase in life span) went on to be tested in a panel of

transplantable tumours representing the major histological types of cancer in the western world. The panel consisted of mouse colon (Colon 38), breast (Mammary Ca CD8F1) and lung (Lewis Lung) tumours, human tumours of the same histological types (Mammary, MX-1, Lung, LX-1, Colon, CX-1) xenografted in athymic nude mice, and two mouse tumours (L1210 and B16 melanoma) which had been found to be of particular value in the past. In addition, where indicated by prior information, eg particular organ specificity of a compound, other ad hoc systems were also employed. If a compound showed activity in any of these eight tumours it then became a candidate for pre-clinical toxicology and subsequent clinical trial. It was hoped that the use of screening models that more closely resembled human solid tumours in histology, metastatic pattern and growth kinetics would improve the detection rate of compounds active in these tumours in the clinic. Moreover results from clinical trials of the agents discovered should provide information on the extent to which a pre-clinical model can predict preferentially for clinical efficacy against human tumours of the same histological type.

At the outset of this tumour panel experiment the impracticality of screening many thousands of compounds against eight tumours was recognised. Thus although some compounds were passed straight to the panel based on "intellectual selection" from structural pattern recognition (Hodes 1981, Hodes 1981a) or some known biological activity, the majority of compounds were subjected to the P388 pre-screen. In retrospect this had the major disadvantage that this screen would continue to detect drugs active in a similar range of tumours to those already discovered rather than agents with a different spectrum of activity. Analyses of the results of the

tumour panel from 1976-1982 by Venditti et al (1984) and Staquet et al (1983) confirmed that the pre-screen had a low false -ve rate of approximately 1% (the low end point - increase in life span greater than 20% - was selected for the pre-screen in order to limit false negative results) and in addition there was a close correlation between the quantitative level of pre-screen activity and the probability of activity in the panel. Further analysis showed that 95% of compounds active in at least one panel assay were active against L1210, B16 or MX-1 and thus it was suggested that the panel be reduced to these three tumours with consequent major cost savings.

However during the past 2-3 years the shortcomings of screening programmes relying entirely on transplanted tumours in mice were becoming ever more apparent. Since 1955 over 600,000 natural and synthetic substances have been tested in the NCI system with a very low yield of clinically useful drugs. Only 12 of the currently available anti-cancer drugs have been developed under direct NCI sponsorship. These include Mithramycin, BCNU, CCNU, DTIC, Cytosine Arabinoside, Bleomycin, Asparaginase, Adriamycin and Cis-DDP. Moreover since the introduction of Cis-platin in 1973 and VP16-213 in 1976 no new agents have made a significant impact on improved survival in any malignancy. Furthermore the emergence of a number of agents, eg Mitozolamide (Newlands et al, 1985) with excellent preclinical activity which later proved to be inactive in the clinic was particularly disappointing and raised serious doubts about the value of the animal based systems used in the selection of anticancer drugs. As a result of these misgivings several organisations including the European Organisation for the Research on Treatment of Cancer (EORTC), the Cancer Research Campaign Phase I/II group and

the NCI itself are now in the process of changing their screening programmes in a radical manner.

The panel of murine tumours has been discarded concentrating instead on the use of specific disease-orientated groups of human tumour cell lines in complementary in vitro and in vivo test systems. Panels of cell lines representing the major categories of human cancer and including multi-drug resistant lines are being acquired and developed and methods of automating the drug testing explored. This latter facet of the programme is essential since it is envisaged that eventually each drug will be tested in 100 cell lines at several different concentrations. The tests are carried out as growth inhibition assays in plates of mico-wells with extensive use of computer controlled robots to prepare the plates and of computers to record and analyse data.

It has been decided that drugs showing 3 log differences of response in different cell lines are of interest whereas those demonstrating uniform activity or inactivity are not. Such "interesting" compounds will then be tested in a range of human tumour xenografts. At present the system is able to cope with approximately 20 compounds per week tested in 50 cell lines although it is hoped to increase this to 50-100 compounds per week. Thus even with the degree of automation described above the system will only be able to put through 25-50% the volume of compounds previously managed by the P388 screen. However it is hoped that this radical departure will allow the identification of new classes of anti-tumour agents with activity against the common solid tumours.

The use of in vitro screening has relied on the development of the human tumour stem cell assay (HTSC) (Salmon et al, 1978; Von Hoff

et al, 1981; Hamburger et al, 1978) in which cell culture colonies formed from freshly explanted human tumour tissue are tested for sensitivity to drugs. This has involved the establishment of growth of excised human tumours in conditioned soft agar culture media and determining the inhibition of colony forming units (CFU) with drug concentrations selected on the basis of clinical pharmacology. This is a rapid and inexpensive method compared with human xenografts. However a significant problem lies in the drug concentrations and times of exposure which with new compounds must be chosen arbitrarily. Some guidance on this might be provided by pre-screening against P388 in vivo. Ultimately the use of human tumour cultures as screens will depend on the ability to establish assays that are stable and reproducible. This might be achieved by transferring cells in vitro but they may lose their intrinsic heterogeneity in this way. Perhaps a more promising technique is to maintain the tumours in nude mice providing a constant source of cells for in vitro screening. Work with anthracycline analogues tested in this way has been reported (Guiliani et al 1980).

The establishment of human tumours in vivo has been achieved in two main ways. In the first method immune deprived mice, either genetically thymus deficient (the nude mouse) or thymectomised at birth, are used as recipients (Giovanella et al 1974). The "takes" in this system have been shown to vary considerably depending both on the origin of the tumour and the condition and strain of the animals used (Sharkey and Fogh 1984). The second method, the renal capsule method (Bogden et al 1978), uses the fact that the kidney is an immune sanctuary site and many tissues can be established by implantation just below the capule of the kidney of the mouse.

It is unlikely that a perfect non-human model of human cancer will be found and consequently all models will have limitations. Nevertheless pre-clinical models have played a major role in developing the currently available anti-cancer drugs (De Vita 1979). Finding new drugs will depend on continued progress in three areas of research; the elucidation of the principles underlying drug action and drug resistance; the continued improvement in the intellectual selection of compounds to be tested pre-clinically; and the continued development of improved pre-clinical models that have greater predictive value for the clinic. However the next two years may prove to be a watershed in mass screening of compounds for anti-tumour activity. The annual budget for the NCI drug development programme is currently 40 million dollars and if the new in vitro screening initiative does not show promise within 2-3 years the US government has threatened to withdraw funding.

Formulation of new chemotherapeutic agents

The formulation of a compound that appears promising in pre-clinical screening is an often underplayed but vital part of the development of the drug. All pre-clinical toxicology must be carried out with the formulated preparation that will eventually be used in the clinic and thus before a drug is presented for toxicology an appropriate formulation must be found. Failure to do this may result in expensive repetition of toxicology if a drug has subsequently to be reformulated.

The formulation should be relatively easy to manufacture in amounts suitable for early clinical trials and it must be stable under normal conditions of storage. In addition the chosen vehicle should itself be free from biological effects.

The main factors to be considered in developing a suitable formulation are the stability and aqueous solubility of the drug. Other factors to be taken into account include the pH, pKa and oil/water partition coefficient.

Solubility

Drugs that are administered intravenously must interact immediately with the predominantly aqueous environment of the body and thus water solubility is extremely important. A formulation must be found that allows the drug to be dissolved in a volume of aqueous solution that can be administered both safely and conveniently.

Drugs of low aqueous solubility may be rendered more soluble by first dissolving in polar solvents such as Dimethylsulphoxide (DMSO) and Dimethylacetamide (DMA). In some cases it may not be possible to achieve an aqueous solution and such drugs may be formulated as

molecular suspensions using surfactant materials such as Cremophor EL or Solutol HS 15.

Drugs for intravenous use that are soluble in water or polar solvents are normally presented as a freeze dried powder eg cytosar, cyclophosphamide, adriamycin, Cis-Platin, methotrexate, mustine, vincristine and others. Freeze dried preparations have the advantage of small volume and are normally very stable. Prior to administration they are reconstituted with the appropriate solvent, usually water or saline.

Aqueous solubility is less important for orally administered drugs and some relatively insoluble compounds such as Busulphan are well absorbed when given in this manner. These drugs however must be sufficiently lipophyllic to enable them to be absorbed across the cell membranes of the GI tract. The oil/water partition coefficient is a measure of a compound´s lipophylicity.

pKa/pH

In addition to lipid solubility the degree of ionisation is also important for absorption. It has been shown that unionised molecules diffuse preferentially across the GI mucosa. Acidic drugs will be predominantly unionised in the stomach and basic drugs predominantly unionised in the more alkaline environment of the intestine. However principal sites of absorption also depend on factors such as surface area of the relevant site. For parenteral drugs the pH at the time of administration should be close to physiological pH.

Stability

A drug must be sufficiently stable so that storage under normal conditions results in an adequate shelf life. In addition it is important to know the stability of the agent once any reconstitution procedures have taken place. Drugs that decay rapidly in aqueous solution will not be suitable for use as prolonged infusions. Stability to heat will affect the method of sterilisation of a product. Drugs that are unstable at high temperatures cannot be autoclaved and will have to be sterilised by other methods eg ultra-filtration. The effect of pH on stability is important for both oral and parenteral drugs. Acid labile drugs intended for oral administration must be protected from the highly acidic environment of the stomach. Buffer selection for parenteral drugs will be largely based on the stability characteristics of the drug.

An exhaustive review of the problems involved in formulating new drugs is beyond the scope of this thesis however it is hoped that this brief outline may serve as an indication of the complexity of the task. The importance of this facet of new drug development cannot be over estimated. A compound may be extremely active in pre-clinical testing but without an adequate formulation further progress to toxicology and clinical trial cannot take place.

Pre-clinical Toxicology

The aims of pre-clinical toxicology are to identify a safe starting dose for human trials and to characterise potential toxicities. However the requirements for the animal testing of new drugs has changed considerably over the years. In 1899 aspirin was approved for use in humans after toxicology had been performed in one fish and two frogs! Since those halcyon days a number of countries, notably the USA, have accumulated such rigorous mandatory pre-clinical testing requirements that the cost of these procedures has become a serious barrier to the introduction of new agents. In 1983 the cost of pre-clinical toxicology in the USA for a single drug was estimated to be $380,000.

The identification of a safe starting dose for phase I clinical trials has been made easier for anticancer agents by the recognition that the maximum tolerated dose (MTD) in terms of mg/unit surface area is surprisingly consistent between species. This fact was highlighted by Freireich et al (1966) in a study of 18 anticancer drugs in the mouse, rat, hamster, dog, monkey and man. As a result of this study it was concluded that on a mg/kg basis the maximum tolerated dose in man is about 1/12 the LD10 in mice, 1/9 the LD10 in hamsters, 1/7 the LD10 in rats, 1/3 the MTD in rhesus monkeys and 1/2 the MTD in dogs. They suggested that it would be reasonable to study pre-clinical toxic effects in the mouse, rat, dog, monkey and hamster, to estimate the MTD (mg/m^2) in man, and to start clinical trials at approximately 1/3 of this dose. This would have produced a safe starting dose for all 18 drugs involved in this study. However a subsequent analysis by Homan (1972) of the data of Freireich et al and that of Schein et al (1970) indicated that if

this rule was applied to all the drugs studied there was a 5.9% probability of exceeding the human MTD. In 1973 the NCI Laboratory of Toxicology revised its protocol for large animal toxicology (Prieur et al 1973) to provide values for the highest non toxic dose (HNTD), the lowest dose to produce toxic effects (toxic dose low or TDL) which when doubled caused no mortality, the lowest toxic dose which when doubled produces mortality (toxic dose high or TDH) and the LD which is the lowest dose to produce drug-induced death. The recommendation was that phase I clinical trials should commence at 1/3 TDL in the most sensitive large animal species.

In 1975 Goldsmith et al published an analysis of mouse, dog and monkey toxicity data on 30 drugs. They concluded that by using 1/3 TDL in the most sensitive large animal species 5 of the 30 drugs would have produced significant toxicity in the first patient. In addition they suggested that closer attention ought to be paid to rodent data since the LD10 and LD90 in mice were found to offer good quantitative prediction of human toxicity. However they concluded that the major emphasis should remain on the monkey and the dog.

In 1981 Rosencweig et al reported an analysis of toxicology for 21 drugs for which experimental results in mice and dogs and clinical results all using the same schedule were available. The data confirmed that mice and dogs are equally relevant for predicting the MTD in man. Thus the ratios of MTD in man to 1/6 LD10 in the mouse and of MTD in man to 1/3 TDL in the dog were similarly distributed. However for a limited number of agents there was a significant difference in the information provided by the mouse and the dog and thus a combination of data from both species could be useful for determining acceptable starting doses in man. In order not to lose

the economic and time advantages of concentrating largely on the mouse it was proposed to use 1/10 LD10 in the mouse (in no case in the drugs tested did this exceed the TDH in the dog) as the starting dose for phase I clinical trials providing this dose was entirely non-toxic in the dog. If toxicity was seen in the dog further work in this species would have to be done to determine the starting dose. During the same period similar initiatives were taking place in Europe with the exception that large animal testing has been completely dropped and the toxicity check of the mouse 1/10 LD10 dose is undertaken in the rat.

Thus it appears to be possible to arrive at a safe starting dose using a system involving only rodents. However it is important to know if exhaustive testing with large animals is more likely to alert the clinicians to the potential hazards of the drugs. The answer is no. It is now appreciated that marked inter-species variation exists in the sensitivity for certain drug side effects. Thus testing in the monkey and the dog failed to predict important toxicities such as cardiotoxicity in the anthracyclines and over predicted others notably renal and liver damage in various other compounds (Goldin et al 1980). It became clear that the predictive value of a test for any type of toxicity depended largely on the prevalence of that toxicity so that the predictability will be relatively high for common toxicities eg myelosuppression and relatively low for rarer ones. In a study of 25 anticancer drugs Rosencweig et al (1981) found that the predictive value for common toxic effects in man was greater than 0.85 compared to 0.05-0.54 for rarer side effects. Moreover there was no clear superiority of animal findings over the mere knowledge of the prevalence of these toxicities in man. Experienced clinicians

will already be anticipating the likely occurrence of common side effects and have at their disposal curative measures for the majority of these. In addition the close monitoring of patients in phase I clinical trials should alert the investigators rapidly to any unusual toxicities thus minimising harm to the patients.

In the light of these observations the requirement for exhaustive large animal histopathology prior to human use has been dropped in some countries. In Europe pre-clinical toxicology now relies entirely on testing in mice with a check that the dose selected for phase I study is entirely non-toxic in the rat. This concentration on rodents for pre-clinical toxicology allows testing to proceed much more rapidly and at greatly reduced cost than was previously possible. The cost of this procedure for the most recently tested drug in the CRC programme completed in May 1986 was approximately $20,000. However despite the scientific and economic arguments in favour of a rodent based toxicology programme the FDA in America still requires large animal testing to be performed.

In summary it has been established that animal toxicology is a relatively inefficient predictor of unusual toxic effects in man and that extensive pre-clinical testing is therefore not cost effective. In addition all animal species tested including rodents are similarly accurate in quantitative prediction of toxicity in man and therefore the use of mice for determining the starting dose for phase I clinical trials provides a major saving in both time and money. In Europe the present policy is to use 1/10 LD10 in the mouse as the starting dose, provided this is totally non toxic in the rat.

The Phase I Clinical Trial

As has been outlined in the previous sections a great deal of time (often over 10 years), money and effort is involved in bringing a new compound through to clinical trial in man. However once the clinical trial commences most of the pre-clinical data becomes irrelevant and the performance of the drug in terms of toxicity and therapeutic effect determines future development (Von Hoff et al 1977).

The main (some would say only) aims of the phase I trial are to determine the spectrum of toxicity of the agent in man and to identify a suitable dose for phase II testing. A further goal is to record any antitumour activity although at this stage of drug development this is of lesser importance (Carter 1977, Schein 1977, Gottleib 1974, Williams and Carter 1978, Karon et al 1973). These three basic aims of the phase I trial can be stated as follows:

(1) To establish a safe starting dose for phase II trials for a new drug on a given schedule via a given route of administration both for previously treated and previously untreated patients.

(2) To determine the qualitative toxicity (organ systems involved) as well as the quantitative toxicity (predictability, grade, duration and reversibility).

(3) To look for any antitumour activity of the drug.

All phase I studies should achieve these aims and in addition where the appropriate assay techniques are available the collection of basic pharmacokinetic data is becoming increasingly important.

In the past phase I trials have attempted to determine the maximum tolerated dose (MTD) of a drug for a given schedule. A difficulty arises however in the definition of MTD. Some investigators describe

it as "maximum deliverable" dose which is not associated with lethality while for others it represents the first evidence of "treatment-limiting toxicity" (Wooley and Schein 1979). To an extent the maximum deliverable dose will depend on the supportive care that is available. For example it is now possible, with the advent of autologous marrow transplantation, to administer considerably higher doses of alkylating agents than was previously possible. In practice such attempts to further escalate doses is only carried out with drugs of proven value when used under conventional conditions. A further point about the MTD is that it will almost always be higher for patients who have not received prior chemotherapy. This applies particularly to myelosuppressive agents. In recent years previously untreated patients have been increasingly entered in phase II studies of diseases where existing therapy is of little or no benefit. Thus it has now become standard practice to define a maximum safe dose for phase II testing for both types of patient.

In practice the dose limiting toxicity for the majority of drugs is myelosuppression and in this case reversible grade 3 WHO (WHO 1979) toxicity (WBC 1.0-1.9 x 10^9/l, platelets 25-49 x 10^9/l) represents an acceptable starting point for phase II trials.

For agents which are non-myelosuppressive the end point for the phase I trial can be much more difficult to determine and is not usually dependent on the extent of prior chemotherapy. In such cases comparison of clinical pharmacokinetics with preclinical data at antitumour doses in animals may provide a valuable guide to the target dose for the trial.

Patient Selection

Patients entered in phase I studies must be carefully selected if the quality of the data generated by the trial is to be high. The experience of the investigator is often the best arbiter as to whether a particular patient will be suitable for the study. This is especially true when assessing performance status and life expectancy. For example a patient with relapsed small cell lung cancer may have a performance status of 1 (WHO) but a life expectancy of only a few weeks whereas a patient with liver metastases from colon carcinoma may have a similar performance status but a life expectancy of several months. The investigator must also decide whether the patient is likely to to default from follow up since if this happens much effort may be wasted. The following are generally accepted criteria for inclusion of patients in phase I trials (Carter 1977, Gottleib 1974, Wooley and Schein 1979, Williams and Carter 1978, Karon et al 1973):

(1) There must be a histological diagnosis of malignancy.

(2) The patient must have failed on all established forms of treatment or have a tumour for which no effective therapy exists. In this context there may be some argument as to what constitutes effective therapy. While no patients with breast cancer, lymphoma or ovarian carcinoma would be entered in a phase I trial without having had extensive prior treatment some would argue that for other conditions eg renal carcinoma, colon carcinoma and possibly non-small cell lung cancer no effective chemotherapy exists and would include these patients in the trial. Other investigators would argue that there are regimens that have given response rates of the order of 20-30%

in these conditions and would therefore not feel able to deny patients such treatment. These response rates have not however been associated with increased survival compared to untreated controls and therefore it is doubtful if they are of great value. In practice during the early stages of a phase I trial all patients entered have normally had prior chemotherapy. Towards the conclusion of the trial when potentially therapeutic doses are being studied a higher proportion of untreated patients will be entered.

(3) Since the dose limiting toxicity of the majority of drugs is myelosuppression patients with compromised marrow function due to their disease should not be included. Thus those with leukaemia and often lymphoma are not suitable. In addition some patients with solid tumours particularly breast cancer may have heavy marrow infiltration and these should also be avoided. In general only patients with solid tumours are entered, separate phase I studies being undertaken in leukaemia once the initial data are known.

(4) It is not necessary for patients to have evaluable disease since the study is basically a toxicity trial but, as mentioned previously, evidence of activity in the phase I trial is likely to stimulate greater interest in the drug.

(5) Most investigators stipulate a lower age limit of 16-18 years. This is because retrospective analyses have shown that paediatric patients may tolerate considerably higher doses of drugs than adults (Von Hoff et al 1977). Separate phase I trials are therefore carried out in children. An upper age limit of 70 years is often laid down in phase I and phase II

protocols. However it is the biological rather than chronological age that is important and once again the experience of the investigator is of more value in determining the suitability of a patient than a rigid age limit.

(6) Although it is unlikely that patients with advanced malignancy would be pregnant it is usually stated that such patients should be excluded on the grounds of possible teratogenicity.

(7) The anticipated life expectancy of the patient is an important consideration. In order to allow sufficient time to assess drug related toxicity a minimum life expectancy of 12 weeks is required. Unfortunately physicians frequently overestimate survival (Wooley and Schein 1979, Parker 1972).

(8) Sufficient time should have elapsed to allow recovery from prior chemotherapy. This is normally taken as four weeks or six weeks for drugs such as the nitrosoureas and mitomycin-C that have a delayed nadir and slow recovery of marrow function. Extensive radiotherapy will also compromise marrow function and time must be allowed to recover from such treatment.

(9) The majority of drugs are excreted by the liver or kidneys or a combination of both. Major dysfunction of these systems will lead to prolonged systemic drug exposure resulting in increased toxicity and therefore phase I patients should have normal renal function and normal bilirubin although some rise in hepatic transaminases is allowed (usually less than 50% above the upper limit of normal. Major degrees of heart failure, symptomatic dysrhythmias or other serious medical conditions also disqualify patients from phase I trials.

(10) Patients should be on as few additional drugs as possible

although many will require analgesics or other symptomatic therapy.

Dose Escalation

The process of dose escalation is governed by a fundamental conflict. On the one hand there is a need to go slowly in order to avoid a sudden jump from a dose with no obvious toxicity to a lethal dose. On the other hand there is a need to proceed rapidly so that large numbers of patients are not treated at doses of no potential therapeutic benefit. Although the phase I trial is primarily a toxicity study it is ethically difficult for investigators to treat patients at doses with little or no likelihood of a therapeutic effect. Moreover inefficient escalation procedures slow the overall pace of new drug development and limit the opportunities for patients with unresponsive tumours to be treated with potentially effective agents in phase II trials. Moreover following the completion of a phase I study the valuable information is that obtained at or near the maximum tolerated dose and the wealth of data on patients treated at lower doses is largely irrelevant. For these reasons it is important therefore to look for ways of escalating doses as rapidly as possible.

The most widely used method of dose escalation is the so called modified Fibonacci search scheme (Carter et al 1977, Selawry and Hansen 1969), and in order to explain this there follows a brief historical digression.

Leonardo Fibonacci, also known as Leonardo of Pisa, was the most distinguished mathematician of the middle ages. His father was a Pisan merchant who sent the young Leonardo to study calculation in

North Africa. There he learnt the art of the nine Indian figures (9, 8, 7, 6, 5, 4, 3, 2, 1) and became increasingly interested in mathematics. His major work, Liber Abacci, appeared in 1202 and described in detail the use of this counting system (also called Hindu-Arabic numerals). The sequence of numbers that now bears his name derived from a problem set out in Liber Abacci:

> " A certain man put a pair of rabbits in a place surrounded on all sides by a wall. How many pairs of rabbits can be produced from that pair in a year if it is supposed that every month each pair begets a new pair which from the second month on becomes productive?"

The resulting number sequence:

1, 1, 2, 3, 5, 8, 13, 21, 34 etc

describes the number of rabbits present at each month. Each term of the series is obtained from the sum of the two preceeding terms and it was the first recursive sequence known in Europe. The relationship between the members of the series was not however stated in a formula

$$(U_{n+2} = U_{n+1} + U_n)$$

until the development of algebraic notation in the early 17th century. In 1753 Robert Simson at Glasgow university noted that as the numbers increased in magnitude the ratio between succeeding numbers approached the constant phi.

The term Fibonacci series was coined in the 19th centuary by the French Mathematician Edouard Lucas and in the ensuing years several interesting properties of the sequence were discovered. For example if each number is divided by its right hand neighbour a series of fractions is obtained which are found widely in natural phenomena. When new leaves grow from the stem of a plant they spiral

round the stem and the spiral turns as it climbs. The amount of turning from one leaf to the next is a fraction of a complete rotation around the stem and this fraction is always one of the Fibonacci fractions. These fractions are also found in the arrangement of petals on a sun flower, of whorls on a pine cone and of segments on a pineapple.

The Fibonacci series was modified empirically in the following manner for use in phase I trials:

Drug Dose	**Percentage increase above preceeding dose level**
n*	
2.0n	100
3.3n	67
5.0n	50
7.0n	40
9.0n	33
12.0n	33
16.0n	33

This scheme allows rapid initial escalations with smaller increments as the toxic range is approached. In effect only the first three terms bear any resemblance to Fibonacci´s original series of numbers but the concept of the ratio between successive members of the series approaching a constant is retained.

Using this scheme the maximally tolerated dose of a new agent has usually been reached in 2-12 (median 6) escalation steps (Carter et al 1977, Penta et al 1979, Greishaber and Marsoni 1986)). It follows that on average 50-75% of patients in a phase I trial using this escalation programme will receive sub-therapeutic doses. Moreover

since each dose level will take 6-8 weeks to complete the average study will last 9-12 months and many considerably longer. It is important therefore to examine alternative escalation procedures in an effort to by-pass the lower doses. A number of investigators have used 100% dose increments until toxicity is encountered with progressively smaller increments thereafter (Gottleib 1974). Although this method often allows a more rapid escalation compared to the Fibonacci scheme the choice of dose increments remains largely empirical.

A different approach is to use pre-clinical pharmacokinetic data from animal experiments as a guide to dose escalation. This arose from the observation that for many drugs the MTD in man is close to the LD 10 in the mouse expressed as mg/m^2 (Collins et al 1986). However a number of agents do not conform to this rule and suggested explanations for this variation in toxicity between mouse and man include:

(1) Species differences in drug metabolism/elimination/binding

(2) Schedule dependency

(3) Species differences in target cell sensitivity.

Differences in metabolism and distribution, ie pharmacokinetic differences, can be overcome by looking at the area under the concentration x time curve rather than relying solely on the dose. This should allow a more accurate inter-species comparison of systemic drug exposure. This theory was tested retrospectively on a series of eight drugs by Collins et al (1986). They found that in 5 cases the AUC was an effective predictor of toxicity and for these drugs the number of escalation steps could be reduced by between 20-50% using pharmacokinetic data. In one case, Adriamycin, the ratio

of doses at the mouse LD10 and human MTD was 5 whereas the ratio of AUCs was 0.8. This was the most striking example of the AUC providing a more accurate correlation with toxicity (Green et al 1983). For a further drug, PALA, the AUC ratio was over conservative and would not have reduced escalations significantly. For two others, F-ara-AMP and Dihydroazacytidine, man was less tolerant than mice but since toxicity was observed in both cases at the initial dose level no problems with patient safety were encountered. However such cases could be potentially dangerous if the dose was escalated above the MTD on the basis of AUC information.

It is instructive that all three drugs where the correlation of dose and AUC with toxicity between species was least secure were antimetabolites and therefore it appears that this is a class of compounds where the use of pharmacological data to aid dose escalation may not be applicable. However even if such data been used in dose escalation in no case would patients have been put at higher risk.

Although pharmacokinetic differences may be the main reason for the poor inter-species correlation of dose with toxicity for some compounds, other factors must also be considered. As idicated above schedule dependency must be excluded. The importance of this arises from the observation that for drugs with equal clearance values in mice and men a bolus dose of equal mg/m^2 will have somewhat different time courses (Skipper et al 1970). Thus in mice the peak drug level tends to be higher with a more rapid fall compared to man. This would lead to greater toxicity in mice if a threshold existed that was at the limit of the level reached in man. Conversely if toxicity depended on duration of exposure then effects would be more

marked in man. This is likely to be the problem with the antimetabolites. Some indication of schedule dependency can be obtained from comparison of the single and daily x 5 pre-clinical toxicology data. If the LD10 values are of the same order then major schedule dependency can be ruled out.

Differences in target cell sensitivity is another area that could account for variations in toxicity between mouse and man. However testing for such differences is difficult and would have to rely on tissue culture experiments. At present limited testing of marrow toxicity using a bone marrow progenitor colony inhibition assay is being evaluated.

Drugs that are metabolised to active compounds may also cause problems where the metabolite is a major factor in producing toxicity. Such compounds are unlikely to be suitable for dose escalation based solely on the AUC of the parent compound.

There are clearly several factors to be taken into account before opting to employ pharmacological data to guide dose escalation but once this has been decided upon the procedure is as follows:

(1) Determine mouse LD10

(2) Determine mouse AUC at LD10 and two other doses.

(3) Begin phase I at 1/10th mouse LD10

(4) Measure human AUC at the starting dose

(5) Choose escalation strategy based on how close initial human AUC is to target AUC.

It is important to determine the kinetics in at least three doses in the mouse in order to detect any evidence of non-linearity which would make rapid escalations in the subsequent clinical trial potentially more dangerous. It is also important to ensure that the

LD10 in the mice used for the pharmacology experiments is similar to that found during pre-clinical toxicology. Ideally therefore these mice should be of the same strain. In addition it is desirable that the pre-clinical and clinical pharmacology should be carried out in the same laboratory to avoid another potential source of error.

The escalation strategy should attempt to achieve an AUC in patients of a similar order to that at the LD10 in mice. Thus if the AUC at the starting dose was well below the target AUC then rapid early escalations would be indicated. Collins and his colleagues proposed two methods of escalation. The first, the square root or geometric mean method, in which the first dose escalation factor is equal to the square root of the ratio of the AUC at the mouse LD10 to the AUC at the phase I entry dose. Where there is a large difference in the AUC at the mouse LD10 and the AUC at the phase I starting dose as in the example of adriamycin, this method will result in the first escalation being seven or more x the starting dose. The second scheme was the more conservative extended factors of two method. This employs 100% escalations until the range of the target AUC is reached. Subsequently escalations can be completed using a modified Fibonacci style series. A combination of these two schemes may prove to be the most efficient. An initial escalation of the order of 100% would allow the detection of non-linearity in the kinetics. If the kinetics appeared to be linear more rapid escalations might be contemplated depending on the difference of the initial AUCs from thew target AUC.

Several advantages accrue from more efficient dose escalation in phase I trials. Firstly the patients benefit from having a greater chance of receiving a potentially therapeutic dose of drug. Secondly

a great deal of time and resources may also be saved. However this may be offset by the extent of pre-clinical testing that is carried out. An exhaustive search for schedule dependency and target cell sensitivity will be expensive and time consuming and may outweigh any advantage gained. It is important that any new approach to dose escalation should be relatively simple and should not incur great additional expense. The scheme suggested above fulfils these criteria.

In addition to the above approaches to dose escalation in phase I studies I felt that a review of the published literature might offer further insight into the problem. I have therefore researched phase I trials published since 1970 and there follows an analysis of the data obtained from this review.

Phase I Trial Survey

Methods

The following journals were scanned for reports of phase I trials conducted since 1970:

Cancer

Cancer Treatment Reports

Cancer Research

European Journal of Cancer and Clinical Oncology

Cancer Chemotherapy and Pharmacology

Clinical Oncology

Investigational New Drugs

In addition a literature search was performed on the Mezline data base to identify any missing trials.

Only the first phase I study performed in man was included ie reports of studies of alternative schedules where the starting dose was based on previous human data were not acceptable. Studies of biological response modifiers and non-cytotoxic agents eg differentiating agents were also excluded.

The following information was abstracted from each study, where this was provided:

The total number of patients treated

The starting dose

The method by which the starting dose was derived

The dose escalation scheme

The escalation point at which toxicity was first noted

The escalation point at which the MTD was reached

The number of escalations to the recommended phase II dose

The dose limiting toxicity

The number of toxic deaths

The number of responses

The number of patients receiving the dose recommended for phase II trials.

Results

Sixty-one phase I clinical trials were identified that were published between January 1971 and June 1986 and which fulfilled the above criteria. The literature references to these studies are contained in Appendix II. The number of trials published by year were as follows:

Table 1 Phase I Trials by Year of Publication

1986	-	4
1985	-	9
1984	-	3
1983	-	10
1982	-	7
1981	-	6
1980	-	6
1979	-	4
1978	-	4
1977	-	-
1976	-	1
1975	-	5
1973	-	1
1971	-	1

As can be seen the majority of the studies that contained sufficient information for inclusion in this review were conducted in the second half of this sixteen year period. This is probably due to the progressive adoption of accepted methods of conducting such studies and a trend to more uniform reporting of the results. During the 7 years from 1977 - 1983 applications were filed with the Food and Drug Administration in America to conduct investigational clinical trials with 30 new compounds (Venditti 1983). The present survey includes data from the trials on 23 of these drugs. Studies on a further 4 of the drugs could not be used because of non-standard methodology or insufficient data. It would appear therefore that this survey includes almost all the drugs that have come through the NCI screening programme and in addition has picked up most of those from other sources. Approximately six new compounds per year currently undergo phase I clinical testing.

Starting Dose

As discussed previously there has been considerable debate as to the most relevant animal species for arriving at a safe starting dose in man. This uncertainty is reflected in the methods used in the 61 trials under review. The animals used were:

Table 2 Animal Species used to Determine Starting Dose

Animal	Number of Drugs
Mouse	22
Dog	24
Monkey	2
Unspecified	13

13/22 trials relying on mouse data used 1/10th LD10 as the starting dose with the remainder using fractions varying from 1/30th to 1/2 LD10. 18/24 trials using information from the dog to calculate the starting dose used 1/3 Toxic Dose Low.

Table 3 Numbers of Patients Treated at Therapeutic Doses

Number of Trials	61	
Total number of patients	2101	
Number of patients per trial, median (range)	30	(12-73)
Number of patients in whom no toxicity was seen	610	(29%)
Number per trial with no toxicity, median (range)	10	(0-27)
Number of drugs recommended for phase II testing	54	(87%)
Number of patients in these 54 trials	1873	
Number of patients receiving the dose recommended for phase II :		
total	812	(39%)
median, (range) per trial	14	(5-30)

Table 4 Escalation Procedure

Modified Fibonacci series	41	(67%)
100% increments	8	(13%)
25-50% increments	4	(6%)
Fixed dose increments	5	(8%)
Based on animal data	1	(2%)
Dose reduction	2	(3%)

Table 5 Escalations Required to Reach First Toxic Dose and MTD

All 61 Trials

No. of escalations to lst toxic dose, median (range)	3 (0-7)
No. of escalations to MTD	7 (0-15)

41 Trials using Fibonacci escalation

No. of escalations to lst toxic dose	3 (0-7)
No. of escalations to MTD	7 (2-15)

I have estimated the number of escalations that would have been required to reach the MTD using the 100% increment scheme for the 41 trials employng the Fibonacci series. This was done by using 100% increments from the starting dose until the first toxic dose was reached and then 50% increments thereafter.

No. of escalations to MTD with alternative scheme, median 5 (3-8).

Among these 61 drugs there were 4 (6%) where the MTD was less than 5 x the starting dose. This represents the third escalation of the modified Fibonacci series. These drugs are listed in table 6.

Table 6 Drugs where toxicity occurred at first dose level

	1st toxic dose level	DLT	MTD dose level	Starting dose
S.A.G.A	1n *	CNS	3n	1/10 LD10 mouse
Tiazofurin	1n	CNS	3n	"
F-ara-amp	1n	Marrow	1n	"
Hycanthone	1n	Liver	3n	1/3 TDH Dog

* : n = the starting dose

Table 7 Dose Limiting Toxicity for all 61 Trials

Marrow	31 (51%)
CNS	7 (11%)
Hypotension	5 (8%)
Renal	4 (6%)
Phlebitis	3 (5%)
Hepatic	3 "
Stomatitis/mucositis	3 "
Vomiting	2 (3%)
Pleuropericardial pain	2 "
Haemolysis	1 (2%)

Toxic Deaths

Toxic deaths occurred in 13 trials (21%). There were 23 deaths recorded representing 1% of all patients treated in the 61 studies.

Table 8 **Objective Responses**

Drug	PR	CR
Homoharringtonine	1	
4-Demethoxydaunorubicin	4	2
Bisantrene	2	
GANU	1	
Spirogermanium	1	
PALA	1	
DHAD (Mitoxantrone)	1	
Cis-Platin	5	4
CBDCA	2	
Mitozolamide	2	
TGU	3	
4´-THP-adriamycin	1	
4-Deoxydoxorubicin	6	
4´-Carboxy Platinum	2	
JM 40	2	
Dihydro-5-azacytidine	2	
4-Epiadriamycin		1
Gallium nitrate	1	
Dianhydrogalactitol	1	1
Isophosphamide	2	
Guanazole	2	
Inosine Dialdehyde	2	1

Objective Responses

Fifty three responses that satisfied the WHO criteria for partial or complete response were seen in a total of 22 trials. Thus objective tumour regression occurred in only 3% of all patients treated but were seen with 36% of the agents tested. There was insufficient data in the majority of reports to say whether these responses occurred predominantly in patients who had had previous chemotherapy or those who had not. The objective responses seen are listed in table 8. Of the drugs where responses occurred in phase I testing:

5 are currently undergoing phase II study.

3 have been discarded because of excessive toxicity or lack activity in phase II.

5 have been licensed for use.

The fate of the remaining nine is at present unknown.

Of the 40 agents where no activity was seen in phase I testing only one, VP16 213, is currently in routine clinical use. There was thus a significantly greater chance of a drug entering routine clinical use if responses were seen in phase I testing ($p < 0.01$ using Chi Sq test with one degree of freedom).

Discussion

Several interesting conclusions can be drawn from this survey. Firstly in only 4 trials (6%) was toxicity seen at the starting dose and in only one of these was the initial dose subsequently shown to be the MTD. In all other trials (94%) where no toxicity was seen at the starting dose for no drug was the MTD less than 5 x the starting dose. Indeed the median number of escalations to the MTD was 7,

equivalent to 9 x the starting dose in the modified Fibonacci series. These data suggest that it would be safe to treat the first patients at the equivalent of 1/10 Mouse LD10 and if this was entirely non-toxic to then escalate to 5 x this dose. This information could be usefully incorporated into the geometric mean dose escalation schemes proposed by Collins et al (1986) for use with pharmacological monitoring. In cases where there is a great discrepancy between the AUC at the mouse LD10 and the AUC at the phase I starting dose this procedure would produce a first dose escalation which might be 7-10 or more x the starting dose. Such an initial escalation might be considered hazardous since no data on the linearity of the kinetics in man would be available. On the other hand the alternative escalation suggested of 100% increments may be over conservative. The information from this survey implies that a maximum initial escalation of 5 x the starting dose (5n) is entirely safe provided no toxicity occurred at the starting dose. Using the geometric mean method a 5n escalation would have resulted if the AUC at the starting dose had been 4% of the target AUC. Thus it would appear safe to use a 5n escalation providing the starting dose is entirely non-toxic and the AUC at the starting dose is less than 5% of the target AUC. Once pharmacokinetic data is obtained at at least two dose levels subsequent escalations can be modified taking into account the linearity of the kinetics.

This scheme would miss out 2 early steps on the Fibonacci series and possibly more later in the trial as kinetic data became available and would therefore significantly reduce the numbers of patients in the study and its duration.

The survey also suggests that escalation procedures based on 100%

increments are more efficient, reaching the MTD in a median of 2 escalations fewer than the modified Fibonacci method. The greatest reduction occurs where the MTD is more than 30 x the starting dose. In these cases the number of escalations may be reduced by up to 50% by using the 100% scheme.

The range of dose limiting side effects was similar to that seen in the drugs in routine clinical use apart from a slight excess of CNS toxicities. This probably reflects the reluctance of physicians to use such agents unless they are clearly very effective. Perhaps these drugs should be tested in patients with brain tumours since is possible that CNS toxicity indicates high concentrations of drug reaching the brain.

As might be expected the total number of responses seen in this survey was very low amounting to 3% of all patients entered. There are several reasons for this. Firstly a considerble proportion of the patients entered in phase I trials do not have measurable disease and therefore by definition a partial response cannot occur (clearly a complete remission could still be recorded). Information on the numbers of patients with measurable disease is rarely included in published reports. The other major factors contributing to the low response rates are the extent of disease and prior chemotherapy. Most patients are entered in a phase I trial at a relatively late stage in their disease and will often have received several chemotherapeutic agents. Such patients are well known to be unlikely to respond to further drugs particularly since over 60% are treated at doses below those subsequently recommended for phase II testing. However although the actual number of responses was low they occurred in a relatively high proportion of the studies, 36% (22 trials). Of

these 22 drugs 5 have been licensed for clinical use (CBDCA, 4-Epiadriamycin, Ifosfamide, Cis-Platin, Mitoxantrone) whereas only one of the 39 drugs in which no activity was seen in phase I is currently in use, VP16-213. Statistical comparison of these results showed that there was a significantly greater chance of a drug eventually entering routine clinical use if responses were seen in the phase I trial, $p < 0.01$. Whether or not this merely reflects the greater interest stimulated by an apparently effective agent is open to debate. Estey et al (1986) recently analysed the responses seen in 54 agents introduced into NCI-sponsored phase I trials from 1974-1982. Objective responses were reported in 4.2% of patients and 39% received doses greater than or equal to the dose recommended for phase II, results very similar to those reported above. They went on to compare the response rates in the phase I and phase II trials of the 38 drugs completing at least one phase II trial. The median phase I response rate for those considered active in phase II was 4.3% and for those considered inactive was 2.7%. This difference was not statitically significant. These data lend weight to the proposal that no drug should be withheld from phase II purely on the grounds of inactivity in phase I.

In summary this survey confirms that a high percentage (39%) of patients in phase I trials are treated at doses with little chance of any therapeutic activity. These numbers may be reduced by employing escalation schemes based on 100% increments which appear to be more efficient than the traditional modified Fibonacci series. In addition the dose of drugs showing no toxicity at the starting dose can immediately be escalated to five times this level providing the AUC at the starting dose is less than 5% of the target AUC with

consequent further savings in time and patient resources.

Numbers of Patients Required In a Phase I Trial

The usual practice is to enter three patients at each non-toxic dose level (Carter et al 1977, Gottleib 1974, Williams and Carter 1978, Rosencweig et al 1985). The first patient at each dose level should be observed for at least three weeks in order to exclude dose limiting effects at that dose before further patients are entered. It has also been suggested that six patients should be entered at doses showing any toxicity. These recommendations have been put forward in the past to ensure that no harm comes to patients from rapid dose escalations before a previous dose has been adequately studied. However I believe that rigid adherence to "rules" such as those set out above has led to to phase I trials being unnecessarily slow and involving too many patients at sub-therapeutic doses. In my view it is unnecessary to study more than two patients at non-toxic doses. We are not really interested in non-toxic doses in a phase I trial so why waste time and resources and deny patients the opportunity of potentially more effective treatment by entering further patients. A similar argument can be advanced for reducing the number of patients studied at doses resulting in non-limiting toxicity. Normally the appearance of nausea and vomiting or WHO grade 1 haematological toxicity is the first sign that a dose with demonstrable biological activity has been reached. However gastrointestinal toxicity is rarely dose limiting and should not in itself be a barrier to further dose escalation. Grade 1 haematological toxicity is also not dose limiting. Obviously the worry is that in other patients grade 2 or 3 toxicity might be seen. However if nothing worse than grade 1 effects occur in 1/3, 2/3 or even 3/3 patients at any dose level the likelihood of this

representing the MTD are remote. I would therefore recommend that two patients are entered at each non-toxic dose level and at levels producing types of toxicity, eg nausea, vomiting, alopecia, that are unlikely to be dose limiting. Three patients should be entered at doses causing grade 1 haematological toxicity and 6-10 patients at the dose recommended for phase II trials. This would normally be a dose causing reversible grade 2/3 toxicity.

Although haematological toxicity is dose limiting for the majority of drugs, renal, hepatic and neurotoxicity may also cause problems. In such cases it is less easy to be dogmatic regarding numbers of patients studied and the pace of dose escalation. Renal toxicity is primarily objective, causing few symptoms in its early stages, and only becomes limiting if recovery does not occur. In addition it is often possible to ameliorate the effects by measures such as hydration (cis-platin) or alkalinisation (methotrexate). If consistent disturbance of renal function is noted at any dose level (2/3 or 3/3 patients) that fails to recover, then further patients should receive the same dose with appropriate hydration procedures and other measures considered necessary. Further dose escalation should proceed with circumspection. Hepatic and neurological damage are less easy to measure. In the case of hepatic toxicity patients often describe a flu-like syndrome comprising fever, lethargy and malaise. The severity of these symptoms often correlates with the degree of disturbance of the transaminases but inter-patient variation and the similarity of the symptoms to those that are often present in cancer patients may make determination of the MTD difficult. Clearly if transaminases do not return to pre-treatment levels then no further escalations should be undertaken.

Neurological toxicity in many ways is the most distressing of all types of toxicity both for the patient and the physician. Peripheral neuropathy can be disabling and may be permanent. Central effects, if serious, can preclude further use of a drug. At present there is only one drug in regular use, Ifosfamide, that results in CNS toxicity in a significant proportion of patients. Most investigators would agree that neurotoxicity exceeding WHO grade 1 (transient lethargy) would be unacceptable for phase II trials unless there was evidence of excellent therapeutic activity.

In general it is better not to re-enter patients at higher dose levels. This policy allows a more accurate determination of cumulative toxicity at any particular dose. Alternatively it can be argued that intra-patient escalation reduces the numbers of patients in a trial and allows each patient more chance of receiving a therapeutic dose. A compromise might be to treat each patient with at least two courses at any one dose level but thereafter allow intra-patient escalation first ensuring that all previous toxic manifestations had resolved.

In summary the requirements for patient entry to a phase I study should minimise the the number of patients treated at non-toxic and minimally toxic doses. The majority of patients should be studied at or near the dose that will subsequently be recommended for phase II trials.

Ethical Considerations

It is the duty of the physician conducting the trial to ensure that before entering a phase I study all patients have a clear understanding of the purpose and limited expectations of the trial. They should realise that the chances of a therapeutic response are small and that they may suffer unforseen toxic side effects.

There is much debate as to whether consent should be in written or verbal form. Some physicians are very much opposed to written consent arguing that the use of a single explicit explanation may be inappropriate for some patients. However all patients entered in a phase I study should receive a similar explanation and be able to fully understand the trial. Those who cannot ought not to be included.

In practice it is unlkely that obtaining written consent for phase I trials would be a problem. If the trial has been properly explained to the patient and they were assured that whatever happened they would continue to receive all appropriate supportive care then very few would refuse to sign a consent form. Moreover in the current climate of increasing litigation it may be prudent to introduce formal written consent for new drug trials. However at present the MRC guidelines require only verbal consent.

The ethics of the structure of the trial should also be considered. Although a phase I trial is primarily a toxicity study I believe that it is unethical to treat large numbers of patients at non-toxic and therefore almost certainly sub-therapeutic doses. Efforts such as those outlined above to facilitate rapid dose escalation and reduce patient numbers at each dose level should be pursued. Some might argue that this will lead to patients being exposed to unacceptable

risks of dangerous toxicity. However if the trial is properly conducted this risk should only apply to one patient at most. Moreover since effective supportive measures exist for the common life threatening toxicities encountered their effects can be minimised. It can also be argued that patients experiencing the highest degree of toxic side effects have the greatest chance of a therapeutic response. It would seem therefore to be ethically preferable to use a trial design that enters limited numbers of patients at low doses and that employs a rapid dose escalation procedure together with very careful monitoring. A further advantage of this scheme is that effective agents will move more rapidly into routine clinical practice. It is surely unethical to withold potentally effective therapy from patients due to inefficient phase I procedures.

Whatever the design of a phase I trial it is recommended that approval for the trial is sought from the local ethical committee.

The Phase II Trial

The phase II trial represents another vital step in the development of a new drug. It takes many years and a great deal of expense to bring a drug to phase II testing but the agent may be discarded in a few short months eg Triglyceryluridine (TGU), if therapeutic activity is not demonstrated. It is therefore of the utmost importance that the design and conduct of phase II trials is correct if active agents are not to be discarded (false negatives).

Selection of Drugs

In theory all agents that demonstrate tolerable and reproducible biological effects should proceed to phase II testing. However many agents do not receive full phase II evaluation for a variety of reasons (Marsoni et al 1984). These include unmanageable toxicity, side effects that are not clearly dose dependent and lack of activity in the phase I trial. Drugs with dose limiting side effects other than myelosupression are particularly likely to be evaluated incompletely (Marsoni et al 1984). The reasons for this are likely to be pragmatic for it is often more difficult and time consuming both for the patient and physician to undertake phase II studies with these drugs. Paradoxically there is a great need for such drugs particularly for use in combination therapy where the addition of agents with similar toxicities, especially myelotoxicity, is a problem. Clearly dose limiting toxicity other than bone marrow should be an incentive rather than a disincentive to phase II testing.

The absence of responses in phase I trials should also not impede progress to phase II testing. This is true for a number of reasons. The majority of patients included in phase I trials at present do not

have evaluable disease and thus cannot be assessed for response. In addition these patients are usually heavily pre-treated and in the early stages of the trial receive very small doses. The likelihood of a response under these circumstances is low.

Thus except for severe unpredictable toxicity there are no good medical or scientific reasons why a drug's clinical development should not proceed to phase II testing.

Selection of Diseases

The phase II evaluation of a new drug is essentially a screening process to determine whether the drug is worthy of further clinical evaluation in phase III trials. In the 1960s such studies were conducted on a drug orientated basis with a large number of patients with different tumour types entered in each trial. However these trials were wasteful of patient resources and often produced results that were difficult to interpret principally because of a lack of information on patient characteristics. It is now recognised that a more efficient method of evaluating a new drug is to perform a number of separate trials in a series of selected tumour types. Since it is rarely possible to test a drug in all the tumour types in which activity might occur the concept of "signal" tumours has arisen to allow extrapolation of the results in a small number of trials to cover the majority of human cancers. A study of the 22 agents that have come through the NCI screening programme to phase II study since 1975 (Wittes RE et al 1985) has shown that activity occurred only in acute leukaemias, the lymphomas, small cell lung cancer and carcinoma of the breast and ovary. In addition all drugs currently marketed for indications other than chronic myelogenous leukaemia are active in at

least one of these 5 tumour categories. These five tumours could form the basis of a phase II clinical screening panel but there are several problems with this approach. The first is that using a group of tumours to find drugs active in that group will produce a self fulfilling prophecy and leave little room for the discovery of novel agents perhaps with a totally different spectrum of activity. This is a similar argument to that advanced for the change in pre-clinical screening discussed previously. In addition as therapy improves for the panel tumours patients available for inclusion in phase II studies will become progressively more heavily pre-treated making meaningful phase II results more difficult to obtain. Moreover as the techniques for growing human tumour cells in vitro become more refined it may be possible to predict which tumour types an individual drug will be active in and thus to target phase II trials towards these cancers. It would appear therefore that the use of a panel as a basic minimum for phase II testing is warranted but there should be room for additional studies possibly based on the known action of the drug or in vitro testing.

Selection of Patients

It is now appreciated that a number of factors relating to the disease and the patient population can markedly influence response rates. The patient´s performance status has been shown to have a large effect on survival in lung cancer (Zelen, 1974) and on response rate in colon cancer (Moertel et al 1974). Patients with a poor performance status are often unable to tolerate full doses of chemotherapy and are more likely to develop serious toxicity which in part accounts for the negative effect on response and survival. In

addition these patients are likely to have a large tumour burden and therefore there is a greater chance of resistant cell lines being present (Goldie et al 1982) which again will reduce the response rate.

The extent of prior chemotherapy also has a major bearing on the outcome of a phase II trial. Heavy pretreatment affects the objective response rate in both solid tumours (Mathe 1973, Wittes 1985) and hamatological malignancies (Frei et al 1973). Again such patients are less likely to tolerate full dose chemotherapy and are at high risk of having developed resistant lines.

When a drug enters phase II trials it should be tested in that patient group which is most likely to show a favourable effect provided it is ethically permissible to do so. This criterion is best fulfilled by patients with maximum performance status, minimum extent of disease and minimum prior chemotherapy. That which is judged to be ethically permissible may vary from centre to centre depending on the viewpoint of individual clinicians. For example some may wish to exhaust all the available active drugs in breast cancer before using phase II agents while others may believe they can be used after failure of a single front line combination. It is clear however that if active agents are not to be lost through poor patient selection, the clinicians involved in phase II trials must be prepared to use new agents much earlier in the course of many diseases than has been the practice. Thus most clinicians would agree that in the majority of solid tumours eg, colon, non-small cell lung and melanoma only patients who have not received prior chemotherapy should be entered in trials. Moreover there is a case for using untreated patients in certain sub-groups of responsive cancers. Thus the prognosis of

patients with extensive small cell lung cancer, stage IV ovarian carcinoma and slowly progressive breast cancer will probably not be altered by the primary use of a new agent. Careful monitoring of the trial with a design allowing early change to a standard regimen in the event of progression should be acceptable to most clinicians.

It is also important that patients entered in phase II trials should have measurable disease otherwise the response rate cannot be determined and the trial will be meaningless. This may seem obvious but for many tumours measurement presents a major problem. For example in lung cancer many patients have masses arising from the mediastinum which cannot be accurately assessed unless prior normal X-rays are available. Prostatic cancer metastases occur largely in bone and are notoriously difficult to measure as are tumours normally confined to the abdominal cavity such as ovarian, pancreas and gastric carcinoma. The more widespread availability of CT scanning and the promise of NMR scanning may in future help to overcome some of these problems.

The use of survival as an end point for phase II studies has been entertained but there are a number of problems with this. Firstly it is difficult to standardise the time from when survival should be assessed. There are several options here. Some would argue that it should be from the date of starting therapy but different clinicians tend to start therapy at different stages in the natural history of a tumour and thus merely starting a drug earlier might give a false impression of activity. For an individual tumour type survival might therefore be assessed from the date of diagnosis but this would have to be closely linked to the stage of the disease since as we have seen tumour burden has a considerable influence on the chance of a

response and survival. A further problem with the use of survival as an end point is the influence of other therapies on the outcome. To enable valid conclusions to be drawn all patients in the trial would have to be treated in a similar fashion following relapse or progression. One way of overcoming this problem would be to incorporate a crossover treatment arm in the protocol design. This would help to avoid some of the heterogeneity of subsequent therapy.

Statistical Aspects of Phase II Trials

The main statistical problems involved in phase II trials are concerned with determining the true response rate of a new agent. There are two main types of error that can occur, false positives and false negatives. These two types of error can be controlled and therfore we must decide which is more important to minimise. Many believe the false negative result to be the more serious error (Williams and Carter 1978) since it may discourage further use of an effective agent and waste a great deal of pre-clinical and phase I effort. On the other hand false positive results will be discovered and rectified at a later date through additional trials.

The false positive and false negative errors can be related to type I and type II errors in statistical hypothesis testing (Colton 1974). Thus a type I error, rejecting the null hypothesis when true, is equivalent to a false positive error, and a type II error, accepting the null hypothesis when false, is equivalent to a false negative error. The power of the hypothesis test is the probability that the null hypothesis is rejected for a given response rate under the alternative hypothesis. The power may be computed for any given response rate under the alternatve hypothesis (ie that the drug is

effective). Thus the significance level of a hypothesis test is equivalent to the probability of a false positive error and the complement of the power of the test is equivalent to the probability of a false negative error.

The greater the sample size the greater the precision in the estimation of the response rate and the smaller the probability of a false negative result. Biostatisticians can compute the minimum sample size requirement according to investigator specifications for precision and false positive and false negative error probabilities. Several different trial designs can be used.

Single Stage Design

In a single stage design the activity of the new agent is assessed on a pre-determined fixed sample size regardless of results in the early part of the trial. To determine the minimum sample size the investigators must specify the minimum acceptable response rate, the maximally tolerated coefficient of variation for this response rate, the maximally tolerated significance level for rejection of the null hypothesis and the minimally tolerated power against a false negative error. This generally requires a sample size of 20-30 to detect a response rate of 20-30% with a coefficient of variation of 30%, a significance level of 5% and a power of 0.80.

Thus if a drug produced a response rate of greater than 20% under these conditions there would be a 95% probability that the result was a true reflection of the drugs activity and a 5% chance of a false negative error.

Multi-stage Designs

In recent years investigators have become aware of the need for early termination of phase II trials for ethical reasons when early data tend to indicate that the treatment is ineffective or to report results if it appears particularly promising. One method for achieving early termination is to use a sequential design. In such a case there is no fixed sample size and investigators decide after each patient is entered whether to continue with the trial. The methodology allows for control of overall false positive and false negative error probabilities but there are several problems in the context of cancer therapy (Byar et al 1976, Pocock 1977). The main drawbacks are the administrative burdens of maintaining a constant vigil over the data and delays between patient entry and response determination. A compromise between the strictly sequential and the single stage design is the multi-stage design. Patients are entered in batches of predetermined size and a decision is made to continue the trial after each batch depending on the number of responding patients observed up to that point. Multi-stage methods allow for control of overall false positive and false negative error rates. A number of different multi-stage designs have been proposed (Schultz et al 1973, Fleming 1982, Herson 1979, Pocock 1977, Sylveter and Staquet 1977, Elashoff and Beal 1976, Lee et al 1979) however the most widely used has been that based on Gehan's rule (Gehan 1961). In this scheme the trial is discontinued if no responses are seen in a preliminary sample of n_1 patients. If at least one response occurs in the preliminary sample an additional n_2 patients are entered. The size of the preliminary sample n_1 is determined by the specified significance level for the hypothesis test and n_2

represents the number of additional patients needed to estimate the response rate with the required precision.

A two stage design based on these principles is currently employed by the EORTC and the CRC for phase II studies of new chemotherapeutic agents. An initial sample size of 14 patients is studied. If there are no responses in this group the trial is stopped. The additional sample size, n_2, depends on the number of responses in the first 14 patients. The number of additional patients is calculated so that the standard error of the observed response rate will be less than or equal to 0.10. :

Responses in the first 14 patients	**Additional patients**
0	0
1	1
2	6
3	9
4 or more	11

This scheme ensures that if the drug is active in 20% or more of patients the chance of erroneously rejecting the drug after the first 14 patients is 0.044. and provides an efficient method of early termination for ineffective drugs.

Randomised Phase II Trials

A phase II design that compares the response rate and toxicity of two or more new agents in a particular tumour has been proposed (Simon et al 1985). Such a design can have a number of advantages. Where phase II trials are being conducted on a multi-centre basis, randomisation helps to ensure that all patients are registered before treatment starts. This is essential for checking patient eligibility,

and terminating accrual when sufficient patients have been entered. A further advantage of this design is that is that differences in activity and toxicity between agents are more likely to be real rather than reflecting differences in patient selection and response evaluation between institutions. In order to undertake a randomised phase II trial two conditions must be satisfied. Firstly patient accrual should be rapid and secondly two or more drugs must be available for testing. With the current pace of new drug development it is unusual for drugs to become available for phase II trials simultaneously and this may prove to be the limiting factor in the widespread adoption of the randomised design. However this methodology is also applicable to the study of different schedules of the same drug and its use in this setting is particularly attractive.

In determining sample sizes for randomised phase II trials direct methods of statistical ranking and selection theory are useful (Gibbons et al 1977). With the statistical selection theory criterion, one always selects for further study the treatment with greatest response rate (or lowest toxicty), regardless of how small or "non-significant" its advantage over the other treatments appears to be. Conventional statistical designs used in clinical research select a treatment as superior only when the data are incompatible with the hypothesis that the treatments are equivalent and thus require much larger sample sizes than selection theory designs which which always select a treatment as superior even if they are actually equivalent.

Reporting Treatment Results

The quality of the response data generated by a phase II trial will depend on the trial design, careful patient selection and strict adherence to the protocol.

A major problem lies in assessment of response. Even when a standard method for calculating tumour size is used there can be a wide inter and intra-physician variation. This was clearly shown by Moertel and Hanley (1976). This study was designed to simulate the conditions encountered in oncological practice when measuring tumours in the abdomen, lymph nodes and subcutaneous tissues. Sixteen experienced oncologists each measured 12 simulated masses composed of solid spheres with diameters ranging from 1.8-14.5cm. The oncologists deviated in their measurements with a range of 3.4-33.6%. Two of the spheres were of the same size. Using data from the assessment of the size of these spheres Moertel and Hanley calculated that false positive regressions of greater that 25% occurred in 24.9% of cases and of greater than 50% in 6.8% of cases. On the other hand false negative progression of greater than 25% occurred in 31.5% and of greater than 50% in 17.8%. This study was conducted under ideal conditions and larger error rates can be anticipated in the clinic when pain and the site of the mass often make measurement more difficult. Such errors may be compounded by an unconscious bias of investigators to choose on the positive side when assessing response. Thus despite carefully stated criteria in the protocol for determining responses there may be a significant error in the eventual numerator (number of responses).

In addition to problems with the reliability of the numerator the denominator (the total number of patients at risk) may also be

altered by the exclusion of "non-evaluable" patients from the analysis. The major reasons for declaring a patient non-evaluable are:

1. Early death
2. Lost to follow-up
3. Failure to complete therapy due to toxicity
4. Refused further therapy
5. Inadequate data
6. Major protocol violation

The largest category is normally those patients who die following a single course of treatment or who have become too ill to tolerate further therapy. If it is known that the deterioration or death is not due to drug toxicity then clearly most early deaths or progressions represent treatment failures. Despite this there has been a great deal of debate as to whether such patients should be included in the analysis of the trial. In practice if protocol stipualations for patient eligibility in terms of life expectancy and performance status are strictly adhered to the number of non-evaluable patients should be greatly reduced. The use of an eligibility check list can be of value in helping to concentrate investigators minds. Such a list should be completed **before** the patient receives any treatment and is most effectve if administered by some third party eg the hospital pharmacy or the data centre. Unfortunately it is a fact of life that investigators often have to be protected from themselves from entering ineligible or borderline patients. Under these circumstances the only valid reason for removing a patient is a major protocol violation such as the wrong dose of drug.

Reports of results of cancer treatment should include:

1. Total number registered
2. Total evaluable
3. Comprehensive details of reasons for non-evaluability

If this is done rigorously for all trials then readers would be able to critically assess the stated response rates and comparisons of different results in the literature would be more valid.

Tumour Measurement

It is now generally agreed that two categories of tumour are acceptable for assessing response to therapy. These have been labelled Measurable and Evaluable.

1. Measurable

Tumours that are measurable can be accurately measured in two perpendicular diameters. These include superficial lymph nodes, subcutaneous masses and pulmonary metastases completely surrounded by aerated lung tissue.

2. Evaluable

Tumours that can only be measured accurately in one diameter are considered to be evaluable. This group includes most palpable abdominal masses and pulmonary metastases that are incompletely surrounded by aerated lung. Disease in some difficult sites that is measured in a particular specified way is also said to be evaluable eg:

Hepatomegally - the sum of three measurements from the xiphoid

and both mid-clavicular lines to the liver edge. Should be accompanied by scan or biopsy evidence of liver involvement.

Mediastinal or hilar mass - These can only be accurately assessed when a pre-involvement X-ray is available for comparison.

Not Measurable or Evaluable

Certain types of tumour involvement are now known to be so difficult to assess accurately that their measurement cannot be accepted as a reliable indicator of response. Principal amongst these is bone disease. This makes trials in patients with metastatic prostatic carcinoma very difficult to perform and excludes a significant number of breast cancer patients from study.

Lung cancer is another common malignancy that can be difficult to assess. This is particularly true of non-small cell tumours where patients often have mediastinal lesions complicated by partial collapse of distal lung segments. Due to the absence of a high rate of complete remission in this disease response judgements can be almost impossible. Other difficult sites include lymphangitic pulmonary metastases, pleural effusions and ascites.

Lesions that have previously been irradiated are also considered to be not evaluable. This is because they may undergo a slow response to radiation and thus give a false impression of response to subsequent chemotherapy. Alternatively the altered blood supply to irradiated areas may not allow adequate perfusion of the tumour and reduce the chance of a response.

Patients often have a combination of evaluable and inevaluable

disease. When this occurs it is important to estimate the percentage of the tumour burden that is not evaluable and include an overall assessment of whether this disease is progressing, regressing or remaining static.

Categories of Response

The guidelines proposed by the Breast Cancer Task Force in the USA (Hoogstraten et al 1977), those proposed by the UICC project (Hayward et al 1979) and the report by Miller et al (1981) and the WHO guidelines (1979) form the basis of the response criteria in general use today.

Complete Response

Complete disappearance of all known disease. All abnormal X-rays and scans must have returned to normal. For the reasons mentioned above it is virtually impossible to define a remission in bone disease and for this reason complex definitions involving sclerosis and isotope scan appearances are no longer considered appropriate.

The mediastinum also represents an area where it is difficult to assess whether or not a complete remission has occurred. This problem often occurs in patients treated for Hodgkins disease who had massive mediastinal disease at presentation. Short of biopsy the only way of resolving this problem is to wait and see if subsequent relapse occurs. With the advent of more sophisticated investigations such as CT scanning and NMR the numbers of patients with residual minor abnormalities will increase. This problem of when is a CR a CR will remain difficult to resolve as long as the imaging techniques available cannot distiguish between tumour and normal tissue.

Under certain circumstances eg testicular teratomas and ovarian cancer it may be advantageous to obtain histological confirmation of complete remission. This is only justifiable when further treatment is available that will influence the prognosis. Patients with a biopsy proven CR are said to be in pathological complete remission.

Unfortunately a complete response is not synonymous with cure. Indeed virtually all patients with breast cancer who obtain a CR and most of those with lung cancer will eventually relapse and die from their disease. However since without a complete response there is no prospect of cure the erradication of all detectable tumour remains the main aim of oncologists, with progressive extension of the durability of the CRs the ultimate goal. At present a CR must last a minimum of four weeks.

Partial Response

At least a 50% reduction in the sum of the products of all measurable lesions + the sum of the diameters of all evaluable lesions. If non-evaluable disease represents the bulk of the tumour burden then there should also be a subjective improvement in these lesions. If non-evaluable disease is a minor feature it should have remained static. No lesion may have progressed and no new lesions appeared. A partial response must last at least four weeks.

No Change

No significant change in measurable or evaluable disease for at least four weeks (defined as less than 50% decrease and less than 25% increase in the dimensions of any single evaluable or measurable lesion). In addition no new lesions must have appeared.

Progression

At least 25% increase in the measurements of any single measurable or evaluable lesion or the appearance of a new lesion. If non-evaluable disease clearly progresses while evaluable lesions do not then this must obviously be coded as a progression.

Duration of Response

In estimating the duration of a respose the main difficulty lies in deciding when the response began rather than when progression occurred. Some authors have used the time that the criteria for a response are first fulfilled (Hoogstraten et al 1977) while others suggest that a response begins as soon as treatment starts and therefore use date of commencement of therapy. The WHO compromised and defined the onset of a complete response as the date when it was first recorded but used the first day of treatment as the onset for partial response. This is the method most widely used today.

Assessment of Treatment Toxicity

A phase I trial is primarily a clinical toxicity study and thus accurate assessment of toxicity is of vital importance. In addition the further characterisation of the side effects of a particular drug forms an important part of the work in a phase II trial. The information gained from these studies allows investigators to determine the correct dose and schedule of a drug and how best it might be combined with other therapies. In cancer treatment the acceptable level of toxicity generally depends on the likely benefit. For example one is more ready to accept potentially life threatening

side effects if there is an opportunity for cure in an otherwise uniformly fatal condition whereas if palliation of symptoms is the best possible result then the level of acceptable toxicity is much lower.

The severity of toxicity at a particular dose of a drug will largely depend on the type of patients treated. Patients who are older, have a low performance status or who have received prior chemotherapy or extensive radiotherapy tend to experience more severe toxic effects than younger, fitter patients who have had minimal or no prior treatment. Thus in assessing toxicity it is important first to define the characteristics of the patient population.

Acute Toxicity

The majority of toxic effects produced by chemotherapeutic agents are acute or sub-acute in nature. Since patients are normally seen at frequent intervals in phase I and II studies a great deal of data is collected. In order to allow more convenient management of the data together with computerised storage and analysis there has been much effort directed towards numerical toxicity grading systems. An additional benefit of the use of a standardised grading system is that results from different centres may be more readily compared. Unfortunately there are a number of such grading systems in current use and the variations in grades for certain important toxicities was pointed out in an analysis by Vietti (1980). Thus in an attempt to standardise the reporting of the results of cancer treatment the World Health Organisation convened two meetings in 1978 and 1979 the results of which have been published (WHO 1979). The WHO system for toxicity grading is now the most widely used and it demonstrates

several usefull features. Firstly there are no vague clinical terms such as mild or moderate. Instead wherever possible specific objective statements are used. The aim of this is to reduce observer error as far as possible. In addition a number of parameters such as biochemical values are assigned grades according to multiples of the normal range. This is intended to facilitate comparisons between results from different laboratories.

There are also drawbacks to toxicity grading scales. The most important of these is that they measure only the severity of the side effects and take no account of time of onset or duration. This may be of particular importance in relation to neutropenia. A neutrophil count below $1x10^9/l$ may be of little importance if it lasts only 4-5 days but may result in serious infections if it continues for 2-3 weeks. An area where toxicity grading has been especially difficult is nausea and vomiting. There are a number of problems with the grading of this toxicity. The first is once again that there is no room for assessment of duration of the vomiting. The second is the problem of giving anti-nausea therapy. The administration of any such therapy immediately increases the WHO grade to 3. However most cancer patients, particularly those who have received previous treatment, know very well that chemotherapy causes nausea and vomiting and request prophylactic medication. Putting all patients who receive prophylactic anti-nausea agents into grade 3 is likely to overestimate the degree of these effects. A further problem is whether nausea is indeed less severe than vomiting. Many patients will say that prolonged nausea is a much worse symptom than one or two episodes of vomiting unaccompanied by nausea. Since these are such common side effects it might be better to separate them with one

grade for nausea and another for vomiting. In addition a more appropriate grading system might be based on duration eg.

Grade		Duration
0	-	none
1	-	1-6 hours
2	-	7-24
3	-	24-48
4	-	48+

The section could be preceeded by a yes/no box for antiemetic therapy and the subsequent grade interpreted taking this into consideration. We must be very careful however not to make grading too complex since it will then begin to lose its value as a more easily handled form of data. It is also important that oncologists continue to report their results according to a uniform system. This does not preclude individual groups experimenting with changes so that when the WHO reconvenes to discuss the reporting of cancer treatment their toxicity grading system can be improved.

Chronic and Late Toxicities

Because of the nature of early clinical trials chronic or late toxic effects are rarely detected at this stage of drug development. Such toxicities come to the attention of investigators after extensive experience with a drug in phase III studies and may only become evident once a significant number of patients have been cured using a regimen including the drug. As the numbers of long term survivors increases the problem of late effects also becomes more important. There has been considerable interest in the sequelae of the treatment of childhood leukaemia particularly in relationship to

effects on growth and intellectual impairment. The major late side effects of the treatment of adult cancer are infertility and the risk of inducing a second malignancy.

Chronic and late toxicities are the price we have to pay for curing or inducing long term remissions in patients with otherwise rapidly fatal conditions. However by the manipulation of chemotherapeutic regimens it should be possible to minimise these effects eg there is a suggestion that the use of ABVD rather than MOPP may allow patients to be treated for Hodgkins disease with reduced likelihood of infertility and the development of second malignancy (Bonadonna et al, 1984). Continued research is necessary to reduce as far as possible the long term morbidity of chemotherapy.

High Performance Liquid Chromatography

The science of pharmacokinetics depends on an ability to detect and quantify drugs and their metabolites in biological samples. In order to do this the compounds of interest must be separated from the other constituents of the sample and then passed through an appropriate detection system. Over the past 40 years chromatography has emerged as the most flexible and efficient method of achieving such separation.

Although chromatographic-like processes occur in nature eg the migration of solutions through soils, clays and porous rocks, their value was not recognised until the 19th century. In 1850 a German chemist, Runge, developed methods for testing dyes and bleaches produced from coal tars. He demonstrated the composition of dye colours by spotting the mixtures on to special paper producing colour separations. However Runge and others could not explain the processes involved in the separation and it was some 50 years before the Russian scientist Tswett described the underlying principles and coined the term chromatography.

Tswett separated pigments from a plant extract by washing a sample of the extract through a column of chalk. He observed coloured bands moving down the column as solvent was passed through and because of this he described the method as chromatography (from the Greek for colour - chroma, and write - graphein). Tswett stated that, "Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system". Recently the International Union of Pure and Applied Chemistry (IUPAC) has defined chromatography as: "A method used primarily for the separation of the components of a sample, in which the components are

distributed between two phases, one of which is stationary and the other moves. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread in a layer, or distributed as a film etc; in these definitions chromatographic bed is used as a general term to denote any of the different forms in which the stationary phase may be used. The mobile phase may be gaseous or liquid." (IUPAC, 1974).

Liquid chromatography (LC) is the generic name used to describe any chromatographic procedure in which the mobile phase is a liquid and includes paper chromatography, thin-layer chromatography (TLC) and variants of traditional column chromatography. In the latter solvent is gravity fed on to a column of large particles (150-250nm) and the components of the mixtures are then carried through the packed column with the passage of the eluent. Separation is achieved by differential distribution of the sample components between the stationary and mobile phases. However classical liquid chromatography suffers from a number of disadvantages:

(1) Column packing is tedious and columns are often used only once.

(2) The efficiency of large particle columns is low and analysis times lengthy.

(3) Detection of eluent solutes is by labour intensive manual analysis.

Thin layer chromatography offered an answer to some of the above problems but automation is difficult and the procedure therefore slow and not always easily reproducible. The development of gas-liquid chromatography in the early 1950s overcame many of the problems of LC and TLC. This technique relies on each of the components of a sample

having an appreciable vapour pressure at the operating temperature of the column. Glass or metal columns are packed with a material such as PTFE which is coated with the stationary phase. The stationary phase must be thermally unreactive and non-volatile and materials such as methyl silicone are normally used. The sample is carried down the column by an inert gas eg nitrogen or helium and the separation processes involves an equilibrium being established by the solute between the stationary and mobile phases. The partition coefficient involved depends on solute vapour pressure and the thermodynamic properties of the bulk solute. The component with the longer retention time will have the higher heat of solution in the stationary phase. Altering the amounts of stationary phase on the column and the operating temperature will alter retention times. Detectors used with GC columns include flame ionisation, nitrogen phosphorous and electron capture. The principle limitation of GC is volatility of the sample and although this can be overcome in many cases by conversion of solutes to suitable volatile derivatives there remain a large number of compounds, notably many drugs and proteins, that cannot be analysed by GC.

The key to improving column efficiencies in LC lay in increasing the rate of mass transfer and equlilibration of solute molecules between the mobile and stationary phases. This was eventually achieved by using much smaller particle sizes and increasing the pressure drop across the column. Such changes required the concomitant development of equipment capable of delivering and withstanding such pressures and detectors of greater sensitivity. Thus it was not until the late 60s that Horvath et al (1967) constructed one of the first practical HPLC systems for use in their

work on nucleotides. Over the past 15 years HPLC has become firmly established as the most widely used of chromatographic techniques. It is not limited by component volatility or thermal stability and in addition many HPLC detectors are non-destructive allowing the opportunity for sample recovery for subsequent spectroanalytical analysis.

HPLC can be carried out in any of the classical modes of chromatography:

- liquid-solid adsorption
- partition
- ion-exchange
- ion-pair
- size-exclusion
- affinity

Of these various modes partition chromatography has emerged as the most widely applicable technique. Classical liquid-liquid partition chromatography was limited mainly by the problem of the stationary solvent stripping off the packing material however this was overcome by the development of packing materials where the stationary phase is chemically bonded or organo-bonded to an insoluble matrix. The most common stationary phase is octadecylsilane (ODS) which is bonded to a silica support. The ODS reacts with silanol groups on the surface of the silica producing a stable siloxane (Si-O-Si) linkage. Residual silanol groups are able to adsorb molecules and therefore interfere with the separation characteristics of the bonded phase. These sites may therefore be "capped" using trimethylsilyl chloride. The advantage of such supports is the stability of the siloxane linkages to column inlet pressures and hydrolysis thus allowing use of

solvents at up to 6000psi in the pH range 2.0-8.5.

The bound organic moiety is usually a straight chain hydrocarbon which imparts a hydrophobic character to the silica surface. Aqueous eluents are generally used with such stationary phases for the separation of substances encompassing a very wide range of polarity and molecular size. This technique is termed reverse phase chromatography. The hydrophobic ligates provide a "soft" nonpolar surface for eluite retention and also protect the support from degradation by contact with aqueous elements.

There are three types of bonded phase depending on the characteristics of the the organic moiety that is bound to the silica surface: polar, nonpolar and ion exchange.

Chromatography utilising a polar bonded phase has been termed "normal phase" to differentiate it from reverse phase HPLC. Columns with a glycerol type function attached to a large pore silica can be used in size exclusion of biopolymers and other water soluble polymers.

Bonded phases with fixed ionic functions such as amino and sulfonic acid groups attached to the surface via an organic moiety are used as anion and cation exchangers respectively in ion-exchange chromatography. These columns are useful for the separation of large organic molecules

Bonded-phases can be quite simply packed into HPLC columns to give efficiencies of up to 20,000 theoretical plates per 25cm if the 5um particle size is used. The larger particle size materials give proportionally smaller numbers of theoretical plates and consequent reduction in the resolution power of the column. Columns are packed using a slurry packing procedure with methanol, isopropanol or

methyl-iodide/methanol as the slurrying liquid. The slurry is driven into the column at pressures of up to 10,000lb/sqin using hexane. After packing the column is conditioned to accept eluent. For normal phase chromatography more hexane is used while for reverse phase chromatography mthanol is used to remove the hexane.

Reversed Phase Chromatography

Reversed phase chromatography is a branch of HPLC that employs hydrocarbonaceous bonded phases such as octadecyl-silica in conjunction with a water/organic mobile phase. The origins of reversed phase chromatography date back to Howard and Martin (1950). They treated kieselguhr with dimethyldichlorosilane vapor. With the non-polar porous support thus obtained they used for the first time a non-polar liquid such as paraffin oil or octane as the liquid stationary phase in conjunction with a polar eluent such as methanol-water. This chromatographic system was the reverse of conventional systems in terms of phase polarity and retention order. In reversed phase HPLC the polarity of the mobile phase is higher than that of the stationary phase. Non-polar solutes will tend to be retained on the column and their retention times will increase as the chain length of the bonded-phase increases. Longer solute retention generally enhances resolution and thus varying the chain lengths of the bonded phase is a further aid to optimizing the resolution. Conversely polar solutes are usually separated better on short chain alkyl phases. The percentage carbon loading (chain length) is not however as important a consideration as the percentage coverage of the silica. As noted above residual silanol groups and their adsorption effects can cause tailing of the peak and thus it is

preferable to use a reverse phase packing with a high percentage coverage or one in which the residual silanol groups have been treated with trimethylsilyl chloride.

It is the very broad range of eluent strength together with the employment of specific solvent effects that is responsible for the wide scope of the technique. In general the lower the polarity the higher the "strength" of the eluent under a given set of conditions. In most cases water-methanol and water-acetonitrile mixtures are used and the strength of such eluents increases dramatically with the concentration of the organic phase. It is advisable to use a buffer in the eluent in order to maintain an ionic strength greater than 0.01 and a constant pH when ionogenic substances are chromatographed.

The Mobile Phase

The mechanism of solute retention on bonded-phase materials is not fully understood, but the organo-bonded layer may be considered to act as a thin film of fluid. In reverse phase HPLC water is used as the base solvent. The eluent "strength" is adjusted by using organic modifiers usually methanol or acetonitrile.

The use of polar solvents as the mobile phase is as much a characteristic feature of reversed phase chromatography as that of non-polar bonded stationary phases. The ideal eluent which is compatible with the materials used in the chromatograph and the column has low viscosity and high optical transparency at low wavelengths and the appropriate chemical properties as far as solvent power and elutropic strength are concerned. Low viscosity combined with high UV transparency make acetonitrile, methanol and tetrahydrofuran the most popular organic solvents. Most experimental

situations require an eluent that is stronger than water but weaker than methanol or acetonitrile. Such eluents can be obtained by mixing an organic solvent with water so that a very wide range of eluent strength can be obtained.

Highly polar and ionisable molecules such as acids have a high affinity for aqueous eluents and wash through the column without being retained at all by the packing material. Modifications can however be made to the technique to allow even highly dissociated molecules to be successfully chromatographed by BPC. This has been achieved by using ODS-silica materials with aqueous buffer solutions containing no organic modifier. Solute retention is influenced by eluent pH which controls the degree of dissociation of the solute and hence its partition between the bonded organo-phase and the aqueous mobile phase. Non-ionisable compounds show little change in retention with variation in pH. The ion-suppression technique can be used for the analysis of weak acids or bases. For the analysis of acidic compounds small amounts of acetic or phosphoric acid are added to the mobile phase. By reducing the pH dissociation of the sample molecules is suppressed and they thus have decreased affinity for the eluent and are retained to a greater degree by the ODS phase. The range of BPC is considerably extended using techniques such as ionic suppression and this mode of LC using ODS bonded phases finds wide application.

Detectors for HPLC

A liquid chromatographic detector is a device for continuously locating the presence of solutes in a flowing liquid eluate coming from a chromatographic column. The electrical signal from the detector should be proportional to the concentration or mass of the solute emerging from the column. Most of the commercial detectors available produce a signal that is proportional to the concentration of the solute and give a linear response over a wide concentration range.

Fluorescence Detectors

Many compounds display the property of luminescence, that is, when they are irradiated with light in the UV region of the spectrum they undergo electronic excitation and emit light of a higher wavelength, either instantaneouly (fluorescence) or after some finite time delay (phosphorescence). In most cases the fraction of the absorbed energy that is re-emitted (known as the quantum efficiency) is rather low, but for a few compounds values of 0.1-1.0 are obtained and these compounds are suitable for fluorescence detection. A valuable feature of the fluorescence phenomenon is that the irradiating light can be filtered out so that the photometer only measures the emitted light and thus the fluorescence signal starts virtually from zero intensity. In contrast other photometric detectors such as the UV absorbance detector rely on measuring small differences between the light attenuated by the sample and that transmitted by the eluent.

To obtain the maximum signal to noise ratio with fluorescence detectors it is essential to ensure that the eluent has a low background fluorescence and that its composition is conducive to

giving the highest quantum yield for the compound of interest. For example some compounds only fluoresce well in non-aqueous solutions, others at certain pH ranges in aqueous solutions etc. The light source in most commercial fluorimeters is a xenon arc which provides a continuum over the the UV range of interest. Excitation filters are used to allow single wavelength bands to irradiate the photometric cell and emission filters to filter out all but the light of interest.

Fluorescence detection is unfortunately the least widely applicable of all the detection methods for few compounds display quantum yields of the required magnitude. It is sometimes possible however to impart fluorescence to compounds by coupling with fluorigenic agents.

Electrochemical Detectors

Monitoring by this principle is only possible when the compound of interest is electrochemically active under the experimental conditions used and the eluent has sufficient conductivity. In general it is only those compounds which can readily undergo oxidation at the electrode surface that are conveniently detectable. This is because electrochemical reduction in aqueous solution is complicated by high background currents generated by the presence of dissolved oxygen, hydrogen and trace metal cations all of which undergo reduction. Thus amines and phenols frequently occur as detected species whereas nitro-containing compounds do not.

In an electrochemical detector the eluent flows through a cell to which a potential is applied. This potential generates a background current from the eluent and the current increases if the solute undergoes oxidation or reduction as it passes through the detector.

The magnitude of the response obtained depends on the cell design, the chemical properties of the solute, the solute concentration, the applied potential and the eluent composition.

The most successful material for the working electrode of HPLC detectors has been glassy carbon. This is a pyrolytically prepared form of carbon that combines electrical conductivity with low chemical reactivity and the ability to accept a highly polished surface. This latter feature is important since it allows a rapid flow of solvent across the face of the working electrode thus preventing build up of electrochemical products.

UV Absorbance Detectors

Chromatographic monitoring by measuring the UV absorbance of the eluate is the most popular HPLC detection method. The principle of the procedure is that the light absorbance of a solution in a cell of fixed length is directly related to the concentration of the absorbing species. The Beer-Lambert law applies:

$$\log Io/I = abc = \text{absorbance}$$

where a = molar absorptivity or molar extinction coefficient

b = the path length of the cell

c = concentration of the absorbing species

Io = the intensity of the incident light

I = the intensity of the transmitted light

The measurement of absorbance requires the sensing of the difference between the levels of incident and emitted light intensity with considerable accuracy. Variable wavelegth detectors use a deuterium lamp which provides a continuous band of emitted light over the range 180-400nm. The operating wavelength is selected by means of a manually operated grating monochromator.

Mass Spectrometric Detectors

Mass spectrometers can be coupled to a liquid chromatograph to obtain spectral identification of a specific compond. However due to the comparitively large volumes of eluate and solvent the technique is difficult and more applicable to gas chromatography.

IR Absorbance Detectors

These operate in an analagous manner to UV absorbance detectors over the region of 2.5-14.5um. Water must generally be absent from eluents for use with IR detectors.

Refractive Index Detectors

Refractive index detectors operate by measuring the difference in refractive index between the eluent and the solvent modified eluent. The type of response therefore is very dependent on the choice of solvent. All RI detectors are highly sensitive to temperature changes and hence thermostatic control is essential for operation at the most sensitive range for these detectors.

Mass Detectors

This type of detector utilises a novel principle. The eluate is nebulised and carried by an air stream through a heated column in which the solvent vaporizes and a fine mist of solute particles is formed. The suspension of particles is then passed through a monitored light path where scattering occurs and by having a photomultiplier mounted at right angles to the particle stream and at a 120^{o} angle to the collimated light source a response

proportional to the mass of the solute is produced. The response is not dependent on the the chemical composition of the solute although the compounds must be involatile and the solvent volatile.

HPLC Method Development

Selection of the column and initial mobile phase conditions for the separation of a particular compound is governed by past experience with similar compounds or compounds containing similar chemical groups. The important factors to be taken into account are the size, degree of polarity and solubility in aqueous and organic solvents of the compound under study. Subsequently optimum resolution is achieved by experimenting with changes in the organic strength and pH of the mobile phase. For example an increasing organic strength will reduce the retention time for non-polar solutes while adjusting the pH will affect the resolution of polar compounds.

Pharmacokinetic Methods

Pharmacokinetics is the study of the time course of drug and metabolite levels in different fluids, tissues and excreta of the body, and of the mathematical relationships required to develop models to interpret such data. The discipline of pharmacokinetics evolved in the 1930s with the publication of the first papers dealing with the subject by Professor Teorell (Teorell 1937 a,b). During the ensuing years there was a rapid development of the concepts, methods and applications involved and in 1972 the NIH formally recognised the existence of pharmacokinetics by sponsoring an International Conference on Pharmacology and Pharmacokinetics.

Pharmacokinetics originated as a predominantly theoretical discipline with the formulation of models to fit experimental data considered an end in itself. However more recently it has been recognised that pharmacokinetics can play an important part in determining optimal doseage regimens for individual drugs. Thus knowledge of elimination half life and clearance indicate that certain drugs eg Atenolol and phenytoin need only be administered once daily to maintain an adequate therapeutic level while others eg propranolol and sodium valproate need to be taken more frequently. The contribution of pharmacokinetics to cancer therapy is more difficult to define. For the majority of the 40 or so commonly used chemotherapeutic agents doses and schedules have been arrived at by trial and error. Cancer therapy is unique in that drugs are often used at or close to their maximum tolerated dose principally because there is evidence that by increasing dose rate and intensity greater cell kill can be achieved (Connors et al 1984). Thus appropriate doses can only be arrived at through careful clinical

trials. There are however a number of situations where phamacokinetic data have been usefull. It is important to know the major site of excretion of a drug since organ dysfunction can lead to greatly enhanced systemic exposure and therefore toxicity. For example the toxicity of cis-Platin is directly related to the glomerular filtration rate and hepatic dysfunction can significantly reduce the speed of anthracycline detoxification. Under these circumstances dose reductions are necessary to avoid excessive toxicity. In addition the timing of re-infusion of marrow for bone marrow transplantation depends on the rate of clearance of the cytotoxic drugs used.

In certain situations a knowledge of metabolite excretion has allowed safer use of agents. Following the administration of Ifosfamide active alkylating metabolites, notably acrolein, are excreted in the urine and cause severe dose limiting haemorrhagic cystitis. Recognition of this problem was followed by the development of Mesna for local detoxification of these metabolites in the bladder. The rational administration of mesna depends on a knowledge of the pharmacokinetics of Ifosfamide metabolism. A further situation in which pharmacokinetic measurements have been valuable is in the use of loco-regional chemotherapy. For example important studies have been necessry to demonstrate a pharmacological advantage at the peritoneal surface for drugs administered intraperitoneally (IP). Such an advantage has been demonstrated for a number of drugs (Ozols 1985) thus promoting interest in the use of IP chemotherapy for diseases such as ovarian cancer that are largely limited to the peritoneal cavity.

In recent years pharmacokinetic studies have become recognised as an essential part of phase I clinical trials. In addition to

providing useful data for the subsequent deployment of the drug in phase II trials the information gained may also be used, in conjunction with animal data, to facilitate more efficient dose escalation in the phase I trial itself. This will allow more rapid completion of phase I studies with fewer patients treated at doses unlikely to have any therapeutic benefit.

The Concept of Compartments

The most commonly employed approach to the pharmacokinetic characterisation of a drug is to represent the body as a system of compartments, even though these compartments have no physiological or anatomical reality. The one compartment model depicts the body as a single homogenous unit. This does not necessarily mean that the drug concentrations in all body tissues are the same at any given time but it does imply that changes in the plasma concentration quantitatively reflect changes occurring in tissue drug levels. In addition a one compartment model requires that drug elimination is a first order process ie rate of elimination at any time is proportional to the drug concentration at that time.

Most drugs entering the circulation require a finite time to distribute fully throughout the body space. During this distributive phase drug concentrations in the plasma will fall more rapidly than in the post-distributive phase. If distribution is related to blood flow highly perfused organs and tissues such as liver and kidney should be in rapid equilibrium with the blood. The blood and all readily accessible tissues and fluids may be treated kinetically as a homogenous unit referred to as the central compartment. Following iv administration the levels of drug in all tissues and fluids

associated with the central compartment should decline more rapidly in the distributive than in the post distributive phase. In contrast drug levels in poorly perfused tissues (eg muscle, lean tissue, fat) will increase first to a maximum and then begin to decline during the distributive phase. As with the one compartment model drug elimination and transfer between compartments in multicompartment systems are assumed to be first order processes.

At some point pseudo-equilibrium is reached and subsequent loss of drug will occur in a first order fashion indicating kinetic homogeneity with respect to drug levels of all the fluids and tissues of the body.

If the plasma concentration is plotted against time for a drug whose kinetics conform to a one compartment model it can be seen that the concentration follows a single exponential decay pattern falling rapidly at first and then progressively more slowly. This is is a reflection of the first order nature of the elimination processes. Thus a constant fraction of the drug is eliminated in unit time. This fraction is determined by the elimination rate constant (kel).

Because the drug concentration declines exponentially with time a plot of log conc against time is linear. This line can be described by the equation:

$$\log C = \log C0 . - kt$$

where : k is the slope of the line

C is the drug conc at any time t

C0 is the drug conc at time zero

C0 can be estimated by extrapolating the line back to the conc axis.

The relationship between drug concentration and time may also be

described by the exponential function:

$$C = C0.e^{-kelt}$$

where kel is the elimination rate constant

The exponential function can be derived from the log10 plot in the following manner:

(1) Transpose the relationship to the base of the natural $\log_e$.

For any number x : $2.3\log_{10}x = \log_e x$

Thus: $2.3\log_{10}C = 2.3\log_{10}C0 - 2.3kt$

or : $\log_e C = \log_e C0 - 2.3kt$

(2) Taking the antilogs of both sides:

$$C = C0 \cdot e^{-2.3kt}$$

Thus the elimination rate constant is equivalent to 2.3 x slope of the straight line obtained from the semi-logarithmic plot.

For drugs exhibiting multicompartment kinetics a plot of plasma concentration against time reveals a multi-exponential decay pattern, the number of exponentials being equivalent to the number of compartments. Such a line can be described by the equation:

$$C0 = Ce^{-\alpha t} + Be^{-\beta t} \text{ etc}$$

A plot of log conc against time of this line results in a decay curve with a series of linear segments again equivalent to the number of theoretical compartments. For each linear section of the decay curve events taking place in the relevant compartment are dominant. For any model, however, a point is reached where equlibrium between the various compartments is attained. Subsequent loss of drug from the plasma is then described by a monoexponential process indicating that the body is now behaving as a single compartment (the terminal elimination phase).

Although compartmental analysis provides an important insight into

the behaviour of a drug several useful parameters may be calculated without recourse to a model. These model independent parameters include the apparent volume of distribution, clearance, area under the curve and the terminal elimination half life or biological half life.

The Elimination Half Life

The elimination half life is the time taken for the concentration and amount of drug in the body to fall by one half eg from C0 to 1/2C0. Using the exponential equation of the curve this can be expressed as:

$$0.5C0 = C0 \cdot e^{-keltl/2}$$

or $$0.5 = e^{-keltl/2}$$

or $$e^{keltl/2} = 2$$

Taking antilogarithms

$$kel.tl/2 = \log_e 2$$

or $$keltl/2 = 0.693$$

or $$tl/2 = 0.693/kel$$

where kel = 2.303.k (calculated from the $\log_{10}$ vs time plot)

The Volume of Distribution

The apparent volume of distribution is a theoretical value obtained by dividing the amount of drug in the body by the plasma concentration. This calculation requires equlibrium to be reached between plasma and other tissues. The amount of drug is known immediately after an intravenous bolus but equilibrium is not reached until the start of the elimination phase ie the terminal linear portion of the log conc vs time plot. An estimate of the plasma

concentration that would have resulted had all the drug spontaneously distributed into its final volume of distribution is obtained by extrapolating the line delineating the elimination phase back to time zero. Using this value:

$$\text{Vol of Dist} = \text{Dose}/C_o$$

The value for the volume of distribution obtained by this method is often a good estimate but occasionally it is not. The best method is to divide clearance by the elimination rate constant. As shown below:

$$\text{Clearance} = k \cdot V$$

Thus $$V = \text{Clearance}/k$$

For a multi-compartment model the clearance from any individual compartment will be the same once equilibrium has been established thus:

Clearance from central compt. = kc x Vc

= Total body clearance

= kel x Vd

where kel = the terminal elimination rate constant

kc = the elimination rate constant from the central compart.

Vc = the volume of the central compartment.

Vd = the total vol of distribution at time zero.

A number of deductions can be made once the volume of distribution is known. A small volume implies that the drug is largely confined to the vascular space whereas larger volumes, often exceeding the total real body volume by many times, indicates extensive tissue distribution or binding to to cellular elements.

Clearance

Plasma clearance is the volume of plasma cleared of drug in unit

time. Clearance must therefore be related to the volume of distribution and the rate of elimination. In fact clearance is defined as:

Volume of distribution x elimination rate constant

The clearance may be altered by changes in the volume of distribution for example a fall in plasma protein concentration for a highly protein bound drug, or changes in kel. Saturation of metabolism is a method by which kel can be altered.

If the clearance is known then the actual rate of drug elimination can be calculated by multiplying clearance by concentration:

Rate of elimination = Clearance . Conc

The rate of elimination can be expressed in another fashion by returning to the exponential function:

$$C = C0.e^{-kelt}$$

Differentiating with respect to time yields:

$$dC/dt = -kel.C0.e^{-kelt}$$

Since $C = C0.e^{-kelt}$ it follows that:

$$dC/dt = -kel.C$$

Multiplying both sides by V, the volume of distribution gives:

$$V.dC/dt = V.kel.C$$

The rate of change of concentration with respect to time multiplied by the volume of distribution is equivalent to the rate of change of drug in the body and therefore also to the rate of elimination.

Thus Rate of elimination = Clearance . C = kel.V.C

or Clearance = kel.V

or Clearance/V = kel

or V = Clearance/kel

Thus since t1/2 = 0.693/kel

$$t1/2 = 0.693.V/Clearance$$

During a small time interval dt the amount of drug eliminated is equal to Clearance.C.dt, where C.dt is the corresponding small area under the plasma concentration time curve. For an intravenous injection the total amount of drug eliminated is equal to the dose and this can be calculated by adding up the amount in each time interval from zero to infinity, therefore,

$$Dose = Clearance \; . \; AUC$$

Clearance can therefore be calculated from a knowledge of the dose and the AUC. In addition the AUC can be used to calculate the amount absorbed when a drug is given by an alternative route by comparison with the AUC following iv administration.

Area Under the Concentration x Time Curve (AUC)

The intensity and duration of any tissue response is probably in most cases a function of the concentration and persistence of the drug in the blood. Thus we would expect the size and duration of the biological effect to be related to the area under the concentration x time curve.

Assessment of AUC

The simple numerical estimation of area using the trapezoidal rule requires only a table of experimental data and is applicable to all methods of drug administration. This method involves calculating the sum of the products of each individual time interval with the average drug concentration during that interval. The average concentration is obtained from:

$$(conc \text{ at start time} + conc \text{ at end time})/ 2$$

When a drug is given as an intravenous bolus and the decline is exponential the total area under the curve is calculated most rapidly by dividing the extrapolated zero time concentration Co by the elimination rate constant, kel.

Proof: Total area = $\int C \cdot dt$

since $C = Co \cdot e^{-kelt}$

$$\text{area} = \int Co \cdot e^{-kelt} \cdot dt$$

and since Co is a constant

$$\text{area} = Co \int e^{-kelt} \cdot dt$$

Which upon integrating between time zero and infinity yields:

$$\text{Total area} = Co/-kel.^{-kelk} = Co/-k \cdot 0-1 = Co/k$$

For a drug demonstrating multi-compartment kinetics:

$$AUC = A/\alpha + B/\beta + C/\gamma \text{ etc}$$

The relative amounts that each phase contributes in terms of drug elimination can be estimated by comparing the relative areas.
If A/α is less than B/β then the a phase is largely distribution. If A/α is equal to or greater than B/β then significant drug elimination takes place during this phase.

The Two Compartment Model

Normally a one compartment model is not sufficient to describe the kinetics. As mentioned previously there is often an initial distribution phase followed by a terminal elimination phase. During the elimination phase the graph of log conc vs time moves into a log linear phase. This phase represents elimination from the central compartment in equilibrium with the second compartment. The slope of this line can be used to determine the rate constant β in a similar fashion as for kel in a one compartment model. When the time

is plotted against $\log_{10}$ then:

$$\beta = 2.3 \times \text{slope}$$

When time is plotted against $\log_e$ then:

$$\beta = \text{slope}$$

This rate constant is a hybrid rate constant related to several individual constants. It governs the overall elimination of the drug. This can then be used to calculate the half life, sometimes referred to as the biological half life:

$$T1/2 = 0.693/\beta$$

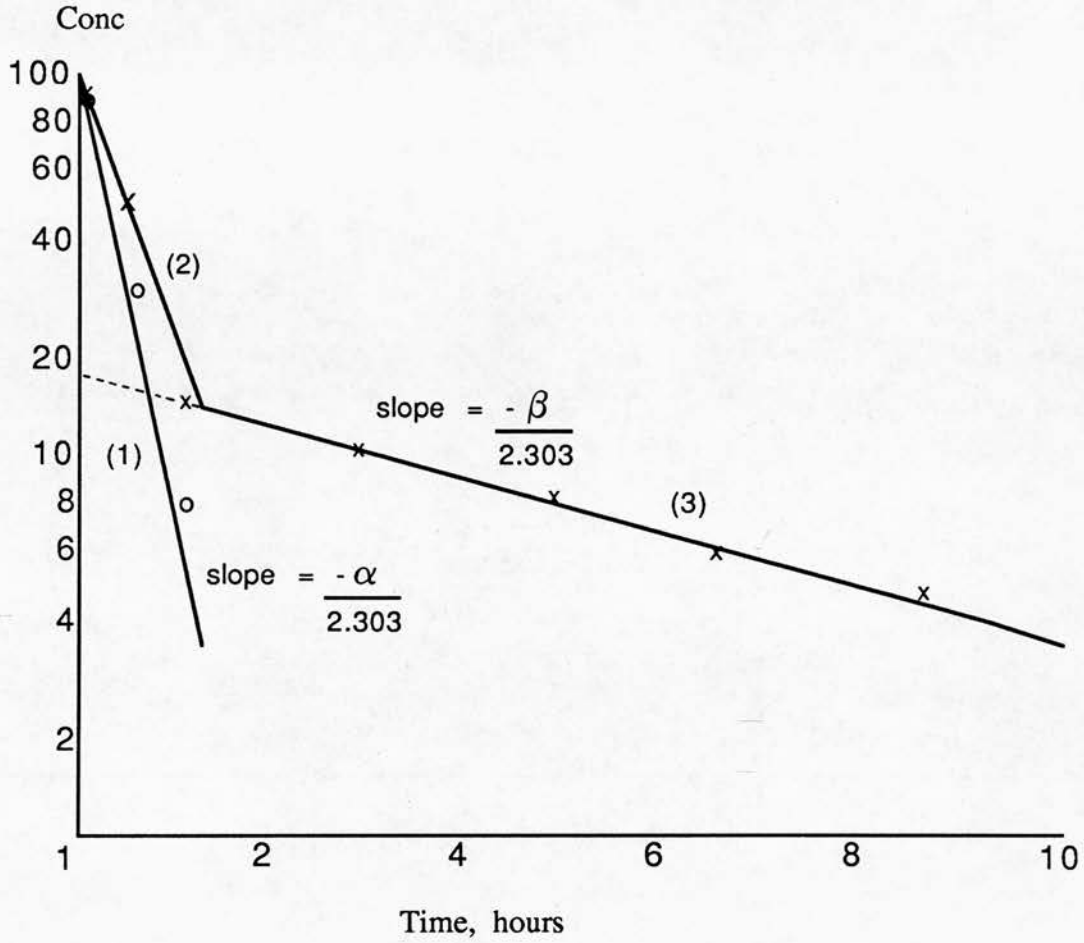
The rate constant β should not be confused with Kel the rate constant governing elimination from the central compartment. For the one compartment model the rate of elimination is governed by the total amount of drug in the body and thus β and kel are identical. In a two compartment model the two constants are closely related but will not be identical. In such cases elimination often takes place only from the central compartment and thus the rate of elimination at equilibrium will be governed by the amount of drug in the central compartment rather than the total amount in the body. Thus kel would be greater than β. It is from β that the biological half life can be obtained. Although β or the biological half life obtained there from is a highbrid parameter it is probably the most important functional pharmacokinetic parameter. kel may be calculated from:

$$kel = A + B \; / \; (A/\alpha + B/\beta)$$

In this equation B represents the apparent concentration if the drug has been distributed instantaneously throughout both the central and peripheral compartments. Thus intercept B can be used to calculate an apparent volume of distribution. A and α are the

fig 1

Method of Residuals



Line (1) is obtained by subtracting the values on the extrapolated line (--------) from the actual experimental values giving the residual concentration.

intercept and slope of line (1) (fig 1). This line results when Cp values which lie on the extrapolation of line (3) are deducted from the real values on line (2). This is known as the method of residuals (Gibaldi and Perrier, 1975). The slope of the line is used to determine the rate constant α which is another highbrid value governing the distribution of the drug into the second compartment. If A is added to B we obtain Co the theoretical concentration of the drug in plasma at time zero. This can be used to calculate the volume into which the drug was initially introduced ie the central compartment.

$$Vc = Dose/A+B$$

The elimination phase is characterised by two parameters, the elimination half life and the apparent volume of distribution. The elimination half life is the time taken for the plasma concentration as well as the amount of drug in the body to fall by one half. All the drug (97%) will in practice be eliminated in 5 half lives. The elimination half life is calculated as above:

$$T1/2 = 0.693/\beta$$

Practical Applications

The experimental data should first be plotted on semilog graph paper. This will enable a visual assessment of the decay curve allowing an estimate of which kinetic model is likely to be applicable. If the data appear to fit a one compartment model the slope of the line may be calculated using the linear regression programme on a scientific calculator. The correlation coefficient will give an indication as to the linearity of the line.

(1) $T1/2\,\alpha = 0.693/kel$ (kel = 2.303 x slope)

(2) AUC is calculated using the trapezoidal rule

(3) Clearance = dose/AUC

(4) Vd = Clearance/kel

If the data appear to fit a two compartment model the slope of the elimination phase is determined as for the one compartment model using only the points applicable to that phase. The correlation coefficient is a useful check as to whether the correct starting point for the elimination phase has been chosen.

The points on the first phase are calculated using the method of residuals described previously. Again linear regression is used to find the slope of the line.

Tl/2 = 0.693/2.3 x slope

Tl/2 = 0.693/2.3 x slope

AUC is calculated using the trapezoidal rule

Clearance = Dose/AUC

Vd = Clearance/kb

The intercepts A and B can be determined by extrapolating lines a and b back to the y axis. These values can be used to calculate the volume of the central compartment:

$$Vc = Dose/A+B$$

and as an alternative way of calculating AUC

$$AUC = A/ + B/$$

Computer Modelling

Computers can be used to calculate what the ideal concentration values would have been if the kinetics had conformed perfectly to the model. The computer performs a series of calculations using the experimental data and the initial estimates for the slopes and

intercepts of the linear segments of the curve as a starting point.

At the completion of the calculations new values for A, α, B and β are determined which can be used to calculate intercompartmental rate constants, half lives and volumes of distribution. It is common for experimental data not to fit a model.

For a two compartment model the elimination rate constants and intercompartmental rate constants (fig 2) are calculated as follows:

kel	=	$A + B / (A/\alpha + B/\beta)$
k21	=	$(A.\alpha + B.\beta)/(A + B)$
k12	=	$\alpha + \beta$ - k21 - k10
AUC	=	$(A/\alpha) + (B/\beta)$
Vc	=	Dose / A + B
Vd	=	Dose / (β.AUC)
Vp	=	Vc (k12.k21)
Vss	=	Vl (1 + k12/k21)
Clearance	=	Dose / AUC
	or	Vc . k10
	or	Vd . β

Where A and B are the intercepts on the concentration axis and α and β are the respective elimination rate constants.

Vc is the volume of the central compartment

Vd is the volume of distribution at time zero

Vp is the volume of the peripheral compartment

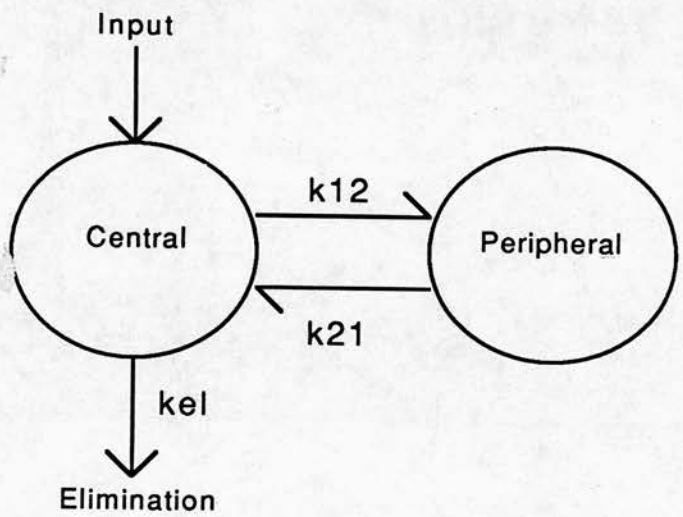
Vss is the volume of distribution at steady state

kel is the elimination rate constant

k12 is the costant governing movement of drug from the central to the peripheral compartment

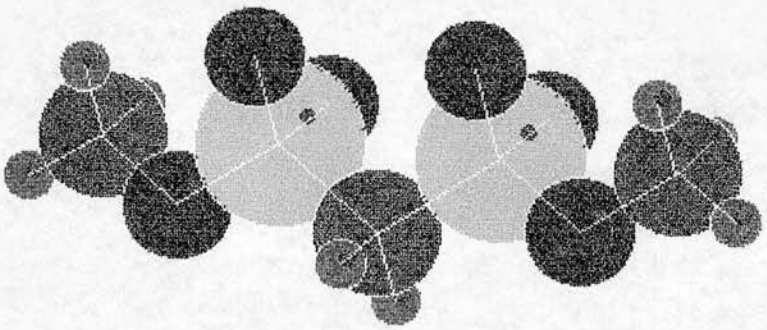
k21 is the constant governing movement of drug from the peripheral to the central compartment

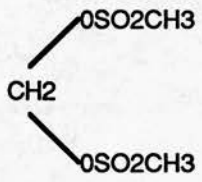
fig 2 **Two Compartment Model**



A Phase I Study of Methylene Dimethane Sulphonate

fig 3 **Methylene Dimethane Sulphonate**





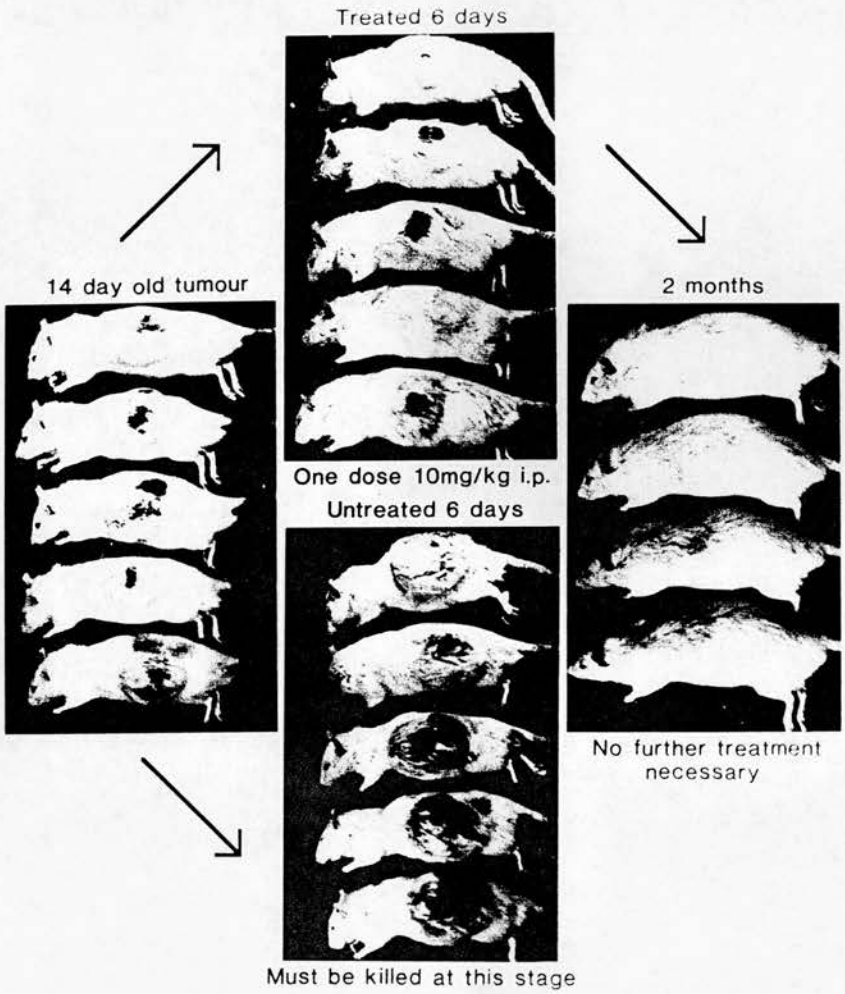
Introduction

Methylene dimethane sulphonate (MDMS) is the first member of the homologous series of straight chain dimethane sulphonic acid esters. Members of the series 2-10 were first synthesised in 1950 in an effort to identify new alkylating agents following the success of the nitrogen mustards. They were formed by the reaction of methane sulphonyl chloride with the appropriate alkanediol under anhydrous conditions (Emmons and Ferris, 1953). These compounds were tested for their antitumour activity against the Walker 256 carcinoma (Haddow and Timmis 1951), their neutrophil depressing activity (Elson 1958) and their immunosuppressive activity (Berenbaum et al 1967). In all cases optimal biological activity was observed with the 4-carbon chain member of the series, 1,4-dimethanesulphonyloxybutane (Busulphan). Further studies showed Busulphan to be most selective in producing a greater depression in neutrophils than lymphocytes (Elson 1958) and this drug soon became established as the treatment of choice in the management of chronic myeloid leukaemia. During these studies the n=2 compound showed a lack of activity and probably for this reason the n=1 diester was not investigated until later.

MDMS was synthesised by the reaction between silver -methanesulphonate and dibromomethane in 1953 (Emmons and Ferris). However it was not until 1965 that Fox and Jackson reported the activity of this drug in the Walker carcinoma. Subsequently Fox (1969) demonstrated that single doses of MDMS administered to rats bearing established 14 day old (20-30g) tumours resulted in cures in approximately 90% of animals (fig 3). Comparable doses of Busulphan failed to produce complete regression of the tumour and the level of inhibition was much less than with methylene dimethane sulphonate.

fig 4

Effect of MDMS on Yoshida Sarcoma in Rats



Although it has demonstrated significant activity in rat tumours MDMS is largely inactive in murine tumours other than the TLX/5 ascites tumour.

In 1976 Nias and Fox in experiments with HeLa and Chinese Hamster (ovary) cells showed that MDMS exerts its maximal cell kill at the end of G1 and the beginning of S phase with the lowest activity at the end of S and during G2. In more recent years studies of the first 9 straight chain dimethane sulphonic acid esters have demonstrated that MDMS produces approximately three times more DNA-DNA crosslinking than Busulphan at a comparable treatment level in cell culture (Bedford and Fox 1983). Moreover there appeared to be a correlation between cytotoxicity in these systems and the degree of crosslinking caused by different members of the series. MDMS is a small molecule (molecular wt. 204) and it has been estimated that the distance separating the alkylating moieties following the elimination of methane sulphonic acid is of the order of 2.3A (Bedford and Fox 1983). This distance is too small to span the more usual nucleophile distances but is compatible with that between the atoms involved in the DNA interstrand hydrogen bonds. It is possible therefore that MDMS may act by replacing such a bond.

The high activity in the Yoshida tumour, apparent greater formation of DNA crosslinking compared to Busulphan and possible novel site of alkylation stimulated interest in bringing MDMS forward for clinical testing.

Formulation

The formulation was carried out by Dr. Vezin, CRC Formulation unit, Dept. of Pharmacy, Strathclyde University, Glasgow. Before embarking on the formulation the main considerations to be taken into account were:

(1) Likely dose range

(2) Aqueous solubility

(3) Stability

Doseage

Preliminary work indicated that the LDl0 in mice was of the order of l0mg/kg. Thus for the average adult a single dose might be expected to range from 5 - 80mg.

Aqueous solubility

The aqueous solubility is calculated to be 7 - 8 mg/ml at 21^{o}C. However the drug has a strongly hydrophobic surface requiring fine pulverisation to increase the rate of dissoloution. Solubility in aqueous solution is therefore slow.

Stability

MDMS hydrolyses rapidly in aqueous solution to formaldehyde + methane sulphonic acid. The half life of this reaction is 22min at 37^{o}C.

The instability of the drug in aqueous solution suggested that iv bolus rather than infusion would be the appropriate method of administration. Instability also meant that a simple aqueous formulation was not possible and therefore the drug would have to be presented in non-aqueous solution or as a powder with reconstitution in an aqueous vehicle immediately prior to administration.

MDMS was found to be readily soluble in alcohols, lipophyllic and

polar solvents. The most elegant formulation proved to be a freeze dried preparation. MDMS (1.25g) was first dissolved in Dimethylsulphoxide (DMSO, 50ml) containing less than 1% w/v water. Appropriate aliquots of this solution were placed in vials and these freeze dried under vacuum until all visible trace of DMSO had gone.

This preparation appeared to be stable for over 12 months when stored at $4^{o}C$. The drug is particularly soluble in dimethyl acetamide (over 100mg/ml) and thus this agent was chosen for reconstitution. Dimethylacetamide (DMA) has been used in the formulation of several chemotherapeutic agents including AZQ, mAMSA, VM26, PCNU, N-methylformamide and Hexamethylmelamine. Although there was a suggestion of hypersensitivity to DMA in one trial (Posada et al, 1984) and elevation of hepatic transaminases in another (Earhart et al, 1981) these have not been confirmed by subsequent studies and the compound appears to be safe and free from biological effects at the doses used. A potential problem with polar solvents is that they may react with plastic containers. However a study by Vishnuvajjala and Cradock (1984) demonstrated negligible dissolution of PVC bags by 5 and 10% DMA solutions when stored for periods of up to 20 hours. Significant reaction with the plastic only occurred when the concentration of DMA exceeded 60%. In addition we have compared the Infra-red spectra of pure DMA with that of pure DMA that had been stored in a standard polypropylene syringe for four weeks and there was no evidence of any reaction. Syringes manufactured from the more reactive polystyrene should not however be used for drawing up pure DMA.

Vials for clinical use have been prepared containig 20mg, 50mg and 100mg MDMS. These are rconstituted with 0.5ml DMA; 10-20ml sterile

water is then added to each vial providing a final solution containing 2.0mg/ml MDMS and 5% v/v DMA for injection. Reconstituted drug must be used immediately to avoid significant hydrolysis.

Formal testing of the vials for clinical use have shown them to be over 99% pure and entirely free from contaminants and pyrogens.

Preclinical Toxicology

Preclinical toxicology was carried out according to the requirements of the CRC Phase I Committee by Life Science Research. These requirements included :

(1) To identify the LD50 and LD10 with 95% confidence limits for IV and IP schedules.

(2) To identify target organs and systems

(3) To assess the time course of any toxic effects

(4) To identify delayed or irreversible effects at sub-lethal doses

Acute Intravenous Toxicity in Male Mice

The acute intravenous toxicity was investigated in groups of male mice of the Charles River CD-1 strain at doses in the range 54-150mg/kg. Mortality and signs of reaction to treatment were recorded during a 28 day observation period. All decedents and mice killed on day 29 were subject to necropsy.

The principal sign of reaction to treatment was an ungroomed appearance which persisted until death or day 13. Deaths occurred in all doseage groups usually between day 6 and 9. No animal survived a dose of 100 or 150mg/kg. There were no consistent findings on necropsy of either decedents or animals surviving to 28 days.

Under the conditions of the study the acute median LD50 and LD10 were as follows:

	median	**95% confidence interval**
	mg/kg	mg/kg
LD50	61.7	33.8-65.1
LD10	46.8	44.4-49.3

Acute Intraperitoneal Toxicity in Male Mice

The acute intraperitoneal toxicity of MDMS was investigated in groups of the Charles River CD-1 strain at doseages in the range 20-101mg/kg. MDMS was administered at a constant volume of 20ml/kg in 0.5% w/v carboxymethylcellulose.

Overt signs of toxicity were similar to those with the acute iv schedule. Death occurred in groups given a dose of 45mg/kg or above, usually between six and eight days after treatment.

Necropsy of decedents and animals surviving to 28 days again showed no consistent changes.

The acute intraperitoneal median LD50 and LD10 were as follows:

	median	**95% confidence interval**
	mg/kg	mg/kg
LD50	50	49-51
LD10	43	42-44

Subacute Intraperitoneal Toxicity in Male Mice

MDMS was administered at seven doseages in the range 1.4-43mg/kg at a constant volume doseage of 20ml/kg on 5 days per week for four weeks in 0.5% carboxymethylcellulose in water.

As with iv doses overt signs of treatment were confined to

non-specific signs of ill health. Death occurred in groups treated at 5.4mg/kg or above with no animal surviving treatment at 10mg/kg or above.

No significant lesions were found on necropsy of decedents or mice surving to 29 days.

The median LD50 and LD10 were as follows:

	median	**95% confidence interval**
	mg/kg	mg/kg
LD50	5.4	3.9-7.4
LD10	2.6	1.9-3.6

Supplementary Intraperitoneal Study

A supplementary study was performed to assess any temporal changes in haemograms and bone marrow cytology in male mice following a single intraperitoneal administration of MDMS at a dose approximately equal to 1/10th LD10; and to detect any changes in bone marrow cytology or histological abnormalities following single intraperitoneal administration of a dose approximately equal to the acute median lethal dose.

The haemograms demonstrated a treatment related reduction in total leukocyte count largely attributable to a reduced lymphocyte population and an absence of eosinophils among treated mice during the first week of observation. Bone marrow cytology revealed no consistent treatment related changes.

Histological examination showed peritonitis involving several abdominal organs together with degeneration of the germinal epithelium and interstitial cell hyperplasia of the testes in animals killed 21 and 28 days after treatment. There were no other consistent

histological changes.

Toxicity Check in the Rat

Groups of male rats were treated with MDMS using a 4x5 day intraperitoneal schedule at a dose equivalent to 1/10 mouse LD10. No toxic effects attributable to MDMS were seen.

Summary

The LD10 in mice using the acute iv schedule was 47mg/kg. This is equivalent to 141mg/m^2 and thus the starting dose for phase I testing was chosen as 1/10th of this dose ie 14mg/m^2.

Haematological monitoring revealed a possible treatment related reduction in circulating leukocytes during the first week after treatment but no consistent marrow changes were seen. In addition there were no significant histological changes other than in the testes and local sclerosant effects on the peritoneum. These studies therefore indicate that MDMS may be marrow toxic and may produce infertility.

Clinical Trial

The phase I clinical trial of MDMS commenced in January 1985. The major points from the protocol were as follows.

Patient Eligibility

(1) Histological diagnosis of malignancy

(2) Any solid tumour type

(3) Age between 16 and 75 years

(4) Performance status 2 or less, life expectancy over 3 months

(5) Patients should have received conventional therapy where this exists

(6) Patients must have had no chemotherapy in the 4 weeks prior to commencing MDMS (6 weeks for nitrosoureas and mitomycin-C) and must have recovered from the toxic effects of prior treatment (alopecia excluded).

(7) Minimal haematological requirements were WBC above $3x10^9/l$, platelet count above $100x10^9/l$.

(8) Minimum renal and hepatic function were, serum creatinine 0.12mmol/l, normal bilirubin, hepatic transaminases less than 50% above the upper limit of normal.

(9) Patients with CNS involvement were excluded.

(10) Patients with serious co-existing medical conditions were excluded.

Consent

In line with the current policy at this institute patients were informed verbally of the experimental nature of the treatment and the limited therapeutic expectations of the study. They were given every oportunity to decline to take part and were assured that if they decided not to enter the trial they would continue to receive all available supportive therapy.

Following such an explanation 2/41 patients declined to take part.

Serial Observations During the Trial

	On + Off study	Prior to each course	Weekly
Height	+		
Weight	+	+	
Fbc	+	+	+
Biochemical profile	+	+	+
LFTs	+	+	
CXR	+		
ECG	+		

Starting dose, patient entry and escalation procedure

The starting dose for the trial was 14mg/m^2 using an intermittent bolus schedule with an initial interval between doses of 21 days. It was intended to enter 3 patients at each non-toxic dose with increasing numbers at doses exhibiting toxic effects. Dose escalation was by the modified Fibonacci series. There was no within patient dose escalation in order not to obscure data on cumulative toxicity. It was planned that patients would receive at least 2 courses of therapy. Further courses could be given in the event of a response occurring.

Prior to commencing the trial the protocol was submitted to the Christie Hospital Protocol Review Committee, the CRC Phase I Committee and the South Manchester Ethical Committee. In addition permission to use MDMS in this trial was obtained from the DHSS.

Results

All platelet and leukocyte counts are expressed as the value x 10^9/l.

Dose Level

14 mg/sqm Date start 15:1:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
KM	SCLC	61	2	Liver
BT	SCLC	63	1	Mediastinum
PM	Melanoma	34	2	Peritoneum

	Prior XRT	Prior Chemotherapy
KM	Spine	Ifosfamide, VP16
BT	Mediastinum	Ifosfamide, VP16
PM	Mediastinum	DTIC, Vindesine

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
KM	7.3	14	-			
BT	5.0	14	21	4.9	7	14
PM	8.5	14	21			

	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
KM	243	7	-			
BT	309	7	14	378	14	21
PM	259	21	28			

	Other Toxicities	Transfusion
KM	none	0
BT	none	0
PM	none	0

Comment

This dose was entirely nontoxic and no anti-tumour activity was seen.

28mg/sqm Date start: 14:2:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
DG	Ca. Ovary	52	1	Local rec.
AA	SCLC	35	2	Mediastinum
DF	Melanoma	74	2	Vulva

	Prior XRT	Prior Chemotherapy
DG	None	Cis-Plat, Bleomycin, Cyclo., Chlorambucil
AA	Mediastinum	Ifosfamide, VP16
DF	Vulva	DTIC

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
DG	5.5	14	21			
AA	4.5	21	21	3.7	14	21
DF	7.2	21	21			

	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
DG	494	14	21			
AA	229	14	21	233	14	21
DF	175	21	28			

	Other Toxicities	Transfusion
DG	None	0
AA	None	0
DF	None	0

Comment

Again this dose was entirely non-toxic and there was no evidence of therapeutic effect.

46mg/sqm Date Start: 14:3:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
RR	SCLC	57	2	Mediastinum
JB	NSCLC	57	2	LSCF
JJ	Ca.Ovary	36	2	Peritoneum

	Prior XRT	Prior Chemotherapy
RR	Mediastinum	Ifosfamide, VP16
JB	Left neck	Ifosfamide, Cyclophosphamide
JJ	None	Cis-Plat, Bleo, Cyclo, CHIP, Chlorambucil

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
RR	9.6	14	21			
JB	7.8	7	14	5.4	14	21
JJ	1.8	14	28			
	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
RR	150	21	-			
JB	365	21	-	394	-	-
JJ	24	21	35			

	Other Toxicities	Transfusion
RR	Venous pain	0
JB	Venous pain	0
JJ	None	0

Comment

Patient JJ experienced grade 3 nuetropenia and grade 4 thrombocytopenia at this dose. This lady had a history of being very sensitve to chemotherapy and had had a great deal of prior treatment. Since the remaining two patients treated demonstrated no haematological toxicity it was decided not to delay escalation by studying further patients at this dose.

Patients RR and JB both experienced intense burning pain along the

vein radiating up the arm and over the anterior chest wall as the injection was given. The pain cleared rapidly when the injection was stopped but reappeared on restarting. The pain was minimised by injecting very slowly. There had been no delay in administering the drug following preparation but subsequently it was noticed that saline instead of water had been used in reconstitution. When JB had a second dose with saline used to reconstitute he had no discomfort on quite rapid bolus administration. The MDMS solution in saline was later analysed extensively but no reason such as more rapid pH change could be found to account for the pain.

70mg/sqm Date start: 15:4:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
WA	Mesothelioma	66	2	Pleura
IT	Ca. Ovary	64	1	Peritoneum
WM	NSCLC	54	2	Liver

	Prior XRT	Prior Chemotherapy
WA	None	Cyclophosphamide
IT	None	Chlorambucil
WM	None	Cyclo, Ifosfamide, Adriamycin, VP16, MTX

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
WA	7.3	14	21			
IT	2.9	14	21	2.8	21	28
WM	4.9	14	21			

	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
WA	170	21	28			
IT	81	21	28	58	35	49
WM	320	21	28			

	Other Toxicities	Transfusion
WA	none	0
IT	none	0
WM	none	0

Comment

There was no non-haematological toxicity at this dose. In addition two patients had no haematological toxicity. Patient IT developed grade 2 platelet and WBC toxicity and there was a suggestion of cumulative platelet toxicity. There was no evidence of anti-tumour activity.

100mg/sqm Date start: 16:5:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
WH	Ca.Rectum	63	1	Liver
PS	SCLC	41	1	Liver
MH	Ca.Ovary	52	2	Peritoneum

	Prior XRT	Prior Chemotherapy
WH	none	none
PS	mediastinum	Ifosfamide, VP16
MH	none	Cyclo, Cis-Plat, Melphalan, CHIP

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
WH	3.9	21	28	3.1	14	21
PS	3.0	21	28			
MH	2.1	21	42	1.2	14	35

	Course 3		
	WBC nadir	Time to nadir	Time to recovery
WH	2.9	21	28

	Course 1			Course 2		
	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
WH	111	21	28	103	14	21
PS	171	21	28			
MH	90	28	35	46	14	42

	Course 3		
	Platelet nadir	Time to nadir	Time to recovery
WH	71	21	35

	Nausea/Vomiting	Alopecia
WH	1	1
PS	0	0
MH	1	0

Comment

The first signs of GI toxicity occurred at this dose with mild

nausea in two patients. In addition WH developed grade 1 alopecia following the second dose of MDMS. There was more consistent myelosuppression with at least grade 1 leukopenia in all three patients. There was further evidence of cumulative platelet suppression in the two patients receiving more than one cycle of treatment. No responses were seen.

125mg/sqm Date Start: 13:6:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
HL	SCLC	65	1	Mediastinum
TL	Mesothelioma	54	2	Pleura
FH	STS	53	2	Peritoneum
WC	Ca.Ovary	58	1	Peritoneum
DH	NSCLC	51	2	Mediastinum
GJ	STS	64	1	Retroperitoneum
EW	Ca.Ovary	68	1	Pelvis
BE	SCLC	48	1	Lung
GS	Mesothelioma	58	1	Pleura
JT	STS	61	1	Pelvis
AN	Hepatoma	67	1	Liver
AS*	STS	54	2	Lung
GK*	Ca.Ovary	48	0	Peritoneum

	Prior XRT	Prior Chemotherapy
HL	Mediastinum	Cyclo, VP16, Methotrexate
TL	Ant. Chest	Cyclo, Adriamycin
FH	none	Ifosfamide, Adriamycin
WC	none	Cyclo, Cic-Plat, Bleomycin, Melphalan
DH	Mediastinum	Ifosfamide, Cyclo
GJ	none	Ifosfamide, Adriamycin
EW	none	Melphalan
BE	Mediastinum	Ifosfamide, Etoposide
GS	none	Cyclo
JT	none	Ifosfamide, Adriamycin
AN	none	none
AS *	Pelvis	Ifosfamide, Adriamycin
GK *	none	Cyclo, CHIP

	WBC nadir	Course 1 Time to nadir	Time to recovery	WBC nadir	Course 2 Time to nadir	Time to recovery
HL	3.7	28	28			
TL	1.7	28	died			
FH	2.0	14	28			
WC	1.5	14	28	1.8	21	28
DH	2.9	21	died			
GJ	2.3	14	21	2.4	28	35
EW	0.8	21	56	1.2	28	56
BE	3.0	21	28	2.5	14	died
GS	1.0	21	35			
JT	2.8	21	35	2.7	21	35
AN	3.0	14	21			
AS*	3.1	21	28	3.0	14	21
GK*	2.3	21	35	3.6	21	28

	WBC nadir	Course 3 Time to nadir	Time to recovery
WC	1.7	7	28
AS*	4.8	21	28

	Platelet nadir	Course 1 Time to nadir	Time to recovery	Platelet nadir	Course 2 Time to nadir	Time to recovery
HL	189	21	28			
TL	26	28	died			
FH	95	28	35			
WC	116	14	21	139	21	28
DH	36	21	died			
GJ	131	21	28	95	28	35
EW	34	21	42	26	28	died
BE	162	21	28	152	14	died
GS	21	21	35			
JT	154	21	35	32	21	died
AN	136	21	28			
AS*	84	14	21	45	14	28
GK*	45	21	28	96	28	35

	Platelet nadir	Course 3 Time to nadir	Time to recovery
WC	25	7	42
AS*	47	14	42

	Nausea/Vomiting	Alopecia	Venous Pain	Transfusions
HL	0	0	0	0
TL	0	0	0	0
FH	0	0	0	1
WC	2	3	0	1
DH	0	0	0	0
GJ	0	0	0	1
EW	1	1	0	1
BE	0	0	0	0
GS	1	1	0	1
JT	1	2	0	1
AN	0	0	0	0
AS*	2	3	mild	2
GK*	2	3	0	0

Comment

Thirteen patients were treated at this dose of whom twelve had had prior chemotherapy. Seven experienced grade 3 or worse haematological toxicity and ten grade 2 or worse haematological toxicity. In addition 7 patients required blood transfusions as a result of treatment. It thus appeared that this would be an appropriate dose for future phase II trials in patients who have received prior chemotherapy. 6/13 patients had either grade 1 or 2 nausea and vomiting. When vomiting occurred it commenced 2-4 hours after treatment and lasted 2-12 hours. 6/13 patients developed a degree of alopecia in 3 cases grade 3, requiring a wig. No anti-tumour activity was seen at this dose.

Two patients (*) had dose reductions performed according to nadir platelet counts as follows:

	nadir platelet count	
	50 - 75	25 - 49
% of previous dose	75%	50%

This scheme prevented the occurrence of grade 4 thrombocytopenia in subsequent courses.

170mg/sqm Date start: 4:7:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
RS	NSCLC	57	1	Lung
BB	Ca.Ovary	55	1	Pelvis
DW	NSCLC	54	1	Lung
TB	NSCLC	56	1	Peritoneum
HP	NSCLC	62	1	Lung
JP	NSCLC	64	1	Lung

	Previous XRT	Previous Chemotherapy
RS	none	Ifosfamide
BB	none	Melphalan
DW	none	none
TB	none	none
HP	none	none
JP	none	none

	Course 1			Course 2	
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
RS	2.5	21	28	2.0	21	28
BB	2.6	21	35	2.9	21	35
DW	2.7	14	21	5.4	14	21
TB	1.6	14	28			
HP	1.0	21	35	0.6	28	63
JP	2.8	14	21	2.5	21	28

	Course 3		
	WBC nadir	Time to nadir	Time to recovery
BB	2.0	35	49

	Course 1			Course 2	
	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
RS	175	21	28	32	21	35
BB	38	21	35	49	28	49
DW	135	21	28	112	21	28
TB	221	21	28			
HP	34	21	28	10	42	56+
JP	32	21	28	10	28	56+

Course 3

	Platelet nadir	Time to nadir	Time to recovery
BB	11	21	20 weeks

	Nausea/Vomiting	Alopecia	Venous Pain	Transfusions Blood	Plts
RS	0	2	none		
BB	0	2	none	1	1
DW	0	2	none		
TB	2	1	none		
HP	2	2	none	2	1
JP	1	3	none	1	1

Comment

At this dose the gastrointestinal side effects were similar to the previous dose but now all patients developed a degree of alopecia. Haematological toxicity was again more severe with marked cumulative thrombocytopenia. In two patients, both of whom had received no prior chemotherapy, the platelet count did not fully recover before they died 2 months after the second course of MDMS. In a third patient who received 3 courses of treatment the platelet count took 5 months to reach $100x10^9/l$. Three patients whose platelet counts fell to $10x10^9/l$ were given prophylactic platelet transfusions but no evidence of haemorrhage was seen other than minor spontaneous bruising.

225mg/sqm Date start:29:10:85

Patient	Diagnosis	Age	Performance Status	Major Disease Site
JS	NSCLC	63	1	Mediastinum
MM	NSCLC	49	2	Lung

	Prior XRT	Prior Chemotherapy
JS	none	none
MM	none	none

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
JS	2.1	14	28	1.1	14	35
MM	0.8	21	28	1.0	14	died
	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
JS	73	21	28	14	28	6 months+
MM	18	14	35	7	14	died

	Nausea/Vomiting	Alopecia	Venous Pain	Transfusions Blood	Plts
JS	1	3	0	1	0
MM	2	3	0	4	6

Comment

The first patient treated at this dose level tolerated the first cycle of treatment with mild nausea only and grade 2 haematological toxicity. A second patient was therefore entered. This patient developed grade 4 platelet and WBC suppression. This was complicated by recurrent haemoptysis and pneumonia together with elevation of the bilirubin and hepatic transaminases. The disturbance of liver function resolved when the fbc returned to normal and the infection cleared. No specific cause for this could be found. On recovery the patient was considerably better than before treatment with less dyspnoea and cough and improved appetite and general well being. Moreover a repeat chest X-ray demonstrated a significant reduction in

the size of the tumour mass although not reaching a partial remission. In view of this it was decided to proceed with a further course of MDMS but with a 50% dose reduction because of the previous grade 4 toxicity. Unfortunately despite the dose reduction the patient once again developed grade 4 haematological toxicity and died 3 weeks following therapy from a combination of pulmonary infection and haemorrhage.

Patient JS developed grade 4 thrombocytopenia following his second cycle of therapy but did not require platelet transfusion. By 6 months after treatment his count had reached $46x10^9/l$.

It was now becoming increasingly clear that whereas most patients who had not had prior chemotherapy could tolerate an initial dose of $170mg/m^2$ the administration of subsequent doses was restricted by cumulative thrombocytopenia. It was therefore decided to investigate an intermediate dose level of $150mg/m^2$ in previously untreated patients.

150mg/sqm Date start: 27:3:86

Patient	Diagnosis	Age	Performance Status	Major Disease Site
DT	Renal Ca.	54	1	Retropritoneum
HM	Colon Ca.	51	0	Lungs
RF	Renal Ca.	56	2	LSCF

	Prior XRT	Prior Chemotherapy
DT	none	none
HM	none	none
RF	none	none

	Course 1			Course 2		
	WBC nadir	Time to nadir	Time to recovery	WBC nadir	Time to nadir	Time to recovery
DT	7.4	14	21	2.1	14	21
HM	2.9	14	21	4.1	14	21
RF	2.1	14	21	2.2	21	28
	Platelet nadir	Time to nadir	Time to recovery	Platelet nadir	Time to nadir	Time to recovery
DT	156	14	21	42	14	28
HM	137	14	21	56	21	42
RF	128	14	21	35	28	56

	Nausea/Vomiting	Alopecia	Venous Pain	Transfusions Blood	Plts
DT	1	3	0	2	0
HM	0	3	0	0	0
RF	3	3	0	2	0

Comment

The results at this dose level indicate that grade 2-3 thrombocytopenia can be expected after two cycles of therapy. Platelet counts recovered at 3 weeks after the first cycle and at 4-8 weeks after the second cycle. The WBC had recovered by 4 weeks after both cycles in all 3 patients. This dose therefore appears an appropriate level to commence phase II trials in patients who have received no prior chemotherapy. It should be possible to administer two courses without difficulty but for subsequent cycles appropriate dose reductions, such as those suggested above, will have to be made according to nadir platelet counts.

Table 9 Median values for WBC and platelet nadirs at each dose level

Median nadir count (range)*

Dose	First Course		Subsequent Courses		
mg/m	WBC	Platelets	Total	WBC	Platelets
14	7.3	259	1	4.9	378
	(5.0-8.5)	(243-309)			
28	5.5	229	1	3.7	233
	(4.5-7.2)	(175-494)			
46	7.8	150	1	5.4	394
	(1.8-9.6)	(24-365)			
70	4.9	170	1	2.8	58
	(2.9-7.3)	(81-320)			
100	3.0	111	3	2.9	71
	(2.1-3.9)	(90-171)		(1.2-3.0)	(46-103)
125	2.3	95	10	2.6	54
	(0.8-3.7)	(21-189)		(1.2-4.8)	(25-152)
150	2.9	137	3	2.2	42
	(2.1-7.4)	(128-156)		(2.1-4.1)	(35-56)
170	2.5	86	6	2.2	21
	(1.0-2.8)	(32-221)		(0.6-5.4)	(10-112)
225	0.8, 2.1	18, 73	2	1.0, 1.1	7, 14

* Values= $x10^9$cells/l

Table 10 **Blood transfusion requirements**

Dose, mg/m^2	Patients	No. requiring transfusion
14	3	0
28	3	0
46	3	1
70	3	0
100	3	1
125	13	7
150	3	2
170	6	3
225	2	3

Table 11 **Non-haematological toxicity**

Dose, mg/m^2	Patients	Nausea/Vomiting	Alopecia	Venous pain
14	3	0	0	0
28	3	0	0	0
46	3	0	0	2
70	3	0	0	0
100	3	1	1	0
125	13	7	6	1
150	3	2	3	2
170	6	3	6	0
225	2	2	2	0

Table 12 **Differential WBC Toxicity**

	Pre-treatment	Nadir
Total WBC	8.3 (4-13.6)	2.3 (0.6-7.5)
% granulocytes	75% (50-95%)	52% (75-91%)
% lymphocytes	18% (3-43%)	37% (5-73%)

Differential WBC data for 23 courses in patients treated at doses ranging from $125mg/m^2$ to $225mg/m^2$.

Discussion

MDMS was introduced for phase I testing because of its high activity in the rat Yoshida sarcoma system and a possible unique site of alkylation. Pre-clinical toxicology suggested that the drug might have a myelosuppressive action and some depressant effect on testicular function but no other specific toxicities were noted. The dose limiting toxicity in this phase I trial proved to be thrombocytopenia which was cumulative with lower more prolonged nadirs following second and subsequent courses. Thus although the maximum tolerated single dose was 170mg/m^2 lower doses are recommended for phase II trials to allow additional courses to be administered safely. These doses are 150mg/m^2 for patients without prior chemotherapy and 125mg/m^2 for patients who have received prior chemotherapy.

Neutropenia was not a major problem with MDMS. Grade 4 neutropenia (wbc less than $1.0x10^9$/l) was recorded following only 3 of the 68 courses administered during the study and there were only two episodes of infection (in the same patient) requiring intravenous antibiotics. Moreover in contrast to platelet toxicity neutropenia did not appear to be cumulative. The median WBC nadirs at the doses recommended for phase II testing were $2.3x10^9$/l for 125mg/m^2 and $2.2x10^9$/l for 150mg/m^2 thus it is not anticipated that infections will be a major problem in such trials.

At all dose levels above 70mg/m^2 limited nausea and vomiting occurred in 30-50% of patients. These symptoms began 2-6 hours after treatment and in only one patient, who received 150mg/m^2, continued for more than 24 hours. When vomiting occurred it was normally limited to 1-2 episodes.

The only other non-haematological side effects encountered were alopecia and venous pain. Alopecia was clearly dose related, affecting 100% of patients at doses of 150mg/m^2 and above but only 50% of patients at 125mg/m^2. Two patients experienced severe venous pain during drug administration. The only possible explanation for this appeared to be the use of saline instead of water for reconstitution but despite all injections being given into a fast running saline infusion only 3 other patients experienced significant pain. In these cases fresh drug was prepared and injected without further difficulty implying that delay in administration following reconstitution with consequent formation of methane sulphonic acid and alteration in pH was responsible for the pain.

Busulphan is the only member of the dimethane sulphonic acid ester series that has entered routine clinical use. Following the first synthesis of the drug in 1951 (Haddow and Timmis, 1951) it was subsequently shown to have a marked depressive effect on the myeloid series in experiments in rats (Haddow and Timmis, 1953). It was thus selected for clinical trial in chronic granulocytic leukaemia and rapidly became established as the treatment of choice for this condition. Busulphan is used as an oral preparation and is normally given in a continuous daily regimen until control of the white cell count has been achieved. However if the drug is continued for too long severe irreversible bone marrow aplasia may result. Busulphan is therefore considered to be a stem cell poison affecting all marrow elements but particularly the granulocytic series. Although a very large number of patients have been treated with continuous daily Busulphan over the past 20 years, there is very little data on the

effects of single large doses of this drug. Thus in order to compare the toxicity of MDMS with that of Busulphan following a single bolus dose I have abstracted data from the records of patients treated in this manner in the department of Medical Oncology, Christie Hospital, Manchester.

The patients involved had all received induction chemotherapy for the treatment of Acute Myeloid Leukaemia and had remained in remission for a period of 12 months. Busulphan was then given orally in a single dose of 100mg/m^2 as a "late intensification" style therapy. All patients had a normal bone marrow and peripheral blood count prior to Busulphan. Remission induction therapy consisted of 2 or 3 courses of marrow ablative therapy using Daunorubicin, Thioguanine and Ara-C.

Comparison of these data on Busulphan (table 13) with the data on MDMS shows a number of similarities between the two agents. In both cases there is a greater degree of thrombocytopenia than neutropenia with delayed marrow recovery. In addition for both drugs the percentage fall in neutrophils is greater than for that of lymphocytes, the figures being very similar in each case. Anaemia affected up to 60% of patients at higher doses following MDMS therapy but was not seen in the Busulphan group. However the MDMS induced anaemia was mainly a cumulative effect and was therefore unlikely to occur after only one course of Busulphan.

Non-haematological toxicity was mild following oral Busulphan. Only two patients noticed any gastro-intestinal side effects and one experienced some hair loss. This compares to 25-50% of patients complaining of nausea and vomiting with MDMS at doses over 100mg/m^2 and 50-100% developing alopecia. None of the more

Table 13 Busulphan in AML in remission

Number of patients	17
Age at diagnosis, yrs: median (range)	48 (22-70)
Dose of Busulphan, mg: " "	170 (100-190)

	Prior to Busulphan	Nadir
Total WBC	4.2 (3.0-11.3)	2.5 (1.0-6.8)
% granulocytes	59% (37-86%)	44% (6-68%)
% lymphocytes	29% (6-53%)	43% (24-63%)
Platelet count	173 (107-324)	39 (11-210)
Haemoglobin	13.8 (11.9-16.8)	12.3 (7.9-15.5)

Time to WBC nadir	(weeks)	4 (3-11)
Time to WBC recovery	"	8 (3-12+)
Time to platelet nadir	"	4 (3-11)
Time to platelet recovery	"	8 (6-12+)

unusual side effects of Busulphan eg pulmonary fibrosis (Leake et al 1963) were seen in either study.

Busulphan is generally considered to be a stem cell poison with cumulative effects on leukocyte and platelet counts. It exhibits a greater effect on granulocyte precursors than other members of the white cell series and for this reason was introduced for the treatment of chronic myeloid leukaemia. The data presented above suggest that MDMS has very similar effects on the bone marrow and in this respect may be considered to be an intravenous form of busulphan. 100mg/m^2 Busulphan orally appears to be equitoxic with 125mg/m^2 MDMS with respect to marrow tolerance. However at these doses MDMS results in nausea and vomiting and alopecia in a significant propotion of patients whereas Busulphan does not. Perhaps these differences are related to the alkylating range possible with both molecules (Bedford and Fox, 1983) which will determine their respective sites of action.

Unfortunately agents producing delayed, cumulative thrombocytopenia can be difficult to use effectively in the clinic. Unless it is possible to administer a drug in at least a q 21 or 28 day schedule many tumours will escape control before the next dose is due. Moreover there is mounting evidence that in certain conditions, particularly high grade lymphomas, results can be improved by increasing the dose rate and intensity of treatment (Connors and Klimo, 1984). It will be difficult to fit drugs, such as MDMS, into programmes of this type. However it is possible that, as a presumed stem cell poison, MDMS might find a place in the treatment of leukaemia and related disorders prior to bone marrow transplantation.

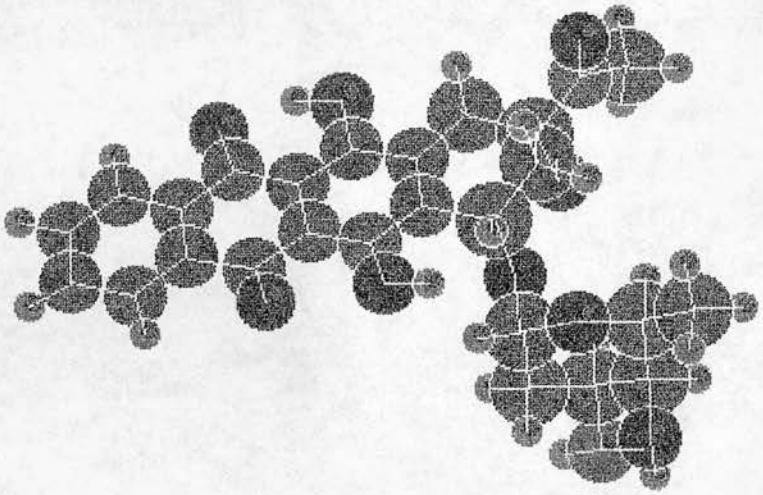
In common with many phase I studies the therapeutic activity seen

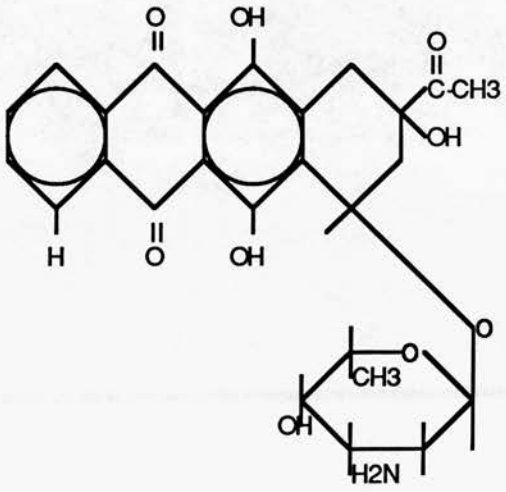
in this trial was minimal with only a single minor response (less than 50% reduction in tumour size). However as has been demonstrated by Estey et al (1986) therapeutic response rates in phase I trials are poor predictors of activity in phase II and thus a negative phase I should not prevent entry to phase II. At present phase II trials with MDMS have commenced in Soft Tissue Sarcomas within the EORTC and in Ovarian carcinoma within the CRC.

This phase I trial was conducted according to classical guidelines using a modified Fibonacci escalation series and entering three patients at non-toxic doses. 15/39 patients (38%) treated in the trial received a dose below that subsequently recommended for phase II study. In addition the slow early escalation through non-toxic doses contributed to the long duration of the trial which took 18 months to complete. However this was also due to the delayed recovery of myelosuppression. The importance of avoiding within patient dose escalations was seen in this trial. If these had been employed the cumulative nature of the thrombocytopenia would have taken longer to become apparent and the data on non-haematological effects blurred.

Clinical Pharmacology of 4-Demethoxydaunorubicin

fig 5 **4-Demethoxydaunorubicin**





Introduction

The anthracyclines are amongst the most important chemotherapeutic agents. Adriamycin is included in programmes for the curative treatment of Hodgkins disease, high grade lymphoma, small cell lung cancer and a number of childhood malignancies including acute lymphatic leukaemia while daunorubicin remains an important part of remission induction therapy in acute myeloid leukaemia. In addition adriamycin is the most active available drug in several solid tumours including breast cancer and soft tissue sarcomas and is also used in the management of gastric cancer, myeloma, ovarian cancer and many other tumours.

However the parent drugs, adriamycin and daunorubicin, have a number of unwanted side effects. Both cause nausea and vomiting and alopecia in a high percentage of patients, side effects that can be very distressing particularly if the drugs are being used in a palliative setting. In addition they produce myelosuppression but the duration of neutropenia is short and serious infective complications uncommon unless combinations are being used. A further problem that may occur with these drugs is cardiomyopathy. This toxicity is cumulative and affects a significant proportion of patients at total doses above 550mg/m^2. The cardiac lesion is irreversible and the clinical effects may range from mild heart failure to death. The problem of cardiomyopathy applies more to palliative than curative treatment. In situations such as the leukaemias and lymphomas the total doses employed in treatment programmes usually fall well short of toxic levels. However in the palliative management of eg breast cancer treatment is usually continued to the 550mg/m^2 limit if a response has been obtained.

Clearly new anthracyclines that retain the therapeutic activity of the parent compounds but with reduced nausea and vomiting, alopecia and cardiomyopathy would be extremely useful. Over the past 15 years a great deal of effort both in Europe and America has been directed towards developing anthracycline analogues that might combine these properties. During this time over 400 anthracyclines have been presented to the NCI screening programme but less than 10 have entered phase I clinical testing. Many claims have been made for these analogues and although several appear to cause less gastrointestinal toxicity and alopecia they all cause cardiotoxicity and as yet none have been shown to have superior therapeutic activity to the parent drugs.

One of the more interesting anthracycline analogues to be developed is 4-Demethoxydaunorubicin. In contrast to existing anthracyclines this drug can be administered orally, a feature of particular value in the palliative situation. This compound arose out of a study by Arcamone et al (1976) of the effects of altering the substituent at the C4 position of the tetracyclic aglycone. Although the substituent at this position in both adriamycin and daunorubicin is a methoxyl group this substituent is not present in other analogues such as Carminomycin which had shown excellent activity in preclinical testing (Gause et al,1974). In order to investigate the properties conferred by the C-4 methoxyl substitution Arcamone et al synthesised 4-demethoxydaunorubicin (4-DMDNR) and compared its activity against L1210 and Sarcoma 180 with that of daunorubicin. The results showed that 4DMDR was 5-8 times more potent than the parent compound on a mg for mg basis, although their therapeutic ratios were similar. Subsequently it was demonstrated that, in contrast to the

parent drug, 4DMDNR was equally effective when administered orally to mice bearing Gross leukaemia and Sarcoma 180 albeit at doses 3-4x the iv dose (Di Marco et al, 1977). In addition it appears that the major metabolite of 4DMDNR, 13-OH4DMDNR, is of similar antitumour activity to the parent compound in animal models (Casazza et al, 1983) and that this metabolite has a particularly long serum half life, with significant amounts still present 7 days after drug administration.

There is evidence that adriamycin is equally effective but less toxic when administered in a weekly low dose schedule rather than an intermittent high dose regimen (Chlebowsky et al 1980, Weiss et al 1976, Mattson et al 1982). An oral preparation would be particularly suitable for use in this manner since it avoids repeated clinic visits and injections. Moreover as a result of the long biological half life of the cytotoxic metabolite, 13-OH4DMDNR, a weekly schedule would mimic a continuous infusion of antitumour activity. Continuous infusion therapy is of theoretical advantage in tumours with slow doubling times where the growth fraction represents a relatively small proportion of the total cell population. Prolonged exposure to the active agent should therefore allow greater cell kill as new cells are recruited to the cycle during the infusion.

4-Demethoxydaunorubicin had shown activity in advanced breast cancer (Bonfante V et al 1983) and we therefore decided to test the oral preparation in a weekly schedule in such patients. This pharmacokinetic study was conducted in conjunction with the clinical trial to compare the pharmacokinetics of oral and iv 4-DMDNR and to determine whether its metabolism alters significantly with prolonged continuous administration.

Methods

All patients entered in the study had advanced breast cancer, a Karnofsky performance status of 60% or more and no significant hepatic (bilirubin less than 25mmol/l, transaminases less than 50% above upper limit of normal) or renal dysfunction (creatinine less than 0.15mmol/l). 4-DMDNR was administered as a single agent at a dose of 15mg/m^2 weekly. Treatment was given for at least 8 weeks and continued until progression occurred. It was planned to assess serum drug and metabolite profiles at 0, 4, 12, 24 and 52 weeks and to measure the 7 day 13-OH4DMDNR level at each four weekly clinic visit. In addition the pharmacokinetics of oral 4-DMDNR were compared with the same dose given iv in five patients.

Blood and Urine Sampling

4-DMDNR was administered following a light breakfast. For the oral study samples were taken at 0, 0.5, 1.5, 2, 3, 6, 9, 12, 24, 48 and 72 hours and for the iv study additional samples were obtained at 2, 5, 10, 20, and 60 minutes. Blood samples were centrifuged for 10 min at 500G, the serum separated and stored at -20^{o}C prior to assay.

Urine was collected during time intervals 0-6, 6-12, 12-24, 24-48 and 48-72hrs after drug administration. Total volumes were recorded and 5ml aliquots stored at -20^{o}C.

Analytical Method

Analysis of 4-demethoxydaunorubicin and 13-OH4DMDNR in blood and urine was carried out by a modification of the reverse phase HPLC method described by Israel et al (1978) for adriamycin. Prior to the

development of this technique the anthracyclines had been measured by thin layer chromatography. However the light and air sensitivity of the anthracycline chromophore led to underestimation by these methods. Moreover the acidic conditions of the silica gels used in TLC resulted in formation of aglycone artefacts due to the acid-sensitivity of the glycosidic linkage. The new HPLC technique allowed the pH of the mobile phase to be carefully controlled to avoid this problem. In addition the native fluorescence of the anthracycline chromophore meant that sensitivities of the order of 1ng/ml could be achieved.

Extraction Procedure

Stored serum was thawed and 2ml removed. To this was added 3 ml methanol and the resulting solution centrifuged at 33,000G for 15 mins at $4^{o}C$. The supernatant was removed and 4ml chloroform added. The mixture was then shaken until the chloroform was completely dispersed and further centrifuged at 500G for 10mins at $15^{o}C$. The upper layer was then discarded and the chloroform solution passed through a phase separation filter to remove any remaining aqueous phase. The resulting solution was evaporated to dryness and the residue resuspended in 100ul methanol prior to assay.

Urine was prepared for analysis by the addition of 450ul mobile phase to 50ul from each sample. This solution was centrifuged at 11,600G for 10mins at $4^{o}C$. 50ul aliquots of the resulting supernatant were used for analysis.

Standards were prepared by the addition of known amounts of 4-DMDNR and 13-OH4DMDNR to blank serum and urine. The mean correlation coefficient of the standard curves thus obtained was 0.997 +/- 0.012

with an inter-assay variation of 5.6%. The extraction efficiency was 15% for serum and 60% for urine.

HPLC Method

The HPLC assay was performed using a Waters Associates U6K injector and 6000A pump in conjunction with a Technicol ODS Hypersil column (5u particle size and 120A pore size). The column was isocratically eluted with an acetonitrile/ammonium formate buffer pH4 (45/55) at a flow rate of 1.5 mls/min. UV fluorescence was monitored with a Schoeffel FS970 detector using an excitation wavelength of 250nm and a 550nm emission filter.

Retention times for 13-OH4DMDNR and 4DMDNR were 3 min 40 secs and 4 min 30 secs respectively (fig 4). The limit of sensitivity of the assay was 0.25ng/ml.

Pharmacokinetic Analysis

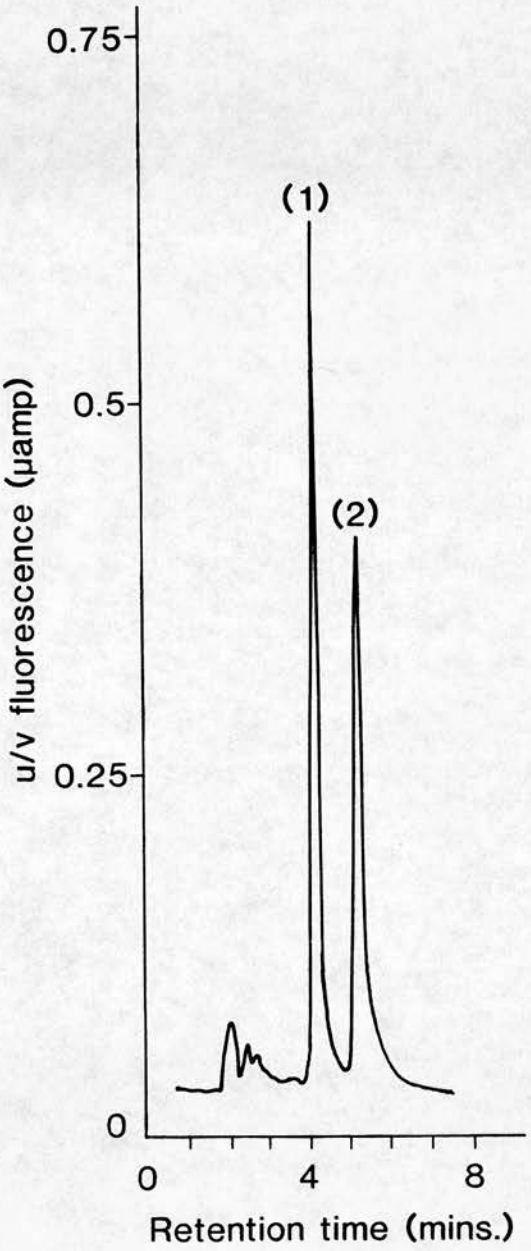
Data from the iv study were analysed using a Nelder and Mead Non-Linear Optomisation computer programme (Box et al 1969).
The AUC from time zero to infinity was calculated by :

$$AUC = A/\alpha + B/\beta + C/\gamma$$

Where A,B, and C are the intercepts on the y axis and α, β and γ the rate constants governing the triple exponential decay curve calculated by the computer programme. The clearance was obtained from the expression:

$$Clearance = dose / AUC$$

fig 6 **Chromatogram of 4-Demethoxydaunorubicin**



(1) 4-Demethoxydaunorubicin

(2) 13-OH 4-Demethoxydaunorubicin

The apparent volume of distribution (Vd) was obtained from the equation:

Vd = clearance/elimination rate constant

The elimination rate constant was the constant governing the terminal elimination phase.

For the oral study the elimination rate constant (kel) was calculated from post peak concentration data using least squares regression analysis. This constant was used to calculate the biological half life by the equation:

$$T1/2 = 0.693/kel$$

Total area under the curve to infinity (AUC) was calculated using the trapezoidal rule + C/kel where C represents the concentration at the final data point. Statistical comparison of the mean values for AUC and elimination half life at 0 and 24 weeks therapy was by the paired t test.

Results

Intravenous Study

The experimental serum concentrations of 4-DMDNR together with the values calculated by the model are shown in table 14.

The plasma concetration of 4-DMDNR following iv bolus administration described a triple exponential decay curve with equation:

$$Ct = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

In addition the data fitted a three compartment model with input into the central compartment and excretion from one of the peripheral compartments. The pharmacokinetic parameters derived from the model together with the AUC and clearance are shown in table 15.

The AUC and elimination half life following the same dose administered orally are shown in table 16 together with the bioavailability for the oral preparation calculated from the ratio of AUC oral/AUC iv.

fig 7a **Three Compartment Model**

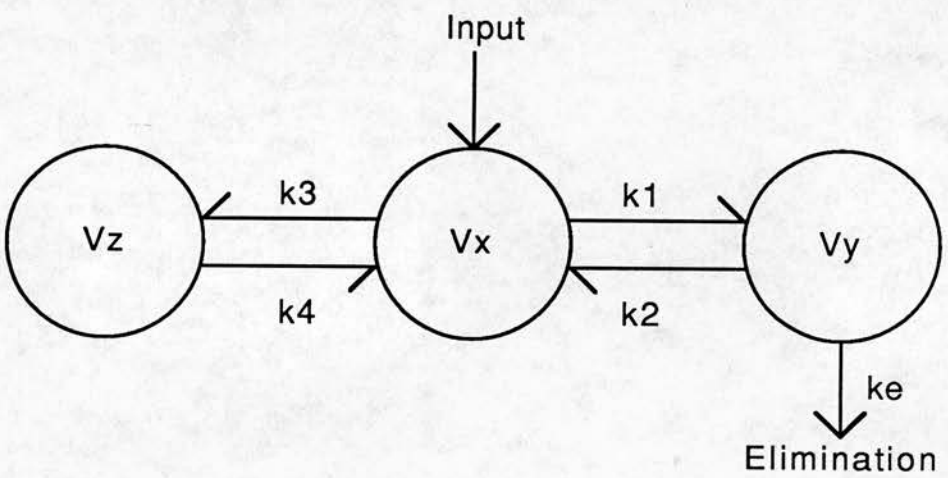


Table 14 Serum concentration (ng/ml) of 4-DMDNR experimental (E) and computed (C) values in patients 1-5

	(1)		(2)		(3)		(4)		(5)	
Hours	E	C	E	C	E	C	E	C	E	C
0.083	110.0	98.8	78.0	78.1	30.2	29.0	109.0	106.1	43.1	45.3
0.16	54.4	72.6	61.5	63.0	48.3	22.3	78.7	82.8	33.5	38.1
0.33	51.2	45.1	45.0	44.4	11.0	14.2	51.0	54.0	26.3	28.5
0.5	35.8	32.8	38.0	34.2	8.8	10.0	36.3	38.2	23.9	22.5
1.0	18.1	22.6	24.4	23.7	4.8	6.2	28.5	23.3	19.0	15.5
1.5	21.2	19.1	20.2	20.5	6.8	5.6	22.5	20.4	13.5	13.4
2.0	18.0	16.6	16.7	18.5	3.8	5.5	19.8	19.3	11.3	12.3
3.0	-	-	14.1	15.3	6.5	5.3	18.0	17.6	10.8	10.8
6.0	8.4	8.0	9.6	9.1	5.3	4.7	13.3	13.5	6.2	7.5
9.0	5.5	6.4	5.4	6.0	5.0	4.2	7.8	10.5	5.8	5.4
12.0	5.2	5.8	4.9	4.3	3.8	3.8	6.7	8.3	4.0	4.0
24.0	6.2	4.6	2.8	2.4	3.2	2.5	4.5	3.8	2.1	1.9
48.0	2.7	3.0	1.4	1.6	0.7	1.0	1.8	1.5	0.9	1.0
72.0	1.9	1.9	1.1	1.1	0.5	0.4	0.7	0.8	0.7	0.7

Table 15 Pharmacokinetic parameters of 4-DMDNR following iv bolus

Patient	A	B	C	α	β	γ	k1	k2	k3	k4	ke
(1)	109.10	23.51	7.01	5.50	0.43	0.017	29.2	4.5	65.4	161.1	1.26
(2)	71.52	24.00	3.48	4.05	0.23	0.015	16.8	2.6	57.6	106.7	1.93
(3)	32.39	0.004	5.93	4.08	0.66	0.035	25.1	4.1	201.5	22.1	5.69
(4)	115.18	18.66	4.51	3.92	0.11	0.023	11.1	2.3	59.7	32.2	5.22
(5)	38.66	13.46	2.58	3.26	0.15	0.018	19.6	4.2	87.2	382.3	4.75

Patient	AUC	Cl	Vd	Vx	Vy	Vz	Tl/2α	Tl/2β	Tl/2γ
	ug/l.h-1	l/hr	lt	lt	lt	lt	hr	hr	hr
(1)	486.4	61.6	489.4	214.8	613.7	1074.5	0.12	1.6	39.6
(2)	353.8	73.4	380.3	252.4	1150.3	844.7	0.17	3.0	46.2
(3)	204.6	122.1	214.5	652.1	152.3	329.2	0.16	1.0	19.3
(4)	394.3	76.0	145.5	216.8	1930.6	439.5	0.17	6.3	30.1
(5)	244.5	102.2	215.1	456.9	2307.0	3640.5	0.21	4.3	38.5

Vd - apparent volume of distribution

ke - elimination rate constant

Vd - apparent volume of distribution

Vx - volume of central compartment

Vy - volume of first peripheral compartment

Vz - volume of second peripheral compartment

kl-4 - intercompartmental rate constants

ke - elimination rate constant

A,B,C - intercepts on the y axis

α,β,γ - slopes of α,β,γ phases

Table 15 (cont)

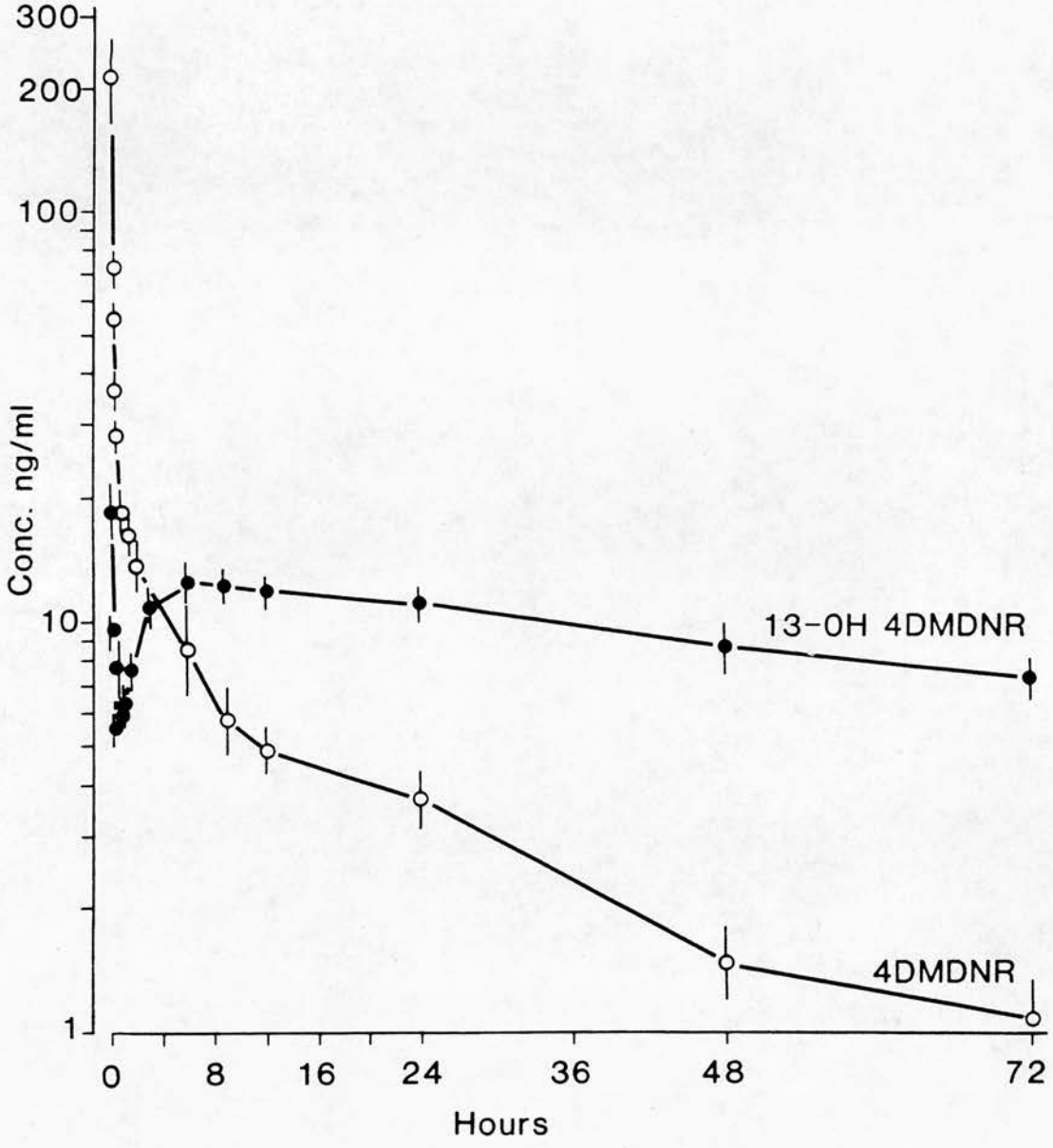
		13-OH4DMDNR	
Patient	Dose of 4-DMDNR	Tl/2el	AUC
	mg	hr	ug/l.h-l
(1)	30	10.6	623.3
(2)	25	73.7	814.1
(3)	25	69.3	596.7
(4)	30	49.5	839.6
(5)	25	57.7	609.4

Table 16 Pharmacokinetic Parameters Following Oral 4-DMDNR

	4-DMDNR			13-OH	
	Tl/2el	AUC	Bioavailable	Tl/2el	AUC
Patient	hr	ug/l.h-l	%	hr	ug/l.h-l
(1)	18.7	190.6	38.9	21.6	259.7
(2)	21.6	79.3	23.5	63.0	599.4
(3)	18.3	36.1	18.9	49.5	236.5
(4)	16.1	35.2	8.9	33.4	200.2
(5)	27.7	69.7	28.9	34.6	358.3
mean	20.4	82.2	23.8	40.4	330.8
+/-SEM	2.0	28.5	5.0	7.1	72.0

fig 7

Plasma Decay Following IV 4-DMDNR



Mean +/- SEM for five patients receiving 15mg/sqm

The mean short, intermediate and long half lives for 4-DMDNR were $T1/2\alpha$= 9.6min, $T1/2\beta$ = 3.24h and $T1/2\gamma$= 34.74h, while the mean elimination half life for 13-OH4DMDNR was 52.1h. For these five patients the mean elimination half lives for 4-DMDNR and 13-OH4DMDNR following oral administration were 20.4h and 40.4h respectively.

The mean bioavailability for the oral preparation calculated as the ratio of AUC following oral administration to AUC following iv administration was 23.7% (range 8.9-38.9%). The mean ratio of oral 4DMDNR AUC + 13-OH4DMDNR AUC to iv 4DMDNR AUC + 13-OH4DMDNR AUC was 40.6% (range 19.1-58.9%). The decay curves for 4-DMDNR and 13-OH4DMDNR after iv bolus are shown in Fig 7.

Urinary recovery of unchanged drug was 0.41-0.67% (mean 0.53%) after oral and 2.01-3.31% (mean 2.62%) after iv administration (table 17). Recovery of 4-DMDNR following oral dosing was therefore 20% of that following iv.

Table 17 72 Hour Urine recovery of 4-DMDNR and 13-OH4DMDNR

		oral			intravenous		
Patient	Dose	4-DMDNR	%dose	13-OH	4-DMDNR	%dose	13-OH
	mg	ug		ug	ug		ug
(1)*	30						
(2)	25	102.7	0.41	778.0	715.0	2.86	2374.0
(3)	25	127.0	0.50	613.0	515.0	2.06	1898.0
(4)	30	203.6	0.67	1190.5	683.0	2.27	1968.0
(5)	25	135.6	0.54	726.1	828.0	3.31	2436.0

* : urine volumes unreliable

The mean ratio of 13-OH4DMDNR : 4-DMDNR detected in the urine was 3.1:1 for the iv and 6.0:1 for the oral route. The corresponding values for metabolite and parent drug detected in the serum were 2.06:1 for iv and 4.02:1 for oral administration. These results indicate that there is an increase in 13-OH4DMDNR by a factor of two when 4-DMDNR is given orally compared to iv.

Study of Repeated Oral Administration

Serum profiles were assayed in ten patients at the start of therapy. Subsequent measurements were obtained in two patients at 4, 12, 24 and 52 weeks, in two at 4, 12 and 24 weeks and in 4 at 4 weeks only. Tables 18 and 19 show the AUC and elimination half lives for 4-DMDNR and 13-OH4DMDNR at these times.

Figures 8 and 9 show the change in Tl/2el and AUC with time for 4-DMDNR and 13-OH4DMDNR in patients receiving 6+ months treatment. For these patients the mean AUC for 4-DMDNR at week 0 was 57ug/l.h^{-1} and at week twenty four 64ug/l.h^{-1} ($p>0.1$). The corresponding elimination half lives were 23.1hrs and 22.9hrs ($p>0.5$). The AUCs for 13-OH4DMDNR at 0 and 24 weeks were 366.3ug/l.h^{-1} and 378.1ug/l.h^{-1} ($p>0.5$). The elimination half lives at these times were 43.5hrs and 44.4hrs ($p>0.5$).

Following oral administration the peak serum 4-DMDNR concentration was 3.1 +/- 1.5 ng/ml occurring 2.7 +/- 1.0hrs after ingestion. Peak 13-OH4DMDNR was 7.1 +/- 3.7 ng/ml occurring 3.6 +/- 1.8 hrs after ingestion.

The decay curves for 4-DMDNR and its metabolite after oral administration are shown in Fig 10. The 13-OH4DMDNR seven day level was measured every 28 days in 24 patients. The mean value was

0.8ng/ml (range 0.18-2.48ng/ml) and there was no evidence of accumulation (Fig 11, table 20).

There was no correlation between peak drug concentrations or 7 day metabolite levels and response to therapy in this group of patients.

Table 18 4-DMDNR AUC and Tl/2 following repeated weekly administration

	wk 0		wk 4		wk 12		wk 24		wk 52	
Patient	AUC	Tl/2	AUC	Tl/2	AUC	Tl/2	AUC	Tl/2	AUC	Tl/2
(1)	49.3	24.7	50.8	22.3	52.6	10.5	56.1	23.8	54.5	15.0
(2)	49.4	13.0	35.6	18.2	41.4	15.7	58.2	19.2	44.5	24.7
(3)	66.5	20.3	68.1	20.3	70.2	23.1	71.3	19.8		
(4)	64.1	34.6	32.1	16.5	50.4	23.8	72.6	28.8		
(5)	76.6	31.5	73.2	33.0						
(6)	51.7	17.7	50.8	23.1						
(7)	56.6	28.8	78.0	28.8						
(8)	47.6	21.6	45.7	26.6						
(9)	71.8	19.2								
(10)	76.3	21.6								

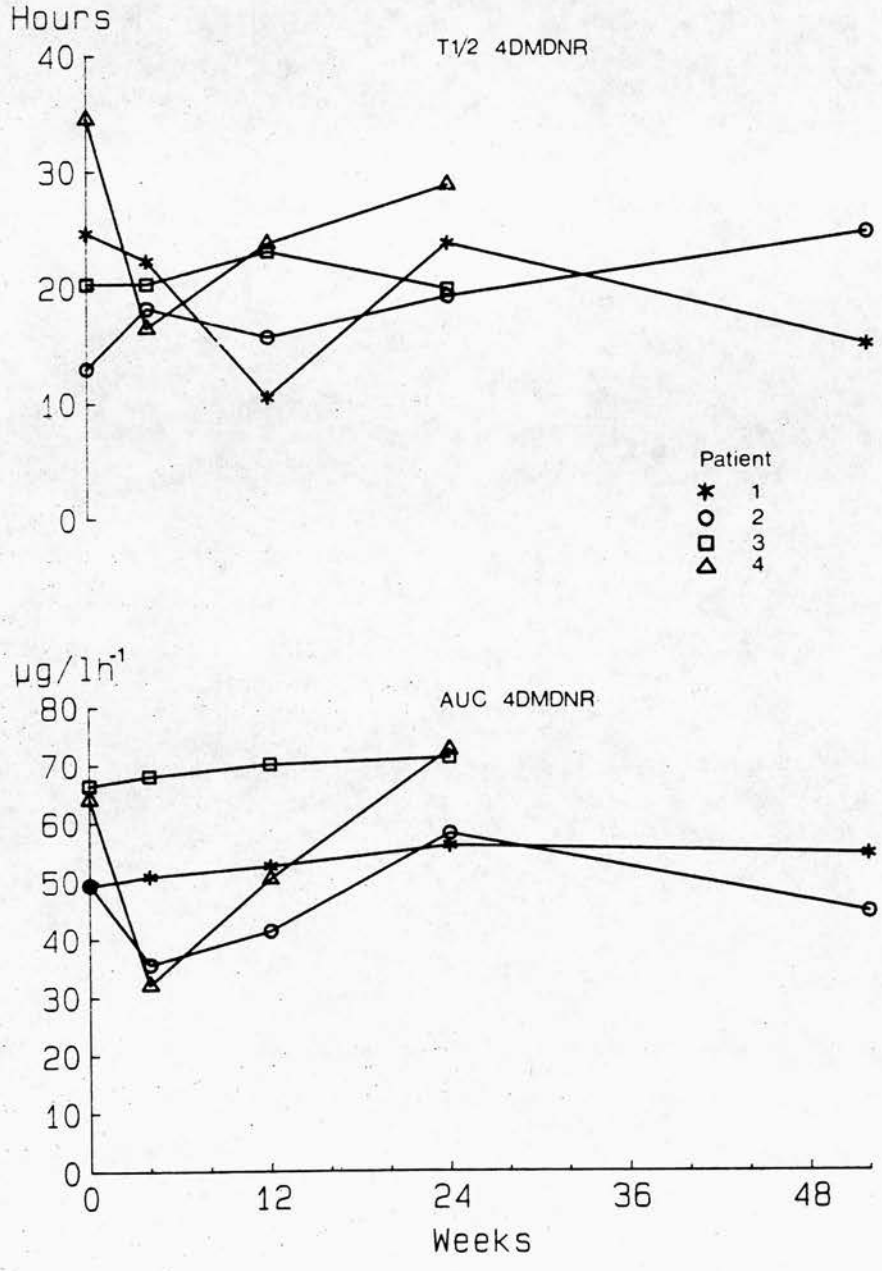
Table 19 13-OH4DMDNR AUC and Tl/2 following repeated weekly administration

	wk 0		wk 4		wk 12		wk 24		wk 52	
Patient	AUC	Tl/2	AUC	Tl/2	AUC	Tl/2	AUC	Tl/2	AUC	Tl/2
(1)	305.5	43.3	417.1	53.3	404.9	39.3	311.3	38.5	462.6	40.7
(2)	485.3	38.5	275.9	49.5	402.1	46.2	349.9	40.7	327.4	43.3
(3)	503.2	34.6	414.6	40.7	403.4	46.2	469.5	57.7		
(4)	171.3	57.2	170.7	40.7	478.3	53.5	382.3	40.7		
(5)	316.3	46.2	404.8	57.7						
(6)	363.0	53.3	458.8	63.0						
(7)	314.6	69.3	538.5	57.7						
(8)	212.4	40.7	484.5	69.3						
(9)	366.7	40.7								
(10)	330.6	63.0								

Table 20 7 day 13-OH4DMDNR levels (ng/ml) during continuous treatment

	Week									
	2	4	8	12	16	20	24	28	32	36
Patient										
(1)	0.72	0.31	1.35							
(2)	0.61	1.65								
(3)	0.23	0.69	0.61							
(4)	0.59	0.40	0.43							
(5)	0.74	0.24		0.70	0.70					
(6)		0.72		0.40	0.40					
(7)	1.20	1.10				0.96				
(8)	2.30	2.02	1.27	1.12	1.60	0.99				
(9)	0.24	0.21	0.18	0.18		0.33		0.60		0.30
(10)	0.27	0.37	0.62		1.23	0.99		1.20	1.30	1.00
(11)	0.72	0.39	0.62	0.52	0.72	0.66		1.09	0.79	
(12)	0.56	0.65	0.78					0.79		
(13)	0.64	1.16	0.88	0.56	0.37	0.35	0.10			
(14)	1.54	1.96	1.90							
(15)	0.50	0.69	0.41	0.43	0.40					
(16)	0.76	0.56								
(17)	0.80	0.30	1.02							
(18)	1.97	1.66	2.48	1.62	2.12	1.84	1.37	1.11	1.36	1.01
(19)		0.63	0.73	0.61	0.42					
(20)	0.80		1.66	1.54	2.13	2.32	1.00			
(21)		1.54	0.49	0.79		1.06	1.34			
(22)		0.44	1.23							
(23)	0.35	0.22	0.36	0.91						
(24)	0.56	0.55	0.40	0.53	0.53	0.51				

fig 8 **4DMDNR AUC and T1/2el in Patients Receiving at least 6 months Therapy**



B040207J+K

fig 9 **13-OH-4DMDNR AUC and T1/2el in Patients Receiving at least 6 months Therapy**

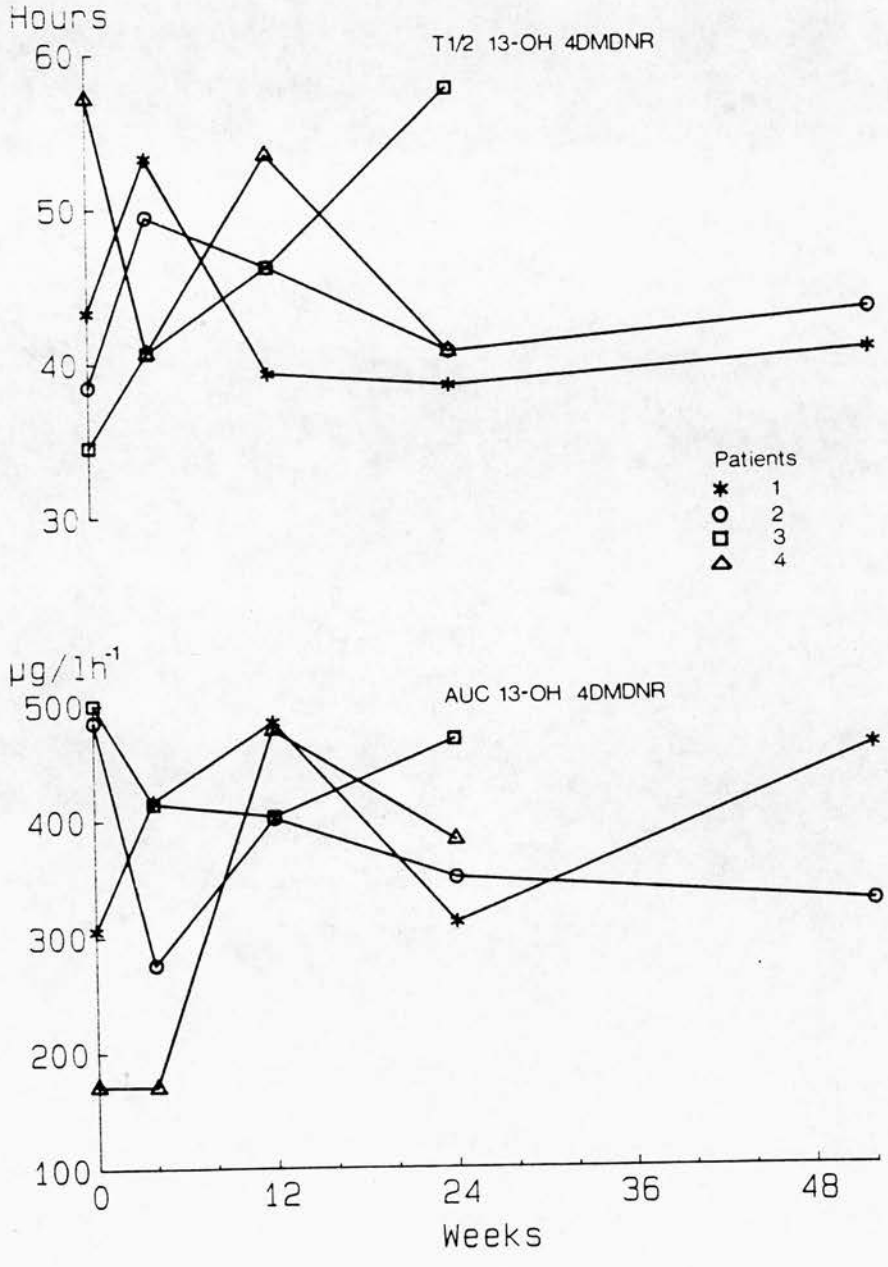
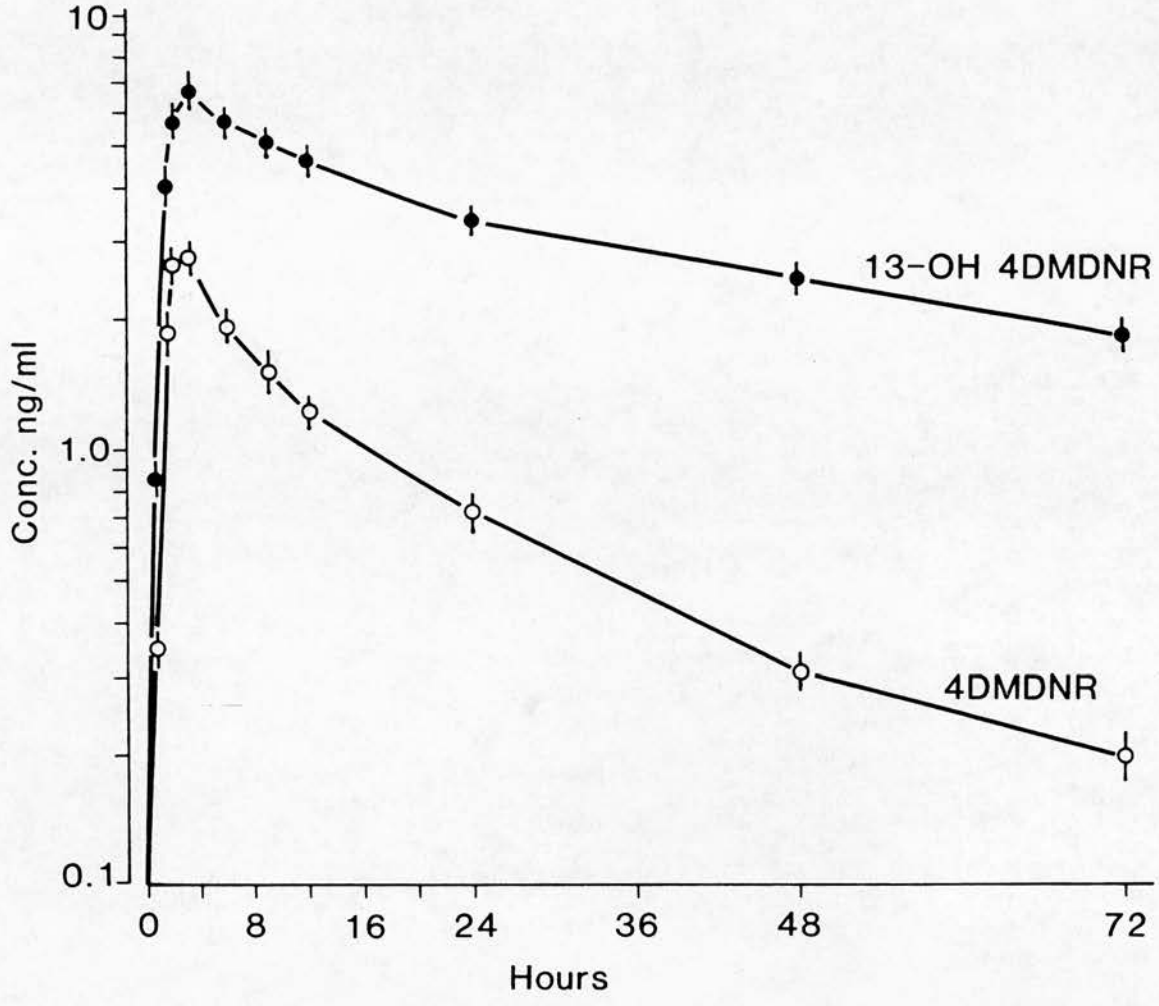
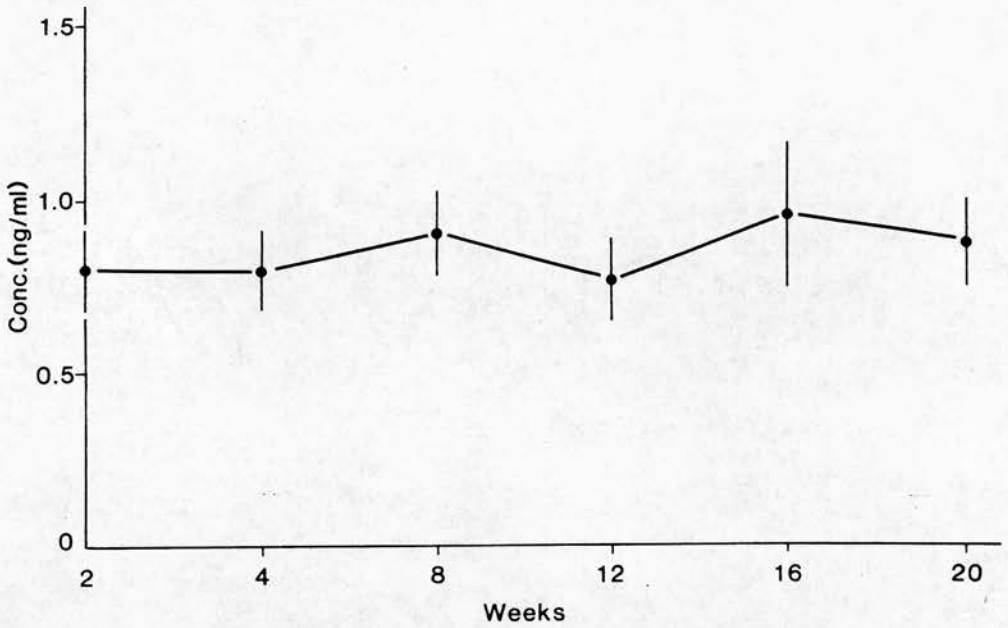


fig 10 **Plasma Decay Following Oral 4DMDNR**



Mean +/- SEM for 5 patients receiving 15mg/sqm

fig 11 **7 Day Levels of 13-OH-4DMDNR During Continuous Weekly Therapy**



Mean +/- SEM for : 20 patients at 2 weeks
23 patients at 4 weeks
19 patients at 8 weeks
13 patients at 12 weeks
11 patients at 16 weeks
10 patients at 20 weeks

Discussion

The assay method used in this study was a modification of that developed for adriamycin by Israel et al (1978). The method was relatively simple to perform, in particular the use of phase separation filters avoided tedious manual removal of the aqueous phase. In addition the combination of an acetonitrile/ammonium formate mobile phase at pH 4 with a flow rate of 1ml/min resulted in retention times of under 5 minutes for both 4-DMDNR and 13-OH4DMDNR. Other authors using alternative buffer systems have reported retention times of 10, 15 and 24 min (Daghestani et al, 1985, Berman, et al 1983, Moro et al 1983). The method described here therefore achieves a considerable time saving by allowing samples to be processed more rapidly. Moreover the limit of sensitivity for the HPLC system used in this study was 0.25 ng/ml. This represents a 10 fold improvement over previous techniques where measurement has not been possible below 2ng/ml (Daghestani et al, 1985, Berman et al 1983, Moro et al 1983). Moreover In these studies it was not possible to detect the parent compound in the blood after 24 hours but we have been able to show that 4-DMDNR is present for up to 72hrs after both oral and iv administration. It was also possible to detect 13-OH4DMDNR in the blood 7 days after treatment at levels consistent with the observed elimination half life of 44 hours.

Previous reports comparing the AUC for 4-DMDNR administered orally with that following iv injection have suggested that the drug has an oral bioavailability of approximately 30%. (Berman et al 1983, Kaplan et al 1984, Lu et al 1984). In the present study a similar calculation produced a mean value of 25% for the bioavailability of the parent drug. This figure is slightly lower

than the previous estimates but may be more accurate since comparisons of oral and iv administration were made in the same patients to minimise the known inter-patient variability in the handling of anthracyclines.

Although the boioavailability of 4-DMDNR assessed in this way appears to be of the order of 25-30% the actual absorption of the drug is likely to be approximately 40%. The discrepancy arises from the rapid hepatic metabolism of 4-DMDNR. Following iv administration significant amounts of 13-OH4DMDNR are present in blood samples taken 2 minutes after injection. When the drug is given orally absorption into the portal circulation results in a higher proportion being converted to this metabolite. Thus the ratio of AUC for 13-OH4DMDNR iv to oral was 2.1 : 1 compared to a ratio of 4.0 : 1 for the parent compound. In addition the sum of the AUC 4-DMDNR + AUC 13-OH4DMDNR for oral dosing gave a value of 40% of that for iv injection. These results indicate that the absorption of 4-DMDNR is somewhat higher than previously thought but that rapid hepatic metabolism prevents 10-15% of the absorbed dose reaching the systemic circulation.

A number of phase I trials have shown that the the maximum tolerated single dose of 4-DMDNR was 15mg/m^2 by the iv route and 50mg/m^2 by the oral route (Kavanagh et al 1985, Kaplan et al 1984, Daghestani et al 1985, Berman et al 1983). The dose limiting toxicity in these studies was myelosuppression and thus in terms of marrow tolerance the oral preparation has a biological activity 30% that of the iv preparation. This suggests that while the parent drug and metabolite appear to be of equal cytotoxic activity in pre-clinical testing (Casazza et al 1983) this is not bourne out in clinical trials. If this were the case the MTD for the oral dose

would be nearer 50% that of the iv dose than 30%.

In common with others (Berman et al 1983, Daghestani et al 1985) it was found that the blood levels of 4-DMDNR followed a triphasic decay pattern ($T1/2\alpha$ =9.6min ,$T1/2\beta$ =3.2h and $T1/2\gamma$ =34.7h), the kinetics conforming to a 3 compartment model. The short alpha half life and high volume of distribution are probably due to the rapid tissue binding, known to occur with anthracyclines.

The results of the oral study show that there was no significant alteration in the metabolism of 4-DMDNR with continuous weekly treatment. In particular there was no accumulation of 13-OH4DMDNR and no reduction in biological half life for the parent drug or metabolite. Although there was some intra-patient variability the absorption of 4-DMDNR, as assessed by the AUC, the bioavailability remained constant in the majority of patients.

Studies of the pharmacokinetics of doxorubicin and 4´epiadriamycin during successive courses of therapy at 21 day intervals have demonstrated a small increase in clearance and a shortening of the alpha half life but no change in the elimination half life for these drugs (Robert et al, 1983, Gil et al 1983, Vrignaud et al, 1985). The present study shows that despite using a weekly schedule the pharmacokinetics of 4-DMDNR also change little with time.

A Phase II Study of Oral Weekly 4-Demethoxydaunorubicin in Advanced Breast Cancer

Introduction

In recent years the chemotherapeutic management of advanced breast cancer appears to have entered a plateau phase. Despite the existence of several active single agents and the early promise of high response rates with combination regimens it is clear that at present this is not a curable disease (Harris et al, 1985). Even the introduction of high dose therapy with autologous marrow rescue has not had a significant impact on survival. Moreover it appears that while responses can be obtained and symtoms improved in many patients only those with life threatening visceral disease, particularly liver involvement, can be shown to have a survival advantage conferred by the use of chemotherapy. This realisation has prompted cancer physicians to search for less toxic chemotherapy in an effort to palliate symptoms with minimal side effects.

One method of reducing subjective toxicity is by administering drugs orally. This avoids the necessity for repeated injections and may allow a reduction in the frequency of clinic visits. Three of the most active single agents in breast cancer cyclophosphamide, methotrexate and 5-fluorouracil are available in oral formulations and they have been combined in a recently reported study (Wagstaff et al 1985). In this trial the drugs were given on a continuous weekly basis, cyclophosphamide on Monday, methotrexate on Wednesday and 5-FU on Friday. The response rate of 53% was similar to that obtained in other trials with classical CMF and the median duration of response of 11 months and median survival of 16 months also did not differ from previously reported figures. Moreover the subjective toxicity of oral CMF was low with minimal nausea and vomiting and no significant episodes of alopecia. The use of oral chemotherapy in this manner

therefore appeared to be an effective way of reducing toxicity without prejudicing response or survival.

Another method of reducing toxicity is the use of more frequent low dose chemotherapy. A number of studies have been conducted with Adriamycin used in this way (Chlebowsky et al 1980, Weiss et al 1976, Mattson et al 1982). The rationale for these studies is that while adriamycin is the most active single agent in advanced breast cancer with response rates of around 50% in untreated patients the toxicity is substantial. Nausea, vomiting and alopecia occur in most patients and treatment eventually has to be discontinued because of cumulative cardiotoxicity. However the severity of these side effects appears to be related to peak blood concentrations rather than total dose administered. Adriamycin used in a weekly schedule at doses of 20-25mg/m^2 has very little subjective toxicity. Gastrointestinal side effects are unusual, significant alopecia umcommon and the threshold for cardiotoxicity rises to over lgm/m^2. Moreover there does not appear to be a significant loss in therapeutic effect. The major disadvantage of weekly adriamycin is the frequency of clinic visits. This puts an additional strain on all the hospital services involved in managing patients being treated with intravenous chemotherapy. In addition the patients have the inconvenience, expense and psychological morbidity of frequent hospital attendence and an increased risk of drug extravasation. Moreover there may be a tendency for new problems not to be detected in patients who are seen too frequently.

4-Demethoxydaunorubicin (Idarubicin, 4-DMDNR)) is a new synthetic analogue of daunorubicin and is the first anthracycline to be available in an oral formulation. For the reasons outlined above it

would therefore be particularly suitable for use in a weekly schedule. Moreover in view of the antitumour activity and long serum half life of the 13-OH metabolite it was possible that a weekly schedule might mimic a continuous cytotoxic infusion which may be of benefit in tumours with relatively slow doubling times. 4-Demethoxydaunorubicin had demonstrated activity in advanced breast cancer during phase I testing (Bonfante et al 1983) and we therefore decided to test this drug in a weekly oral schedule in such patients.

Patients and Methods

A phase I study by De Sloover et al (1984) using a weekly x 4 oral schedule demonstrated minimal toxicity with doses up to 15mg/m^2 but increasing nausea, vomiting and neutropenia at higher doses. Since one of our aims was to use a continuous therapy we did not wish to choose a dose that would result in frequent delays. 4-Demethoxydaunorubicin was therefore given at a dosage of 15mg/m^2 in a weekly oral schedule. It was planned to escalate the dose by 5mg/m^2 increments until toxicity was seen that necessitated treatment delays in the majority of patients. Patients were seen at 14 day intervals for the first 4 weeks and subsequently every 28 days. The dose was reduced by 5mg/week when the wbc fell below $2.5x10^9$/l and treatment was delayed by one week if the wbc was less than $2.0x10^9$/l. Following a delay 4-Demethoxydaunorubicin was not restarted until the wbc was above $3.0x10^9$/l. Treatment was given for at least 8 weeks, unless progression of life threatening disease dictated a change to intravenous chemotherapy, and continued until relapse. When progression occurred it was our intention to use Adriamycin

60mg/m^2 in a q21 day schedule in order to obtain data on cross resistance between these two agents.

Patient entry was according to the two stage design described previously with an initial sample of 14 patients. The trial protocol was submitted to the South Manchester Ethical Committee and the Christie Hospital Protocol Review Committee.

Verbal informed consent was obtained from all patients according to current MRC guidelines.

Patients

Thirty eight evaluable patients with a median age of 63.5 yr, a WHO performance status of 0 or 1, and an anticipated survival of at least 3 months were entered into the study. Patients with cardiac failure, major rhythm disturbances or significant ECG abnormalities were excluded. Patient characteristics are summarised in Table 21. No patient had received prior chemotherapy but 32 had one or more trials of endocrine treatment. Reasons for not using endocrine therapy included rapidly advancing liver disease (3) and receptor negative inflammmatory carcinomas (3). Among the 18 patients with soft tissue metastases as the dominant site of disease there were 10 with locally advanced primary tumours, 3 with involved lymph nodes and 5 with cutaneous disease. In the group of 8 patients with dominant bone disease only 2 did not have evaluable disease elsewhere.

All patients were assessed according to UICC criteria (Hayward et al 1977) with duration of remission measured from the date of starting chemotherapy. Toxicity has been graded using the WHO scale (Miller et al 1981).

Table 21 Patient Characteristics - 4-DMDNR Phase II

Characteristic				%
Age, median (range)			63.5 (33-79)	
Menopausal staus : pre			1	3
post			37	97
Post surgery radiation			11	29
Disease free interval,				
months median (range)			19 (0-204)	
Time from first relapse to 4-DMDNR,				
months median (range)			10 (0-132)	
Receptor status:				
oestrogen +	progesterone +		15	48
+	-		3	10
-	+		2	6
-	-		11	35
Response to hormone therapy:				
Progression			18	56
Static (6 months+)			4	12
PR			8	25
CR			2	6
Dominant site of disease:				
Soft tissue			18	47
Bone			8	21
Lung			6	16
Liver			4	11
Retroperitoneal			2	5

Results

The 38 patients received a median of 12 weeks treatment (range 2-60 weeks). Three patients with rapidly progressing liver disease had to be switched to Adriamycin after 2-4 weeks therapy. Six patients received in excess of 450mg/m^2 total dose of 4-DMDNR. In two patients, neither of whom had bone disease, the dose was escalated to 20mg/m^2/week. In both cases the WBC fell below 2.5x10^9/l after 3 weeks treatment necessitating delay. When the dose was reduced to 15mg/m^2 neither patient required further delay of therapy. 15mg/m^2 was therefore considered to be the maximum tolerated dose using this schedule.

One CR (cutaneous) and 5 PRs were seen providing an overall response rate of 15.7%. (95% confidence limits 6-31%). Partial remissions occurred in patients with the following dominant sites of disease. Inflammatory carcinoma (1), lymph nodes (1), retroperitoneal infiltration + bone (1) and cutaneous deposits (2). In addition six patients had static disease for a period of at least six months. The duration of the remissions was 13 months for the CR and 5+,5+,9+,10+ and 14+ months for the PRs. The median time to objective remission was 8 weeks (range 4-10 weeks) and responses were evenly distributed between patients with receptor positive and receptor negative tumours.

Toxicity

The toxicity of 4-Demethoxydaunorubicin administered in this schedule was mild. Seven patients (18%) required treatment delays, six due to neutropenia (grade 3) and one to a severe radiation recall reaction. Dose reductions were necessary in 13 patients (33%), 9 because of haematological toxicity (grade 2/3 neutropenia) and 4 as a result of unacceptable nausea and vomiting (grade 3). Bone marrow examinations were performed in six of the nine patients who had dose reductions following episodes of neutropenia and in all cases the marrow was involved with tumour. Two of the remaining three patients had widespread bone disease.

Gastrointestinal toxicity was variable. Twelve patients (31%) experienced no nausea or vomiting while 14 (37%) had mild nausea (grade 1) affecting less than 25% of their doses. Six patients also reported grade 1 nausea but affecting 25-75% of doses and a further six (16%) had nausea and vomiting (grade 2-3) with every treatment, in 4 cases requiring dose reductions. Three patients developed mild alopecia not requiring a wig (grade 1) and there were no episodes of cardiac failure or ECG abnormalities seen during the study. One patient developed a radiation recall reaction. This occurred in a lady who had received postoperative radiotherapy to the chest wall and lymph node drainage areas and became apparent after the second dose of 4-DMDNR. The reaction consisted of an extremely painfull erythematous eruption in the area of previous irradiation together with dyspnoea and a cough productive of clear sputum. These symptoms resolved in 7 days with the aid of steroid therapy.

Treatment on Progression with Adriamycin

Thus far 32/38 patients have progressed on treatment with 4-Demethoxydaunorubicin and fourteen of these have received Adriamycin. Reasons for not using Adriamycin in the remaining patients included : too ill to warrant further therapy (6), fear of alopecia (2), elderly and frail (5), asymptomatic (2), radiation recall with 4-Demethoxydaunorubicin (1), cirrhosis with rising bilirubin (previously normal) (1) and prolonged pancytopenia (1).

Patient characteristics of these 14 patients are shown in table 22. Six patients had rapidly advancing liver disease in 2 cases occurring as a new site after 12 and 17 weeks 4-DMDNR.

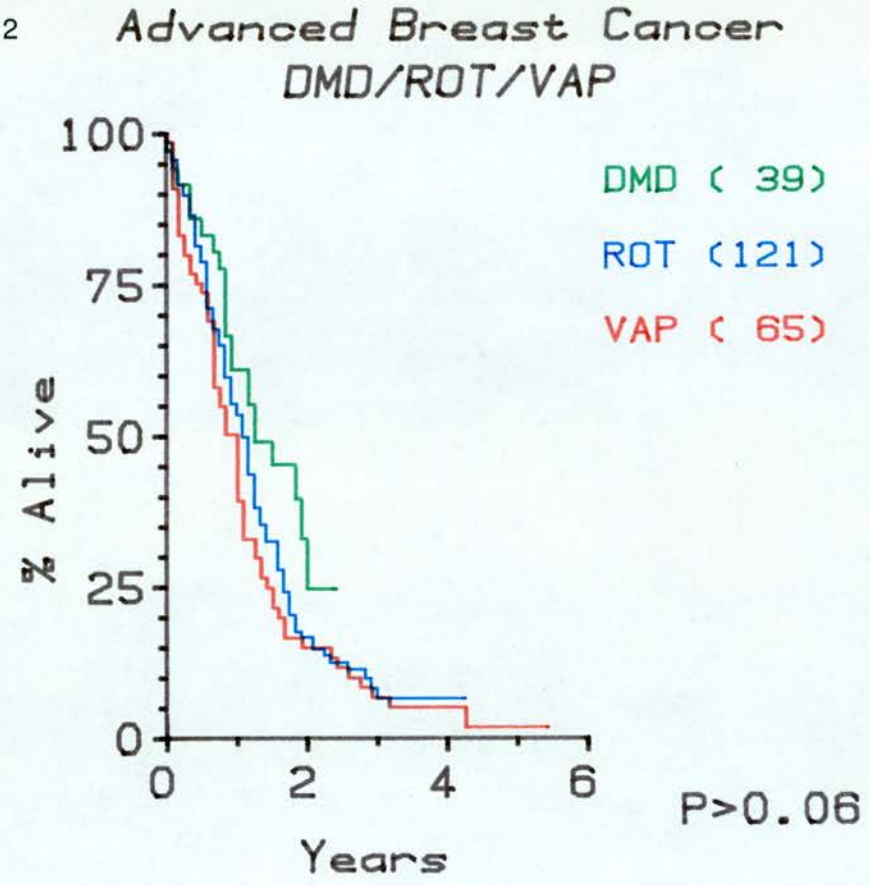
There were 5 PRs (36%) in this group of patients. A further 3 patients had less than 50% regression of their disease with good symtomatic response for 5, 5, and 8+ months. One responding patient has relapsed after 5 months but the remaining 4 continue in remission at 6+months after commencing Adriamycin.

Two patients who responded to Adriamycin had previously had static disease on 4-Demethoxydaunorubicin for 6 months+. These were patients with lung metastases as the major site. The remaining 3 all had liver metastases and in two cases were switched to Adriamycin after less than 4 weeks 4-Demethoxydaunorubicin because of rapidly advancing disease.

Table 22 Characteristics of Patients Treated with Adriamycin

Characteristic				%
No.			14	
Age, median (range)			60	(33-65)
Number of weeks 4-DMDNR therapy, median (range)			14	(2-32)
Response to 4-DMDNR :	Static (6 months+)		2	14
	Progression		12	86
Dominant site :	Soft tissue		4	28
	Bone		1	7
	Lung		3	21
	Liver		6	43
Number of courses of Adriamycin, Median (range)			4	(2-8)
Reason for stopping Adriamycin :	Toxicity		1	7
		Reached maximum dose	4	28
		Progression	8	57
		Continues on therapy	1	7

fig 12



Retrospective comparison of the survival of three groups of patients treated with three different chemotherapy regimens.

DMD - 4-Demethoxydaunorubicin

VAP - Vincristine, Adriamycin, Prednisolone

ROT - Oral weekly Cyclophosphamide, Methotrexate, 5-FU

Discussion

The majority of trials with new agents in advanced breast cancer are difficult to interpret because the drugs are tested in heavily pre-treated patients, a situation where even drugs of proven value in first line management are largely ineffective (Harris et al 1985). One method of overcoming this problem is to use new agents in previously untreated patients but with a trial design allowing early cross-over to an active regimen in the event of progression. Such a procedure may be criticised as being unethical in that patients are denied optimal therapy. However if these patients are observed carefully and transferred to alternative treatment at an early stage if no response is seen, their chances of responding to such therapy should not be significantly diminished. Moreover if a response is seen then these patients will benefit from all the first line agents still being available if and when they relapse. In the initial stages of such a trial the patients entered would have to be limited to those without immediately life threatening disease. Using trial designs of this type would save a great deal of the time and effort currently expended on trials in pre-treated patients and would yield valuable data allowing early rejection of ineffective agents and preventing false rejection of active compounds.

In the present study 4-DMDNR, previously untested in breast cancer, was used as first line therapy in patients who had not received prior chemotherapy. The trial design incorporated a cross-over to adriamycin in the event of progression and was justified by the activity of the parent drug and probable reduction in toxicity of the new therapy. The objective response rate of 16% was low but with the addition of the patients with static disease for 6+ months this rises

to 29%. This is thought to be justifiable since patients with static disease for this length of time have been shown to have an identical survival to those who obtain a partial remission (Howell et al 1985). Perhaps low dose chemotherapy of this type may act by controlling tumour growth rather than producing significant reductions in measurable disease.

Comparison of the survival of the total patient group to that of previous groups of patients treated initially with VAP-Cyclo-AC and CMF at this institute showed no significant difference (fig 12). This is likely to be due in part to the use of adriamycin on progression, particularly in the patients with liver disease, where a response rate of 38% was obtained. Thus the use of an unproven drug as first line treatment did not appear to prejudice survival in these patients and delayed the use of more toxic chemotherapy in a significant proportion of patients. Where responses occurred these were of good quality and obtained with minimal adverse side effects.

The main subjective toxicity was limited GI toxicity affecting 59% of patients and there was no significant alopecia. There were no episodes of cardiac failure seen during the study despite six patients receiving more than 450mg/m^2, a cumulative dose that might be expected to result in myocardial dysfunction allowing for an oral bioavailability of 30%. This suggests that 4-DMDNR is less cardiotoxic than the parent drug although the weekly schedule may also have contributed to a reduction in this side effect.

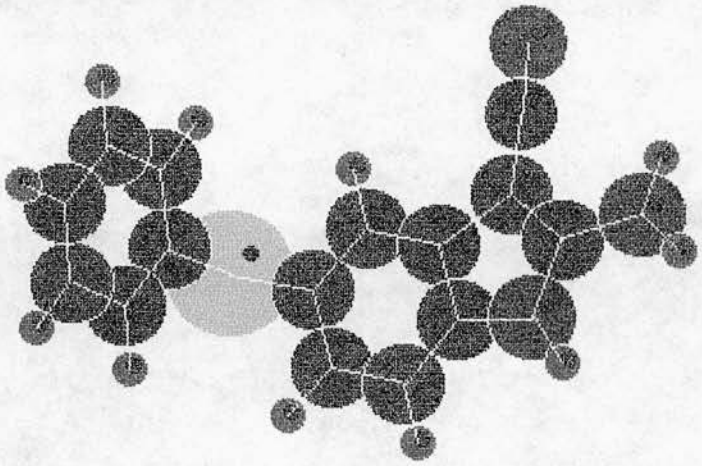
Haematological toxicity was also mild. There was no thrombocytopenia and neutropenia only occurred in patients with marrow infiltration or widespread bone disease. There was no evidence of cumulative myelosuppression.

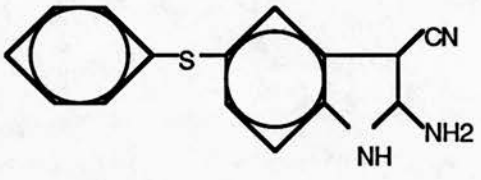
For the reasons outlined above the number of patients in the Adriamycin arm of this study was too small to draw any firm conclusions on cross resistance between these two drugs. Moreover two of the five patients who responded to Adriamycin had only had two weeks therapy with 4-Demethoxydaunorubicin due to rapidly advancing liver diseese. However there were three patients who progressed after more than 8 weeks 4-Demethoxydaunorubicin therapy (lung- 2, liver- 1) who subsequently achieved a partial remission with Adriamycin. Although this suggests that there may be a degree of non-cross resistance between these two agents the different scheduling could also be important. Indeed since this study was activated a number of authors have published preliminary results of the use of 4-DMDNR in advanced breast cancer used at doses of 40-50mg/m^2 in a q21 day schedule. These trials have obtained response rates of 30-50% (Pannuti et al 1985, Bastholt et al 1985) although GI toxicity was more severe than for the weekly regimen. Thus it appears likely that in contrast to Adriamycin there is a loss of therapeutic effect when 4-Demethoxydaunorubicin is used in a weekly schedule. The inference that may be drawn from these data is that high peak levels of 4-Demethoxydaunorubicin or 13-0H4DMDNR or both are important for antitumour activity rather than the more continuous lower levels achieved by weekly administration. In the light of these findings it would be interesting to know whether patients progressing on weekly 4-Demethoxydaunorubicin would respond to higher dose three weekly 4-demethoxydaunorubicin Moreover in order to answer the question of cross resistance between the parent anthracyclines and 4-Demethoxydaunorubicin a randomised cross-over trial using similar scheduling would have to be carried out.

In summary 4-Demethoxydaunorubicin used in a weekly schedule is well tolerated and has activity in advanced breast cancer. However this mode of administration appears to be inferior, in terms of response, to giving the same total dose of drug in a q21 day regimen, although the weekly schedule is probably less toxic and may be a more acceptable option in frail elderly patients. Low dose weekly treatment was clearly inadequate therapy for rapidly progressive liver disease. Thirty six percent of patients treated with adriamycin in a q 21 day schedule after failing 4-DMDNR achieved a partial remission.

A Phase I and Pharmacokinetic Study of Amphethinile

fig 13 **Amphethinile**





Introduction

Amphethinile (ICI 134154, CRC 82/07) was first synthesised in the ICI laboratories at Mereside in Cheshire and aroused interest when it was shown to terminate pregnancy in rats. This effect occurred following two injections on day 9 of gestation or one on day 10 and the qualitative appearance of the degenerating implants suggested that it might be due to cytotoxic damage. The drug was fully effective both orally and subcutaneously at a dose of 5mg/kg and produced other toxic effects at a dose of 25mg/kg. In this test vincristine was fully effective at a dose of 0.25mg/kg but lethal at 0.5mg/kg. Amphethinile, which has been shown to be a spindle poison in dividing Hela cells, thus appears to have a better therapeutic ratio than the vinca alkaloids.

The compound was submitted to the NCI pre-clinical screen and was shown to be active in L1210, ADJ/PC6 and the Walker carcinoma but inactive in the TLX/5 and GHS-Pit tumours. Additional testing by Harold Jackson at Manchester University confirmed the activity in the Walker and ADJ/PC6 tumours but showed the drug to be inactive in a Chondrosarcoma and a chlorambucil resistant Walker tumour.

In view of this pre-clinical activity, the possible improvement in therapeutic ratio compared to existing spindle poisons and the apparent oral absorption the drug was taken up by the CRC Phase I/II committee for formulation and pre-clinical toxicology.

Formulation

Amphethinile is a relatively simple synthetic compound with a molecular weight of 265 and melting point of 190.5^{o}C. Investigation of the bulk compound showed that it contained

approximately 1% inorganic material by ash analysis and less than 2% impurities on TLC and HPLC.

The main problem with amphethinile is that it is extremely insoluble in aqueous solution, 1.7 ppm at 20°C and 6 ppm at 37°C. It is highly soluble in polar solvents such as DMSO and DMA but the solubility in these diluents drops rapidly when the solvent/water ratio falls below 60/40, and an extremely coalescent precipitate forms. The volumes of solvent that would be required to prevent this happening on contact with blood made such a formulation impractical. Another option was the use of a surfactant like material such as Cremophor EL. These agents have a hydrophobic "head" and a hydrophylic "tail" and can surround individual molecules of a compound forming a molecular suspension in aqueous solution. Cremophor EL has been used in the formulation of several antitumour agents (Echinomycin, Teniposide, Diaziquone, Taxol) and also the systemic antifungal miconazole. Cremophor is the reaction product of castor oil and ethylene oxide and the main problem associated with its use is hypersensitivity. A significant proportion of patients treated with Cremophor containing preparations experience allergic reactions up to and including anaphylaxis and deaths have occurred. Some drugs, notably the anaesthetic induction agent Althesin, have had to be withdrawn for this reason. Solutol HS 15 is a development of Cremophor that is prepared from 12-hydroxystearic acid, one of the components of castor oil, + ethylene oxide. It is thus a much "cleaner" compound than Cremophor and in experiments on Beagle dogs has been shown to cause 10-20 x less histamine release and is therefore thought to be potentially less allergenic. The LD 50 for Solutol in dogs is in excess of

3.1g/kg.

Solutol is a relatively recent development and has not been used previously in pharmaceutical preparations. For this reason special permission had to be obtained from the DHSS for its use with amphethinile. This was granted and Dr Vezin at the CRC Formulation Unit, Department of Pharmacy, University of Strathclyde was able to produce an elegant formulation consisting of:

Amphethinile 50mg

+

Solutol 500mg

+

1ml 30% Citrate buffer/70% Alcohol

Citrate buffer was prepared from Citric acid 0.1M + Sodium hydroxide 0.1M to pH 6.

The resulting formulation contains 50% weight/volume solutol (500mg/ml Solutol). This is a very stable product with no detectable degradation after four months at 57^{o}C. The solution is a rather viscous straw coloured fluid and requires 1:10 dilution with N saline prior to administration.

Preclinical Toxicology

Preclinical toxicology was carried out by BIBRA Ltd., Carshalton, Surrey according to CRC Phase I Committee protocols.

Acute Intravenous Toxicity in Male Mice

The acute intravenous toxicty was investigated in groups of ten MF1

strain male mice. These animals were treated with doses ranging from 0 - 225mg/kg as a bolus injection into the tail vein. At doses over 200mg/kg all mice exhibited an immediate catatonic reaction and half died within 20 minutes of receiving the injection. Following these observations further mice were treated using Solutol alone and similar catatonic reactions and deaths occurred. It was also found that these reactions and deaths could be completely avoided by administering the solutol slowly over thirty seconds rather than as a rapid bolus. In addition none of the other effects that were seen with the amphethinile preparation occurred when the solutol alone was given. As a result of these findings all toxic effects reported below with the exception of the catatonic reaction were considered to be due to amphethinile. All subsequent doses of amphethinile were given as a slow injection and no further acute catatonic reactions or early deaths were seen.

Effects on the mice at higher doses included tail necrosis distal to the injection site, uncoordinated movements and low body temperature. All deaths occurred within 3 days of treatment but gross macroscopic post mortem examination showed no treatment related effects.

	mg/kg	**95% confidence interval**
LD 10	137	(98-193)
LD 50	213	(171-263)

Acute Intraperitoneal Toxicity

In addition to estimation of LD50 and LD10 values and observation of gross effects this study involved detailed haematological and histological examinations.

Groups of mice were treated with single intraperitoneal injections of amphethinile in doses ranging from 125-260mg/kg. All mice dying as a result of treatment did so within one day. Other effects observed at higher doses included lethargy, reduced body temperature and generalised unsteadiness. There was no reduction in body weight.

In all treated groups there was a rapid fall in the number of circulating granulocytes, lymphocytes and platelets by day 3. The granulocytes and platelets recovered to normal levels by day 10 but only at the lowest dose was there definite recovery in the lymphocyte population by day 28. No effect was seen on the red cells.

Histological examination of the marrow, sleen and lymph nodes showed moderate necrosis in mice dying within 2 days of treatment but no changes in mice sacrificed at 14 days. These changes may be responsible for the slow recovery seen in the peripheral lymphocyte count.

The major histological changes seen were in the testes. Germ cell and spermatogonial necrosis occurred at all dose levels, in severe cases leading to tubular calcification, atrophy and interstitial cell hyperplasia. These changes appeared to be dose related.

A small percentage of the mice dying within two days of treatment had minimal necrosis of the glandular, squamous or sub-mucosal areas of the stomach and of the crypt epithelium cells of the small intestine. No abnormalities in these areas were seen in mice sacrificed at 14 or 28 days.

No additionl changes were seen in any of the remaining organs examined. The LD50 and LD10 calculated were as follows:

	mg/kg	95% confidence interval
LD 10	138	(138-169)
LD 50	200	(178-226)

Subacute Intraperitoneal Toxicity

Groups of animals were given a total of 20 IP injections over 28 days at doses ranging from 18.5mg/kg to 150mg/kg. All mice receiving 150mg/kg died within 5 days. Histological examination of these animals revealed marrow aplasia together with similar changes in the testes and gastrointestinal mucosa seen with single IP doses. No marrow changes were seen in mice surviving to 28 days post treatment. The approximate LD50 from this study was 86mg/kg.

Toxicity Check in the Rat

From the above mouse studies the LD 10 appears to be close to 140mg/kg or 420mg/m^2. The proposed starting dose for phase I clinical trials would therefore be 42mg/m^2 ie 1/10th LD10. A check of this dose was carried out in the rat. Groups of 10 male Wistar rats were given 20 IP injections at 7.5mg/kg. No toxic effects were seen.

Summary

At doses over 200mg/kg rapid iv injection resulted in a catatonic like reaction and in some cases death. However these effects were shown to be due to the solutol vehicle rather than the amphethinile and could be avoided by administering the drug as a slow infusion over 30 seconds. No other solutol related effects were seen Histological damage was limited to the rapidly dividing tissues of

the marrow, testes and GI tract with no obvious effects elsewhere. There was a rapid fall in WBC and platelets with subsequent quick recovery in granulocytes and platelets but slow recovery of lymphocytes. When deaths occurred these were early after treatment coinciding with the time of maximum histological damage to marrow and gut. No late deaths occurred and residual damage at higher doses was limited to the testes.

As a result of pre-clinical toxicology a dose of 40mg/m^2 has been selected as the starting dose for phase I trials.

Phase I Clinical Trial of an Intermittent IV Bolus Schedule

The initial phase I clinical trial of Amphethinile using an intermittent IV bolus schedule was commenced on 21st July 1986 at the Christie Hospital, Manchester. The trial protocol differed in two main areas from that used in the MDMS phase I. Firstly the escalation procedure was modified to take into account both the findings of the survey of phase I trials described earlier, and the concept of using preclinical pharmacological data to guide dose selection. The escalation scheme was as follows :

Escalatiom	**Dose level**
starting dose	n
1	5n*
2	10n
3	20n
	etc

* It was proposed to escalate to 5n if the first dose was entirely

non-toxic and the AUC measured at this dose less than 5% of the target AUC. The target AUC was estimated to be of the order of 300ug/l.h-1 from experiments at the LD10 in mice. These experiments will be fully described later.

If toxic effects were seen and the AUC was less than 20% of the target AUC then a 100% escalation to 2n was permissible. Smaller increases of 25 - 50% or dose reduction would be undertaken depending on the level of toxicity and the pharmacological data. Subsequent escalations would also rely on these considerations although in the early stages of the trial a maximum increase of 100% would be allowed. If it became clear that the AUC was far short of the target AUC and that the kinetics were linear, then larger dose increases would be allowed once this information was available. This escalation scheme is designed to be flexible in order to arrive at the MTD safely with the fewest possible escalation steps.

The second difference in the protocol was in the numbers of patients to be treated at each dose level. It was decided to treat only two patients at each non-toxic dose level to further reduce the numbers of patients treated at doses with no potential therapeutic benefit. As has been the practice in the past increasing numbers of patients would be treated at toxic dose levels with at least six at the dose recommended for phase II testing.

Results

In the following results section the platelet and leukocyte values are given in units of x 10^9/l. Clearance is in ml/min, AUC in ug/l.h-1 and half lives in hours.

Results

Dose : 40mg/sqm Date start : 2 : 7 : 86

Patient	Diagnosis	Age	Performance Status	Major Disease Site
TH	SCLC	69	1	Bone
HM	Colon	43	1	Lung

	Prior XRT	Prior Chemotherapy
TH	Mediastinum	Ifosfamide, VP16
HM	none	MDMS

	Course 1 Platelet nadir	Course 2 Platelet nadir
TH	262	
HM	157	144

	WBC nadir	WBC nadir
TH	5.1	
HM	9.1	9.6

	Other toxicities
TH	none
HM	none

Pharmacokinetic Data

No drug could be detected in the serum at this dose.

Comment

The drug was given as a bolus injection over five minutes. There were no acute or sub-acute effects and in addition amphethinile was present in concentrations too low to be measured in the serum. Since the lower limit of detection of the assay was 0.lug/ml the AUC at this dose was clearly well below 5% of the target AUC. It was therefore decided to escalate the dose immediately to five times the starting dose, 200mg/sqm.

Dose : 200mg/sqm Date start : 31 : 7 : 86

Patient	Diagnosis	Age	Performance Status	Major Disease Site
JC	Ovarian Ca.	43	0	Lymph nodes
RH	Squamous lung	68	1	Primary

	Prior XRT	Prior Chemotherapy
JC	none	VAC, CBDCA
RH	none	Ifosfamide

	Course 1 Platelet nadir	Course 2 Platelet nadir
JC	167	150
RH	663	

	WBC nadir	WBC nadir
JC	5.9	5.7
RH	11.5	

	Other toxicities
JC	none
RH	Grand mal convulsion

Pharmacokinetic data

	AUC	Clearance	$T1/2\alpha$	$T1/2\beta$
JC	11.5	492	.04	1.1
	12.7	444	.15	2.2
RH	9.4	636	.21	2.4

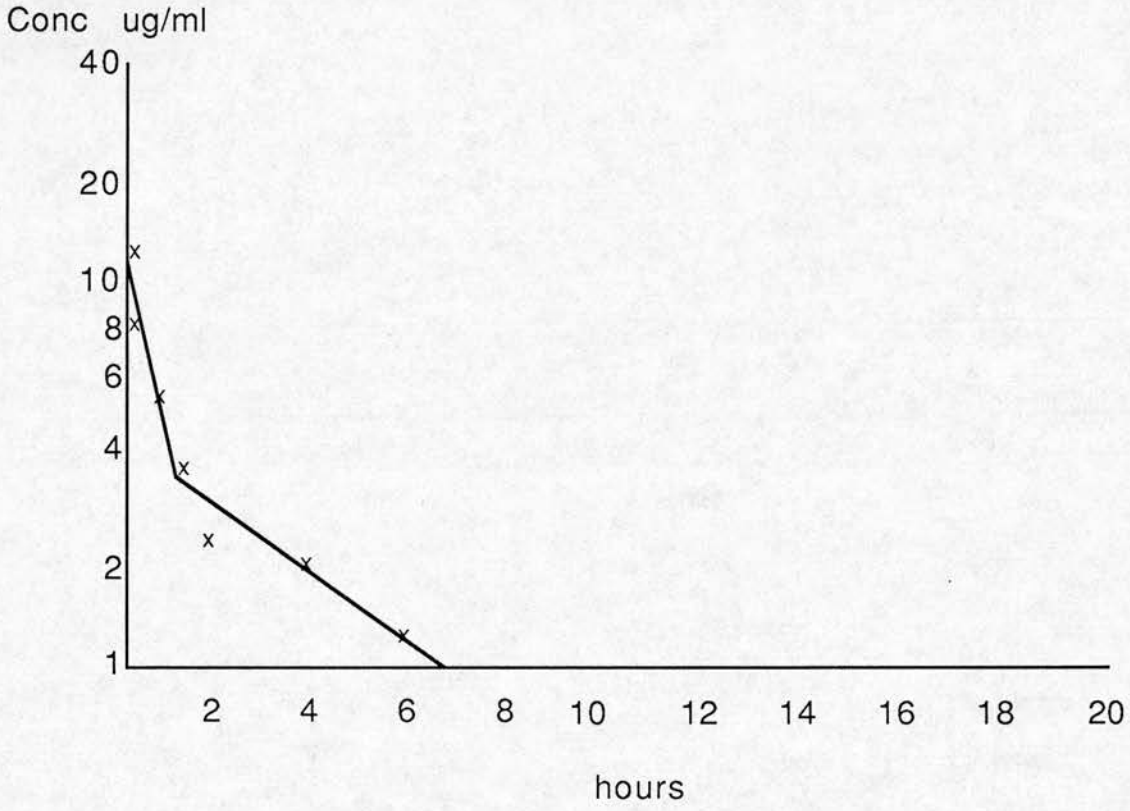
Comment

Patient RH had a grand mal convulsion during the administration of his second dose of amphethinile. This occurred after approximately 50% of the dose had been given. He made a full and rapid recovery. There had been no problems with the first course. Patient JC experienced no side effects. It appeared that this fit was due to a bolus effect and was possibly analagous to the catatonic state seen in mice with rapid iv injections. It was decided that future doses should be given as a short infusion and in view of the lack of other

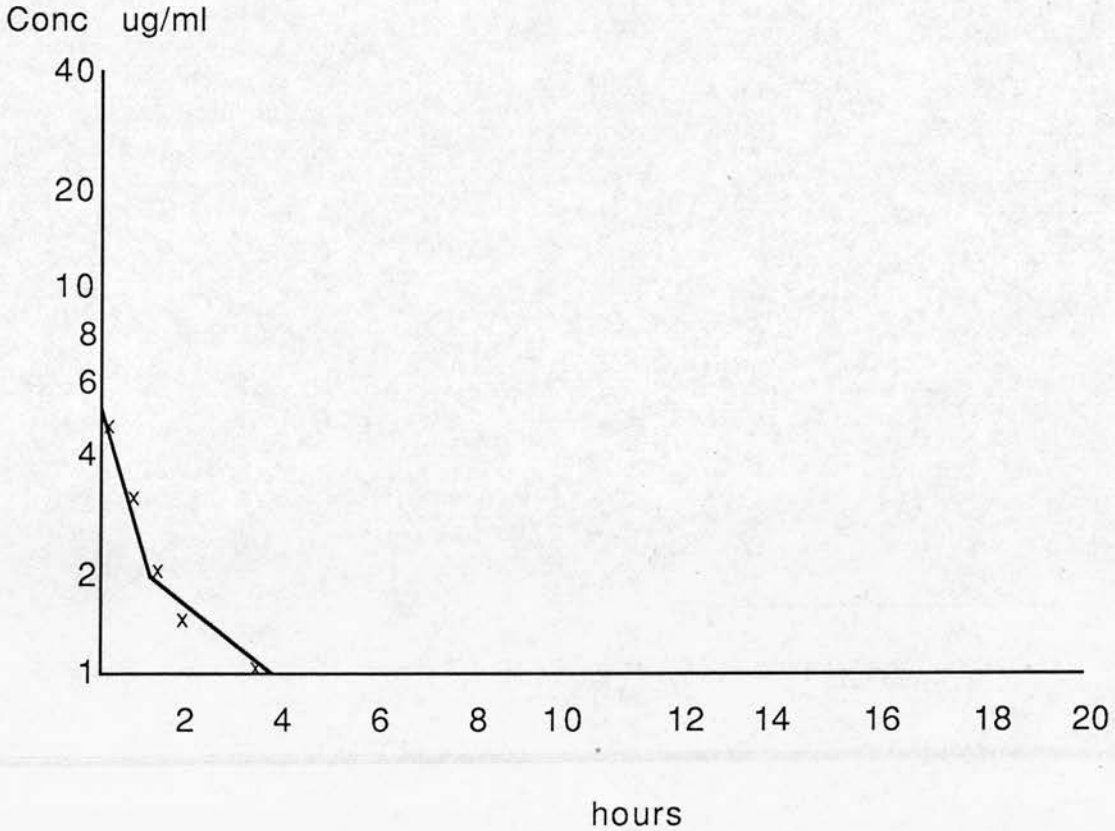
toxic effects to escalate the dose by 100%. This was felt to be safe since the AUC measured at this dose was less than 1/15th of the target AUC. Plasma decay curves are shown in fig 14.

fig 14

200mg/sqm : JC



200mg/sqm : RH



Dose : 400mg/sqm Date start : 17 : 9 : 86

Patient	Diagnosis	Age	Performance Status	Major Disease Site
GG	SCLC	46	1	lung
TL	NSCLC	51	1	lung

	Prior XRT	Prior Chemotherapy
GG	Mediastinum	Ifosfamide, CBDCA
TL	Mediastinum	None

	Course 1 Platelet nadir	Course 2 Platelet nadir
GG	244	182
TL	250	231

	WBC nadir	WBC nadir
GG	10.7	13.7
TL	8.2	9.5

	Other toxicities
GG	Feeling of warmth at end of infusion
TL	" " " "

Pharmacokinetic data

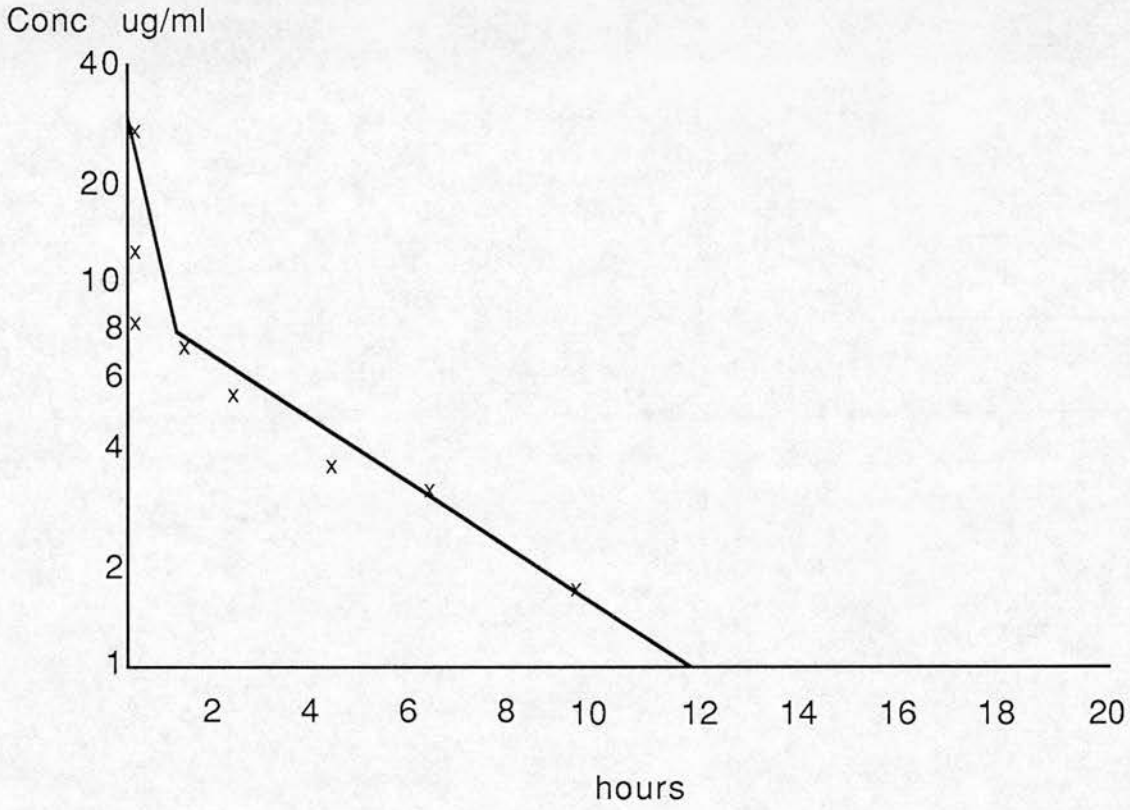
	AUC	Clearance	Tl/2α	Tl/2β
GG	17.5	711	.03	2.3
	25.9	481	.05	2.6
TL	45.5	201	.03	2.7

Comment

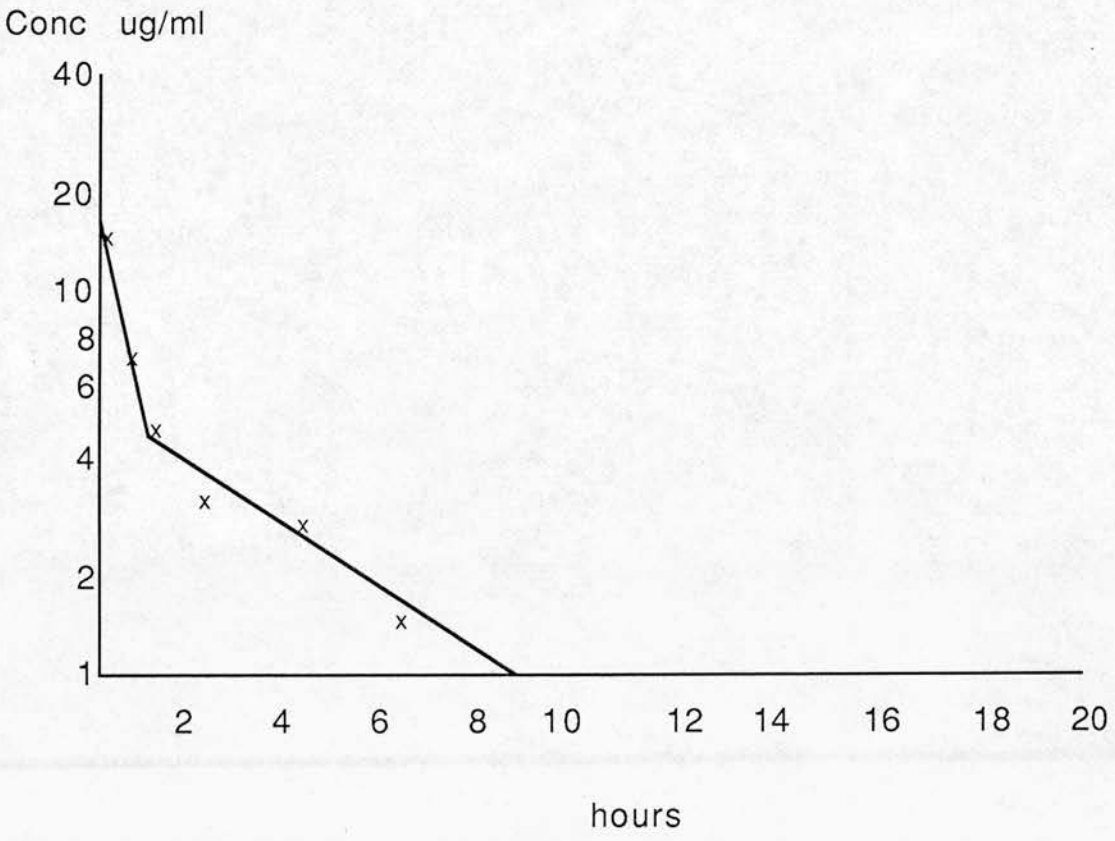
The drug was given as a 20 minute infusion in 250ml saline. Apart from a transient feeling of warmth at completion of the infusion no side effects were seen. The parmacokinetic data indicate non-linear kinetics with an increase in elimination half life and 3-4 fold increase in AUC (fig 15) compared to 200mg/sqm. Allowing for this a further 100% dose increase would still not exceed the target AUC.

fig 15

400mg/sqm : TL



400mg/sqm : GG



Dose : 800mg/sqm Date start : 30 : 10 : 86

Patient	Diagnosis	Age	Performance Status	Major Disease Site
FL	Sarcoma	24	2	Lungs
MG	Ovary	58	1	Abdomen
JB	NSCLC	63	1	Lung
SL	Sarcoma	26	1	Lung
NB	Ovary	58	1	Abdomen
AG	NSCLC	67	2	Lung

	Prior XRT	Prior Chemotherapy
FL	Pelvis	Ifosfamide, Adriamycin
MG	none	Cyclo, Cis-Platin, CBDCA
JB	Mediastinum	Ifosfamide
SL	none	Cis-Platin, Mtx, Adriamycin, Ifos.
NB	Abdomen	CHIP, Chlorambucil
AG	Mediastinum	Ifosfamide

Platelet nadir

	Course 1	Course 2	Course 3	Course 4
FL	209	357*	295	146
MG	168			
JB	592	553		
SL	244			
NB	406	443		
AG	403			

WBC nadir

	Course 1	Course 2	Course 3	Course 4
FL	1.4	5.4	6.7	4.3
MG	4.4			
JB	6.6	7.9		
SL	3.9			
NB	3.8	4.3		
AG	5.7			

	Nausea/vomiting	Alopecia	Pain	Allergy
FL	3	3	3	0
MG	2	0	3	3
JB	1	0	0	0
SL	2	0	3	0
NB	3	0	0	0
AG	2	0	0	0

	Lethargy	Light headed	Diarrhoea
FL	5 days	15 min	2
MG	3 days	-	0
JB	7 days	20 min	0
SL	7 days	-	3
NB	7 days	20 min	0
AG	3 days	15 min	0

Pharmacokinetic data

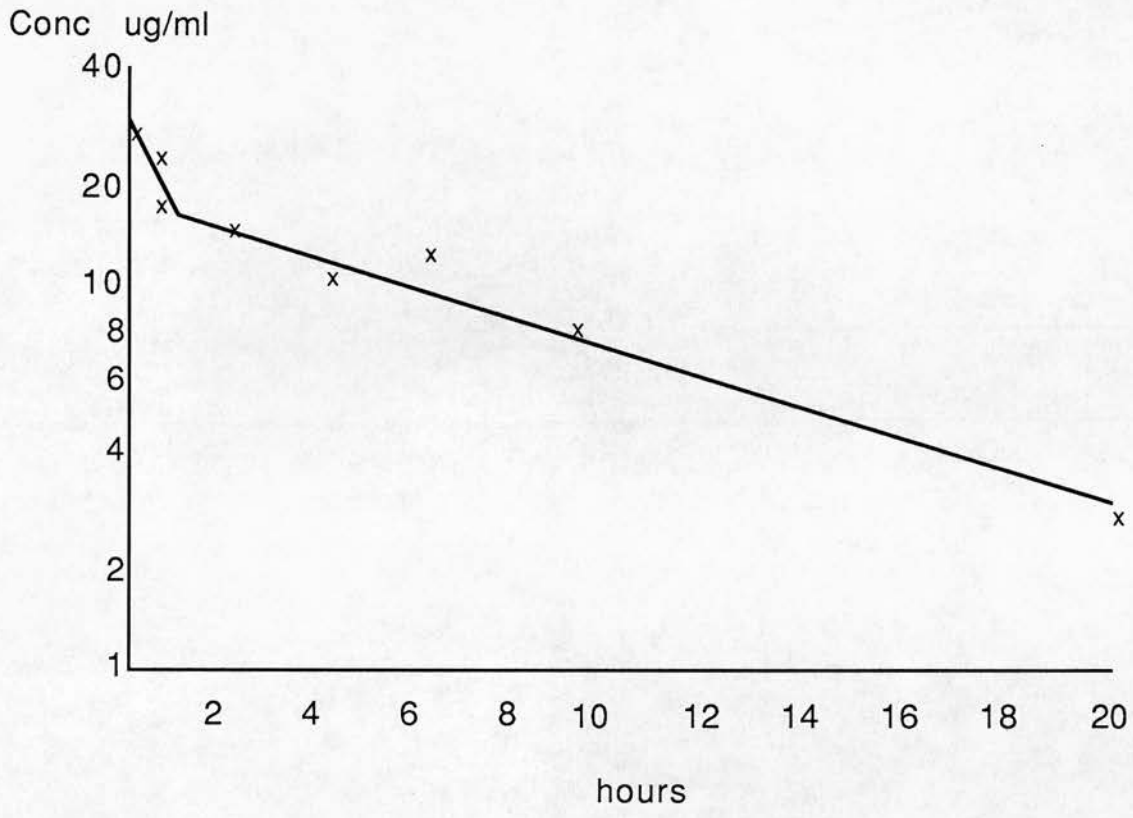
	AUC	Clearance	Tl/2α	Tl/2β
FL	231	79	.33	9.2
	70*	131	.43	7.0
MG	112	200	.41	5.5
JB	81	248	.19	4.6
SL	109	227	.83	7.6
NB	122	174	.52	11.3
AG	223	71	.26	12.1

* Second course given at 400mg/sqm

Plasma decay curves are shown in fig 16.

fig 16

800mg/sqm : FL



800mg/sqm : MG

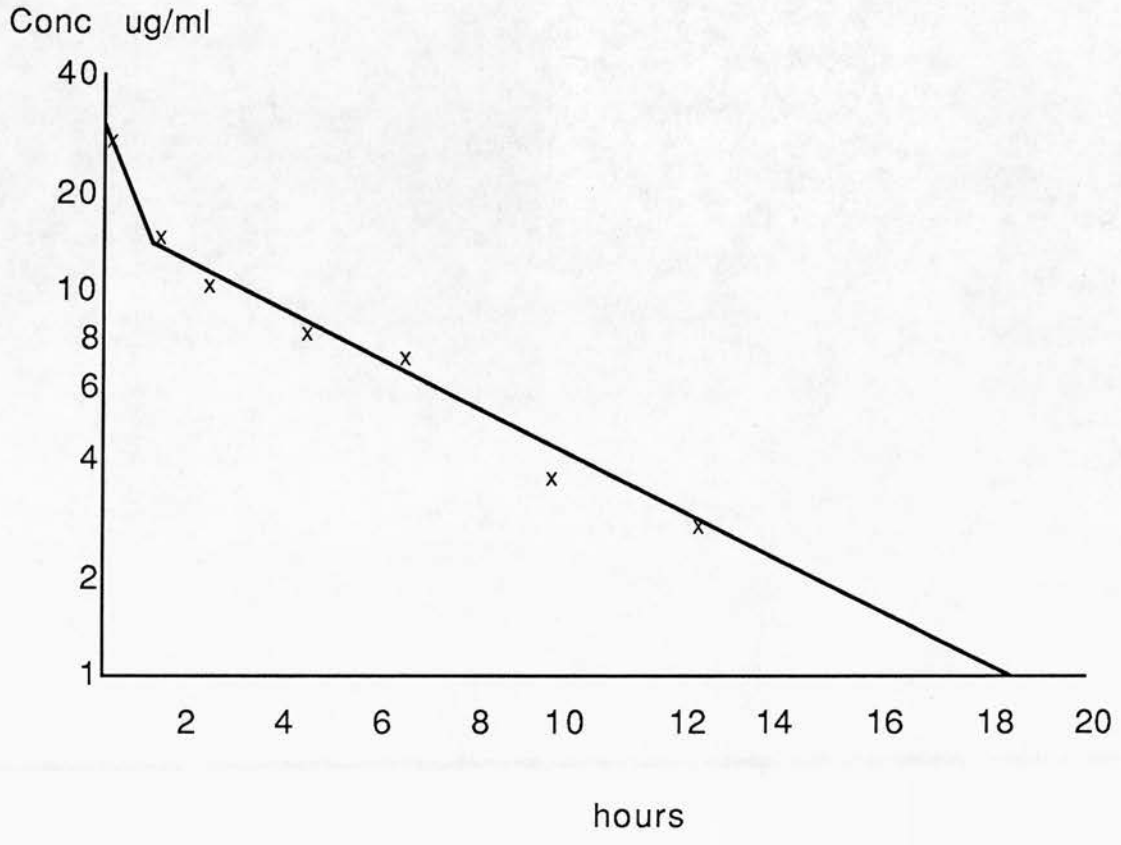
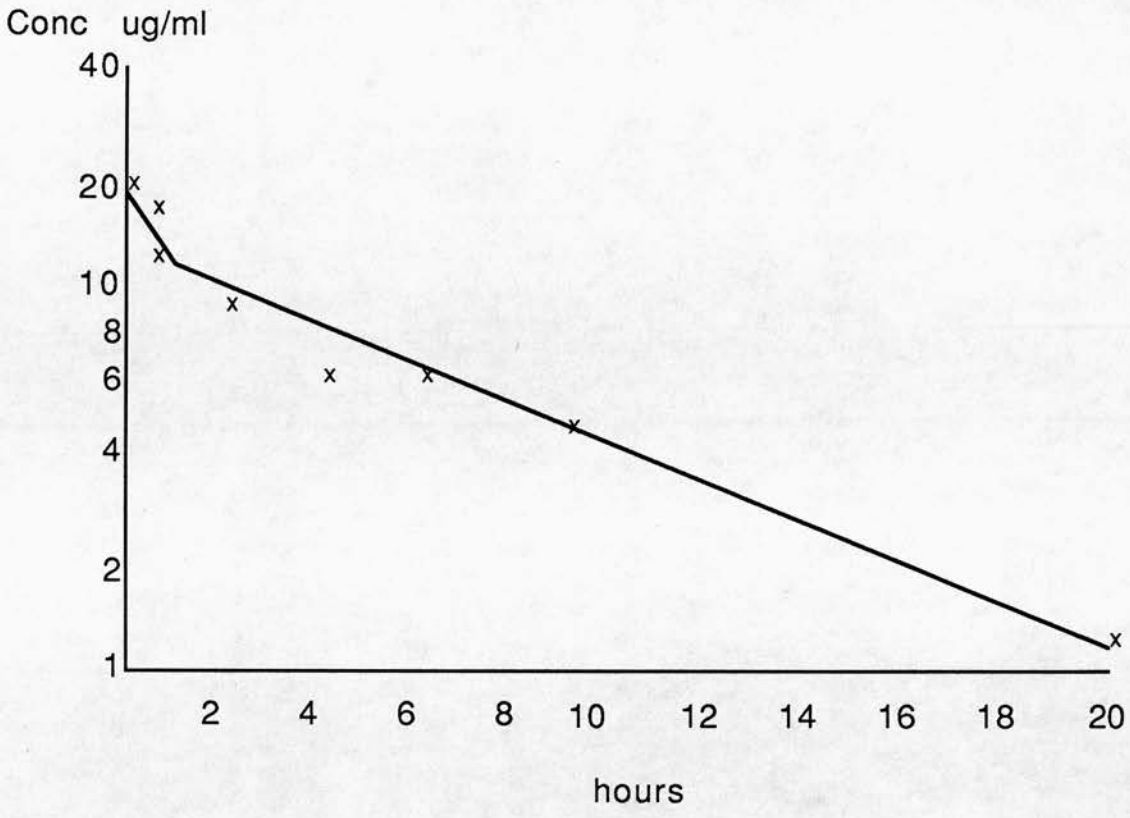


fig 16 (cont)

800mg/sqm : JB



800mg/sqm : NB

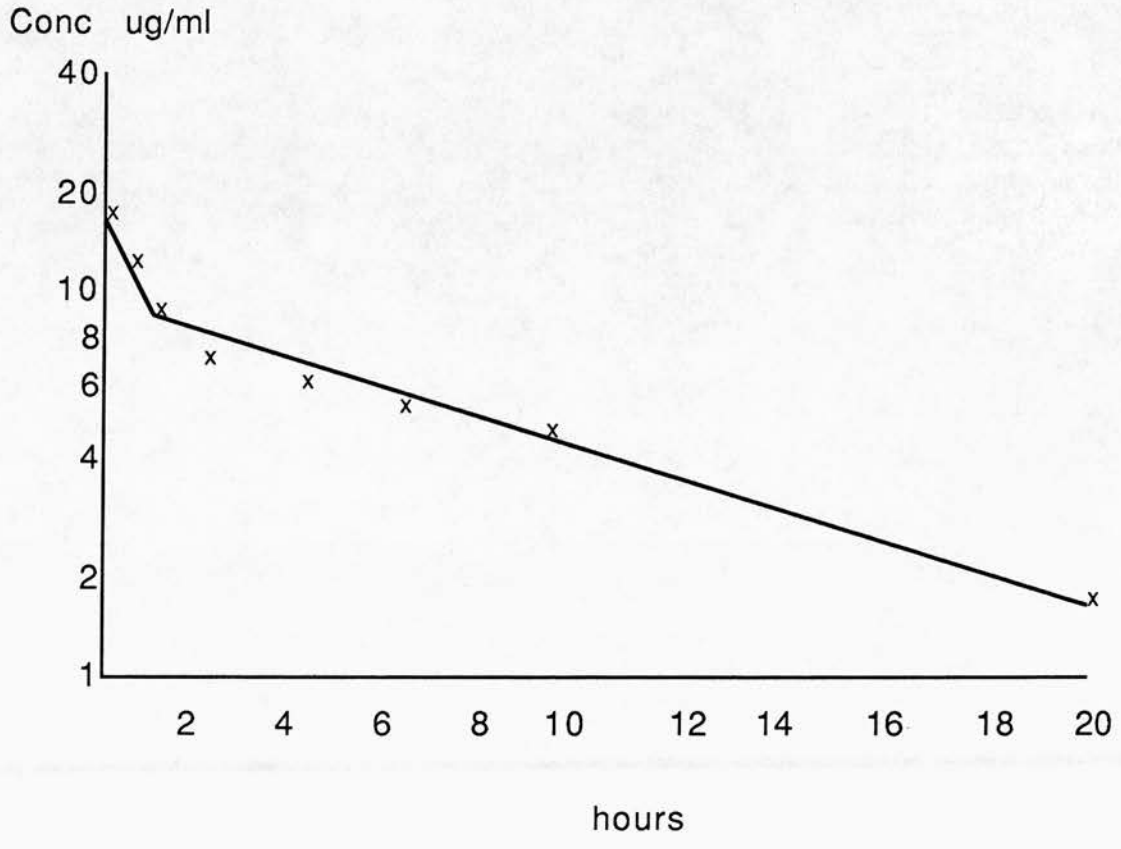
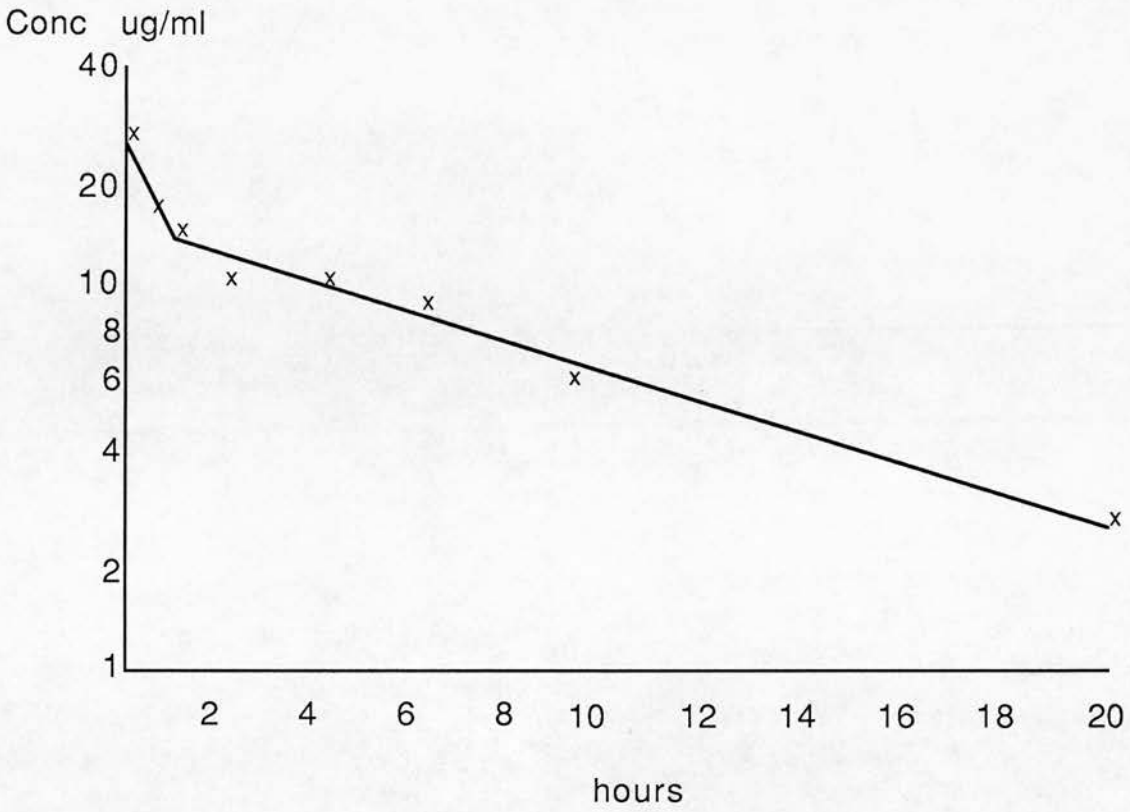


fig 16 (cont)

800mg/sqm : AG



800mg/sqm : SL

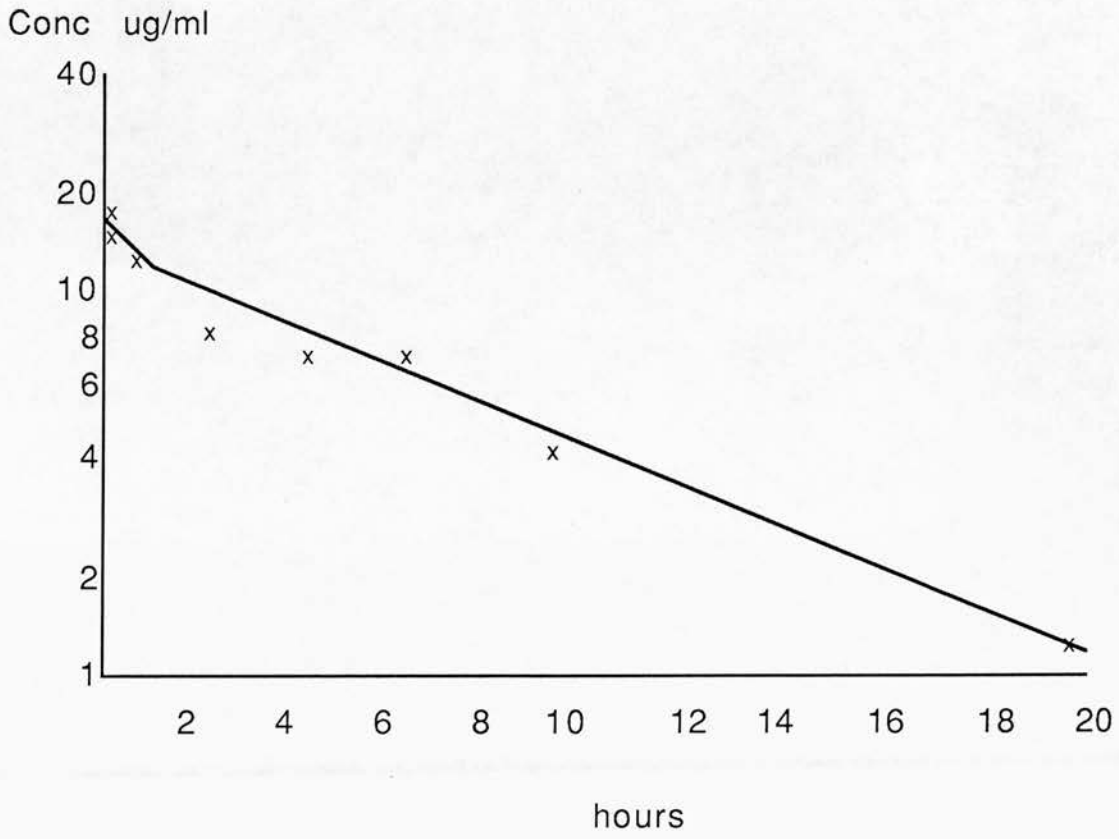
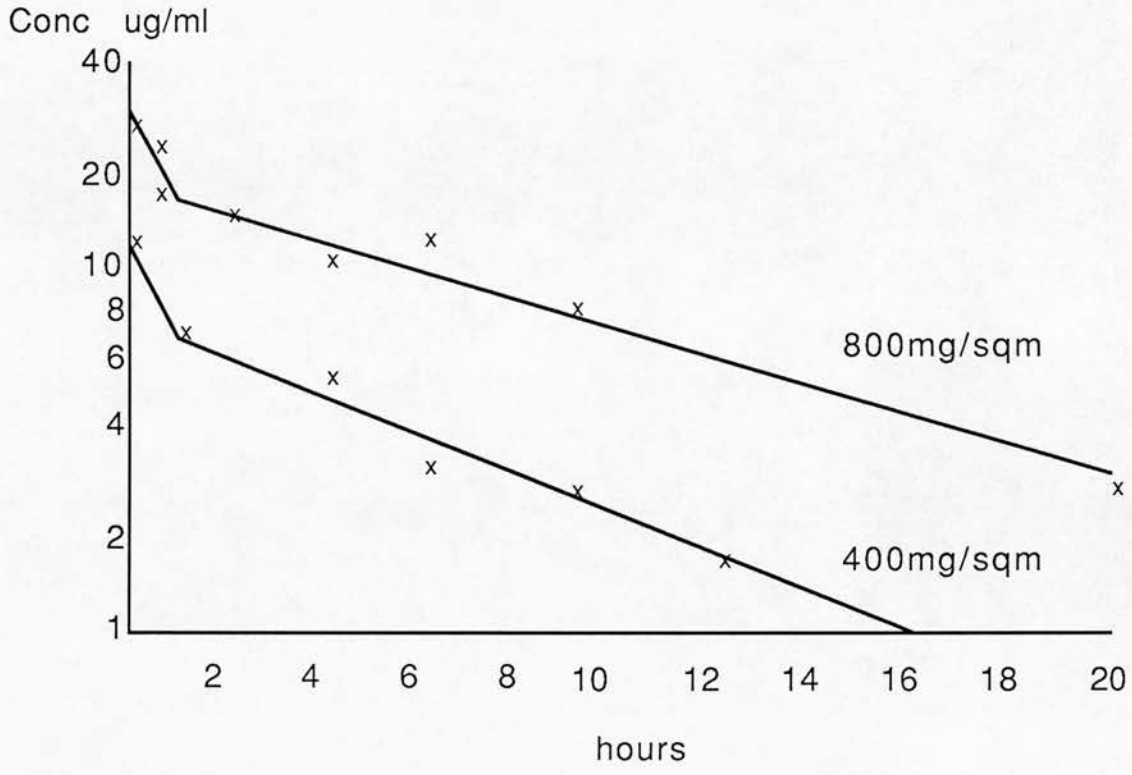


fig 17 **Alteration in kinetics with 50% dose reduction in patient FL**



Comment

A range of non-haematological toxicities occurred at this dose which was given as a one hour infusion in 500ml N saline. Four patients noticed a feeling of light headedness lasting 15 - 20 minutes towards the end of the infusion. This was not associated with an increase in pulse rate, fall in blood pressure or any neurological abnormalities. Three patients developed severe pain requiring opiate analgesia. In two cases this occurred within two hours of the infusion and the pain affected known sites of tumour; buttock in a sarcoma patient and abdomen in a patient with ovarian cancer. In both these cases the pain continued for 2-3 days before fully resolving. In the third case colicky abdominal pain was associated with severe diarrhoea which began 12 hours after treatment and continued for 16 hours.

All patients felt very tired and lethargic for up to 7 days after the amphethinile infusion and all had some degree of nausea and vomiting related to treatment. Patient MG had an episode of breathlessness, sweating and vomiting 40 minutes after the first treatment. This lasted five minutes and was treated with an iv injection of hydrocortisone. When rechallenged with the drug 3 weeks later these symptoms recurred immediately after only a few ml had been infused. The treatment was stopped and she made a full recovery. This was clearly an allergic reaction probably to the solutol vehicle.

Neutropenia and alopecia occurred in one patient treated at this dose. The AUC for this patient was 23lug/l.h-l, a level considerably higher than that of other patients treated at this dose and approaching the target AUC of 300ug/l.h-l. This patient also

experienced severe pain in the buttock area commencing 30 minutes after the amphethinile infusion finished and because of this her second dose was given at 400mg/m^2. At this dose she had no side effects of any kind. Moreover The terminal elimination half life fell by 2.2 hours and the AUC by a factor of 3. The wbc nadir occurred on day 7 following therapy and recovered by day 14. Prior to starting therapy this patient had rapidly progressing pulmonary metastases. While receiving amphethinile the chest X-ray appearances remained static for three months before progressing again. This may indicate an effect of the drug on the tumour.

The pharmacokinetic data appear to confirm that an AUC of 200-300 is required to produce biological effects in terms of haematological suppression, hair loss and possibly anti-tumour activity. In addition it appeared that an increase in dose by a factor of two results in a 3-4 fold increase in AUC. The majority of patients treated at 800mg/m^2 had an AUC of less than 130 and therfore a dose increase of 50% which should approximately double the AUC was selected for the next dose level.

Dose : 1200mg/sqm Date start : 22 : 1 : 87

Patient	Diagnosis	Age	Performance Status	Major Disease Site
DM	Teratoma	27	1	Liver
GM	Sarcoma	58	1	Abdomen
PC	NSCLC	61	2	Lung

	Prior XRT	Prior Chemotherapy
DM	none	POMB/ACE
GM	none	Ifosfamide, Adriamycin
PC	Mediastinum	Ifosfamide

	Platelet nadir	WBC nadir
DM	253	1.9
GM	-	-
PC	-	-

	Nausea/vomiting	Alopecia	Pain	Allergy
DM	3	3	0	0
GM	3	-	0	0
PC	2	-	0	0

	Lethargy	Light headed	Diarrhoea
DM	7 days	60 min	0
GM	2 days	30 min	0
PC	-	60 min	0

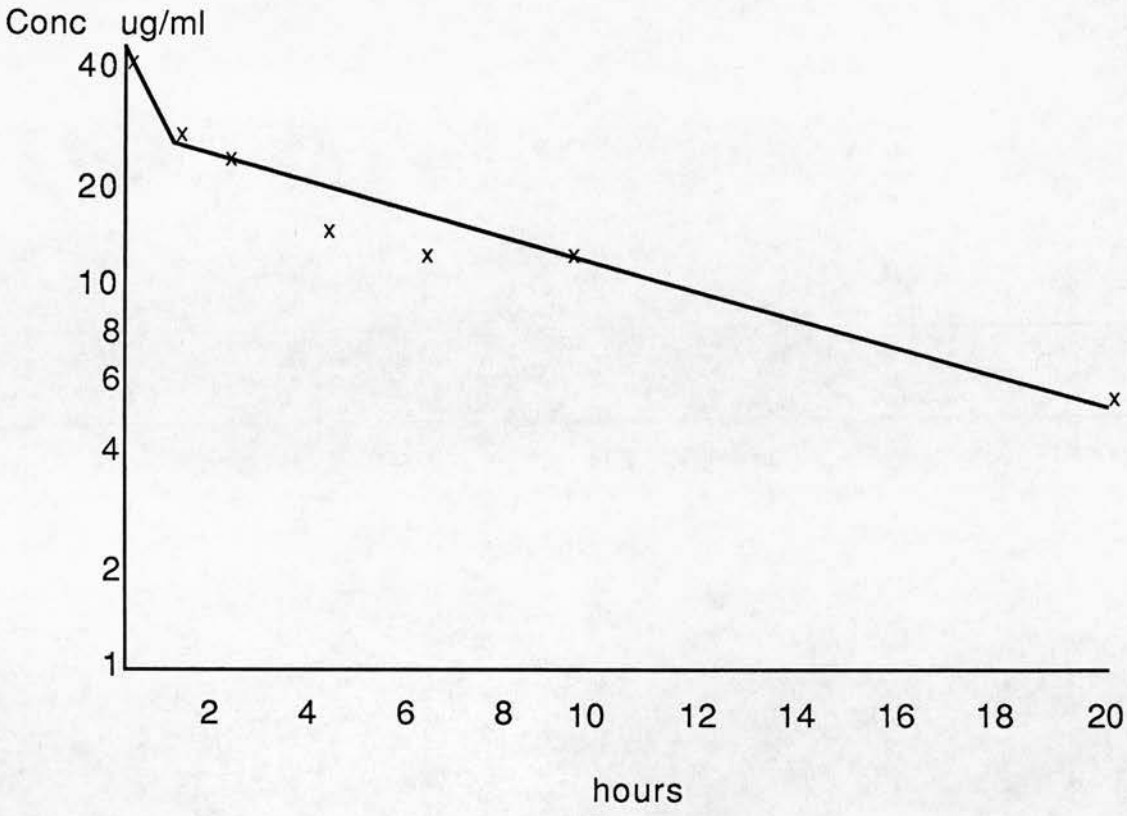
Pharmacokinetic data

	AUC	Clearance	$T1/2\alpha$	$T1/2\beta$
DM	361	92	1.1	13.8
GM	194	188	.06	6.9
PC	184	216	2.3	8.6

Plasma decay curves are shown in fig 18.

fig 18

1200mg/sqm : DM



1200mg/sqm : GM

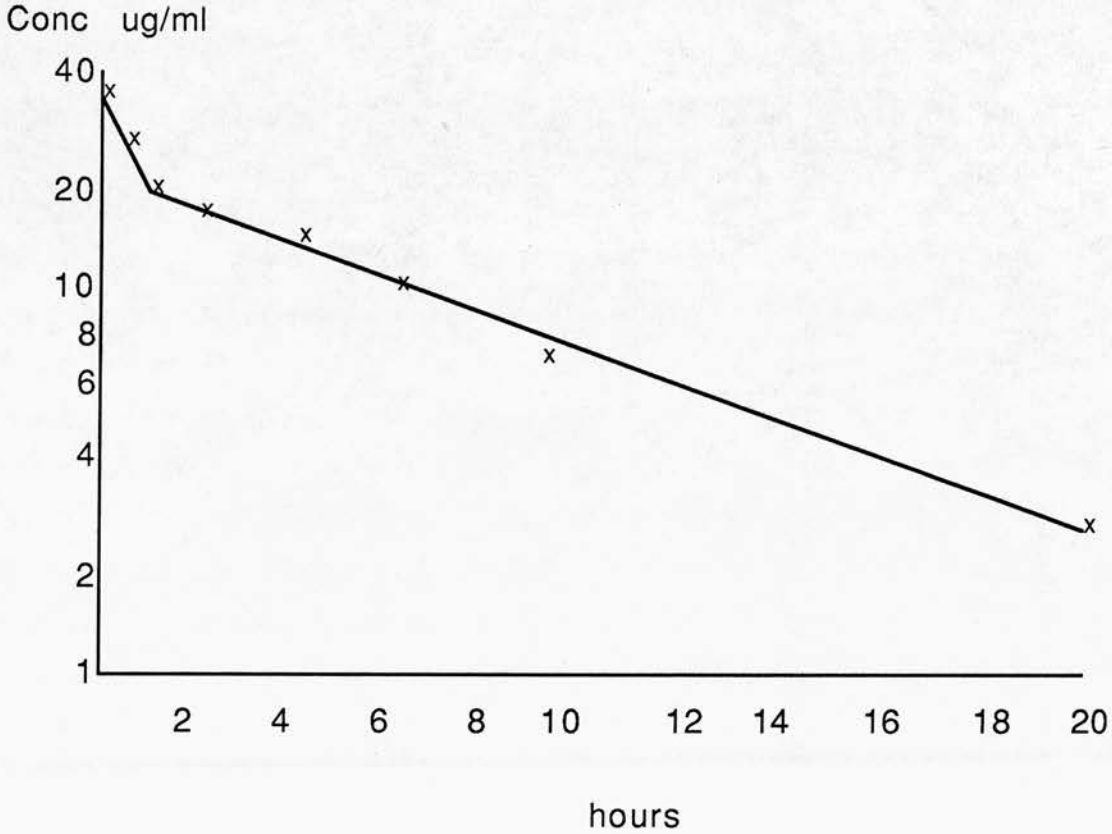


fig 18 (cont) **1200mg/sqm : PC**

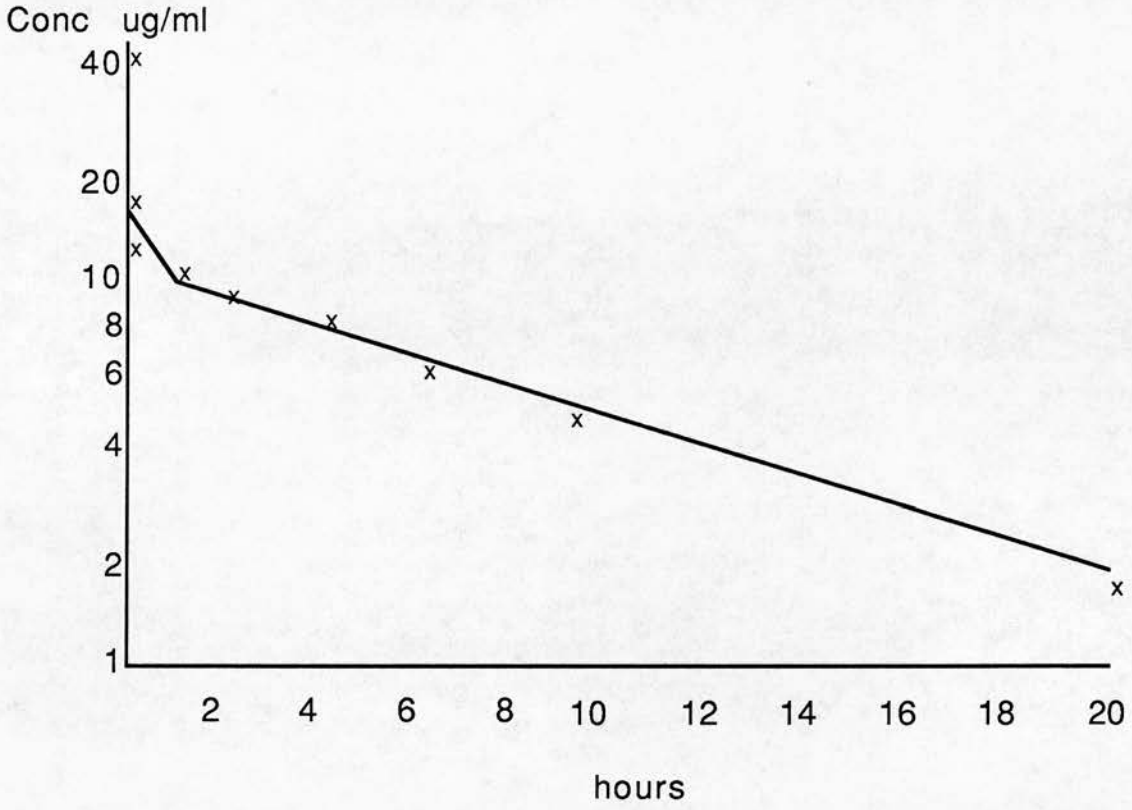
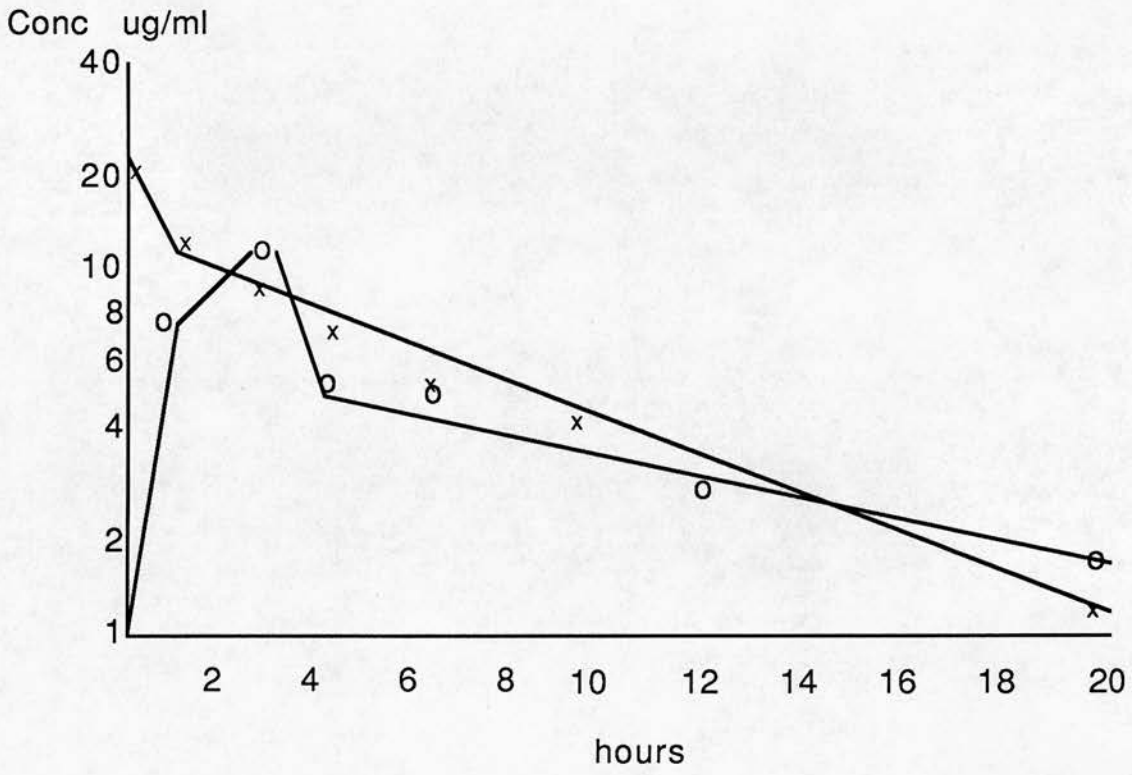


fig 19 **Plasma decay following 800mg/msq orally and IV in patient JB**



o : oral dose
x : iv dose

Comment

This dose was given as a two hour infusion in 500ml saline to patients DM and GM and as a four hour infusion in 1000ml saline to PC. All three patients experienced a feeling of light headedness for 30 - 60 min similar to that seen at lower doses. Again this was not associated with cardiovascular or neurological abnormalities. In addition all patients had varying degrees of nausea and vomiting but no episodes of pain or allergic phenomena were seen. The first patient treated at this dose, DM, experienced an unusual feeling of being unable to focus properly for 4 days in addition to somnolence and profound tiredness for 7 days. This patient also developed alopecia and neutropenia of $1.9 \times 10^9/l$ on day 14 with recovery by day 21. Unfortunately due to rapidly advancing liver disease a second course was not given.

The second patient, GM, was readmitted to hospital 48 hours after completing therapy suffering from haematemesis, melaena and in a state of collapse. The patient subsequently died but permission for a post mortem was not given. The patient had a large abdominal mass measuring 24 x 16 cm and it was considered that an intra-abdominal catastrophe associated with this mass had taken place.

The third patient had minimal initial toxicity following the infusion but 16 hours later suddenly developed a right hemiparesis and died 12 hours later. At the time of the CVA the patient was hypotensive but an ECG was normal. A post mortem examination was performed. Infarcts were seen in the brain, spleen and both kidneys. These were considered to be due to emboli from a loosely adherent area of thrombus in the ascending aorta. Apart from a large mass of tumour in the mediastinum no other abnormalities were seen and in

particular there were no obviously drug related effects.

There were therefore two deaths within 48 hours of treatment at this dose and although these did not appear to be due to the more usual causes of toxic death it is difficult not to attribute these to the drug. One possibility in the second case is that the high dose of solutol administered, 24gm, resulted through its detergent like action in destabilisation of an atheromatous plaque with subsequent embolisation. Moreover the first patient (DM) experienced grade 3 vomiting, grade 3 somnolence and a very disturbing sensation of inability to focus for 3 days. It was therefore felt that further patients should not be entered at this dose.

Discussion

Amphethinile was introduced for phase I clinical trial because of activity in pre-clinical systems, a novel stucture, and apparent non-cross resistance with the vinca alkaloids. However a major problem was the compound's insolubility and this was overcome by using a formulation including Solutol HS15, a surfactant like material that produces a molecular suspension of amphethinile in aqueous solution. Unfortunately Solutol has not been used in man before but it had been shown to be 10 - 20 x safer than the related material Cremophor EL which has. However this meant that the current study was a phase I trial of both amphethinile and solutol.

The highest dose given using the intermittent iv schedule was 1200mg/m^2. At this dose 2/3 patients died within 48 hours of treatment possibly as a result of solutol induced vascular effects. The third patient developed neurological toxicity comprising lethargy, somnolence and visual disturbance which precluded further

treatment. This dose clearly exceeded the MTD.

At 800mg/m^2 all six patients experienced nausea and vomiting lasting a few hours to 3 days and all experienced quite profound lethargy for 3-7 days. In addition four patients noted a feeling of light headedness for 15-30 min at the end of the infusion, three had pain requiring opiate analgesia and two diarrhoea. This dose therefore represents the maximum tolerated dose for the trial although the degree and nature of toxicity varied considerably from patient to patient.

Two patients during the study developed alopecia and neutropenia as a result of treatment. One was treated at 800mg/m^2 and one at 1200mg/m^2 but both had an AUC in excess of 230ug/l.h-1. These effects are presumably due to the amphethinile and indicate that an AUC above this level is required for cytotoxicity to occur. There was however a consistent thread of neurotoxicity running through the trial. At 400mg/m^2 a bolus injection provoked a fit in one man and at 800 and 1200mg/m^2 the majority of patients experienced light headedness and lethargy. In addition one patient at 1200mg/m^2 had visual disturbance and another a CVA although the latter appeared to be embolic in origin. Amphethinile is said to be a spindle poison and therefore neurotoxicity might be expected as a result of treatment. On the other hand solutol produced immediate CNS toxicity in mice at high doses, particularly when the dose was given as a rapid iv bolus. These solutol related effects were not seen with the intraperitoneal injections. It is therefore also possible that the neurotoxicity seen in this trial was due to solutol. Moreover at 800 and 1200mg/m^2 the dose of solutol was 10 - 25 gm. This represents approximately 10% of the LD50 of Solutol in the mouse and

is therefore a considerable dose. Solutol is known to release vasoactive substances such as histamine at high doses in dogs and it is possible that local vasodilation was the cause of the pain in three patients. In addition the detergent-like action of solutol may have had some effect on the vasculature of the patients who died following treatment at 1200mg/m^2. The patient who survived this dose was considerably younger and would therefore have less chance of having degenerative vascular disease on which the solutol might have an effect. The one event that can definitely be attributed to the solutol was the allergic reaction which occurred in one of the 14 patients entered in the trial.

Although it is possible to administer 800mg/m^2 as an iv infusion over 2 hours even this dose was associated with considerable toxicity. Moreover only one of the patients treated at this dose achieved an AUC that produced cytotoxic effects. Thus rather than advocating 800mg/m^2 as an appropriate dose for phase II testing alternative methods of administration should be explored. In particular different schedules should be tested. For example since 400mg/m^2 was virtually non-toxic this dose could be given on successive days starting with 2 doses and escalating to 3, 4, 5 etc depending on toxicity. From the pharmacokinetic data available 400mg/m^2 daily for 5 days would probably be needed to achieve a relevant AUC. Alternatively in a manner analagous to the vinca alkaloids a weekly schedule may be appropriate.

A potentially more interesting avenue for research is the use of a solutol free oral formulation. Amphethinile is known to to be absorbed when administered orally to mice. In addition a preliminary trial using the iv preparation in one patient showed that the

bioavailabilty of amphethinile given orally was close to 100% (fig 19). The development of an oral preparation not containing solutol will allow more accurate determination of amphethinile related toxicity.

Work in progress in the Department of Experimental Chemotherapy, Paterson Institute for Cancer Research indicates that Amphethinile acts at different sites compared to the vinca alkaloids confirming that it may be non-cross resistant with these agents (McGown personal communication). Amphethinile therefore appears to be a very interesting compound and attempts to find a practical formulation and schedule should be pursued.

Escalation Procedure

This trial employed a novel escalation procedure combining two separate concepts. The first, derived from the phase I study survey reported earlier, stated that if the starting dose was entirely non-toxic and the AUC was less than 5% of the target AUC, then this could immediately be escalated to 5 times the starting dose. This proved to be entirely safe in this trial.

The second was based on the work of Collins et al (1986) and involved the use of pharmacokinetic data in the mouse as a guide to speed of escalation. This relies on the observation that the AUC at the LD10 in the mouse is close to the AUC at the MTD in man. The use of this concept allowed rational dose increments to be used based on observed changes in amphethinile clearance with increasing dose. Although it is likely that some of the dose limiting effects seen were due to solutol, amphethinile toxicity appeared to occur when an AUC close to the target AUC was achieved.

As a result of the use of these procedures the final escalation step which was 30 times the starting dose was reached in four dose increments. Using the conventional modified Fibonacci scheme this would have taken nine escalation steps. This therefore represents a saving of 5 dose levels which would have increased the duration of the trial by 9 - 12 months and the number of patients included by 10 - 15. The patient and financial resources saved may be used in exploring alternative schedules.

Pharmacokinetic Study

Introduction

The pharmacokinetic study carried out in conjunction with the phase I clinical trial of Amphethinile had the dual aims of guiding dose escalation and characterising the clinical pharmacology of the drug. In order to fulfil these aims it was necessary to develop an HPLC method for measuring the drug in serum and urine and also to perform pharmacokinetic experiments in mice

Methods

An HPLC method was developed to measure amphethinile in the serum and urine.

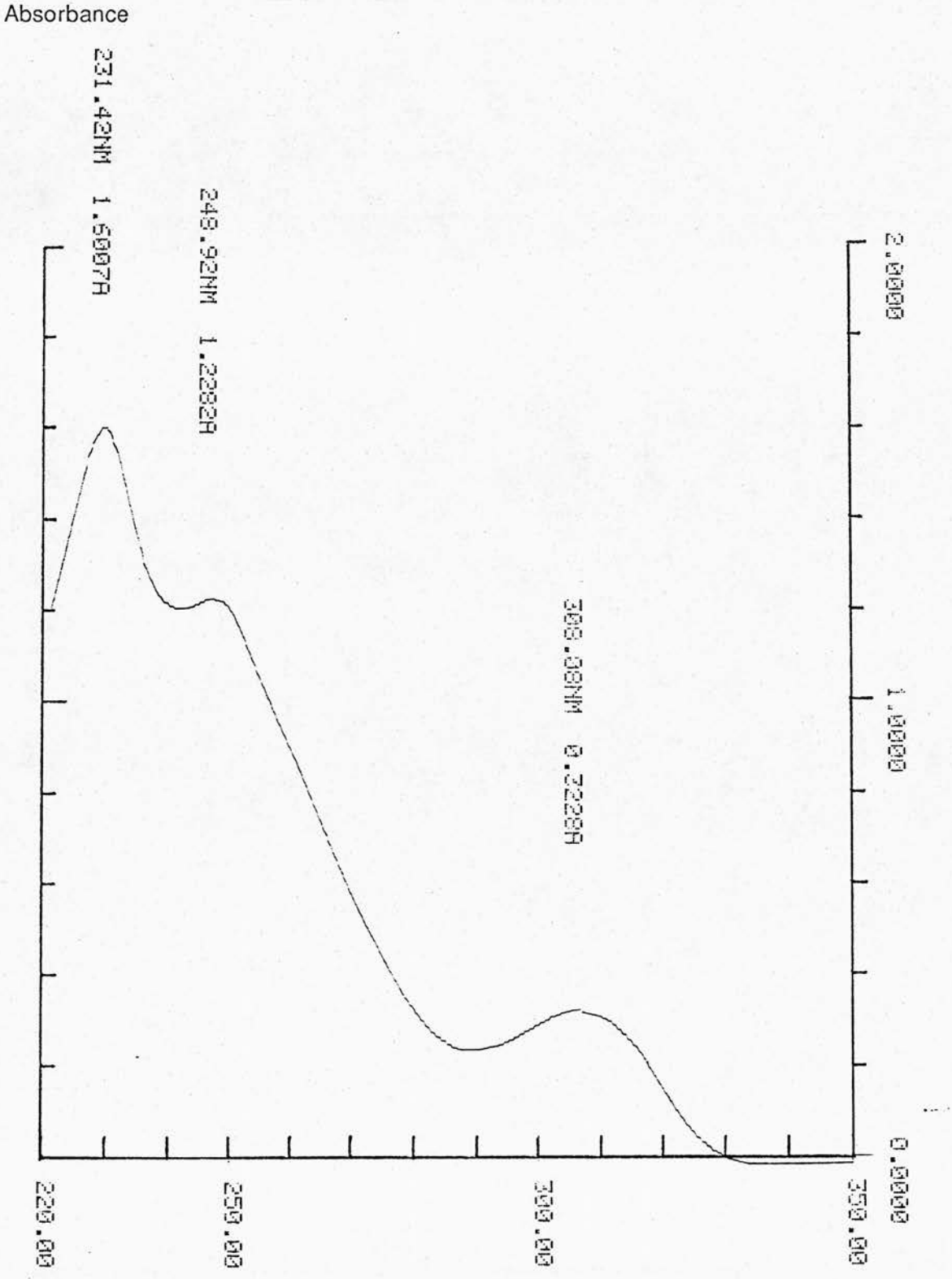
Detector

Amphethinile possesses no native fluorescence but significant UV absorption occurs at 240 and 310nm (fig 20). Due to the considerable background absorption from plasma at 240nm, 310nm was selected as the most efficient wavelegth for this compound. Experimentation with the PYE UNICAM PU4020 UV detector showed that maximum absorbance occurred at 305nm and this was used in all subsequent assays.

Column

Amphethinile is a relatively small molecule, MW 265, alcohol soluble and non-polar. A 5 micron ODS reverse phase column was therefore chosen as most likely to provide adequate chromatography.

fig 20 **Ultraviolet Absorbance Scan of Amphethinile**



Mobile Phase

Amphethinile is highly soluble in most organic solvents and thus a water/methanol buffer was selected. Retention times with varying proportions of water and methanol +/- 0.1% phosphoric acid are shown below. The results for the compound selected as the internal standard for the assay are also shown. This compound, 2-Nitro -5-Chloro Aniline is formed during the synthesis of amphethinile and also exhibited uv absorbance at 305nm.

Table 23 Retention time of Amphethinile with alteration in Mobile Phase

Methanol	Water	H3PO4	Standard retention times in minutes	Amphethinile
0%	100%	0	60+	60+
25%	75%	0	60+	60+
50%	50%	0	28	34
50%	50%	0.1%	24	32
70%	30%	0	4.83	8.33
70%	30%	0.1%	2.33	6
100%	0%	0	2.66	2.66
100%	0%	0.1%	2.66	2.33

The shortest retention time occurred with 100% methanol but this could not separate Amphethinile from the compound selected to be the interal standard for the assay. A solution of 70% methanol/30% water achieved excellent separation of these two compounds. The addition of

0.1% phosphoric acid, by protonating the primary amine group, increases the polarity of both the Amphethinile and the internal standard and therefore reduces their retention times on the column by approximately 100 seconds. The flow rate through the column during these experiments was 1.25ml/min and the working pressure was 270 - 300 bar.

Extraction Procedure

In the first instance a methanol extraction was examined. It was found that over 90% of the drug could be extracted from a 0.5ml serum sample using 3 x 1ml extrctions. However this resulted in a 7:1 dilution of the sample and the lower limit of detection for this method was comparatively high at 5ug/ml. Further studies were therefore carried out using chloroform extraction. Amphethinile is readily soluble in chloroform which can be quickly evaporated once extraction has been carried out. This allows the extract to be resuspended in a much smaller volume of solvent allowing the detection of lower concentrations.

It was found that a single chloroform extraction using 8ml chloroform : 0.5ml serum obtained extraction efficiencies of over 90% at drug concentrations of 0.1ug/ml falling to 70% at 100ug/ml. These figures remained constant over a series of experiments and therefore it was possible to use this method to construct linear standard curves for assessing serum samples. The full extraction procedure developed was as follows:

(1) 0.5ml serum + 8ml chloroform then vortex for 30seconds

(2) remove precipitate + aqueous phase using phase separation filter

(3) evaporate to dryness

(4) resuspend in 50ul methanol

(5) inject 25ul onto column

HPLC Equipment

PYE UNICAM PU4010 pump

PYE UNICAM PU4020 UV detector

PYE UNICAM PU4800 video control centre

5u ODS Hypersil column

Using this method the lower limit of detection of amphethinile was 0.1ug/ml (100ng/ml). 20ul (1mg/ml solution) of internal standard (1-Nitro-5-Chloro Aniline) was added to each serum sample prior to chloroform extraction as a measure of inter-assay variation. The extraction efficiency of the internal standard was 90%.

Pre-clinical Pharmacology in BDF Mice

In order to use preclinical pharmacology data as an aid to dose escalation in the phase I clinical trial (Collins et al 1986) a pharmacokinetic study was carried out in BDF mice. A preliminary experiment was performed to ensure that the LD10 for amphethinile in these mice was similar to that for the MF1 strain used by BIBRA Ltd. during formal pre-clinical toxicology testing.

Groups of 10 mice were treated with control solution, 150mg/kg, 175mg/kg and 225mg/kg amphethinile by intraperitoneal injection. The control solution consisted of a 30% solution of solutol (500mg/ml) in normal saline. The test solutions were given in normal saline to a

total volume of 10ml/kg.

Table 24 Amphethinile LD10 in BDF Mice

	Total	Deaths
Control	10	0
150mg/kg	10	0
175mg/kg	10	9
225mg/kg	10	10

These data show that the LD10 lies between 150 and 175mg/kg and is therfore not significantly different to that found by BIBRA Ltd. using the MF1 mice (138 - 169mg/kg).

Pharmacokinetic Study

The pharmacokinetics of amphethinile were studied in BDF mice at 400mg/m^2, the LD10, 200mg/m^2 and 100mg/m^2. Amphethinile was diluted with normal saline to achieve a dose volume of 10ml/kg. Injections were given into the mouse tail vein using aseptic technique. In order to obtain sufficient blood for assay mice were sacrificed at the appropriate time interval following treatment. Three mice were used for each time point which were: 5min, 15min, 30min, 60min, 2hr, 4hr, 6hr, 9hr. Blood was immediately centrifuged, serum separated and stored at -20^{o}C prior to assay.

Since only 100ul of serum was available from the mice the assay was modified in the following manner:

(1) 100ul serum + 20ul standard + 2ml chloroform, vortex

(2) filter to remove protein + aqueous phase

(3) evaporate

(4) resuspend in 50ul methanol

(5) inject 25ul on column

The limit of sensitivity using these volumes was lug/ml.

The most noticeable acute effect of amphethinile injection was a catatonic like reaction which occurred mainly at 400mg/m^2 but also to a lesser extent at 200mg/m^2. A similar reaction was noted in the pre-clinical toxicology experiments conducted by BIBRA and was ascribed to the solutol vehicle rather than the drug itself.

Pharmacokinetic Analysis

The data from this study were analysed using a non-iterative computer programme. The serum decay of amphethinile in mice conformed to a two compartment model with equation:

$$Ct=Ae^{-\alpha t} + Be^{-\beta t}$$

Results

The experimental data and the pharmacokinetic parameters derived from the computer analysis are shown in tables 25 and 26.

These data show that in the mouse the kinetics of amphethinile are approximately linear with no significant change in elimination half life with increasing dose (fig 21) although the AUC increased by a factor of 3 with each doubling of the dose. The AUC at the LD10 was 313ug/l.h-1 and thus this represents the target AUC for the phase I clinical trial.

During the HPLC analyses an addtional peak was consistently seen that had a retention time of 140 seconds (fig 22). This peak therefore eluted before amphethinile and thus is likely to be a more

Table 25 Serum Amphethinile concentrations in mice at LD10, LD5 and LD2.5

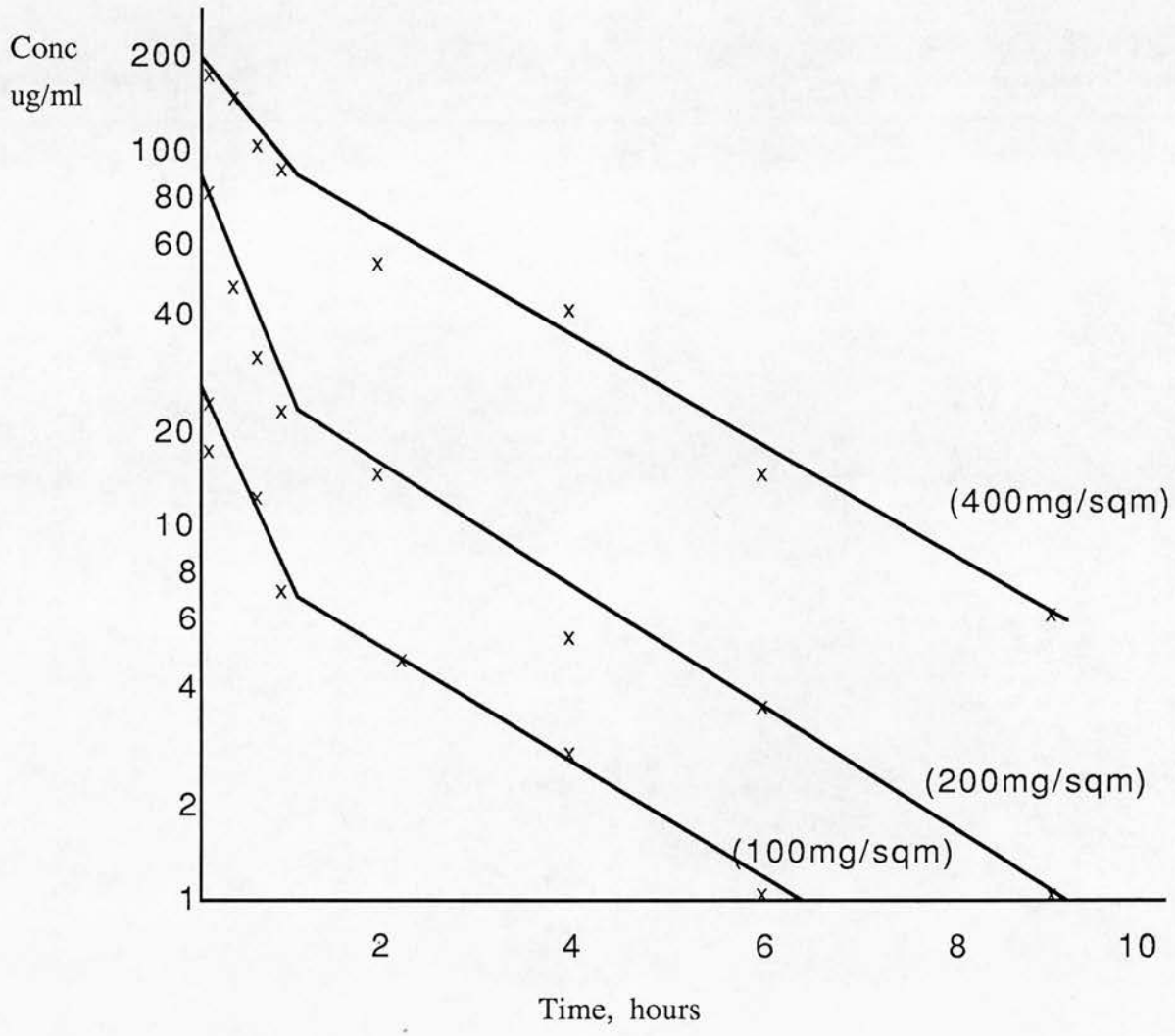
	Hours							
	.083	.25	.5	1	2	4	6	9
LD10	201.1	159.6	131.3	100.8	49.4	19.0	10.2	6.0
	180.0	130.8	118.3	57.3	61.4	21.3	9.7	7.6
	200.7	124.9	117.2	110.6	60.5	12.5	9.9	4.5
Mean	193.6	138.8	122.3	89.3	57.1	17.6	9.9	4.5
LD5	81.6	56.3	53.9	22.1	11.4	2.9	2.4	0.8
	88.3	60.1	43.7	28.0	11.7	3.6	5.1	0.9
	87.6	55.3	15.5	27.7	21.1	7.1	2.6	0.8
Mean	85.7	57.2	37.7	25.9	14.7	4.5	3.4	0.8
LD2.5	26.6	17.5	12.0	8.7	5.8	4.2	1.1	-
	26.9	18.8	9.3	6.8	4.4	2.5	0.9	-
	26.7	14.2	12.9	5.8	4.0	2.1	1.1	-
Mean	26.7	16.8	11.4	7.1	4.7	2.9	1.0	

Table 26 Pharmacokinetic Parameteres of Amphethinile in mice

	KE	K2>1	K2<1	Vl	VDarea	VDSS	VPC
LD10	1.2	8.2	10.3	.01	.01	.01	.01
LD5	1.1	2.1	2.0	.01	.03	.03	.01
LD2.5	0.9	1.4	2.2	.02	.08	.07	.04

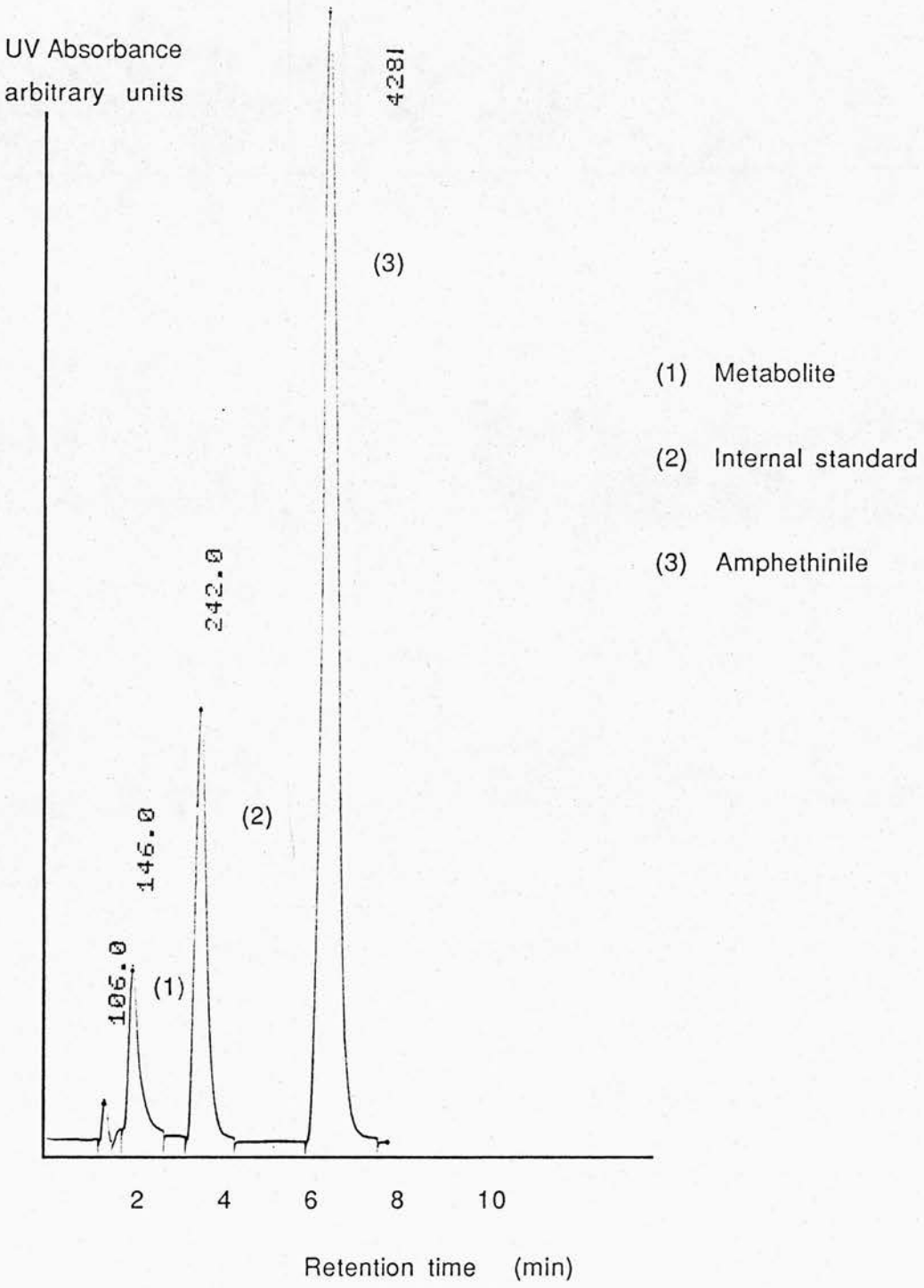
	Tl/2α	Tl/2β	Area	Cl
LD10	.03	1.34	313	.17
LD5	.14	1.36	95	.31
LD2.5	.15	2.06	34	.48

fig 21 **Plasma Decay Curves of Amphethinile in Mice**



Values represent the mean of three mice at each time point

fig 22 **Chromatogram of Amphethinile**



polar compound. This may be a hepatic metabolite of amphethinile, such as a glucuronide derivative. Although the absolute amounts of metabolite formed could not be calculated fig 23 shows the mean excretion in arbitrary peak area units at the three dose levels studied. The AUC at these doses, again in arbitrary units are shown in table 27.

Table 27 Amphethinile Metabolite AUC in Mice

Dose, mg/sqm	AUC, arbitrary units
100	233
200	532
400	1019

The quantity of metabolite formed increases in a linear manner with increasing doses of amphethinile. This suggests that in mice the processes responsible for the metabolism of amphethinile are not saturable and follow first order kinetics.

Pharmacokinetic study in Man

Sample Collection

Prior to drug administration an indwelling catheter was placed in a peripheral vein to facilitate blood sampling. Blood was taken at time zero, 5 , 10, 20, 30, 60 and 90 minutes, and 2, 4, 6, 9, 12 and 24 hours. Samples were centrifuged for 10 minutes at 500G and stored at -20^{o}C prior to assay. Urine was collected during time intervals

0-6, 6-12 and 12-24 hours. Total volumes were recorded and 5ml aliquots stored at -20^{oC}.

Results

At the starting dose of $40mg/m^2$ Amphethinile was was undetectable in the serum five minutes after injection. At the four higher doses studied the plasma decay following bolus injection or short infusion conformed to a two compartment model. The pharmacokinetic parameters for each patient are shown in table 28 and the amounts of unchanged drug recovered in the urine in table 29.

The mean AUC at 200mg/sqm was 11.2ug/l.h-1, at 400mg/sqm 39.7ug/l.h-1, at 800mg/sqm 160.3ug/l.h-1 and at 1200mg/sqm 236.6ug/l.h-1. The mean terminal elimination half lives at these doses were 1.94 hours, 3.66 hours, 9.12 hours and 10.18 hours (fig 23). This increase in terminal half life with increasing dose is reflected in a concomitant fall in clearance from a mean of 524ml/min at 200mg/sqm to 381ml/min at 400mg/sqm, 154ml/min at 800mg/sqm and 180ml/min at 1200mg/sqm.

Recovery of unchanged amphethinile in the urine ranged from 0.012-0.7% of the administered dose, mean 0.12%. In addition there was evidence of a metabolite on HPLC. This additional peak had a similar retention time, 140-180 seconds, to that seen in the mouse experiments. At 200mg/sqm the metabolite peak was too small to be measured accurately. An estimate of the metabolite AUC in arbitrary units at the higher doses is given below (table 30) and the plasma decay curves shown in fig 24.

Table 28 Pharmacokinetic Parameters of Amphethinile in Man

Patient	Dose	Course	KE	K2 1	Kl 2	Vc	Vd	Vss	Vpc	Tl/2α	Tl/2β	AUC	Cl
RH	200	1	0.61	1.49	1.37	62.0	132.6	118.9	56.9	0.21	2.40	9.42	636.5
JC	"	1	2.88	3.38	10.49	10.2	48.9	42.0	31.7	0.04	1.14	11.51	492.3
	"	2	0.96	1.42	2.43	27.8	88.3	75.4	47.6	0.15	2.29	12.74	444.6
Mean			1.48	2.09	4.76	33.3	89.9	78.8	45.4	0.13	1.94	11.22	524.5
sem			0.07	0.64	2.00	15.2	24.1	22.2	7.3	0.04	0.4	0.93	57.6
GG	400	1	0.91	6.40	12.29	46.9	141.3	136.9	90.0	0.03	2.29	17.56	711.6
	"	2	1.24	2.74	9.23	23.2	109.7	101.4	78.2	0.05	2.63	25.98	481.0
TL	"	1	1.89	2.38	13.66	6.3	47.3	42.9	36.5	0.03	2.71	45.53	201.3
FL	"	2	0.16	0.92	0.58	46.6	79.5	76.0	29.4	0.43	7.00	69.84	131.2
Mean			1.05	3.11	8.94	30.7	94.5	89.3	58.5	0.14	3.66	39.72	381.2
sem			0.35	1.16	2.93	9.8	20.1	19.8	15.0	0.09	1.11	11.62	133.5
MG	800	1	0.25	0.79	0.72	46.3	96.2	88.4	42.1	0.41	5.54	112.1	200.6
FL	"	1	0.12	1.26	0.78	38.1	63.4	61.9	23.7	0.33	9.24	231.1	79.3
	"	3	0.11	1.03	1.15	41.9	90.9	88.4	46.5	0.31	13.33	243.2	78.7
JB	"	1	0.24	2.35	1.26	63.1	99.2	96.9	33.7	0.19	4.62	80.6	248.1
NB	"	1	0.14	0.57	0.69	73.1	171.8	161.2	88.1	0.52	11.36	122.1	174.7
AG	"	1	0.10	1.45	1.14	41.5	75.4	74.0	32.5	0.26	12.16	223.3	71.8
SL	"	1	0.15	0.51	0.27	90.9	150.1	139.5	48.6	0.83	7.62	109.7	227.7
Mean			0.16	1.13	0.87	56.4	106.7	101.5	45.0	0.40	9.12	160.3	154.1
sem			0.02	0.24	0.15	7.5	14.9	13.5	7.8	0.08	1.26	26.0	28.9
DM	1200	1	0.09	0.33	0.21	59.9	110.7	103.2	43.2	1.11	13.8	361.1	92.3
GM	"	1	0.20	5.61	5.41	57.0	112.9	111.9	54.9	0.06	6.9	194.7	188.2
PC	"	1	0.11	0.45	0.22	137.7	219.3	206.3	68.6	0.98	9.76	154.0	259.5
Mean			0.13	2.13	1.95	84.9	147.7	140.5	55.5	0.71	10.18	236.6	180.0
sem			0.33	1.74	1.72	26.4	35.8	33.0	7.3	0.33	2.01	63.3	48.4

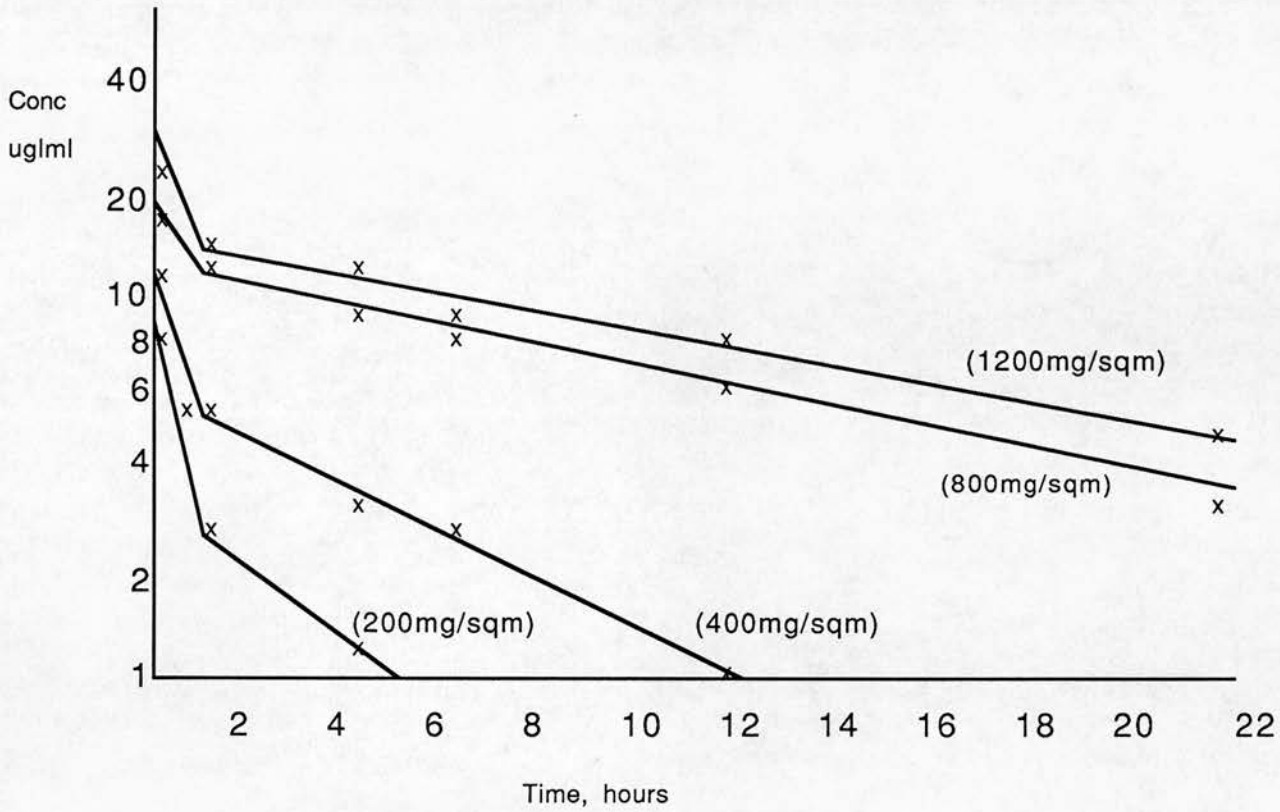
Table 29 Urinary Excretion of Amphethinile

Patient	Dose mg	Course	24hour excretion ug	% dose administered
RH	360	1	192	0.053
JC	340	1	42	0.012
	340	2	58	0.017
GG	750	1	471	0.062
	750	2	705	0.094
TL	550	1	829	0.150
	550	2	491	0.084
MG	1350	1	1350	0.200
FL	1100	1	635	0.050
	550	2	734	0.133
JB	1200	1	8407	0.700
NB	1280	1	513	0.040
AG	960	1	709	0.070
GM	2200	1	522	0.020
PC	2400	1	594	0.020

Table 30 Amphethinile Metabolite AUC in Man

Dose, mg/sqm	AUC, arbitrary units
400	1542
800	5495
1200	6965

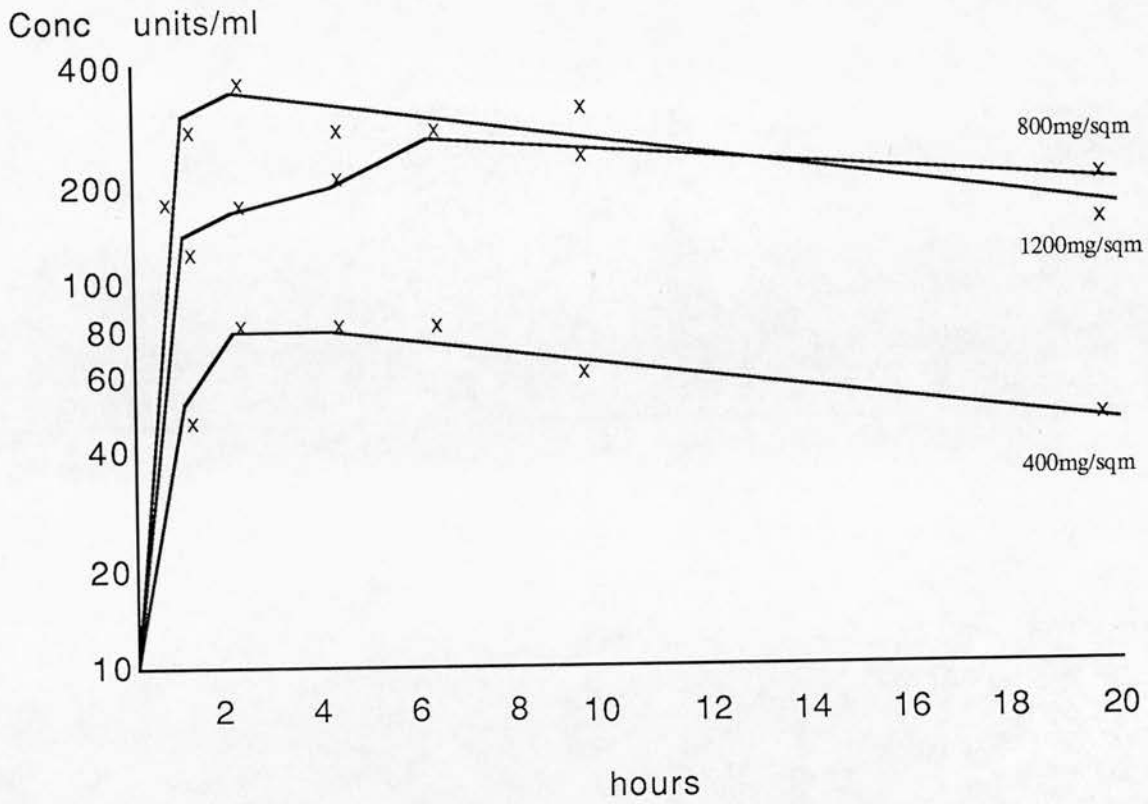
fig 23 **Plasma Decay Curves of Amphethinile in Man**



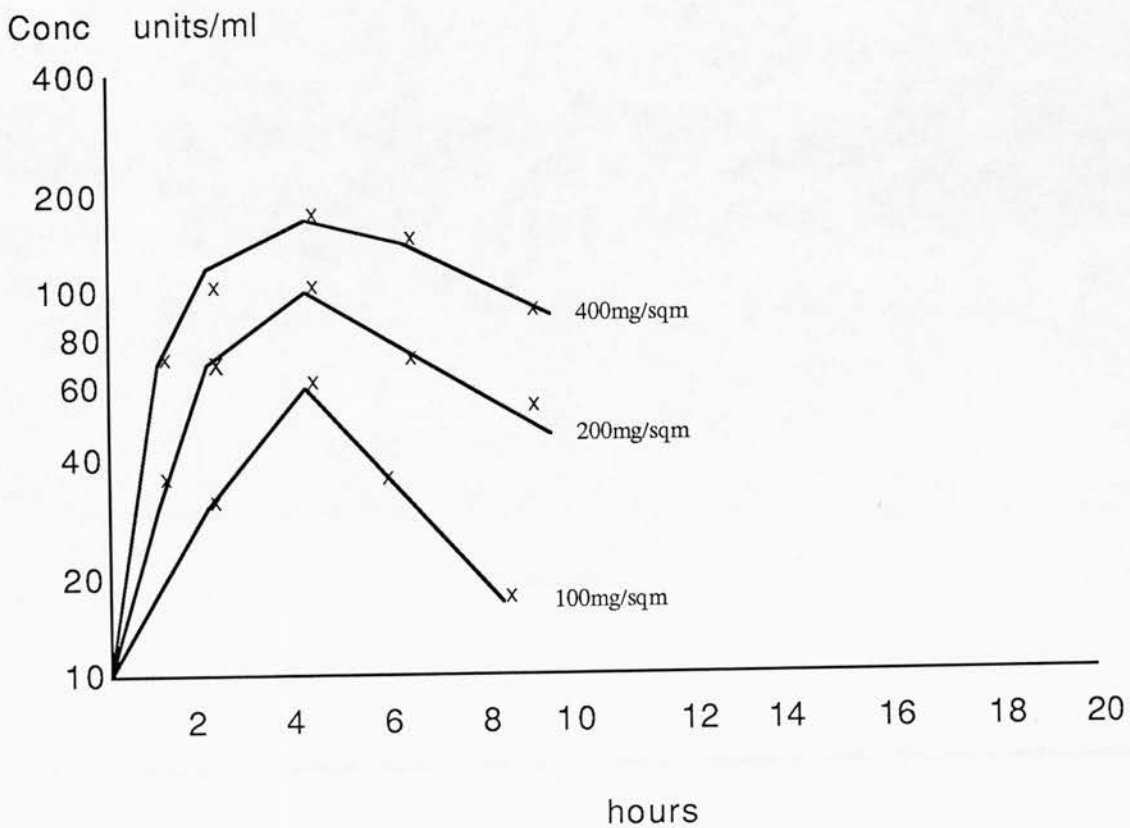
Points represent mean values of all assays at each dose level

fig 24

Amphethinile Metabolite - Man



Amphethinile Metabolite - Mouse



Discussion

The HPLC method developed for the measurement of amphethinile proved to be easily performed and reproducible, with an inter-assay variation of 5.4%. The limit of sensitivity of 0.lug/ml was well below biologically relevant concentrations in the serum and was therefore adequate for this study. At low concentrations the extraction efficiency for the method used approached 100% and this together with the comparatively low volumes of distribution seen in the study in man (89 - 147 litres) suggests that amphethinile does not undergo significant binding to plasma proteins.

In the study of the kinetics in mice there was no increase in the terminal elimination half life of amphethinile with increasing dose. However the AUC increase by a factor of 3 for each 100% increment in dose indicating that the kinetics are not completely linear. Despite this there was a linear increase in metabolite formation implying that this reaction followed first order kinetics. Moreover there was no suggestion that this metabolic process was saturable in the mouse. The AUC at the LDlO in the mouse was 313ug/l.h-l and thus this was adopted as the target AUC for the phase I clinical trial.

The kinetics in man followed a different pattern. There was a progressive rise in terminal elimination half life with an accompanying fall in plasma clearance. Moreover for each 100% increase in dose there was an approximate fourfold increase in AUC. Once again a presumed hepatic metabolite was seen on HPLC which had a similar retention time to that found in the mouse experiments. However in man metabolite formation rose to an early peak at around 2 hours subsequently remaining constant for several hours before declining again (fig 23). Moreover the amount of metabolite produced

in the first 24 hours after treatment did not increase significantly at doses above 800mg/sqm. This evidence suggests that the metabolism of amphethinile is saturable in man and explains the increase in half life with increasing dose.

Although the kinetics of amphethinile in man appear to conform to a two compartment model this is probably an over simplification. In view of the evidence suggesting that the metabolism of amphethinile is saturable it is likely that the the log plot of the post distributive phase should curve towards the x-axis with only the terminal portion, where the kinetics become first order, being truly linear. In order to demonstrate this further time points during the latter portion of the decay curve would have to be examined.

Conclusions

During the past thirty years many hundreds of thousands of compounds have been tested in pre-clinical systems for potential anti-tumour activity. However only a small minority have progressed to clinical testing probably 100 - 200 in total, and a minute fraction have found a place in regular anti-cancer therapy. At present a complete revision of pre-clinical screening systems is being undertaken involving a change from methods relying on rodent tumours to the exclusive use of cultured human tumour cell lines and human tumour xenografts. It is hoped that this radical departure will uncover a new spectrum of agents with activity in the common solid tumours.

An important obstacle to the development of new agents has been the requirements of various liscencing authorities for pre-clinical toxicological testing. The extent and detail of these tests, particularly those in large animals, has meant that a great deal of time and money has had to be invested in an agent before it reaches clinical trial. The delay caused by these procedures has often been of the order of ten years. It is now appreciated, however, that the qualitative predictiveness of such large animal testing for toxicity in man is low and many important toxicities such as cardiotoxicity in the anthracyclines and renal toxicity with platinum drugs were not foreseen. The main aim of pre-clinical toxicology is to determine a safe starting dose for phase I trials and since this can be done with equal accuracy in mice and large animals it is now proposed that 1/10 of the LD10 in the mouse is used as a starting dose provided that this is entirely non-toxic in the rat. The exclusive use of rodents for pre-clinical toxicology dramatically reduces the time and expense involved and thus will allow more drugs to reach phase I testing more quickly.

With these changes and improvements in the pre-clinical development of new agents it is possible that increasing numbers will become available for clinical trial. In order that much effort is not to be wasted there should be certain changes made in phase I and II methodology. Dose escalation schemes for phase I trials must be made more efficient so that these studies can be completed rapidly with more patients treated at potentially therapeutic doses. The most promising way of achieving this is to use pre-clinical pharmacological data as a guide to the speed of escalation. This is based on the observation that the AUC at the LD10 in the mouse is close to the AUC at the MTD in man. Using these principles together with the data from the phase I survey it is possible to reduce the number of dose escalation steps by up to 50% compared to classical modified Fibonacci schemes. This increase in the pace of phase I studies will allow drugs to pass more rapidly to phase II and will also allow the exploration of alternative schedules without requiring additional patient resources.

There must also be a change in the approach to phase II testing. The main problem with this stage of drug development is that trials are often performed in patient groups where the chance of a response is very low. The two factors that militate most strongly against a response occurring are the performance status of the patient and the extent of prior chemotherapy. Investigators must be prepared to enter patients in phase II trials who are in good general condition and who have received no prior chemotherapy. In recent years this has been done increasingly in diseases where existing chemotherapy produces consistent response rates of less than 20% eg non-small cell lung cancer and colon cancer. However it is equally important to identify

new active agents in tumours such as breast cancer where although many durgs are active the impact on survival is minimal. In these conditions alternative protocols should be adopted allowing new drugs to be used for a minimum of 2 courses in untreated patients before crossing to a proven regimen if no response is seen. The evidence from the trial of 4-demethoxydaunorubicin in breast cancer presented in this thesis suggests that such a trial design does not predjudice patient survival. The information obtained from one study conducted in this way will be of greater value than several inconclusive trials in heavily pre-treated patients. Answers to the important questions of the activity of a drug in a range of tumours can be obtained rapidly and with greater certainty and in addition the chance of erroneously rejecting an active compound will be reduced. The use of protocols allowing early termination if no responses are seen in the first 14 patients should ensure that a large number of patients are not treated with inactive compounds.

References

Alpert LK, Peterson SK.
The use of nitrogen mustard in the treatment of lymphomata.
Bulletin of the US Army Medical Dept. 1947; 7: 187-94.

Arcamone F, Bernardi L, Giardino P, Patelli B, Di Marco A, Casazza AM, Pratesi G, Reggiani P.
Synthesis and anti-tumour activity of 4-demethoxydaunorubicin, 4-demethoxy-7, 9-diepidaunorubicin and their beta anomers.
Cancer Treat Reports 1976; 60: 829-34.

Barbieri B, Casazza AM, Penco S, Cassinelli G, Poesta A.
Biological activity of 1-OH-anthracyclines.
Proc 13th Int Cong Chemother 1983.

Bastholt L, Ejlertson B, Dalmark M, Sygenhus RO.
A phase II trial of oral 4-demethoxydaunorubicin (DMDNR) in patients with measurable advanced carcinoma of the breast.
Proc ECCO 1985; 3: 169.

Bedford P, Fox BW.
DNA-DNA interstrand cross-linking by dimethanesulphonic acid esters.
Biochem Pharmacol 1983; 32: 2297-301.

Berenbaum MC, Timmis GM, Brown IN.
The relation between the physico-chemical properties and immunosuppressive effects of an homologous series of sulphonic acid esters.
Immunology 1967; 13: 517

Berman E, Wittes RE, Leyland-Jones B, Casper ES, Gralla RJ, Howard J, Baratz R, Young CW.
Phase I and clinical pharmacology studies of intravanous and oral administration of 4-demethoxydaunorubicin in patients with advanced cancer.
Cancer Res 1983; 43: 6096-101.

Bogden AE, Kelton DE, Cobb WR, Esber HJ.
A rapid screening method for testing chemotherapeutic agents against human tumour xenografts. In: Houchens DP, Ovejera AA (eds). Proceedings of the symposium on the use of athymic (nude) mice in cancer research.
New York, Gustav Fischer Inc 1978: 231-50.

Bonadonna G, Viviani S, Bonfante V, Valagussa P, Santoro A.
Alternating chemotherapy with MOPP/ABVD in Hodgkin´s disease. Updated results.
Proc ASCO 1984; 3: 254.

Bonfante V, Ferarri L, Villani F, Bonnadonna G.
Phase I study of 4-demethoxydaunorubicin.
Investigational New Drugs 1983; 1:161-168.

Box MJ, Davies D, Swann WH.
Non-linear optomisation techniques.
ICI Mathematical and Statistical Techniques for Industry Monograph No. 5 1969: 22-4.

Byar DP, Simon RM, Friedewald WT, Schlesselman JJ, De Mets DT, Ellenberg JH, Gail MH, Ware JH.
Randomised clinical trials: perspectives on some recent ideas.
New Eng J Med 1976; 295: 74-80.

Carter SK.
Clinical trials in cancer therapy.
Cancer 1977; 40: 544-57.

Carter SK, Selawry O, Slavik M.
Methods of development of new anticancer drugs.
US Department of Health, Education and Welfare 1977.

Cassaza AM, Barbieri B, Fumagalli A, Geroni MC.
Biologic activity of 4-demethoxy-13-dihydroxydaunorubicin.
Proc AACR 1983; 24: 251.

Chlebowsky RT, Paroly WS, Pugh RP, Heuser J, Jacobs EM, Pajak TF, Bateman JR.
Adriamycin given as a weekly schedule without loading course: clinically effective with reduced incidence of cardiotoxicity.
Cancer Treat Rep 1980; 64: 47-51.

Collins JM, Zaharko DS, Dedrick RL, Chabner BA.
Potential roles for preclinical pharmacology in phase I clinical trials.
Cancer Treat Reports 1986; 70: 73-80.

Colton T.
Statistics in Medicine.
Boston: Little Brown, 1974.

Connors JM, Klimo P.
MACOP-B treatment of advanced diffuse large cell lymphoma (DLL) with an innovative 12 week intensive regimen.
Proc ASCO 1984; 3: 238.

Daghestani AN, Arlin ZA, Leyland-Jones B, Gee TS, Kempin SJ, Martelsmann R, Budman D, Schulman P, Baratz R, Williams L, Clarkson BD, Young CW.
Phase I and II clinical and pharmacological study of 4-demethoxydaunorubicin (Idarubicin) in adult patients with acute leukaemia.
Cancer Res 1985; 45: 1408-12.

Dameshek W, Weisfuse L, Stein T.
Nitrogen mustard therapy in Hodgkin´s disease. Analysis of 50 consecutive cases.
Blood 1949; 4: 338-79.

DeSloover C, Rosencweig M, Nicaise C, Gerard B, Crespeigne N, Kenis Y
Phase I study of oral Idarubicin with a weekly schedule.
Proc ASCO 1984; 3: 79.

DeVita VT.
Human models of human diseases: breast cancer and the lymphomas.
Int J Radiat Oncol Biol Phys 1979; 5: 1855-67.

DeVita VT, Oliverio VT, Muggia FM, Wiernik PW, Ziegler J, Goldin A, Rubin D, Henney J, Schepartz S.
The drug development and cancer clinical trials programs of the division of cancer treatment, National Cancer Institute.
Cancer Clinical Trials 1979; 2: 195-216.

DeVita VT, Henney JE, Hubbard SM.
Estimation of the numerical and economic impact of chemotherapy in the treatment of cancer. In: Burchenal JH, Oettgen HS, eds. Cancer achievements, challenges and prospects for the 1980s. New York: Grunne and Stratton 1981: 857-80.

Dimarco A, Casazza AM, Pratesi G.
Antitumour activity of 4-demethoxydaunorubicin administered orally.
Cancer Treat Reports 1977; 61: 893-4.

Earhart RH, Koeller JM, Davis HL.
Phase I trial of PCNU administered by 5 day courses.
Cancer Treat Rep 1981; 65: 835-40.

Elson LA.
A study of the nutritional and other systemic factors influencing the response of host and tumour to carcinogenic and therapeutic agents, including both chemicals and ionising radiation.
Rep Brit Emp Cancer Campaign 1950, 300.

Elson LA.
The effects of myleran (1:4-dimethylsulphonyl-oxybutane) and homologous compounds on the blood.
Biochem Pharmacol 1958; 1: 39-47.

Elashoff RM, Beal S.
Two stage screening designs applied to chemical screening problems with binary data.
A Rev Biophys Bioeng 1976; 5: 561-87.

Emmons WD, Ferris AF.
Metathetical reaction of silver salts in solution II. The synthesis of alkyl sulfonates.
J Amer Chem Soc 1953; 75: 2257.

Estey E, Hoth D, Simon R, Marsoni S, Leyland-Jones B, Wittes R.
Therapeutic response in phase I trials of antineoplastic agents.
Cancer Treat Reports 1986; 70: 1105-115.

Farber S et al
Action of pteroglutamic conjugates on man.
Science 1947; 106: 619-21.

Farber S, Diamond LK, Mercer RD, Sylvester RF, Wolff JA.
Temporary remissions in acute leukaemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (Aminopterin).
N Eng J Med 1948; 238: 787-793.

Finlay GJ, Baguley BC.
A semi-automated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially carcinoma cells.
Annal Biochem 1984; 139: 272-77.

Fleming TR.
One sample multiple testing procedure for phase II clinical trials.
Biometrics 1982; 38: 143-51.

Fox BW.
The sensitivity of a Yoshida sarcoma to methylene dimethne sulphonate
Int J Cancer 1969; 4: 54-60.

Fox BW, Jackson H.
The in-vivo effects of methylene dimethane sulphonate on proliferating cell systems.
Br J Pharmacol 1965; 24: 24-8.

Frei E, Luce JK, Gamble JF.
Combination chemotherapy in advanced Hodgkin´s disease: induction and maintenance of remission.
Ann Intern Med 1973; 79: 376-82.

Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE.
Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man.
Cancer Chemother Rep 1966; 50: 219-44.

Gause GP, Brazhnikova MG, Shorin VA.
A new anttumour antibiotic, carminomycin (NSC-180024).
Cancer Chemother Rep 1974; 58: 255-6.

Gehan EA.
The determination of the number of patients required in a follow-up trial of a new chemotherapeutic agent.
J Chron Dis 1961; 13: 346-53.

Gellhorn A, Hirschberg E.
Investigation of diverse systems for cancer chemotherapy screening. Summary of results and general correlations.
Cancer Res 1955; 3: 1-13.

Gibaldi M, Perrier D.
Drugs and the pharmaceutical sciences vol 1: Pharmacokinetics.
New York: Marcel Drekker Inc. 1975.

Gibbons JD, Olkin I, Sobel M.
Selecting and ordering populations: A new statistical methodology.
New York: Wiley, 1977.

Gil P, Favre R, Durand A, Iliadis A, Cano JP, Carcassonne Y.
Time dependency of adriamycin and adriamycinol kinetics.
Cancer Chemother Pharmacol 1983; 10: 120-124.

Giovanella BC, Stehlin JS, Williams LJ.
Heterotransplantation of human malignant tumours in nude mice. II. Malignant tumours induced by injections of cell cultures derived from human solid tumours.
J Natl Cancer Inst 1974; 52: 921-30.

Goldie JH, Coldman A, Gudauskas GA.
Rationale for the use of alternating non-cross resistant chemotherapy.
Cancer Treat Rep 1982; 66: 439-49.

Goldin A, Schepartz SA, Venditti JM, DeVita VT.
Historical development and current strategy of the National Cancer Institute drug development program. In: DeVita VT, Busch H, eds. Methods in cancer research, XVI.
New York: New York Academic Press 1979: 165-245.

Goldin A, Serpick AA, Mantel N.
A commentary. Experimental screening procedures and clinical predictability value.
Cancer Chemother Rep 1966; 50: 173-218.

Goldin A, Johnson RK, Venditti JM.
Usefulness and limitations of murine tumour models for the identification of new antitumour agents. In: Mihich E, Eckhardt S, eds. Design of cancer chemotherapy: Experimental and clinical approaches. Antibiotics and chemotherapy vol 28.
Basel: S. Karger, 1980: 1-7.

Goldin A, Rosencweig M, Guarino AM, Schein PS.
Qualitative and quantitative prediction of toxicity from animals to humans. In: Tagnon HJ, Staquet MJ, eds. Controversies in cancer treatment. Paris: Mason 1980: 83-104.

Goldsmith MA, Slavik M, Carter SK.
Quantitative prediction of drug toxicity in humans from toxicology in small and large animals.
Cancer Res 1975; 35: 1354-64.

Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman AM.
Nitrogen mustard therapy.
JAMA 1946; 132: 126-32.

Gottleib JA
Phase I and phase II clinical trials: a critical reappraisal. In: The pharmacological basis of cancer chemotherapy.
Baltimore: Williams and Wilkins, 1974.

Greene DS.
Preformulation. In: Banker GS, Rhodes CT, eds. Drugs and the pharmaceutical sciences vol 7.
New York: Marcel Drekker Inc., 1979: 211-226.

Green RF, Collins JM, Jenkins JF, Speyer JL, Myers CE.
Plasma pharmacokinetics of adriamycin and adriamycinol: implications for the design of in vitro experiments and treatment protocols.
Cancer Res 1983; 43: 3417-21.

Greishaber CK, Marsoni S.
Relation of preclinical toxicology to findings in early clinical trials.
Cancer Treat Rep 1986; 70: 65-72.

Guiliani FC, Kaplan NO, Coirin AK, Howell SB.
Screening of new doxorubicin (DX) analogs with activity against human colon carcinoma xenografts.
Proc Am Ass Cancer Res 1980; 21: 272.

Haddow A, Timmis GM.
Bifunctional sulfonic acid esters with radiometric activity.
Acta Unio Int Contra Cancer 1951; 7: 469.

Haddow A, Timmis GM.
Myleran in chronic myeloid leukaemia.
Lancet 1953; 1: 207-8.

Hamburger AW.
Use of in-vitro tests in predictive cancer chemotherapy.
JNCI 1981; 66: 981-88.

Hamburger AW, Salmon SE, Kim MB, Trent JM, Soehnien BJ, Alberts DS, Schmidt HJ.
Direct cloning of human ovarian carcinoma cells in agar.
Cancer Res 1978; 38: 3438-444.

Harris JR, Hellman S, Canellos GP, Fisher B.
Cancer of the breast. In: DeVita VT, Hellman S, Rosenberg SA, eds. Cancer. Principles and practice of Oncology 2nd edition.
Philadelphia: JB LIppincott Co., 1985: 1119-178.

Hayward JL, Carbonne PP, Hueson J-C, Kumaoka S, Segaloff A.
Assessment of response to therapy in advanced breast cancer.
Br J Cancer 1977, 35: 292-8.

Hayward JL, Carbonne PP, Hueson J-C, Kumaoka S, Segaloff A, Rubens RD.
Assessment of response to therapy in advanced breast cancer.
Cancer 1979; 39: 1289-93.

Herson J.
Predictive probability early termination plans for phase II clinical trials.
Biometrics 1979; 35: 775-83.

Hodes L.
Computer-aided selection of compounds for antitumour screening. Validation of a statistical-heuristic method.
J Chem Inf Comput Sci 1981; 21: 128-32.

Hodes L.
Selection of molecular fragment features for structure-activity studies in antitumour screening.
J Chem Inf Comput Sci 1981; 21: 132-36.

Homan ER.
Quantitative relationships between toxic doses of antitumour chemotherapeutic agents in animals and man.
Cancer Chemother Rep 1972; 3: 13-19.

Hoogstraten B, Irwin L, Ahmann D, Band P, Fink DJ, Greenspan E, Seara ME, Sedransk N, Smalley R, Talley R, Tormey DC, Carter S.
Breast cancer: suggested protocol guidelines for combination chemotherapy trials.
DHEW Publication No (NIH) 77-1192, Bethesda Maryland, 1977.

Horvath C, Priess B, Lipsky SR.
Annal Chim Acta 1967; 38: 305.

Howard GA, Martin AJP.
The separation of the C12-C18 fatty acids by reversed-phase partition chromatography.
Biochem J 1950; 46: 532-8.

Howell A, Wagstaff J, Harland RNL, Jones M, Wilkinson MJ.
The significance of the "no-change" category of response in patients treted with endocrine therapy for advanced carcinoma of the breast.
Proc ECCO 1985; 4: 635.

Huggins C, Hodges CV.
Studies on prostatic cancer. 1. The effects of castration, of oestrogen and of androgen injection on serum phosphatase in metastatic carcinoma of the prostate.
Cancer Res 1941; 1: 293-7.

Israel M, Pegg WG, Wilkinson PM, Garnick MB.
Liquid chromatographic analysis of adriamycin and metabolites in biolgical fluids.
J Liquid Chromatography 1978; 1: 795-809.

IUPAC
Recommendations on nomenclature for chromatography, rules approved, 1973.
Pure Appl Chem 1974; 37: 447.

Johnson RK, Goldin A.
The clinical impact of screening and other experimental tumour studies.
Cancer Treat Rev 1975; 2: 1-31.

Kaplan S, Sessa C, Willems Y, Pacciarini MA, Tamassia V, Cavalli F.
Phase I trial of 4-demethoxydaunorubicin (Idarubicin) with single oral doses.
Investigational New Drugs 1984; 2: 281-6.

Karon M, Sieger L, Leimbrok S, Finkelstein J, Nesbit MF, Swaney JJ.
Azacytidine: a new active agent for the treatment of acute leukaemia.
Blood 1973; 42: 359-65.

Kavanagh JJ, Yeung KY, Savaraj N, Krakoff IH.
Phase I clinical evaluation of oral and intravenous 4-demethoxydaunorubicin.
Eur J Cancer Clin Oncol 1985; 21: 1187-9.

Leake E, Smith WG, Woodliff HJ.
Diffuse interstitial pulmonary fibrosis after busulphan therapy.
Lancet 1963; 2: 432-4.

Lee YJ, Staquet M, Simon R, Catane R, Muggia FD.
Two stage plans for patient accrual in phase II cancer clinical trials.
Cancer Treat Rep 1979; 63: 1721-6.

Lu K, Savaraj N, Kavanagh J, Benjamin RS, Fuen L, Burgess M, Bodey G, Loo TL.
Clinical pharmacology of 4-demethoxydaunorubicin.
Proc ASCO 1984; 3: 147.

Marshall EK jr.
Historical perspectives in chemotherapy. In: Goldin A, Hawkin IF, eds. Advances in Chemotherapy vol I.
New York: New York Academic Press, 1984: 1-8.

Marsoni S, Wittes R.
Clinical development of anticancer agents - A National Cancer Institute perspective.
Cancer Treat Rep 1984; 68: 77-85.

Mathe G.
Clinical examination of drugs, a scientific and ethical challenge.
Biomedicine 1973; 18: 169-72.

Mattson W, Borgstrom S, Landberg T.
A weekly schedule without a loading course: clinically effective with reduced incidence of cardiotoxicity.
Clin Ther 1982; 5: 193-203.

Miller AB, Hoogstraten B, Staquet M, Winkler A.
Reporting results of cancer treatment.
Cancer 1981; 47: 207-14.

Moertel CG, Schutt AJ, Hahn RG, Reitmeier RS.
Effects of patient selection on results of phase II chemotherapy trials in gastrointestinal cancer.
Cancer Chemother Rep 1974; 58: 257-60.

Moertel CG, Hanley JA.
The effect of measuring error on the results of therapeutic trials in advanced cancer.
Cancer 1976; 38: 388-94.

Moro E, Bellotti V, Jannuzzo MG, Stegnjaich S, Valzelli G.
HPLC method for pharmacokinetic studies on the new anthracycline 4-demethoxydaunorubicin and its B-dihydro derivative.
J Chromatography 1983; 274: 281-7.

Mosmann T.
Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays.
J Immunol Methods 1983; 65: 55-63.

Newlands ES, Blackledge G, Slack JA, Goddard C, Brindley CJ, Holden L, Stephens MFG.
Phase I trial of Mitozolamide.
Cancer Treat Rep 1985; 69: 801-5.

Nias AHW, Fox BW.
The sensitivity of Hela and Chinese Hamster (ovary) cells to methylene dimethane sulphonate.
Chem Biol Interactions 1976; 14: 93-9.

Ohuna T, Rosner P, Levy RN, Cutter J, Moon JH, Silver RT, Blom J, Falkson G, Burningham R, Glidewell O, Holland JF.
Treatment of adult leukaemia with L-asparaginase.
Cancer Chemother Rep 1971; 55: 269-75.

Ozols R.
Intraperitoneal chemotherapy in the management of ovarian cancer.
Semin Oncol 1985; 12: 75-80

Pannuti F, Martoni A, Pacciarini MA, Fruet F.
A phase II trial of oral Idarubicin in advanced breast cancer.
Proc ECCO 1985; 3: 70.

Parker CM.
Accuracy of predictions of survival in later stages of cancer.
Br Med J 1972; 2: 29-31.

Penta JS, Rosencweig M, Guarino AM, Muggia FM.
Mouse and large animal toxicology studies of twelve antitumour agents: relevance to starting dose for phase I clinical trials.
Cancer Chemother Pharmacol 1979; 3: 97-101.

Pocock SJ.
Group sequential methods in the design and analysis of clinical trials.
Biometrika 1977; 64: 191-9.

Posada JG, O´Dwyer PJ, Hoth DF.
Anaphylactic reactions to diaziquone.
Cancer Treat Rep 1984; 68: 1215-7.

Prieur DJ, Young DM, Davis RD, Cooney DA, Home R, Dixon RL, Guarino
Procedures for preclinical toxicological evaluation of cancer chemotherapeutic agents: Protocols of the Laboratory of Toxicology.
Cancer Chemother Rep 1973; 4: 1-30.

Robert J, Hoerni B, Vrignaud P, Lagarde C.
Early-phase pharmacokinetics of doxorubicin in non-Hodgkin´s lymphoma patients.
Cancer Chemother Pharmacol 1983; 10: 115-119.

Rosencweig M, Staquet M, Hansen HH, Pinedo HM, McVie JG, Armand JP, Kenis Y, Fox BW, Harrap K, van Putten LM.
EORTC guidelines for phase I trials with single agents in adults.
Eur J Cancer Clin Oncol 1985; 21: 1005-7.

Rosencweig M, Von Hoff DD, Staquet MJ, Schein PS, Penta JS, Goldin A, Muggia FM, Freireich EJ, De Vita VT.
Animal toxicology for early clinical trials with anticancer agents.
Cancer Clin Trials 1981; 4: 21-8.

Salmon SE, Hamburger AW, Soehlen BS, Durie BGM, Alberts DS, Moon TE.
Quantitation of differential sensitivity of human tumour stem cells to anti-cancer drugs.
N Eng J Med 1978; 298: 1321-7.

Schein PS, Davis RO, Carter SK, Newman J, Schein D, Rall DP.
The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man.
J Pharmacol Therap 1970; 14: 3-40.

Schein PS.
Preclinical toxicology of anticancer drugs.
Cancer Res 1977; 37: 1934-7.

Schultz JR, Nichol FR, Elfing GL, Weed SD.
Multiple stage procedures for drug screening.
Biometrics 1973; 29: 273-300.

Selawry OS, Hansen HH.
Initial clinical trial with chloroethyl-cyclohexyl-nitrosourea (CCNU NSC79037)
Proc AACR 1969, 10: 78.

Sharkey FE, Fogh J.
Considerations in the use of nude mice for cancer research.
Cancer Metastases Rev 1984; 3: 341-360.

Shear MJ, Hartnell JL, Peters VB.
Some aspects of a joint institutional research programme on chemotherapy of cancer. In: Moulton FR, ed. Approaches to tumour chemotherapy.
Washington DC: American Assocn. for the Advancement of Science, 1947: 236-384.

Simon R, Wittes RE, Ellenberg SS.
Randomised phase II clinical trials.
Cancer Treat Rep 1985; 69: 1375-81.

Skipper HE, Schabel FM, Mellet LB, Montgomery JA, Wilkoff LJ, Lloyd HH, Brockman RW.
Implications of biochemical, cytogenetic, pharmacologic and toxicologic relationships in the design of optimal therapeutic schedules.
Cancer Chemother Rep 1970; 54: 431-50.

Smith DB, Margison J, Lucas S, Wilkinson PM, Howell A.
Clinical pharmacology of 4-demethoxydaunorubicin.
Cancer Chemother Pharmacol (in press).

Staquet MJ, Byar DP, Green SB, Rosencweig M.
Clinical predictivity of transplantable tumour systems in the selection new drugs for solid tumours: Rationale for a three stage strategy.
Cancer Treat Rep 1983; 67: 753-65.

Sylvester RJ, Staquet MM.
An application of decision theory of phase II clinical trials in cancer. In: Tagnon HJ, Staquet MJ, eds. Recent advances in cancer treatment.
New York: Raven Press, 1977.

Teorell T.
Kinetics of distribution of substances administered to the body I. The extravascular modes of administration.
Arch Int Pharmacodyn 1937; 57: 205.

Teorell T.
Kinetics of distribution of substances administered to the body II. The intravascular modes of administration.
Arch Int Pharmacodyn 1937; 57: 226.

Timmis GM.
1;4-Dimethanesulphonoxybutane in Myeloid Leukaemia.
Rep Brit Emp Cancer Campaign 1950; 28: 77-78.

Venditti JM.
Relevence of transplantable animal tumour systems to the selection of new agents for clinical trial. In: Pharmacolgical Basis of Cancer Chemotherapy. Baltimore: Williams and Wilkins, 1975: 245-70.

Venditti JM.
The National Cancer Institute Antitumour drug discovery program, current and future perspectives: A commentary.
Cancer Treat Rep 1983; 67: 767-72.

Venditti JM, Wesley RA, Plowman J.
Current NCI pre-clinical screening in vivo. Results of tumour panel screening 1976-1982 and future directions. In: Advances in Pharmacology and Chemotherapy. New York. Academic Press, 1984, vol 20

Vietti TJ.
Evaluation of toxicity: clinical issues.
Cancer Treat Rep 1980; 64: 457-61.

Vishnuvajjala BR, Cradock JC.
Compatibility of plastic infusion devices with diluted N-methylformamide and N, N-dimethylacetamide.
Am J Hosp Pharm 1984; 41: 1160-3.

Von Hoff DD, Rosencweig M, Muggia FM.
Variation in toxicities of antitumour drugs in children and adults.
Clin Pharm Ther 1977; 21: 121.

Von Hoff DD, Rosencweig M, Soper WT, Helman LJ, Penta JS, Davis HL, Muggia FM.
Whatever happened to NSC------? An analysis of discontinued anticancer agents.
Cancer Treat Rep 1977; 61: 759-68.

Von Hoff DD, Casper J, Bradley E, Sandbach J, Jones D, Makuch R.
Association between human tumour colony forming assay results and response of an individual patients tumour to chemotherapy.
Am J Med 1981; 70: 1027-32.

Vrignaud P, Egdbali H, Hoerni B, Iliadis A, Robert J.
Pharmacokinetics and metabolism of epirubicin during repetitive courses of adriamycin in Hodgkin´s disease.
Eur J Cancer Clin Oncol 1985; 21: 1307-13.

Waksman SA, ed.
Streptomycin, Nature and Practical Applications.
Baltimore: Williams and Wilkins, 1949.

Wagstaff J, Bramwell VHC, Wilkinson MJ, Howell A.
Rotating oral cyclophosphamide, methotrexate and fluorouracil (oral CMF) for patients with advanced carcinoma of the breast.
Proc ECCO 1985; 3: 166.

Weiss AJ, Metter GE, Fletcher WS.
Studies on adriamycin using a weekly regimen demonstrating its clinical effectiveness and lack of cardiotoxicity.
Cancer Treat Rep 1976; 60: 813-22.

WHO handbook for reporting results of cancer treatment.
WHO Offset Publication No 48.
Geneva: WHO, 1979.

Williams DJ, Carter SK.
Management of trials in the development of cancer chemotherapy.
Br J Cancer 1978; 37: 434-47.

Wittes RE, Marsoni S, Simon R, Leyland-Jones B.
The phase II trial.
Cancer Treat Rep 1985; 69: 1235-9.

Wooley PV, Schein PJ.
Methods in Cancer Research.
New York: New York Academic Press, 1979.

Zelen M.
Keynote address on biostatistics and data retrieval.
Cancer Chemother Rep 1974; 4: 31-42.

Zubrod GC, Schepartz SA, Carter SK.
Historical backgroung of the NCI drug development thrust.
NCI Monograph 45 Methods of development of new anticancer drugs, 1977: 7-11.

Appendices

Appendix I

Master Phase II Protocol

Index

1.0 **Introduction**

1.1

1.2

1.2.1 **Experimental antitumour activity**

1.2.2 **Mechanism of action**

1.2.3 **Animal toxicology**

1.2.4 **Clinical experience**

(summary of phase I)

2.0 **Objectives of the study**

2.1

2.2

2.3

3.0 **Selection of patients**

Patients must fulfil all of the following criteria.

3.1 Histologically proven diagnosis of malignancy.

3.2 Recurrent or metastatic disease not amenable to curative

surgery and/or radiotherapy.

3.3 Measurable or evaluable lesions (see 4.0) with documented progression within 2 months prior to entry into this study.

3.4 Performance status according to the WHO scale < 2 and a life expectancy of > 3 months.

3.5 Age of 75 or less.

3.6 At the start of treatment
WBC > 3 x 10^9/l, platelets > 100 x 10^9/l
Bilirubin < 1.5 mg/dl (or < 25.6 umol/l)
Creatinine < 1.5 mg/dl (or < 132 umol/l) and/or creatinine clearance > 60 ml/min.

3.7 No prior radiotherapy and/or chemotherapy during the last 4 weeks (6 weeks for nitrosoureas and mitomycin C) and all toxic manifestations of prior treatment must have resolved.

3.7.1 No prior therapy with any specified drugs if appropriate

3.7.2 **Preferably not previously treated patients** should be entered into the study. (If appropriate)

3.8 No prior radiation therapy to all present areas of measurable or evaluable disease.

3.9 No brain involvement.

3.10 No previous or current malignancies at other sites, with the exception of cone biopsied in situ carcinoma of the cervix uteri and adequately treated basal or squamous cell carcinoma of the skin.

3.11 Patients who are poor medical risks because of non-malignant systemic disease as well as those with active, uncontrolled infection are not eligible for the trial.

3.12 Written informed consent is not mandatory. However, oral informed consent will be obtained. This will be noted on the patient case report form. Informed consent procedures for this trial will be in compliance with in-country laws pertaining to patient informed consent and rights of human subjects.

4.0 **Measurability of the disease**

4.1 Are not considered measurable or evaluable

- lesions in previously irradiated fields;
- ascites, pleural effusions, and/or bone metastases;

4.2 The following parameters are considered measurable

- lesions measurable by two perpendicular diameters;
- lung lesions surrounded by aerated lung;
- superficial lymph nodes with positive fine needle biopsy;
- skin nodules with positive fine needle biopsy.

4.3 Are considered evaluable, lesions that are measurable in one diameter as well as lesions that are deeply located and measurable in two diameters

- a lung tumour not completely surrounded by aerated lung;
- a palpable and measurable deep lymph node.
- a palpable and measurable abdominal tumour.

Malignant hepatomegally may be measured if the inferior edge is palpated at 5 cm below the costal margin and the tip of the xyphoid. Measurements below the costal margins will be made in the respective midclavicular lines or at other defined points during quiet respiration;

- lesions demonstrated by CT scan provided that they have a diameter of at least 5 cm and that their malignant nature is pathologically (histology or cytology) proven in case of solitary metastasis.

5.0 **Treatment**

5.1 **Pharmaceutical data**

5.2 **Drug administration**

5.2.1 **Dose schedule**

5.2.2 **Dosage schedule modifications**

Drug administration is postponed by one week if there is not full hematologic recovery (WBC > 3,000/mm^3, platelets

> 100,000/mm^3) from the prior course at scheduled retreatment. If treatment is delayed by more than 5 weeks the patient is withdrawn from the study.

Dosage adjustments are made according to the lowest value of WBC and/or platelets measured weekly in the previous course and according to treatment delay due to myelosuppression.

(If appropriate)

If treatment is delayed because of myelosuppression at scheduled retreatment, drug dosage must be reduced to X mg/m^2 if not already indicated by blood counts in the previous course.

5.3 **Treatment duration**

5.4 **Drug supply and distribution**

5.5 **Other treatments**

5.5.1 Ancillary treatment will be given as medically indicated but must be specified in the flow sheets.

5.5.2 Radiotherapy may be given concomitantly for control of bone pain provided that all evaluable lesions are not included in the irradiated field. The treated area cannot be used as a measurable or evaluable parameter.

5.5.3 Patients must not be on other experimental drugs during the study.

6.0 **Study parameters**

Parameter	Prior to therapy	Weekly	q X weeks
History + Physical Examination	+		+
Performance scale (WHO)	+		+
Assessment of evaluable lesions	+		+(a)
Hematocrit and haemoglobin	+	+	+
WBC and differential	+	+	+
Platelet count	+	+	+
Serum creatinine	+	+	+
Other chemistries (b)	+		+
Creatinine clearance	+		+
Urine analysis (Chem, sed)	+		+
ECG	+		+
Chest x-rays (PA and lateral)	+		+
CT-Scan a/o ultrasound abdomen and liver	+		+(c)

(a) Colour photographs of all visible lesions together with identifications, date and rule (cm) should be repeated every 3 months or sooner if changes occur.

(b) Include BUN, uric acid, electolytes, calcium, glucose, bilirubin, alk. phosphatase, SGOT or AST, LDH.

(c) Only in patients who respond; beginning at the time of response and continuing every two months for the duration of the response.

7.0 **Criteria of Response**

Idealy, all measurable and evaluable lesions should be measured at each assessment. When multiple lesions are present, this may not be possible and, under such circumstances, the most representative lesions may be selected. Neither the development nor the healing of skin or mucosal ulcers should be taken as sole evidence of change.

7.1 **Complete response:** disappearance of all known disease, and no new lesions in other sites, determined by two observations not less than 4 weeks apart.

7.2 **Partial response:** decrease by at least 50% in the sum of the products of the largest perpendicular diameters of all measurable lesions plus the sum of the diameters of all evaluable lesions as determined by two observations not less than 4 weeks apart. See 4.0 for the definition of measurable and evaluable lesions. For the liver, 3 measurements must be considered (see 4.0). It is not necessary for all lesions to have regressed to qualify for partial response, but no lesions should have progressed and no new lesions should appear. There should also be an objective improvement in non-evaluable but clinically evident malignant disease (e.g. pulmonary or skin infiltration). Serial evidence of appreciable change

documented by radiography or photography must be obtained and be available for subsequent review. The assessment must always be objective. If non-evaluable but clinically evident disease represents the bulk of disease and this clearly does not respond, even though significant tumour shrinkage is noted in measurable and/or evaluable lesions, then this is to be considered as "no change" not "objective regression".

7.3 **No change:** a 50% decrease in total tumour size cannot be established nor has a 25% increase in the size of one or more measurable or evaluable lesions been demonstrated.

7.4 **Progressive disease:** a 25% or more increase in the size of at least one measurable or evaluable lesion or the appearance of a new lesion. The occurrence of pleural effusion or ascites is also considered as progressive disease if this is substantiated by positive cytology. Pathological fractures or collapse of bones are not necessarily evidence of progressive disease.

7.5 **Early death:** death during the first four weeks without severe toxicity.

7.6 **Toxic death:** any death to which drug toxicity is thought to have made a major contribution.

8.0 **Duration of Response and Survival**

The duration of response will date from the commencement of

treatment until the documentation of progression. Survival will be dated from the commencement of treatment.

9.0 **Statistical Considerations**

All eligible patients with disease X will be entered into the trial until accrual is completed for patients treated with at least 2 courses of drug Y.

The trial will consist of two stages. In the first stage, 14 patients will be entered. If there are no responses (complete or partial) in 14 patients with no prior chemotherapy the trial will be terminated. If there are one or more responses in the first 14 patients, additional patients will be added (see below) so that the standard error of the observed response rate will be less than or equal to 0.10. This scheme ensures that if the drug is active in 20% or more of patients, the chance of erroneously rejecting the drug after the first 14 patients is .044. The advantage of a two stage scheme is that it allows early rejection of an ineffective drug.

No. of response in the first 14 patients	No. of additional patients required
0	0
1	1
2	6
3	9
4 or more	11

10.0 **Study Administration**

10.1 All eligible patients must be registered with the Data Centre within 24 hours of initial drug administration.

- protocol number
- name of institution
- code number of institution
- name of patient
- name of the responsible investigator
- date treatment started

10.2 Clinical Trials Forms are to be used for this study. The on-study form should be sent within one week of registration of the patient to the Data Centre. Flow sheets and measurement forms should be sent as soon as they are complete to the Data Centre.

10.3 The study chairman is called at once in case of pronounced toxicity, serious side effects or toxic death of the patient.

11.0 **Publication and Authorship**

12.0 **References**

Appendix II

Phase I Survey References

1986

Dorr FA, Von Hoff DD, Kuhn JG et al. Phase I clinical investigation of 7-con-O-Methylnogaril, a new anthracycline antibiotic. Cancer Res 1986; 46: 2526-2565.

Winograd B, Vermorken JB, ten Bokkel Huinink WW et al. Phase I study of Ethylenediamine Platinum (II) Malonate (NSC 146068), a second generation platinum analogue (JM 40). Cancer Res 1986; 46: 2148-2151.

Kerr DJ, Kaye SB, Graham J et al. Phase I and pharmacokinetic study of LM985 (Flavone Acetic acid ester). Cancer Research 1986; 46: 3142-3146.

Kris MG, O´Connell JP, Gralla RJ et al. Phase I trial of Taxol given as a 3 hour infusion every 21 days. Cancer Treat Rep 1986; 70: 605-607.

1985

Melink TJ, Von Hoff DD, Kuhn JG et al. Phase I evaluation and pharmacokinetics of Tiazofurin. Cancer Res 1985; 45: 2859-2865.

Curt GA, Kelley JA Fine RL et al. A phase I and pharmacokinetic study of Dihydro-5-azacytidine (NSC 264880). Cancer Res 1985; 45: 3359-3363.

Fanucchi MP, Leyland-Jones B, Young CW et al. Phase I trial of 1-(2-deoxy-2-fluoro-1-D-arabinofuranosyl)-5-methyl uracil (FMAU). Cancer Treat Rep 1985; 69: 55-59.

Ettinger DS, Orr DW, Rice AP et al. Phase I study of N-Methyl formamide in patients with advanced cancer. Cancer Treat Rep 1985; 69: 489-493.

Newlands ES, Blackledge G, Slack JA et al. Phase I trial of Mitozolamide. Cancer Treat Rep 1985; 69: 801-805.

Harvey JH, McFadden N, Andrews WG et al. Phase I study of Echinomycin administered as an intermittent bolus schedule. Cancer Treat Rep 1985; 69: 1365-1368.

Sorensen JB, Groth S, Nansen SW et al. Phase I study of the Cis-Platin analogue - TNO6. Cancer Chemother Pharmacol 1985; 15: 97-100.

Hansen SW, Bach F, Hansen HH et al. Phase I trial of TGU : a new triepoxide cytostatic agent. Eur J Cancer Clin Oncol 1985; 21: 301-305.

Kavanagh JJ, Krakoff IH, Bodey GP. Phase I study of Fludarabine. Eur J Cancer Clin Oncol 1985; 21: 1009-1011.

1984

Frytak S, Eagan RT, Ames MM et al. Phase I study of Diaziquone. Cancer Treat Rep 1984; 68: 975-978.

Niedhart JA, Derocher D, Grever MR et al. Phase I trial of teroxirone. Cancer Treat Rep 1984; 68: 1115-1119.

Ferrari L, Rossi A, Brambilla C et al. Phase I study with 4´-deoxydoxorubicin. Investigational New Drugs 1984; 2: 287-295.

1983

Curt GA, Grygiel JJ, Corden BJ et al. A phase I and pharmacokinetic study of CBDCA. Cancer Res 1983; 43: 4470-4473.

Currie VE, Warrell RP, Arlin Z et al. Phase I trial of 10-Deaza-aminopterin in patients with advanced cancer. Cancer Treat Rep 1983; 67: 149-154.

Mittelman A, Casper ES, Godwin TA et al. Phase I study of tricyclic nucleoside phosphate. Cancer Treat Rep 1983; 67: 159-162.

Stridhar KS, Ohnuma T, Chahinian AP et al. Phase I study of Acivicin in patients with advanced cancer. Cancer Treat Rep 1983; 67: 701-703.

Greaven PJ, Madajewicz S, Pendyala L et al. Phase I clinical trial of CHIP. Cancer Treat Rep 1983; 67: 795-800.

Niedhart JA, Young DC, Derocher D et al. Phase I trial of Homoharringtonine. Cancer Treat Rep 1983; 67: 801-804.

Nicaise C, Rosencweig M, De Marneffe M et al. Clinical phase I trial of Marcellomycin with a single dose schedule. Eur J Cancer Clin Oncol 1983; 19: 449-454.

Dombernowsky P, Lund B, Hansen HH. Phase I study of TGT. Cancer Chemother Pharmacol 1983; 11: 59-61.

Bonfante V, Ferrari L, Villani F et al. Phase I study of 4-demethoxydaunorubicin. Investigational New Drugs 1983; 1: 161-168.

Ogawa M, Miyamoto H, Inigaki J et al. Phase I trial of a new anthracycline : 4´-O-tetrahydropyranyl adriamycin. Investigational New Drugs 1983; 1: 169-172.

1982

Schilskey RL, Kelley JA, Ihde DC et al. Phase I trial and pharmacokinetics of Aziridinylbenzoquinone in humans. Cancer Res 1982; 42: 1582-1586.

Kelsen DP, Scher H, Alcock N et al. Phase I clinical trial and pharmacokinetics of 4´-Carboxyphthalato-(1,2-diaminocyclohexane) platinum (II). Cancer Res 1982; 42: 4831-4835.

Dosik JM, Stewart D, Valdivieso M et al. Phase I study of L-Alanosine using a daily x 3 schedule. Cancer Treat Rep 1982; 66: 73-76.

Abele R, Alberto P, Seematter RJ et al. Phase I clinical study with 5´ Deoxy-5-fluorouridine, a new fluoropyrimidine derivative. Cancer Treat Rep 1982; 66: 1307-1311.

Ohnuma T, Stridhar KS, Ratner LH et al. Phase I study of Indicine N-Oxide in patients with advanced cancer. Cancer Treat Rep 1982; 66: 1509-1515.

Nasca S, Jezekova D, Coninx P et al. Phase I study of SOAZ. Cancer Treat Rep 1982; 66: 2039-2042.

Kaplan S, Martini A, Varini M et al. Phase I trial of 4-demethoxydaunorubicin with single IV doses. Eur J Cancer Clin Oncol 1982; 18: 1303-1306.

1981

Von Hoff D, Myers WJ, Kuhn J et al. Phase I clinical investigation of Bisantrene. Cancer Res 1981; 41: 3118-3121.

Ihde DC, Dutcher JS, Young RS et al. Phase I trial of Pentamethylmelamine : A clinical and pharmacologic study. Cancer Treat Rep 1981; 65: 755-762.

Earhart RH, Koeller JM, Davis HL. Phase I trial of PCNU administered by 5 day continuous infusion. Cancer Treat Rep 1981; 65: 835-840.

Leismann J, Belt R, Haas C et al. Phase I evaluation of ICRF-187 in patients with advanced malignancy. Cancer 1981; 47: 1959-1962.

Piccart M, Rozencweig M, Abele R et al. Phase I trial with Ametantrone. Eur J Cancer Clin Oncol 1981; 17: 775-779.

Piccart M, Rozencweig M, Dodion P et al. Phase I trial with alpha 1,3,5-Triglycidyl-S-Triazinetrione. Eur J Cancer Clin Oncol 1981; 17: 1263-1266.

1980

Von Hoff DD, Pollard E, Kuhn J et al. Phase I clinical investigation of DHAD, a new anthracenedione. Cancer Res 1980; 40: 1516-1518.

Warrell RP, Chou T, Gordon C et al. Phase I evaluation of Succinylated Acinobacter Glutaminase Asparaginase in adults. Cancer Res 1980; 40: 4546-4551.

Valdevieso M, Moore CE, Burgess AM et al. Phase I clinical study of PALA. Cancer Treat Rep 1980; 64: 285-292.

Schein PS, Smythe T, Hoth D, et al. Phase I clinical trial of Spirogermanium. Cancer Treat Rep 1980; 64: 1051-1056.

Ogawa M. Phase I study of GANU and MCNU. Cancer Treat Rev 1980; 7: 197-203.

1979

Bonfante V, Bonadonna G, Villani F et al. Preliminary phase I study of 4'epi-adriamycin. Cancer Treat Rep 1979; 63: 915-918.

Yap BS, Murphy W. Phase I clinical evaluation of 2,3-Dihydro-1H-imidazole (1,2-b) pyrazole (IMPY). Cancer Treat Rep 1979; 63: 1849-1851.

Garnick MB, Blum RH, Canellos GP et al. Phase I trial of Bruceantin. Cancer Treat Rep 1979; 63: 1929-1932.

Kovach JS, Moertel GC, Schutt AJ et al. Phase I study of Hycanthone. Cancer Treat rep 1979; 63: 1965-1969.

1978

Blum RH, Kahlert T. Maytansine; A phase I study of an ansa macrolide with anti-tumour activity. Cancer Treat Rep 1978; 62: 435-438.

Von Hoff DD, Howser D, Gormley P et al. Phase I study of m-AMSA. Cancer Treat Rep 1978; 62: 1421-1426.

Bedikian AY, Valdivieso M, Bodey GP et al. Phase I clinical studies with Gallium Nitrate. Cancer Treat Rep 1978; 62: 1449-1453.

Murphy WK, Burgess MA, Valdivieso M et al. Phase I clinical evaluation of Anguidine. Cancer Treat Rep 1978; 62: 1497-1502.

1976

Haas CD, Stephens RL, Hollister M et al. Phase I evaluation of Dianhydrogalactitol. Cancer Treat Rep 1976; 60: 611-614.

1975

Lokich JJ, Chawla LP, Jaffe H et al. Phase I evaluation of Cyclocytidine. Cancer Chemother Reports 1975; 59: 389-393.

Cohen MH, Creavan PJ, Jejada F et al. Phase I clinical trial of Isophosphamide. Cancer Chemother Rep 1975; 59: 751-755.

Creaven PJ, Cohen MH, Selawry DS et al. Phase I study of Thalicarpine. Cancer Chemother Rep 1975; 59: 1001-1006.

Kaufman J, Mittelman A. Clinical Phase I trial of Inosine Dialdehyde. Cancer Chemother Rep 1975; 59: 1007 1014.

Caoili EM, Talley RW, Smith F et al. Guanazole - A phase I clinical study. Cancer Chemother rep 1975; 59: 1117-1121.

1973

Higby DJ, Wallace HJ, Holland JF. Cis-diaminedichloroplatinum (NSC 119875) A phase I study. Cancer Chemother Rep 1973; 57: 459-563.

1971

Muggia FM, Selawry OS, Hansen HH. Clinical studies with a new podophyllotoxin derivative Epipodophyllotoxin. Cancer Chemother Rep 1971; 55: 575-581.