CI-921: A CLINICAL, PHARMACOKINETIC AND METABOLIC STUDY OF A POTENTIAL NEW CYTOTOXIC AGENT.

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Janet Rea Hardy

Department of Clinical Oncology

Auckland Hospital

Auckland, New Zealand

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ABSTRACT

CI-921, an analogue of the antileukaemic agent amsacrine, was produced in an attempt to develop a cytotoxic agent with a broader spectrum of activity. CI-921 was selected for clinical trial on the basis of superior in vivo and in vitro solid tumour activity.

Sixteen patients with histologically documented cancer for which there was no conventional cytotoxic treatment were entered into a phase I trial. The dose of CI-921 was escalated from 39mg/m^2 to 810mg/m^2 (total dose divided over 3 days) and repeated 3 weekly. Neutropenia was the major dose limiting toxicity and defined a maximum tolerated dose of 810 mg/m^2.

Pharmacokinetic studies revealed a biexponential pattern of drug distribution with a distribution half-life of 2.6 h. The kinetics appeared linear over the dose range tested. Less than 1% of total drug was excreted in the urine.

Nineteen patients were entered into a limited phase II trial in non-small cell lung cancer using CI-921 at a dose of 648 mg/m^2 in the same 3-day schedule. One of the 16 evaluable patients achieved a partial response lasting five months. Myelosuppression was the predominant toxicity as in the phase I trial, but the degree of toxicity confirmed this dose as being suitable for further phase II trials. One patient had a grand mal seizure temporally associated with three of four courses of CI-921 raising the possibility of neurotoxicity.

Although drug-induced cardiotoxicity has been reported with the parent drug amsacrine, there was no evidence of this in the current study.

It has been suggested that CI-921 undergoes hepatic metabolism and biliary excretion following conjugation with glutathione. There was no fall in whole blood glutathione levels in patients following CI-921 infusion, although a transient decrease in mouse hepatic GSH was demonstrated following both amsacrine and CI-921. The toxicity of CI-921 in mice was markedly increased following depletion of hepatic glutathione with BSO but was not affected by pre-treatment with morphine or the glutathione "protector" N-acetyl cysteine.

CONTENTS

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Dr Baguley is the Director of the Cancer Research Laboratory in which both amsacrine and CI-921 were developed. I am very grateful to have had the opportunity of working in association with Dr Baguley and his team and hope that the "bridging" between clinical and laboratory cancer research will continue.

To Dr J. Paxton and Professor D. Paton who appointed me as honorary research fellow in the Department of Pharmacology, I offer special thanks.

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GLOSSARY OF ABBREVIATIONS

AGC	absolute granulocyte count
ALP	alkaline phosphatase
AST	aspartate transferase
AUC	area under the concentration time curve
AUMC	area under the first moment of the concentration/time curve
BSO	buthionine sulphoximine
ß	slope of the terminal portion of the log concentration/time curve
cf	compared with
CHO	chinese hamster ovary
CCNU	1-(-2-chloroethyl)-3-cyclohexyl-1-nitrosourea
CL	plasma clearance
Cmax	maximum concentration achieved in plasma
CNS	central nervous system
Ct	concentration at last time point
cv	coefficient of variation
DEM	diethyl maleate
DNA	deoxyribose nucleic acid
ECOG	Eastern co-operative oncology group
EORTC	European organization for research and the treatment of cancer
FS	fractional shortening
g/dl	grams per deciliter

GSH	glutathione (reduced form)
GSSH	glutathione (oxidized form)
HPLC	high performance liquid chromatography
%ILS	percent increased life span
i.c.	intra-cerebral
i.p.	intra-peritoneal
I.S.	internal standard
i.v.	intra-venous
Ka	association constant
LD_{10}	lethal dose in 10% of treated mice
LD_{50}	lethal dose in 50% of treated mice
LD_{90}	lethal dose in 90% of treated mice
LVEF	left ventricular ejection fraction
m-AQDI	quinine diimine of amsacrine
m-AQI	quinine monoimine of amsacrine
mg/m^2	milligrams per square metre (surface area)
MIS	misonidazole
MTD	maximum tolerated dose
NAC	N-acetyl cysteine
NCI	National Cancer Institute
NSCLC	non-small lung cancer
P	probability
PCV	packed cell volume

QSAR	quantitative-molecular-structure-activity-relationship
QTc	corrected Q-T interval of electrocardiogram
r	Pearson's correlation coefficient
s.c.	subcutaneous
SC	seeded quality control (plasma)
SCU	seeded quality control (urine)
STI	systolic time interval
TEAP	triethylamine phosphate
$t_{1/2}$å	distribution half-life
$t_{1/2}$ß	terminal half-life
UE	urinary excretion
Tt	last time point
Vss	volume of distribution

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

PREFACE

Historically, the discovery of anti-neoplastic agents has been largely serendipitous, with the chance finding of cytotoxic activity in a natural product, or drug originally developed for some other purpose. Recently, there has been a more logical approach to the development of new anti-cancer agents by the chemical manipulation of compounds already known to have some activity. By changing various parts of a molecule to alter such physico-chemical properties as lipid solubility, stability and DNA binding strength, the aim is to improve the anti-neoplastic activity of the compound whilst preserving those parts of the molecule already known to confer a desired property. This is known as the quantitative molecular-structure-activity-relationship (or QSAR) approach to drug design.

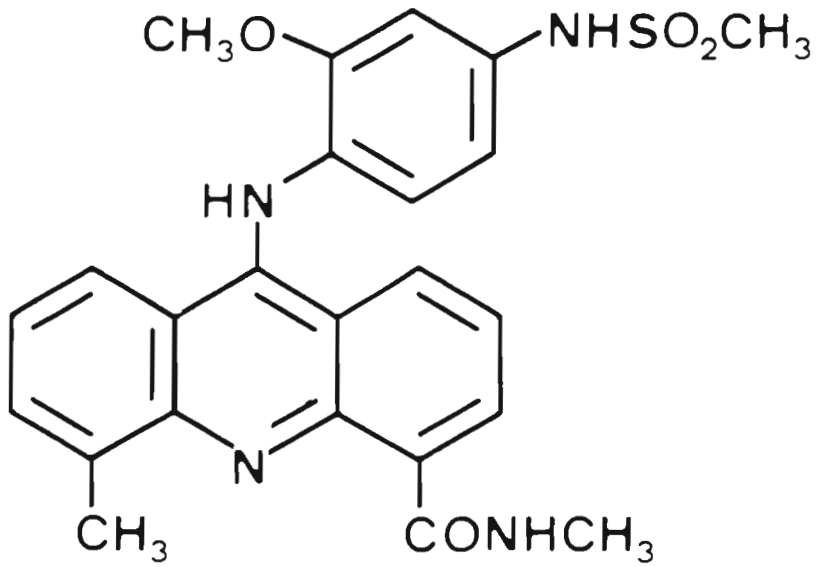
The anilino-acridines were selected as potential anti-tumour agents in the early 1970s and following extensive QSAR studies of this series, amsacrine was developed and entered into clinical trial. By the early 1980s, amsacrine had been shown to be effective against haematological malignancies (Arlin 1983) but had no significant activity against solid tissue tumours in man (Zittoun 1985). Using the QSAR approach, it was hoped to produce a drug with both a wider spectrum of action and physico-chemical properties more suited to solid tumour activity. To this end hundreds of analogues of amsacrine were produced and tested against <u>in vivo</u> and <u>in vitro</u> tumour screens (Denny <u>et al</u> 1984).

The murine leukaemias L1210 and P388 were retained as convenient initial screens of cytotoxic activity but were used in association with the Lewis lung tumour model in an attempt to select out those agents with greater solid tumour activity. The Lewis lung system is a particularly valuable in vivo tumour model. It is one of the few murine tumour lines in the National Cancer Institute's tumour panel which differentiates those agents known to be active against solid tumours (for example cyclophosphamide, doxorubicin, 5-fluorouracil and methotrexate) and those known to be active against haematological malignancies (e.g. daunorubicin, cytosine arabinoside and L-asparaginase) (Goldin et al 1981). The agent showing the most cytotoxic activity against the Lewis lung tumour model whilst retaining its anti-leukaemic activity was the 4-methyl, 5-methyl carboxy derivative of amsacrine, known as CI-921 or "AMSALOG" (Baguley 1984) (Fig.1).

CI-921 is being developed by the Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company. Three phase I and limited phase II studies of CI-921 were undertaken in Puerto Rico, Ohio and Auckland, each centre giving the drug according to a different schedule. The phase I and II clinical and pharmacokinetic studies of CI-921 completed in Auckland form the basis of this thesis.

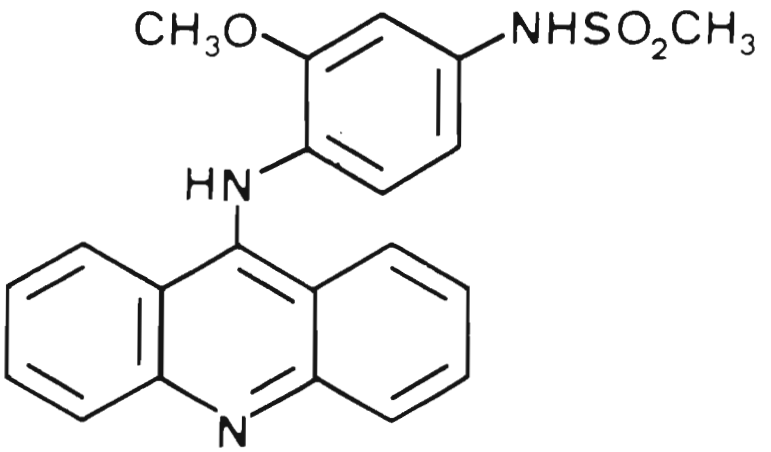
FIG. 1.

CI-921



Empirical formula:	$C_{26}H_{30}N_4S_2O_8$
Molecular weight:	590.5 (monohydrate, 608.7)
Chemical name:	9-[[2-methoxy-4-[(methyl-sulfonyl) amino]-phenyl]amino]-N, 5-dimethyl-4-acridine-carboxamide, 2-hydroxyethane sulfonate (1:1)
Common name:	"AMSALOG"
Other designations:	CI-921 NSC 343,499.

FIG. 2. **Amsacrine**



Empirical formula:	$C_{21}H_{19}N_3O_3S$
Molecular weight:	393.5
Chemical name:	N-[4-(9-acridinylamino)-3-methoxyphenyl]-methanesulfonamide.
Common name:	m-AMSA, acridinyl anisidide.
Trade name:	Amsidine (Europe) Amsidyl (USA)
Other designations:	CI-880 NSC-249992.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

AMSACRINE.

Amsacrine, 4'- (9-acridinylamino)-methanesulphonanisidide (Fig.2) is a cytotoxic agent which has an established role world-wide in the treatment of leukaemia (Arlin 1983). It is the parent drug from which CI-921 was derived. CI-921 differs from amsacrine only by the addition of methyl and methyl carboxamide groups to the acridine nucleus (Fig.3). It is therefore likely to have a similar mechanism of action and pathway of metabolism and may have similar toxicity. An understanding of the parent drug is helpful in studying the analogue CI-921. The following introduction reviews the activity, toxicity and metabolism of action of amsacrine.

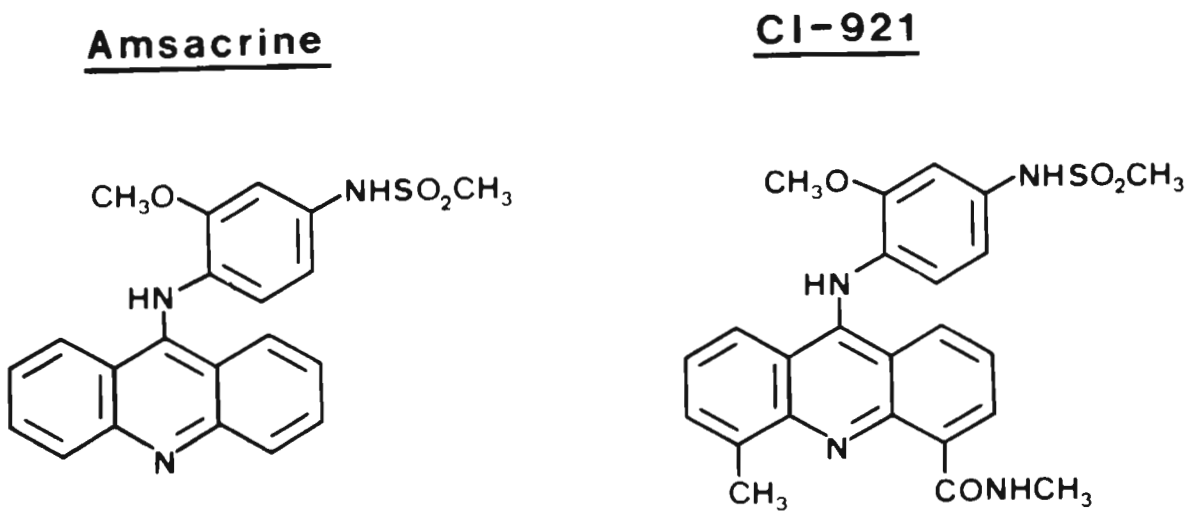


Fig 3. Comparative structures of amsacrine and CI-921

Amsacrine was developed in Auckland in the early 1970s by the Auckland Cancer Research Group, under the directorship of the late Professor Bruce Cain. It is unique in that it is the first pharmaceutical of any type developed in New Zealand to be used here clinically and the first synthetic DNA-intercalating anti-cancer drug to enter clinical use anywhere (Denny 1987). Amsacrine was chosen for clinical trial from a large series of acridine derivatives produced in an attempt to find new DNA-binding antitumour agents. Impressive antitumour activity was shown against a range of experimental tumour cell lines and animal tumour models including murine leukaemias, colon 26 and colon 38 carcinomas, mammary adenocarcinomas and B16 melanoma (Baguley 1984).

Clinical Efficacy.

Although phase I trials had shown some antitumour activity against solid tumours (Bodey et al 1983), Phase II activity against solid tumours was disappointing with overall response rates generally less than 10% (Zittoun 1985). While a low degree of antitumour activity was observed against breast carcinoma (Legha et al 1979), malignant melanoma and non small cell lung cancer (Legha et al 1978, Samson et al 1981), amsacrine was inactive against colorectal, pancreatic, prostatic and testicular carcinomas (Baguley 1984). Occasional responses have been observed in the treatment of sarcoma (Van Echo et al 1979), head and neck, (Crivellari et al 1987) ovarian and bladder carcinomas (Natale 1983, Baguley 1984).

Phase I and II trials in patients with haematological malignancies were more promising however (Legha et al 1980, Arlin et al 1980). The overall response rate in 174 patients

with refractory leukaemia pooled from several phase II trials was 26% (Baguley 1984). Zittoun (1985) has reviewed eight phase II studies of the use of amsacrine since 1980 in patients with acute leukaemia refractory to previous therapies. The overall response rate in these heavily pre-treated patients was 27% with no difference between acute myeloid and acute lymphocytic leukaemia. The duration of remission was relatively short in most cases, with a median of 12 weeks (range 3-59 weeks).

The place of amsacrine in previously untreated leukaemia has still to be determined, but several trials are currently under way to define its role in standard induction regimens (Arlin 1983). It has been suggested that amsacrine should be used in the place of anthracyclines to lessen cardiac toxicity (Zittoun 1985).

A recent study by Keating et al (1987), comparing amsacrine and Adriamycin (both in combination with cytosine arabinoside, vincristine and prednisone) in previously untreated patients with adult acute leukaemia, has found the two regimens to be equivalent in the induction of complete remission.

The role of amsacrine in the initial induction treatment of acute lymphocytic leukaemia is more difficult to define in view of the high remission rates already possible with standard agents (Arlin 1983, Keating et al 1987). As a single agent, amsacrine appears to have definite antitumour activity against malignant lymphomas (Cabanillas 1983, Cabanillas et al 1981, Weick et al 1983). Of 138 patients from pooled phase II trials, 14% achieved a response (Baguley 1984). Further studies should define the value of

amsacrine in combination treatments in various histological types of malignant lymphomas (Zittoun 1985).

Clinical Toxicity

Myelosupression is the major dose-limiting toxicity of amsacrine. Neutropenia is predictable, reversible and non-cumulative. Thrombocytopenia and anaemia are generally mild and rarely dose-limiting (Issell 1980). Gastrointestinal side-effects include nausea, vomiting and stomatitis, especially at doses >600 mg/m^2/course. Phlebitis and venous irritation in the infusion arm has been observed but can be avoided by the use of central indwelling catheters or larger infusion volumes (Arlin 1983, Legha et al 1978). Alopecia is seen but its true incidence has been difficult to determine as many of the patients in the phase I and II trials already had hair loss from previous therapy. Hepatic dysfunction, with increasing serum bilirubin and alkaline phosphatase levels following treatment has been observed (Legha et al 1980, Arlin et al 1980). Appelbaum and Shulman (1982) described a case of fatal hepatotoxicity in a 44 year old woman with acute lymphoblastic leukaemia treated with amsacrine. Mahal et al (1981) suggested that doses should be reduced in patients with abnormal liver function tests to avoid severe and unpredictable myelosuppression.

There are several reports of patients developing grand mal seizures during amsacrine infusions (Legha et al 1980, Stewart et al 1984), but in most cases there was a major underlying metabolic disturbance. One of the patients described by Stewart et al (1984) had the highest cerebral concentration of amsacrine at autopsy out of a series of five

patients and had no other underlying factor or abnormality that may have precipitated a seizure. It was postulated that the seizure reflected neurological toxicity from amsacrine.

The cardiac toxicity of amsacrine is well documented and is reviewed separately (see page 23).

Metabolism

The metabolism of amsacrine has been extensively studied by Shoemaker, Cysyk et al. In 1977 they demonstrated a selective localization of radio-labelled amsacrine in the liver of rats followed by rapid elimination in the bile of a metabolite identified as the amsacrine-glutathione conjugate. A 40% reduction in liver glutathione (GSH) and a 20% reduction in liver GSH transferase activity occurred after amsacrine administration to mice (Cysyk et al 1977). The biliary excretion of labelled amsacrine was significantly increased following pre-treatment of rats with phenobarbital (a known inducer of the hepatic mixed function oxidase system) and decreased following pre-treatment with metapyrone (an inhibitor). Moreover, biliary excretion was significantly decreased following pre-treatment with diethyl maleate (DEM) an agent known to deplete hepatic GSH. These findings support the role of microsomal activity and glutathione conjugation in the metabolism of amsacrine (Shoemaker et al 1980). In 1982, Shoemaker et al isolated the principal biliary metabolite and using nuclear magnetic resonance, showed it to be an amsacrine glutathione conjugate preserving both the acridine and anilino portions in which the thioether linkage occurred at the 5 position of the anilino ring. From further studies using an in vitro rat liver microsomal system,

Shoemaker et al (1984) postulated a pathway of metabolism as shown in Figure 4. Two microsomal oxidation products of amsacrine were isolated and identified as a quinone-diimine (m-AQDI) and a quinone mono-imine (m-AQI). Both were considerably more cytotoxic to L1210 cells in vitro than was amsacrine. m-AQDI reacts with glutathione to form the previously identified primary biliary metabolite which was not cytotoxic to L1210 cells. These results suggested that bioactivation of amsacrine was required to form the active cytotoxic species of the drug, but that the end result of microsomal activation was detoxification.

In contrast, Robbie et al (1988) have questioned whether m-AQDI is responsible for the cytotoxicity induced by amsacrine. They have used radio-labelled amsacrine to investigate the metabolism of amsacrine in AA8 tumour cells. After exposure of tumour cells to labelled amsacrine, greater than 95% of the radioactivity was recovered as the parent drug, with no m-AQDI detected by HPLC analysis. Studies with radio-labelled m-AQDI have shown that m-AQDI is rapidly metabolised in cultured AA8 cells to amsacrine, GSH conjugates and protein adducts (in equal amount). With less than 0.4% of total cell-associated radioactivity associated with GSH adducts following exposure of tumour cells to labelled amsacrine, it seems that intracellular conversion of amsacrine to m-AQDI is less than 1%. These results would suggest that m-AQDI is not responsible for the cytotoxicity induced by amsacrine treatment of tumour cells. Further studies are planned to clarify this contradictory finding.

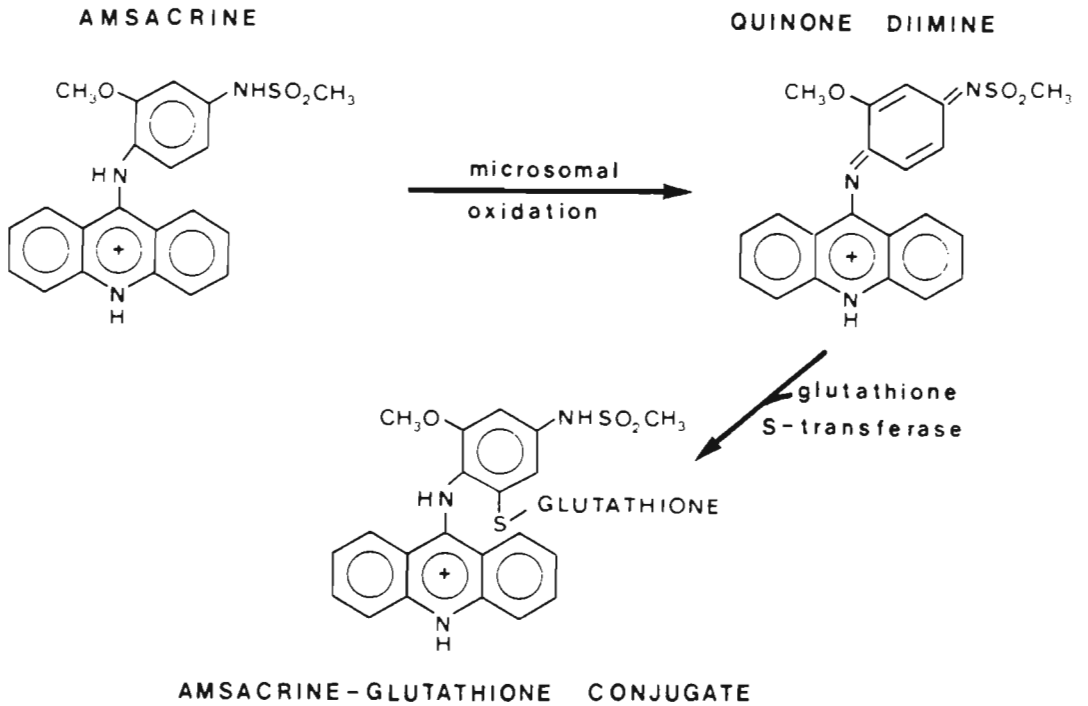


FIG. 4. The metabolism of amsacrine.

Pharmacokinetics

Amsacrine shows biphasic clearance with a short initial half-life in the range 0.2-1.66 hours and a terminal half-life in the range 3-10 hours (Baguley 1984). Biliary excretion following hepatic extraction and metabolism is the major pathway of elimination in humans. In patients with pre-existing hepatic dysfunction, a significant reduction in amsacrine clearance and prolongation of terminal half-life has been reported (Hall et al 1983, Staubus et al 1980). Furthermore, urinary measurements indicate that renal elimination plays a minor role in the total clearance of this drug (Jurlina et al 1985, Staubus et al 1980).

Amsacrine is highly bound (approximately 97%) to plasma proteins (Paxton et al 1986). Human tissue distribution determined by HPLC from autopsy tissue samples from five patients who had received the drug antemortem showed the highest concentrations in gallbladder, liver and kidney. Low levels were generally seen in lung, testicle, muscle, fat, spleen, bladder, pancreas, colon, prostate and brain (Stewart et al 1984). Cerebrospinal fluid concentrations of amsacrine are less than 2% of plasma concentrations, indicating poor distribution across the blood-brain barrier (Hall et al 1983).

THE MECHANISM OF ACTION OF AMSACRINE - TOPOISOMERASE II.

The mechanism of cytotoxic action of amsacrine and CI-921 is thought to involve the inhibition of the enzyme topoisomerase II. Amsacrine was one of the first agents shown to exert its antineoplastic effect via topoisomerase II and has become the model drug for study of this enzyme. The function of topoisomerase II in the cell and the interaction between this enzyme and antineoplastic agents is therefore reviewed.

Amsacrine and topoisomerase II.

The intercalating agents are a class of cytotoxic drugs that bind to DNA by physical insertion into the double helix. They characteristically possess a flat aromatic portion that can "slot in" to DNA between base-pairs,distorting DNA geometry and changing the supercoiling of the molecule (Fig 5). This can be measured as an"unwinding angle" by viscometric studies (Waring 1981).

Fig 5. Intercalation of drug between base pairs and subsequent distortion of the DNA helix.

Lerman first described the process of intercalation to explain the binding of the aminoacridines to DNA in 1961. In 1976, Waring described the intercalative properties and unwinding characteristics of amsacrine and related acridinyl methanesulphonanilides. The development of the acridine series by Cain et al (1976) was based on the assumption that the intercalative binding of these drugs was required for antitumour activity. A positive correlation between affinity for DNA and antitumour activity had been shown in an extensive series of 9-anilino-acridine drugs by Baguley et al (1981). However, Wilson et al (1981) compared the binding of amsacrine and related drugs differing only in their anilino-ring substituents and related this to biological activity. They found that amsacrine ("m-AMSA") binds to nucleic acids with lower affinity than the less active parent drug "AMSA" (4-(9-acridinyl-amino)-methane-sulphonalide) or the inactive ortho-isomer, "o-AMSA". They suggested that the cytotoxic activity of amsacrine involved DNA binding by the acridine moiety acting as an "anchor", while the methanesulfonalide ring interacted with a second macromolecular species. Subsequently, Zwelling et al (1981) demonstrated that amsacrine produced single strand DNA breaks and DNA-protein cross-links in a 1:1 ratio in mouse leukaemia L1210 cells. These breaks were rapidly reversible and temperature dependent. They did not occur at low temperatures despite normal entry and exit of the drug from the cell. Whereas drug entry into the cells was proportional to drug concentration, the maximum number of breaks occurred at relatively low concentrations. These findings suggested an enzyme-mediated process, with the covalent binding of a protein to either terminus of the DNA strand break produced. These workers postulated that the enzyme involved was a topoisomerase stimulated to produce DNA strand breaks by amsacrine. This mode of action was supported by Nelson et al (1984). Using purified mammalian DNA topoisomerase

they demonstrated a marked stimulation of formation of topoisomerase II - DNA complexes by amsacrine with the subsequent production of both single and double stranded DNA breaks. The non-cytotoxic isomer, "o-AMSA", which does not induce a significant number of DNA breaks in cultured cells, showed less stimulation of formation of topoisomerase II - DNA complexes.

Yang et al (1985) gave further support to the hypothesis that topoisomerase II is the target of amsacrine and related drugs. They were able to label the protein associated DNA breaks with polyclonal antibodies specific for type II topoisomerase. Amsacrine has since been recognized as one of the most potent known topoisomerase stimulators and as such has become a "model drug" for the extensive study of this enzyme over the last few years.

The function of topoisomerase in the cell

Topoisomerases are enzymes of critical importance in maintaining the normal structure of DNA. The primary structure of a DNA molecule is the sequence of paired nucleotide bases along the sugar-phosphate "backbone". The secondary structure describes the inter-twining of two strands of DNA to form a double helix with a linear axis. The tertiary structure refers to its three-dimensional form in space, i.e. its topological configuration. In vivo, DNA does not float freely in the cell but exists in the form of closed coils or loops, held at the base with no free ends. Supercoils are introduced when a DNA duplex is twisted in space around its own axis. This twisting or supercoiling places the molecule under torsional stress.

Two molecules of DNA with the same sequence of bases, but differing configurations in space, are defined as topoisomers. Enzymes that change this topological form are topoisomerases (Wang 1982, Lewin 1983, Ladish et al 1986). DNA topology is of critical importance to normal cellular function, as the topological form dictates ease of strand separation and thus affects such processes as DNA replication, transcription, transposition and viral integration. Topoisomerases therefore have a major role in cellular control (Ross 1985).

The topological form of a DNA helix is controlled by the linking number. This is defined as the number of times one DNA strand revolves completely around its pair. In a closed circle of DNA, partial strand separation, or supercoiling of the molecule cannot change the linking number unless one of the strands is broken, allowing one strand to rotate about the other. Therefore, all topological interconversions of DNA require the transient breakage and subsequent re-sealing of the DNA strands which is a function of the topoisomerases.

In eukaryotic systems, there are two distinct types of topoisomerases (Wang 1985). Topoisomerase type 1 (topoisomerase 1) breaks and re-seals single strands of DNA, changing the linking number of the molecule by a factor of one. This enzyme remains bound at the 3' end of the break site following strand cleavage. The rotation of one strand around the other following the "nicking" of one strand results in the relaxation of DNA supercoils, alteration of DNA topology and thus cellular function. Similarly, the enzyme is able to link (catenate) two complementary single stranded DNA rings. The exact biological role of topoisomerase 1 in vivo is poorly understood however (Ross 1985).

Topoisomerase II is characterized by its ability to break and subsequently reseal both DNA strands simultaneously and to allow the passage of a separate strand through the break site. It can alter the topology of a DNA molecule by catalyzing such reactions as the removal of complex "DNA knots", changing the degree of supercoiling, or linking double-stranded DNA circles (Ross 1985). Topoisomerase II exists as a dimer with a subunit molecular mass of about 170 kilodaltons. It remains covalently bound to the 5' end of the DNA break and is only loosely associated with the 3' end. Strand passage through the break is an ATP dependent process (Sullivan et al 1986).

In eukaryotic cells, topoisomerase II appears to be a major structural protein of mitotic chromosomes. Chromatin fibres of both meiotic and mitotic chromosomes are folded into radially projecting loops tethered at their bases to a chromosome "scaffold" (Baguley 1987). The major protein component of these scaffolds as identified by polyclonal antibody studies, is topoisomerase II (Earnshaw et al 1985, Earnshaw and Heck 1985). In this model, topoisomerase II is in an ideal position to act as a swivel, altering the torsional stress of the loop and thus "controlling" such processes as DNA transcription, replication and repair (Baguley 1987).

The interaction between topoisomerase and antineoplastic agents.

Following cleavage of a DNA molecule by topoisomerase II, the two strands are held "in register" on the protein framework, to allow for resealing following strand passage (Fig. 6). This intermediate protein-DNA complex is fragile and if disrupted forms a potentially lethal DNA break (Baguley 1987). In the presence of a variety of

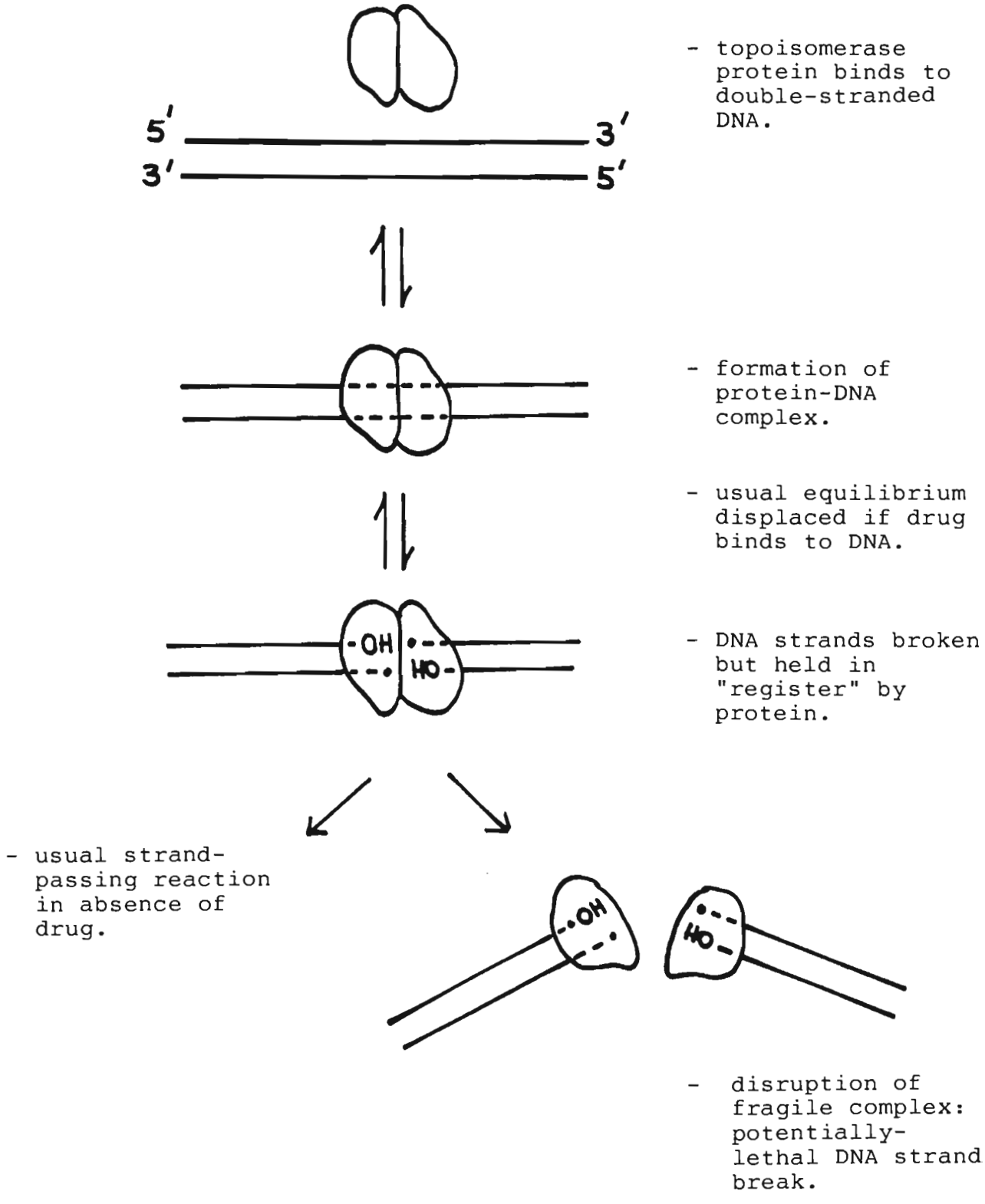


FIG. 6. Mechanism of topoisomerase-mediated DNA cleavage by amsacrine and related drugs.

intercalating agents, site specific DNA cleavage by topoisomerase II is markedly stimulated (Tewey et al 1984). The intercalative agents now known to act via topoisomerase II include the anthracyclines, acridinediones (e.g. mitozantrone) and members of the ellipticine and acridine series (Tewey et al 1984, Baguley 1987). The interaction with topoisomerase II is not dependent on intercalation as shown by the epipodophyllotoxins (etoposide and teniposide) which also exert their antineoplastic effect via topoisomerase, but do not intercalate into DNA (Rowe et al 1985, Ross et al 1984, Ross 1985, Long et al 1984).

Although DNA topoisomerase II appears to be a common target for several antineoplastic agents, it can be affected in more than one way (Tewey et al 1984, Pommier et al 1985a). Whereas amsacrine and the epipodophyllotoxins appear to stimulate the formation of unstable DNA-topoisomerase complexes (Baguley 1987, Rowe 1985), members of the ellipticine series appear to inhibit strand passage or destabilize the DNA-topoisomerase complexes (Pommier et al 1985).

The exact relationship between the topoisomerase-mediated DNA breaks induced by a drug and its antitumour effect is not clear. Although a direct relationship between DNA cleavage and cytotoxicity has been shown with the amsacrine isomers (Zwelling et al 1981, Nelson et al 1984) and for epipodophyllotoxin cogeners (Long et al 1984), the DNA breaks are generally rapidly reversed following drug removal. A small proportion of these lesions are not repaired however, and may result in longer lasting forms of DNA damage (Baguley 1987). Chromosomal aberrations following exposure to a variety of intercalating agents and the epipodophyllotoxins have been demonstrated (Long et al 1984, Pommier et al 1985b, Wilson et al 1984). Ferguson

and Baguley (1984) have shown an inverse relationship between the frequency of chromosomal aberrations and cell survival.

Studies with an epipodophyllotoxin-resistant Chinese hamster ovary line (VpmR-5) which shows cross resistance to unrelated intercalating agents also known to act via topoisomerase II, support the relationship between drug-induced DNA breaks and cell death. The degree of resistance to the cytotoxic effect of any one of these drugs was well correlated with the level of resistance to DNA cleavage activity (Glisson et al 1986a, 1986b).

Topoisomerase activity is not constant but fluctuates, depending on several factors including the proliferative status of the cell (Sullivan et al 1987, Markovits 1987). Topoisomerase mediated DNA cleavage and the cytotoxicity of intercalating agents is markedly reduced in non -proliferating cells (Ross 1985). For example, while log-phase Chinese hamster ovary (CHO) cells exhibit dose-dependent drug-induced DNA breaks with etoposide and amsacrine, plateau cells are resistant to these effects despite equivalent cell-drug uptake (Wilson et al 1981, Sullivan et al 1986). Similarly Zwelling et al (1985) have shown an increased number of DNA breaks in proliferating human fibroblasts following exposure to amsacrine as compared to quiescent cells. Conversely, cells may develop resistance to these cytotoxic agents by reducing either the amount or activity of topoisomerase II (Johnson and Howard 1982, Hofman et al 1986, Glissen et al 1986a, 1986b, Pommier et al 1986).

Fluctuation of topoisomerase II activity raises the possiblity of increasing the cytotoxic activity of agents known to act via topoisomerasee II by increasing the amount or activity of the enzyme in cells. For example, topoisomerase II activity can be increased by phosphorylation of the enzyme either by protein kinase C or a calmodium-activated kinase (Sahyoun et al 1986). Similarly, DNA breaks in human breast cancer cells following treatment with daunorubicin and amsacrine can be stimulated by oestrogen (Zwelling 1983). Studies are currently being undertaken in the Auckland Cancer Research Laboratory to investigate the possibility of stimulating topoisomerase activity by using various culture media and growth factors.

THE CARDIOTOXICITY OF AMSACRINE

Amsacrine has been shown to be cardiotoxic under certain conditions and therefore all patients receiving CI-921 in the phase I and II trials were monitored closely to detect any evidence of cardiac toxicity that could be attributed to the drug. The results of this study are presented in Chapter 5 and the cardiotoxicity of amsacrine is reviewed in this introduction.

The cardiotoxicity of amsacrine is well recognized (Weiss et al 1986). In pre-clinical studies in dogs, high dose amsacrine resulted in prolongation of atrioventricular and intraventricular conduction, prolongation of QRS complexes, a fall in cardiac output and ECG ST-T wave changes (Legha 1983). Similarly, administration of amsacrine to rabbits induced a series of cardiac rhythm abnormalities and a dose-related negative inotropic effect in an isolated rabbit heart model. Dimethylacetamide, the amsacrine vehicle, had no effect either in vivo or in vitro (D'Alessandro et al 1983). Cardiotoxicity of amsacrine in man was first suggested in 1979. Legha et al reported the development of ventricular fibrillation in two patients, temporally related to amsacrine infusion. Both patients had underlying medical conditions however, (hypoxaemia and hypokalaemia), that might have contributed to the cardiac arrhythmias.

Subsequently there have been multiple reports of the development of ventricular fibrillation in patients receiving amsacrine almost always in association with hypokalaemia (Riela et al 1981, McLaughlin et al 1983, Foldes et al 1982). Both Weiss et al (1983) and Shiner and Hasin (1984) have demonstrated prolongation of the QT

interval on ECGs of patients receiving amsacrine infusions. They suggest that QT interval prolongation may be the initial manifestation of the amsacrine effect on the heart and that in a few patients, this abnormality initiates ventricular arrhythmias. Care should be taken to avoid other factors that may prolong the QT interval, (e.g. phenothiazines) during amsacrine administration. ECG changes consistent with ischaemia (Miller and Rajdev 1982) and multiple ventricular extrasystoles (Falkson 1979) have also been related to amsacrine infusion.

Lindpaintner et al (1986) reported a case of myocardial necrosis, presenting clinically as a myocardial infarction in a patient with normal coronary arteries who received amsacrine as treatment for acute myeloid leukaemia.

The development of clinical congestive heart failure was described by Steinherz et al (1982) in seven out of 27 patients treated with amsacrine as part of the phase I and II trials of amsacrine. Four of these patients died with intractable congestive heart failure.

The cardiotoxicity of amsacrine has been assessed by cardiac monitoring, radionuclide ventriculography (Vorobiof et al 1983) and echocardiography (Steinherz et al 1982). Vorobiof demonstrated a marked decrease in left ventricular ejection fraction in two of six patients receiving a total dose of amsacrine of $\geq$580mg/m^2 with no decrease in ejection fraction at lower doses. Similarly, Steinherz et al (1982) demonstrated echocardiographic changes in 18 of 27 patients on amsacrine. Sixteen patients had an increase in left ventricular pre-ejection period (PEP) with respect to ejection time (ET). The PEP/ET ratio increased by 19-33% of baseline (average 46%). Nine patients showed

a decrease in fractional shortening (FS) compared to the baseline value by an average of 33%. The changes were most frequent in those patients who had received a combined anthracycline and amsacrine dose of $>900 mg/m^2$.

Grillo-Lopez and Hess (1983) reviewed the clinical literature involving approximately 3,200 patients and found 74 cases (2.3%) of cardiotoxicity temporally related to the use of amsacrine. Most of the patients described in these studies had been previously treated with anthracyclines (the cardiotoxicity of which may be delayed and of late onset), several of the patients had had radiotherapy to the mediastinum and many were also hypokalaemic.

In conclusion, although many patients have underlying medical or metabolic abnormalities, cardiac changes can follow amsacrine administration. These changes are usually rapidly reversible on stopping the drug or correcting any underlying metabolic abnormalities. There is no evidence of chronic toxicity as found with the anthracyclines (Legha 1983). Correction of the serum potassium is now standard practice prior to amsacrine infusion and some authors have even suggested that the total dose of amsacrine plus anthracyclines should not exceed 900 mg/m^2 if normal cardiac status has not been confirmed by echocardiography and radionuclide angiography (Steinherz et al 1982).

Because of the documented cardiac toxicity associated with amsacrine infusion, the cardiac status of patients receiving CI-921 in the phase I and II trials was closely monitored as described in Chapter 5.

THE DEVELOPMENT OF CI-921

Active cytotoxic agents must show both tumour cell selectivity and pharmacokinetic properties which allow maximum exposure of tumour cells to drugs (Baguley et al 1984). An agent with solid tumour activity should also have favourable tissue distributive properties so that the drug can reach remote tumour sites and poorly vascularized areas. Factors affecting tissue distribution include hydrophilicity, lipophilicity, solubility and base strength.

Although amsacrine had proved to be a valuable agent in the treatment of haematological malignancies it had shown very little activity towards solid tumours. In an attempt to develop a drug with a broader spectrum of activity, multiple analogues of amsacrine were produced. CI-921 was identified as the agent with the greatest solid tumour activity.

The following introduction illustrates the use of the quantitative-molecular-structure-activity-relationship (QSAR) approach during the development of CI-921 to maximize those factors necessary for solid tumour activity whilst retaining those parts of the anilino-acridine molecule known to confer anti-neoplastic activity.

The findings from the extensive preclinical evaluation of CI-921 are also presented.

QSAR in the design of CI-921.

During the development of the 9-anilino-acridine series, an intact acridine ring system was found to be essential for cytotoxic activity (Cain et al 1974) and the disubstituted anilino side-chain of amsacrine was considered optimal (Denny 1987).

Electron-donor functions attached to the 9-anilino ring were necessary to maintain activity (Atwell et al 1972, Atwell et al 1984) but various other substituents either failed to add to the activity of the molecule or were too toxic (Denny et al 1984). Attention was then focused on the acridine moiety with the synthesis of a range of substituted derivatives, differing in those physico-chemical characteristics known to effect tissue distribution and cytotoxic activity.

Physio-chemical properties

i) Lipophilic/hydrophilic balance

A favourable lipophilic/hydrophilic balance is necessary for tissue distribution. Drugs which are either too lipophilic or too hydrophilic do not partition well between lipid and aqueous phases and are therefore unable to diffuse readily through biological membranes (Baguley and Cain 1982).

Using various members of the anilino-acridine series, Cain et al (1974) were able to determine an optimal hydrophilic-lipophilic "balance" that resulted in maximal cytotoxicity to L1210 leukaemic cells. Although greater lipophilicity was necessary for activity against remotely implanted tumours, this resulted in decreasing cytotoxicity and solubility.

Similarly, hydrophilic compounds were less cytotoxic and were unable to penetrate to distant sites (Cain and Atwell 1976).

Of the numerous compounds tested, CI-921 had a near optimal balance, with the combination of a lipophilic methyl substituent at the 4-position of the acridine moiety and the hydrophilic methyl carboxy group at the 5-position (Denny et al 1982, Denny et al 1984). CI-921 is only slightly more lipophilic than amsacrine (lipophilicity (log p) = 0.6 for amsacrine and 1.10 for CI-921) but is readily soluble and was significantly more active against remote solid tumours in preclinical studies (Baguley et al 1984).

ii) Base Strength

Low base strength is a desirable property of agents designed to be active against solid tumours. At a physiological pH, most drugs will be in the neutral uncharged form allowing more rapid distribution into solid tumours. However, in a series of amsacrine derivatives studied by Denny et al (1979), high acridine base strength was associated with greater dose potency. Although CI-921 is a considerably weaker base than amsacrine (pKa = 6.4 compared to 7.43) it has a higher DNA dissociation constant which is thought to compensate for the lower dose potency predicted by the decrease in acridine base strength (Baguley 1984).

DNA Binding

In 1981, Baguley et al demonstrated antitumour potency to be directly related to tightness of drug-DNA binding in a series of acridine-ring substituted amsacrine analogues (Baguley

et al 1981a, Baguley et al 1981b), with dose potency measured by life extension studies in the L1210 leukaemia model (Denny et al 1982).

DNA binding constants can be determined by spectrophotometry, equilibrium dialysis, or by the displacement from DNA of ethidium, a fluorescent intercalating probe molecule.

CI-921 was shown to bind to DNA 16 times more strongly than amsacrine, having a DNA association constant (Ka) of 2.1 x $10^6 m^{-1}$ (compared to Ka = 1.3 x 10^5 m^{-1} for amsacrine) (Baguley et al 1984).

There are at least three aspects of drug-DNA interaction that are important for biological activity: the binding mode or geometry, base-pair selectivity and kinetics (Denny et al 1983 b , Denny et al 1986).

i) Binding Mode

Amsacrine and its derivatives bind to DNA via the intercalation of the acridine moiety between base pairs with the positioning of the anilino ring in the minor groove of the double helix (Waring 1976, Wilson et al 1981, Baguley 1984, Traganos et al 1987, Pommier et al 1987). In this binding mode the 1- and 2- positions of the acridine ring lie close to the sugar-phosphate backbone of the DNA while the 4- and 5- positions are orientated toward the major groove, free of steric restraint (Denny et al 1983 b).

Substitution experiments changing the steric bulk of the molecule support this theory (see Fig 2). Substitution at the 1, 2, 7 and 8 positions of the acridine ring abolished activity

presumably because of steric hindrance to binding (Cain et al 1974), whereas substitution at the 4 and 5 positions (as with CI-921) did not cause such hindrance (Denny et al 1983b). Small groups in the 3-position had a beneficial effect on activity but only up to a limited size. 3, 5-substitution was unfavourable (Denny et al 1983a, Baguley et al 1981a).
In a large series of 9-anilinoacridine derivatives tested for antitumour potency in an in vivo antileukaemic (L1210) study, by far the most significant influence with respect to potency was the steric effects of groups placed at various positions of the 9-anilinoacridine skeleton (Denny et al 1982, Denny et al 1986).

The binding mode of CI-921 was investigated by viscometric titration with closed circular duplex DNA and was found to be consistent with intercalative binding. The DNA unwinding angle of CI-921 was 18° (as compared to 20.5° for amsacrine and 26° for ethidium) (Baguley 1984). Recent studies on the DNA-binding properties of CI-921 by Traganos et al (1987) and Pommier et al (1987) confirm these findings.

ii) Base Pair Selectivity

Sequence selectivity is measured by the reduction of fluorescence of ethidium-bound DNA following addition of the study drug (Baguley et al 1981a). Within the amsacrine derivative series, all the 4-carboxamide derivatives showed selective binding to guanine and cytidine nucleotides (Denny et al 1983b).

For CI-921, the binding constant for repeated sequences of adenine and thymidine nucleotides (dA-dT) was $1.15 \times 10^{6} m^{-1}$ (as compared to $3.7 \times 10^{5} m^{-1}$ for amsacrine) and 4.4

x $10^6 m^{-1}$ for repeated sequences of guanine and cytidine (dG-dC) (4.5 x $10^5 m^{-1}$ for amsacrine) (Baguley et al 1984).

iii) Kinetics

Long residence time of a drug at a particular site on a DNA molecule is important to maximize chromosomal damage, possibly by providing long-lived blocks to the passage of replication and/or transcription enzymes (Denny et al 1983b). Not only does CI-921 bind more tightly to DNA, but it dissociates more slowly (Baguley et al 1984).

Tissue Distribution

In the early QSAR studies of the amsacrine derivatives, Cain et al (1974) attempted to demonstrate those molecular features which provided favourable drug disposition patterns while maintaining high intrinsic selectivity towards target tumour cells. A series of derivatives known to be active against L1210 leukaemia were screened against L1210 cells implanted at various sites: intra-peritoneal (i.p.), subcutaneous (s.c.) and intracerebral (i.c.), employing i.p. drug administration. Those drugs active against the remotely implanted tumours were more lipophilic than those which were only active against i.p. implanted tumours.

A useful structural feature was found to be a 4-CH_3 or 4-OCH_3 group which greatly enhanced drug distribution to remote sites (Cain and Atwell 1976).

As a further test of drug distribution and activity against remote tumours, Baguley et al

(1983) utilized an advanced remote solid tumour model using the Lewis lung line in mice. When inoculated intravenously as a single cell suspension, this tumour locates in the lungs where multiple tumour foci can be identified after the implantation of 10^6 cells. If drug treatment is delayed until 4 days post implantation, about 30 colonies are present, each averaging about 100 cells, providing some representation of a solid tumour (Denny et al 1984). Amsacrine analogues known to be active against murine leukaemia were given i.p. using an intermittent schedule and starting either on day 1, or on day 5 after tumour inoculation. Whereas all those derivatives with amino- or substituted amino substituents at the 3-position of the acridine ring were completely inactive, the 3-halogen,4-methyl and 4- (N-methyl) carboxamide derivatives were highly active and resulted in a significant increase in life span of the mice and a proportion of cures. Although this activity was reduced when treatment was delayed until day 5, several analogues (including CI-921) provided a proportion of long-term survivors and activity comparable to that of cyclophosphamide. Amsacrine displayed low activity in this model.

Oral activity

In order to have activity when given orally, an antitumour drug must possess excellent distributive properties to enable it to reach the tumour site following absorption from the gastro-intestinal tract. It must be stable at gastric pH and not inactivated by gastro-intestinal enzymes. Following absorption through the gastro-intestinal wall it must pass through the liver before reaching the systemic circulation. Derivatives of amsacrine with substitutions at the 4 and 5 positions on the acridine nucleus had all shown high activity when administered i.p. against i.p. implanted P388 leukaemia cells. When tested for oral

activity, the 4-methoxy and 4-carbamoyl derivatives proved inactive, whereas the 4-methyl and 4-methyl carboxyl derivatives retained activity. The 4-methyl, 5-methyl carbamoyl derivative showed exceptional oral activity, presumably because of its increased solubility and low base strength (Denny et al 1984).

Pre-clinical evaluation.

During the development of the 9-anilinoacridine series, CI-921 was recognized as an active analogue, with physico-chemical properties favouring solid tumour activity. It subsequently underwent extensive evaluation in five cancer research laboratories (including the Auckland Cancer Research Laboratory) during its development to clinical trial (Baguley et al 1984, Leopold et al 1987, Investigators Brochure 1985).

In the assessment of antitumour activity, CI-921 was tested against a wide range of tumour cell lines in vitro and experimental tumour systems in vivo. Its activity was compared to that of amsacrine and to a number of antileukaemic and solid tumour cytotoxic drugs. The pre-clinical antitumour activity of CI-921 is summarized as follows.

Anti-tumour activity.

In vitro: Growth inhibition assays.

Using a range of murine, hamster and human cell lines, CI-921 was compared to amsacrine and daunorubicin (both established antileukaemic agents) and to doxorubicin, an agent with

activity in several solid tumours. Cytotoxicity is expressed as concentration of drug required to reduce the cell count of a culture to 50% of untreated controls (ID_{50}). CI-921 showed greater potency (a lower ID_{50}) than amsacrine and daunorubicin in all solid tumour lines tested and was as active or more active than doxorubicin in all but the human breast and melanoma lines. Its activity against murine leukaemia lines was similar to that of amsacrine (Table 1.1).

In vivo studies.

i) Murine leukaemia

The P388 lymphocytic leukaemia line is used by the National Cancer Institute as a pre-screen for potential chemotherapeutic agents (Goldin et al 1981). Mice are inoculated intraperitoneally (i.p.) with P388 cells prior to treatment with the study drug. Cytotoxic activity is expressed as the percentage by which life span is increased in treated animals as compared to untreated controls (%ILS). The activity of CI-921 in this system greatly exceeded that of amsacrine, doxorubicin and daunorubicin and was close to that of cyclophosphamide, one of the most active agents known in this system (Table 1.2).

TABLE 1.1 ID50 Values (mM) for CI-921 and related agents.*

Cell line	Origin	CI-921	Amsacrine	Doxo-rubicin	Dauno-rubicin
L1210	Mouse leukemia	38	33	30	21
P388	Mouse leukemia	12	14	14	18
V-79	Chinese hamster fibroblast	9.5	16	19	18
Lewis	Mouse lung carcinoma	12.6	27	30	150
HCT-8	Human colon carcinoma	25	70	63	220
HT-29	Human colon carcinoma	20	72	25	83
Lo Vo	Human colon carcinoma	12	47	13	75
MCF-7	Human breast carcinoma	18	79	13	56
MDA-231	Human breast carcinoma	31	77	20	73
T-47D	Human breast carcinoma	6.7	28	2.1	22
MM-96	Human melanoma	16	50	6.3	48
Jurkat	Human T-cell leukemia	3.6	12	9.2	38

*adapted from Baguley et al 1984.

TABLE 1.2 Activity against i.p. inoculated P388 leukaemia*

Drug	Optimum dose (mg/kg i.p.)	%ILS	Survivors (50 days)
CI-921	20	200	3/6
Amsacrine	8.9 or 13.3	78	0/6
Doxorubicin	3.9	79	1/6
Daunorubicin	2.7	60	0/6
Cyclophosphamide	225	216	5/6

*Adapted from Baguley et al 1984

ii) Murine lung tumours

a) Lewis lung.

The Lewis lung carcinoma is the least responsive murine tumour in the NCI tumour panel, being unresponsive to most intercalating agents including doxorubicin, actinomycin D, amsacrine, daunorubicin and mitoxantrone (Goldin et al 1981). The tumour is injected intravenously into mice as a single cell suspension and seeds to the lungs where multiple tumour foci can be detected (Baguley and Nash 1981). Cytotoxic activity of test drugs is expressed as %ILS as above. In this system, CI-921 not only gave a highly significant

increase in life span, but a number of "cures" (survived >60 days), even when treatment was delayed for 5 days from tumour inoculation. Amsacrine had only marginal activity in this system and doxorubicin is inactive. The activity of CI-921 was again comparable to that of cyclophosphamide, the agent that has the greatest activity in this model and which is therefore considered as the "standard" against which the activity of other agents is compared.

b) Other murine lung tumours

CI-921 was clearly superior to amsacrine and doxorubicin against the LC-12 squamous cell lung system but showed similar activity against the Madison 109 and Nettesheim lung carcinomas.

TABLE 1.3 Activity against i.v.-inoculated Lewis lung carcinoma*

Drug	Optimum dose (mg/kg)	%ILS	Survivors (60 days)
CI-921	20	168	14/17
Amsacrine	8.9 or 13.3	42	0
Doxorubicin	2.6	25	0/6
Daunorubicin	3.9	12	0/6
Cyclophosphamide	225	216	5/6

*Adapted from Baguley et al (1984).

c) Other tumour systems

CI-921 also showed activity against the murine colon 26 and colon 28 carcinomas, B16 melanomas, M5076 reticulum cell sarcoma and several mammary carcinoma systems, including at least one doxorubicin resistant tumour.

Preclinical toxicology

Preclinical toxicology was performed in mice, rats and dogs.

Mice were given a single intravenous (i.v.) dose of CI-921 over the dose range 20 mg/kg to 107 mg/kg and observed for 28 days. The LD_{10} (lethal dose in 10% of animals tested) was 42 mg/kg with an LD_{50} and LD_{90} of 60 and 86 mg/kg respectively indicating a relatively steep lethality curve. Clinical signs of toxicity observed included tremors, ptosis, hypothermia, decreased motor activity, hunched posture and laboured breathing. The majority of deaths occurred between days 5 and 7.

When administered to rats in single i.v. doses of 10, 20 or 30 mg/kg, dose-related myelosuppression was seen, with no change in biochemical parameters and no gross pathological abnormalities.

In dogs, toxic doses of CI-921 produced myelosuppression (especially leucopenia), gastro-intestinal toxicity, hypocellularity of bone marrow, generalized lymphoid depletion and mild biochemical alterations. A single i.v. dose of 0.31 mg/kg was the minimal toxic dose while doses of 3.125 mg/kg were lethal.

When given according to a five day schedule, a dose of 0.13 mg/kg/day produced no toxic effect, 0.31 mg/kg/day produced a mild, reversible leucopenia, whereas a dose of 1.25 mg/kg/day was lethal. These dosage levels were 1/10, 1/4 and equivalent to the mouse LD_{10} values. No unusual or unexpected toxicity was seen.

Schedule/dose dependency.

Baguley et al (1985) examined the schedule dependency of CI-921 in three tumour models; i.p. inoculated P388 leukaemia, i.v. inoculated Lewis lung carcinoma and s.c. inoculated P388 leukaemia. In all three systems, an intermittent treatment schedule with doses given on days 1, 5 and 9 after inoculation was more effective in prolonging life span than a single dose on day 1 or than multiple daily doses on days 1 to 5, or days 1 to 9.

A highly significant correlation between response and total dose has been demonstrated in advanced tumour models in mice (Baguley et al 1985).

The intermittent schedule may be superior to single dose or daily schedules because it allows a higher total dose of drug to be administered. The maximum tolerated doses administered were in general, greatest for the intermittent schedules. This implies greater host toxicity from daily schedules, or alternatively some host recovery or host "tolerance" with intermittent schedules.

This data was utilized in the design of the phase I clinical trials, which utilized single dose, daily and weekly schedules of drug administration.

Synergism with other antitumour agents.

Preclinical studies have shown strong therapeutic synergism between CI-921 and cisplatin in murine P388 leukaemia and LC-12 squamous cell lung carcinomas. With intraperitoneally inoculated P388 leukaemia cells, the combination of CI-921 with cisplatin gave an optimal cell kill in excess of 8-logs and a 40% cure rate. There were no cures with either drug alone.

Cisplatin as a single agent is inactive against LC-12 lung carcinoma. CI-921 is active and gives a significant increase in life span in treated mice. The two drug combination however was curative in 3/10 mice.

Potentiation in L1210 leukaemia has been reported for CI-921 with selenazofurin, cisplatin, etoposide and cyclophosphamide (Burchanal et al 1985).

Mechanism of action.

CI-921 is thought to act in the same manner as its parent drug amsacrine, by intercalative binding to DNA and interference with topoisomerase II (Rowe et al 1986). As mentioned previously (see page 28) Baguley et al (1984) have studied the DNA binding properties of CI-921 and have demonstrated an intercalative binding mode from viscometric studies with closed-circular DNA. This mode of binding was confirmed by Pommier et al (1987) from a DNA unwinding assay using L1210 topoisomerase I.

The effect of CI-921 on the cell cycle of cultured leukaemic cells was also characteristic of an intercalating agent and similar to that observed with amsacrine. A dose dependent suppression of cell transit through the S phase (DNA synthesis) was seen with an irreversible block in G_2 (post DNA synthesis, pre-mitotic phase) (Baguley et al 1984, Traganos et al 1987, Drewinko et al 1982). Covey et al (1986) have investigated the effects of amsacrine and several 9-aminoacridine derivatives (including CI-921) on DNA integrity in L1210 leukaemia cells. Following incubation with topoisomerase II in vitro, DNA protein cross-links and strand breaks were formed. Their results suggest that CI-921 is similar to amsacrine in its ability to trap topoisomerase II-DNA complexes, resulting in the formation of protein-associated DNA strand breaks and cell kill (see page 14).

Summary.

The data acquired during the pre-clinical evaluation of CI-921 supported its development to clinical trial. CI-921 was found to have significant anti-cancer activity against 84% (16 of 19) of the different tumour models tested, demonstrating a broad spectrum of in vivo activity. The antitumour activity was superior to that of amsacrine in 71% (10 of 14) test systems, with equivalent activity in the remaining four systems. Of special interest was the superior activity of CI-921 against the Lewis lung tumour.

The favourable physico-chemical properties, improved spectrum of activity (relative to amsacrine) and activity following oral administration suggested that CI-921 might have distributive and pharmacokinetic characteristics favouring activity against solid tumours in man.

CI-921 was therefore selected to go forward to clinical trial. The results of a phase I and limited phase II study of CI-921 are presented in this thesis.

EXPERIMENTAL TUMOUR MODELS AND EARLY CLINICAL TRIALS IN THE DEVELOPMENT OF CYTOTOXIC DRUGS.

After extensive pre-clinical investigation, a phase I trial of CI-921 was undertaken at Auckland Hospital in conjunction with sister trials in Ohio and Puerto Rico, each trial centre using a different drug schedule. This was followed by a limited phase II study in non-small cell lung cancer (NSCLC).

The following introduction discusses the use of experimental tumour models and early clinical trials in cytotoxic drug development. It justifies the treatment of NSCLC in a phase II setting and the relevance of associated pharmacokinetic studies.

Experimental tumour models.

CI-921 was introduced into clinical trial on the basis of its superiority to amsacrine with respect to both spectrum and degree of anticancer activity in pre-clinical tumour models. What is not clear at this time, however, is how this might relate to the activity of the drug in the clinical setting. There has been considerable work in recent years in trying to establish reliable drug screening methods by the selection of tumour cell systems which accurately reflect clinical disease. Thus, the rationale for the development of new experimental models is to increase their clinical predictivity (Finlay and Baguley 1981). For example, the mainstay of the NCI's screening programme had been the murine L1210 and P388 leukaemia models (Carter and Goldin 1977) until 1975 when a panel of transplantable rodent tumour screens was introduced, designed to match the histological

types of common visceral cancers (Goldin et al 1981). Whereas the previous screen preferentially selected drugs active against rapidly growing tumours, it was hoped that the use of slow growing murine tumours and human tumour xenografts in nude mice might select drugs more likely to have activity in many of the common solid tumours in man. Human xenografts rate a lower percentage of drugs as active compared to animal tumour systems. It was hoped that the reduced sensitivity of the former could provide an advantage in drug selection. More recently, in its drug screening programme, the NCI has been using disease orientated panels of human tumour cell lines grown in defined media. It is hoped that such in vitro assays using cell lines that retain the appropriate features of the tumours of origin might be of greatest relevance to the problems of clinical cancer (De Vita 1989).

There are obvious problems inherent in each of these systems which can only be tested by clinical experience. The use of murine leukaemia models will tend to preferentially select agents active against similar diseases in humans (leukaemias and lymphomas), with limited relevance to solid tumours (Double and Bibby 1989, Staquet et al 1983). Some of the problems associated with in vitro screening models are discussed on page 56 . Problems inherent in the use of mouse tumour models to define activity include the following:

i) Animal tumours may manifest drug sensitivities which are different to those of human neoplastic cells (Finlay and Baguley 1981).

ii) Routes of distribution, metabolism and elimination of a drug may show inter-species variation (Shoemaker 1986). This is especialy relevant for those drugs requiring metabolic activation e.g. cyclophosphamide.

iii) The relationship between cell kill and tumour response (e.g. growth delay) is complex and varies from drug to drug. Most assays are based on median survival times or

growth inhibition (change in weight of treated tumours as compared to controls). Cell kill however does not necessarily result in a decrease in tumour size (Twentyman 1985).

iv) Cell division times and tumour doubling times in tumours of patients are very different from those in transplantable mouse tumours (Baguley et al 1988).

v) Most preclinical studies are carried out at an early stage of growth whereas phase I clinical studies are conducted in advanced stage carcinoma (Baguley et al 1988).

vi) Recovery time, e.g. of bone marrow, is considerably shorter in mice (Baguley et al 1988).

vii) Previously unrecognized toxicity may be limiting in man (Carter and Goldin 1977).

viii) Novel agents, e.g. flavone acetic acid, may have mechanisms of action dependent on the biology of the tumour system in the animal (Double and Bibby 1989).

ix) Animal models, especially those using nude mice, are expensive and time consuming. They do not allow for the testing of large numbers of compounds (De Vita 1989).

Unfortunately, for solid tumours, where very few clinically active drugs have been identified in recent years, any claim that a given screen is predictive cannot as yet be substantiated (Staquet et al 1983). Furthermore, an unbiased prospective evaluation of the clinical predictivity of a screen would require that random samples of all drugs both screen positive and screen negative be studied clinically. Few clinicians would find this ethically acceptable. In an analysis of the experimental murine tumours and xenografts as used by the NCI, Goldin et al (1981) found on testing established anticancer agents, that the spectrum of activity in the screening panels correlated (but only partially) with antitumour effectiveness in the patients. It has been suggested (Double and Bibby 1989), that the

limited clinical activity of current anticancer drugs results from the methods used in their selection. The yield of clinically effective drugs has so far been extremely low, which may reflect either the inaccuracy of current selection strategies or the fact that only a small number of active compounds actually exist (Staquet et al 1983).

As previously discussed (see page 33), CI-921 was shown to have a high and broad spectrum of activity and was particularly noteworthy because of its high activity against the Lewis lung carcinoma which is considered the most resistant tumour in the NCI panel. While this does not necessarily equate with clinical activity, its activity against this tumour was similar to that of cyclophosphamide, an agent with an established role in the treatment of solid tumours. The opportunity to test drugs such as CI-921 in clinical trials will therefore also provide a test of the screening methods from which they were selected. As stated by Muggia (1987), "regardless of the direction taken by various anticancer drug development programs and their proponents, the loop must be closed through the appropriate feedback from clinical studies."

Phase I trials.

The primary aim of a phase I trial of a cytotoxic drug is to define the maximum tolerated dose (MTD) for a specified mode of administration and to characterize the toxic effects and their reversibility. The MTD is the highest dose that can be safely administered to a patient, i.e. that which produces acceptable, manageable and reversible toxicity. It must be determined in order to perform phase II trials at the highest safe dose to obtain a reliable estimation of the maximum clinical effectiveness of a new agent (EORTC new

drug development committee 1985).

All patients entered into a phase I trial must have a histologically confirmed diagnosis of cancer which is not amenable to established forms of treatment, or which has recurred following treatment. Most patients are therefore heavily pretreated. In a review of 187 phase I trials by Estey et al (1986), 90% of the patients had received prior chemotherapy.

Although there is therapeutic intent at all stages of clinical testing, tumour response in this setting, although gratifying, is not the primary aim of the study. Anti-tumour response, or lack thereof, is not part of the decision-making process to move drugs into phase II (Carter et al 1977). Of the 187 phase I trials of 54 anti-cancer drugs reviewed by Estey et al, there were only 271 objective responses reported in 6447 patients, an incidence of 4.2%.

Historically, the initial starting dose in a phase I trial is one tenth the dose that produces death in 10% of treated mice (the LD_{10}). The dose is then escalated, initially in large increments and then in progressively smaller increments as the MTD is approached as in the modified Fibonacci search scheme (see Table 1.4) (Carter et al 1977). Three patients are treated at each level and the toxicity determined before entering patients at the next higher level. When overt toxicity is observed at least five patients should be entered at each dose level (EORTC new drug development committee 1985). The MTD is defined as that dose which produces dose-limiting toxicity in at least 50% of patients initially treated at that dose level.

A phase I trial utilizing a dose escalation method such as the modified Fibonacci scheme

usually requires about 15-30 patients to reach the MTD (EORTC new drug development committee 1985). This method of dose escalation, while ensuring a safe starting dose inevitably results in a large number of patients being treated at doses well below the MTD and therefore unlikely to be of any benefit to the patient. Although entry into a phase I study is theoretically beneficial to patients since the new drugs selected for study have been chosen because of their expected antitumour activity, it might appear that those patients given the initial doses are nothing more than experimental subjects. This is considered unethical by some authors (Durry and Dion 1986). Even though disease response is not the primary goal in a phase I trial, the investigator's obligation is to maximize the chance that the dose which an individual receives has potential therapeutic value (Grove et al 1987).

TABLE 1.4 Modified Fibonacci search scheme for dose escalation in a phase I study

Drug Dose	per cent increase above preceeding dose level
n*	-
2.0n	100
3.3n	67
5.0n	50
7.0n	40
9.0n	30-35
12.0	30-35
16.0n	30-35

n = starting dose in mg/m^2

In an attempt to minimize the number of patients treated at low dose levels, several modifications in the design of phase I trials have been introduced in recent years. This includes minimizing the number of new patients entered at each dose level, increasing the speed of dose escalation and escalating doses within patients. The danger of the latter is the possibility of unexpected,cumulative, or late drug toxicity (Dodion et al 1986). The advantage to the patient is that they will receive a dose closer to the MTD and therefore theoretically of greater potential benefit. Some workers believe that the only circumstance where retreatment at a higher dose in the same patient can be considered is that of a patient initially treated at a very low, clearly non-toxic dose level, provided that other patients have been treated at higher doses with no toxic effects (Dodion et al 1986).

It is important to evaluate several schedules of administration during phase I trials as the schedule of administration of cytotoxic drugs in animals has only limited predictive value for the schedule of administration of the same drug in man (Dodion et al 1986). This can be done by initiating multi-centre trials, whereby each centre gives the drug according to a different schedule. The total number of patients entered can thus be reduced by skipping dose levels proven to be safe in such companion studies. By the prompt reporting of data by the study sites plus the central co- ordination of data by a central monitoring office, on-going safety data can be shared between the centres (Grove et al 1987).

As myelosuppresion is the dose limiting toxicity for most drugs and effective measures to control this side effect are available, some EORTC centres are evaluating new dose escalation procedures (Dodion et al 1986). Initial escalations are 100% increases.When the equivalent of the mouse LD_{10} is reached, the dose is further increased by steps of 50%.

When toxic effects are observed, further increases are decided according to toxicity.

The utilization of pharmacokinetic data (specifically the area under the concentration time curve (AUC)) to guide dose escalation has received much attention over the last few years and may become standard practice in future phase I trials (Calvert and Balmanno 1987, EORTC pharmacokinetics and metabolism group 1987, Collins et al 1986). The MTD in man usually approximates the LD_{10} in mice in mg/m^2 (Goldsmith et al 1975, Collins and Grieshaber 1987). It has been shown that the correlation between AUCs obtained for the same drug in mice and in man is usually good and allows for a better prediction of the MTD in man than the use of the mg/m^2 method. Following the determination of the AUCs obtained at the first dose concentrations in a phase I trial, subsequent doses can be increased to concentrations designed to achieve the desired AUC in man, (i.e. the AUC of the LD_{10} in mice) thus making it possible to omit several stages of dose escalation. This illustrates one aspect of the importance of pharmacokinetic studies in a phase I trial.

Phase II trials.

In a phase II trial, the efficacy of a drug in specified schedules against specific tumour types is tested. The dose is determined from phase I studies and is usually one dose level below the MTD. These trials act as screens for antitumour activity and determine whether a drug is worthy of further clinical evaluation (Carter and Selawry 1977). They are not designed to give definite answers regarding the ultimate value or role of a given drug but provide information about the degree of efficacy and nature of adverse effects

against a specific tumour type when given according to one particular dose schedule (Marsoni et al 1987). A phase II study can also give further information as to the toxicity of the compound often in a more homogeneous, less heavily pre-treated group of patients. It also allows assessment of toxicity in a larger number of patients treated at the same dose.

To make testing more reliable, the NCI clinical drug development program has established an objective that each drug be evaluated independently in phase II trials in at least six different tumour types. They have also implemented uniform standards for the extent of prior therapy for patients entering these trials (Marsoni et al 1987).

Solid tumours with low growth fractions, e.g. adenocarcinomas of breast, colon, pancreas and ovary and bronchogenic carcinomas are most commonly used when screening for solid tumour activity. Traditionally these are tumours which have proven to be the most resistant to chemotherapy. Complete responses with single agents are rare and partial response rates with the most active agents are in the range of 20-30%. Therefore, the sensitivity level for defining activity can be placed lower than that for leukaemias and lymphomas against which more drugs are active (Carter et al 1977).

Marsoni et al (1987) examined the activity reported in phase II trials for 47 drugs introduced into clinical trial by the NCI since 1970. Of these drugs, 24 were rated active in at least one cancer type. The diseases most commonly responsive included lymphoma, leukaemia, urothelial cancer, small cell lung,ovarian, cervical and breast cancers. Only one drug out of 33 was considered "active" against non-small cell lung cancer, with similar results for colon cancer and melanoma.

The degree of activity of a drug depends not only on the number of patients responding, but also on the total number of patients treated with the drug i.e. the sample size. Statistical methods have been devised that dictate the number of responses and the number of patients necessary to define set levels of activity (Lee et al 1979). For example, it is common to study each new drug in at least 14 patients with a given tumour. A study with this number of consecutive treatment failures permits rejection of a response rate of 20% with a rejection error of 5%. Some authors prefer to treat at least 19 patients as 19 consecutive treatment failures permits rejection of a response rate of 15% with a rejection error of 5%. Similarly, a single response in 30 patients indicates a response rate of $<15\%$ and in 14 patients indicates a response of $<30\%$. Tables are available which allow estimates of overall response rates using the number of responses in a limited sample size (Lee et al 1979).

Rejection error is the chance of failing to send an agent on to further study when it should have received further consideration. It is generally agreed that identifying an active drug as inactive is a much more serious mistake than accepting an inactive drug as active (Lee et al 1979). Investigators will therefore accept a high chance of incorrectly identifying a drug as active, but are reluctant to take a $>5\%$ chance of incorrectly dismissing a drug as inactive (Carter and Selawry 1977).

A major criticism of phase II trials as they are run in standard practice today relates to the chance of missing active drugs because of the current pattern of patient selection (Wittes et al 1985). Those patients entered into phase II trials have usually been heavily pretreated with both conventional and often experimental cytotoxic agents. They often

have advanced disease and low performance status. Drug dosage in a phase II study is often well below the MTD as identified in prior phase I studies because of previous myelosuppressive treatment to which the patients have been exposed. This can result in inadeqate phase II evaluation in view of the steep dose-response relations observed for many cytotoxic agents (Simon et al 1985). Similarly, the tumour types tested are characteristically those generally unresponsive to chemotherapy, or recurrent following initial chemotherapy and therefore very unlikely to show response to further chemotherapy. This is reflected in an analysis of response rates for 83 cytotoxic drugs entering clinical trial under NCI sponsorship from 1970 to 1985 (Marsoni et al 1987). By far the majority of response rates were in the 0-5% range and some of the drugs that are thought of as active have pooled response rates of only 20-25%.

Wittes et al (1985) have stressed that a phase II trial should be an efficient method of detecting new active agents rather than a source of therapeutic alternatives for patients with heavily pretreated progressive disease who have little chance of responding.

To overcome these problems it has been suggested that drugs should only be tested in that patient group that is most likely to show a favourable effect (i.e. those patients with good peformance status, limited disease extent and minimum prior therapy), to maximize the chance of detecting any activity. A history of heavy drug pretreatment exerts a deleterious influence on the probability of response and therefore prejudices the chances of obtaining an accurate assessment of a new drug (Wittes et al 1985).

Not only should a favourable patient group be selected but one should also select for

tumour type. Instead of trialing agents against moderately unresponsive solid tumours (for example NSCLC or pancreatic cancer), one should utilize tumour types that are known to be responsive to chemotherapy at a relatively early stage of disease.

This is clearly not ethical with potentially curable malignancies such as Hodgkin's lymphoma, where no new agent is likely to be trialed until the patient has been exposed to at least 5-7 drugs which have proven activity in this disease, but may well be feasible in those tumours sensitive, but non-curative by chemotherapy (e.g. small cell lung cancer, ovarian or breast cancer following first relapse). Wittes et al (1985) have suggested that phase II agents might be used first line in certain diseases such as slowly progressive metastatic breast cancer, advanced stage ovarian cancer or indolent lymphomas. A carefully monitored trial of a new agent with change to a standard drug combination immediately upon disease progression or evidence of non-response is unlikely to prejudice chances of responding to standard therapy. Aisner (1987) has suggested that new agents might be offered to small cell lung cancer patients who have achieved a stable partial response after first line conventional therapy or to offer phase II agents as initial therapy for patients with extensive disease.

There is frequently great variability in the response rates reported from different phase II studies of the same agent (Simon et al 1985). This can presumably be related to differences in patient selection, response criteria, assessment of response, protocol compliance and dose modification between different institutions and groups. To overcome this problem, Simon et al (1985) have suggested that phase II trials be run according to randomized designs. Patients could be randomized to receive either the phase II agent

under trial, or a single agent already known to have activity. This might overcome the problem of patient selection in that the conclusion about the activity of the new agent would depend on the response rate observed in the control group.Another type of randomized design involves two or three arms, each using an experimental drug. Alternatively, the new drug might be included as part of a randomized study in combination with other agents (Aisner 1987).

It is now possible to develop clonogenic cell lines from many human tumours that are considered to be closely related to tumour stem cells in vivo. The human tumour stem cell assay is an in vitro method proposed as a means of screening both potential anti-cancer agents and assessing the activity of drugs against a given type of tumour (Salmon et al 1978). The sensitivity of a human tumour cell line to a variety of drugs can therefore be tested in a manner analogous to testing for antibiotic sensitivity in patients with bacterial infections (Selby et al 1983). This approach can potentially identify phase I and II drugs suitable for specific patients for the treatment of given tumour types and would theoretically eliminate the need to subject patients (who would be predicted not to respond) to toxic side-effects. Such in vitro phase II trials would give a preliminary projection of the anti-tumour spectrum and "response-rate" of specific tumour types to new agents (Salmon 1984). The human tumour stem-cell assay may thus provide an alternative to the current phase II testing approach (Salmon et al 1981).

However, although a highly significant correlation has been observed between in vitro tumour resistance to specific drugs and failure of patients to respond to the same drug clinically, Salmon (1984) has drawn attention to the significant false positive rate seen,

(those patients whose cells exhibited in vitro sensitivity but failed to show a clinical response). Von Hoff et al (1983) carried out a prospective clinical trial to evaluate the usefulness of human tumour stem cell assays in selecting single agent chemotherapy. There was a 60% true positive and an 85% true negative rate for predicting for response or lack of response of an individual patient's tumour to the single agent. However, only 41% of 604 trials could be directed by the cloning results mainly because of inadequate tumour growth. A number of technical and logistic difficulties limit the routine use of the human tumour stem-cell assay system for selection of chemotherapy, for example limitations in growth rate and cloning efficiency for most tumour types, the lack of effective drugs and the poor clinical condition of many patients whose tumours are submitted (Salmon 1984). As suggested by Selby et al (1983), although such stem-cell assays have great potential value in the study of the biology and chemosensitivity of human tumours, their routine use in place of clinical trials would seem rather premature.

"Since there are no reliable laboratory predictors of efficacy for specific human cancers, drug development will continue to require extensive testing in human subjects, an endeavor that is never without ethical dilemmas" (Marsoni and Wittes 1984).

A limited phase II trial in NSCLC was undertaken in this study to confirm the dose identified in phase I, to gain further information about the toxicity of the agent and to identify early disease activity in patients typical of future phase II studies.

NON - SMALL CELL LUNG CANCER AND PHASE II TRIALS

Carcinoma of the lung is the leading cause of death from malignant disease in males and is steadily overtaking breast and colon cancer to become the leading cause of malignant death in females (Silverberg and Lubera 1987).

Carcinoma of the lung can be divided into 4 major groups according to histological classification: - small cell, squamous, adeno- and large cell carcinomas. The latter three are often grouped together and referred to as non- small cell lung cancer (NSCLC) because of common features in natural history and response to treatment.

Small cell lung cancer is an aggressive malignancy characterized by its rapid course and early widespread dissemination. The median survival of untreated patients is very short, in the order of 6-12 weeks (Harvey and Kolbe 1986). This cancer is highly sensitive to chemotherapy however, which gives significant improvement in medium survival and a small number of long-term disease-free survivors (Hansen et al 1980). Conversely, NSCLC is notoriously unresponsive to chemotherapy (Hoffman et al 1983). The only possibility of cure is by surgical resection of localized disease, or rarely following radical radiotherapy (Mulshine et al 1986). In Auckland, only 20% of patients have resectable disease at the time of presentation and only 30% of patients believed to have had curative surgery survive five years (Harrison 1985). Only 5-10% of patients receiving radiotherapy with curative intent are in fact cured, such that the major role of radiotherapy in NSCLC is in palliation (Harrison 1985).

The frequency of lung cancer, the extent of disease at presentation (which usually precludes local therapy) and the poor results of treatment with surgery and radiotherapy have led to much effort investigating the use of chemotherapy in this disease.

A large number of single agents have been shown to have some activity against NSCLC, but the objective response rates are usually less than 20% (Zinreich et al 1985, Hoffman et al 1983). From pooled trials of the use of single agents in NSCLC, agents showing "some activity" include ifosfamide (reported response rates 7-32%), cisplatin (6-32%), mitomycin C (9-49%), vindesine (6- 31%), adriamycin (6-38%), etoposide (3-21%) and methotrexate (0- 26%) (Bakowski and Crouch 1983). Responses are generally partial with complete responses rarely seen. The response duration is short (2-4 months) and the overall median survival generally less than six months (Zinreich et al 1985, Greco 1986). Although somewhat better results have been claimed for several drug combinations, response rates are still usually less than 20% (Aisner and Hansen 1981, Zinreich et al 1985). For example, an initial response rate of 44% for a regimen of methotrexate, Adriamycin,cyclophosphamide and CCNU was followed by a reported response rate of 12% for the same drug combination in a subsequent study by the Eastern Co-operative Oncology Group (ECOG) (Zinreich et al 1985). Similarly, early response rates using PAC (platinum, Adriamycin and cyclophosphamide) ranged from 28-48% compared to 22% reported by ECOG (Zinreich et al 1985). Takita et al (1979) reported a 66% response rate for the use of PACCO (PAC + lomustine and vincristine) but in a similar study, a total response rate of 17% was reported by Whitehead et al (1980).

The most promising regimens to date are those containing cisplatin in combination with vinblastine, vindesine or etoposide which have resulted in reliably reproducible response rates of between 30-40% (Hoffman et al 1983, Sculier and Klastersky 1984, Klastersky 1986, Elliot 1986).

The marked variability in response and survival rates reported for both combinations and single agents in NSCLC might reflect the variations in pre-treatment characteristics of the patient populations under study (Elliot 1986, Zinreich et al 1985). Patients differ not only with respect to previous treatment, but also in performance status, stage of disease and cell type. These and other prognostic factors e.g. age, weight loss, serum lactic dehydrogenase and prior therapy, can all independently predict for response and survival (Aisner and Hansen 1981, Mulshine et al 1986, O'Connell et al 1986). Very few randomized studies have been carried out in NSCLC to compare different drugs or drug versus placebo in groups of patients matched for such prognostic factors.

The dose and schedules used for individual drugs can also show considerable variation between trials. This is especially relevant for such agents as cisplatin, which appears to have a dose-response relationship in NSCLC (Gralla et al 1981) and etoposide, which has been shown to be highly schedule dependent (Krook et al 1989). Response in NSCLC can also be difficult to assess, especially in those patients previously treated with radiotherapy or in those centres where computerized tomography is not routinely available. Other reasons for the varying response and survival data include inadequate and variable criteria of response, insufficient sample size, different criteria for patient selection and the exclusion of "inevaluable" cases (Staquet et al 1983).

Whilst many studies have claimed benefit for chemotherapy on the grounds that responders live longer than non- responders, this argument is fallacious and may only indicate patient selection (Klastersky 1986). It remains to be shown that response to chemotherapy can be related to survival benefit (Bonomi 1986). As the total response rate for any given drug or combination is well below 50%, it is unlikely that median survival will be affected (Aisner and Hansen 1981, Greco 1986). Randomized studies have not yet proven a survival advantage for patients receiving chemotherapy compared to those who receive only symptomatic and supportive care (Bonomi 1986 , Greco 1986). For example, Woods et al (1985) compared a platinum-vindesine combination to a"non-chemotherapy" arm. Although the response rate in the treated patients was 30% (and 0% in the patients not receiving treatment), the median survival of both groups was similar, at 24 and 21 weeks respectively.

In the face of these low response rates, chemotherapy induced toxicity must be taken into account as most patients will experience toxicity without benefit from therapy (Klastersky 1986) and with the most "active" regimens often containing cisplatin,the toxicity may be considerable.

In summary, none of the most "active" drug combinations currently available can be regarded as having a standard place in the management of NSCLC (Elliot 1986).

The failure of any chemotherapy to date to produce a reliable survival benefit or improved quality of life compared to untreated patients receiving supportive care only, suggests that new drugs may be ethically used as primary therapy in NSCLC

(Aisner and Hansen 1981). NSCLC remains an "investigative proposition" (Elliot 1986). Only by the evaluation of new drugs and when indicated new combination chemotherapeutic regimens, "can we hope to increase the objective and complete response rates to give rise to meaningful survival rates" (Zinreich et al 1985).

It was this rationale which lead to the use of CI-921 in a phase II study in patients with NSCLC.

THE RELEVANCE OF PHARMACOKINETICS IN CLINICAL TRIALS

The primary goal of pharmacokinetic studies is to define the absorption (of orally administered drugs), the extent of distribution, the rate of metabolism and elimination and the total exposure of a compartment to a drug (Erlichman et al 1980). Pharmacokinetic studies give a quantitation of the time course of drugs and active metabolites in the body.

The rational development of new anti-cancer agents should ideally include kinetic analysis during pre-clinical and phase I clinical evaluation in order to develop the optimal clinical schedule and dose for the best therapeutic management of the patient (Tognoni et al 1980, Powis 1985). The reasons for performing pharmacokinetic studies are listed below :

-to determine whether the drug follows linear or saturation kinetics. This is important as the latter can result in severe toxicity with only small increments of dose (Calvert and Balmanno 1987).

-to define a therapeutic range which is particularly important in cancer therapy because of the narrow therapeutic index of most anti-cancer agents (Collins and Grieshaber 1987, Grieshaber and Marsoni 1986).

-to ensure appropriate dosing, as in the case of methotrexate where the administration of "rescue" agents can be tailored according to serum drug levels (Erlichman et al 1980, Dodion 1983).

-the effect of organ dysfunction or drug elimination can be determined and drug doses reduced if necessary in the case of impaired liver or renal function to avoid

toxicity. Alternatively, one may proceed to maximum dose if the organ dysfunction does not influence kinetics (Powis 1982, Erlichman et al 1980, Dodion 1983).

-to relate anti-cancer activity (or lack thereof) to blood concentrations achieved by tolerable doses (Collins and Grieshaber 1987).

-to demonstrate why a drug is inactive in a clinical setting. For example, a drug may be converted to an active form in animals but not in man (Rutty et al 1982).

-to ensure that the drug concentrations obtainable in humans are not vastly lower than those necessary for anti-cancer effects in animals (Calvert and Balmanno 1987).

-to predict or recognize drug interactions.

-to compare drug analogues.

-to ensure patient compliance in taking drugs given orally (Tognoni et al 1980).

-to guide dose escalation in a phase I trial, assuming drug exposure (AUC) to be a better predictor of the MTD than dose in mg/m^2 (Calvert and Balmanno 1987, EORTC pharmacokinetics and metabolism group 1987, Collins et al 1986, Powis 1985).

The rapid growth of pharmacokinetic studies of anticancer drugs in recent years is based on the expectation that a greater understanding of drug disposition and metabolism will lead to more effective drug use. This is especially important with antineoplastic drugs because of the narrow therapeutic index of most agents (Tozer 1983). To this end, the pharmacokinetic characteristics of CI-921 were studied in both the phase I and II clinical trials as discussed in Chapter 4.

THE METABOLISM OF CI-921 - GLUTATHIONE.

CI-921 is structurally similar to amsacrine and is therefore likely to follow similar pathways of metabolism. As previously discussed (see page 10), amsacrine is metabolized via hepatic microsomal oxidation to a reactive quinone diimine prior to conjugation with glutathione to form an inactive product that is excreted in the bile. Glutathione (GSH) plays an important role in the biotransformation of many xenobiotics and its place in the metabolism of amsacrine and CI-921 has been investigated in this study (see Chapter 6).

The following introduction reviews the function of GSH in man and its importance with respect to antineoplastic therapy.

The role of glutathione in the cell.

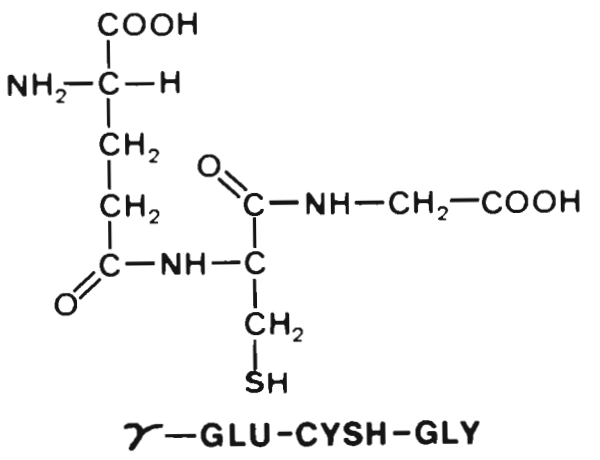


Fig.7 Chemical Structure of Glutathione

Glutathione (GSH) (δ-L-glutamyl-L-cysteinyl-glycine) is a naturally occurring tripeptide (Fig. 7). It is the predominant intracellular non-protein sulphydryl in most cells and has several important physiological functions including the maintenance of cell membranes and cytoskeletal organization, an involvement in protein and DNA synthesis, the metabolic processing of endogenous compounds (e.g. oestrogens, prostaglandins, leukotrines), the maintenance of cellular reduction/oxidation states and protection against oxygen toxicity. One of its most important functions however is in drug and xenobiotic biotransformation (Meister 1983, Meister and Anderson 1983, Orrenius and Moldeus 1984).

The biosynthesis of GSH from its constituent amino acids is catalyzed by the enzymes δ-glutamyl cysteine synthetase and glutathione synthetase (Fig. 8). Under conditions of stimulated GSH consumption, the availability of cysteine appears to be rate limiting for GSH resynthesis (Moldeus 1984). GSH is synthesized in virtually all cells, but the liver is among the organs with the highest content and is the major source of plasma GSH. It is not known whether changes in plasma GSH accurately reflect changes in hepatic GSH (Pyke et al 1986, Kaplowitz et al 1985). In mammalian cells, the majority of GSH exists in the reduced form (0.5-10mM). Oxidation of reduced GSH either non-enzymatically or by the action of glutathione peroxidase (GPO) yields oxidised glutathione (GSSG). NADPH dependent reduction of GSSG by glutathione reductase (GR) maintains the intracellular concentration of GSSG at very low levels (5- 50μM) (Fig.9) (Arrick and Nathan 1984).

A number of factors dictate the intracellular concentration of GSH. Levels decline with

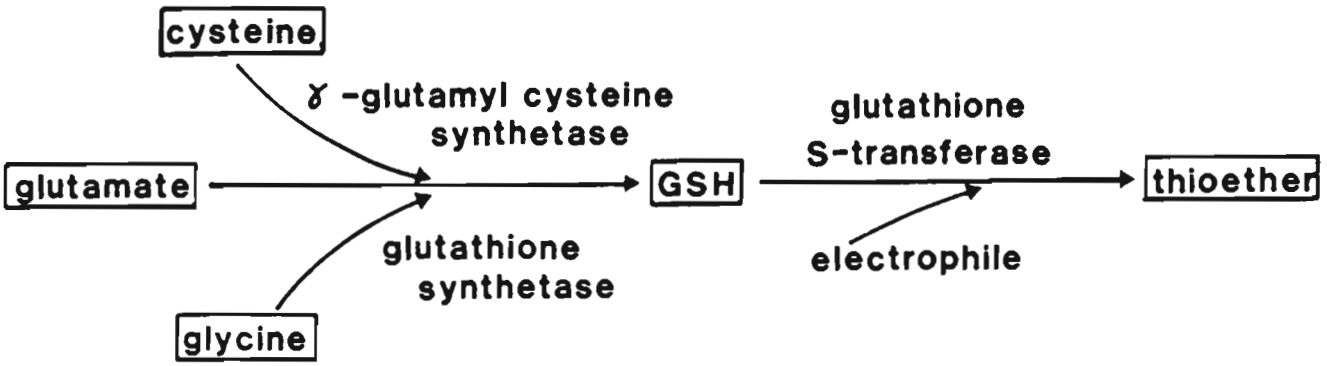


FIG. 8. Pathway of synthesis and metabolism of glutathione.

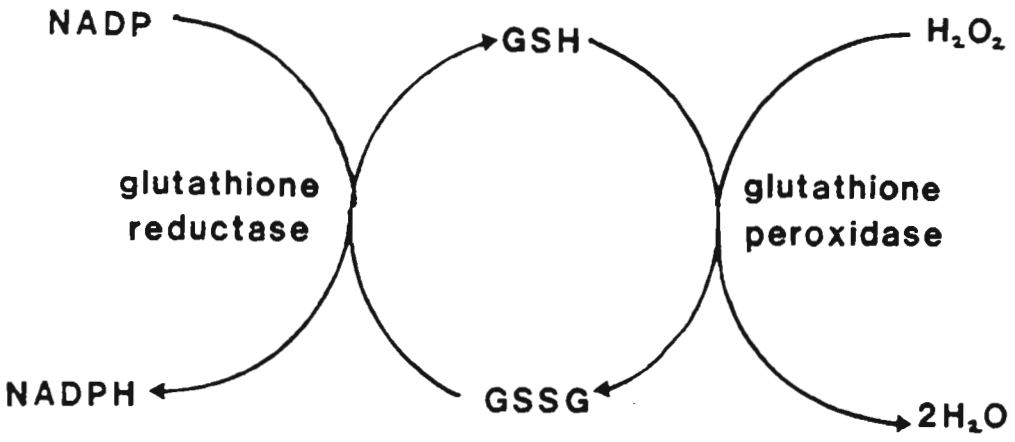


FIG. 9. Glutathione oxidation-reduction cycle.

age (Al Turk et al 1987, Hazelton and Lang 1980) (this has been implicated in the process of ageing), following extreme physical activity (Pyke et al 1986) and with protein deprivation (Bidlack et al 1986).

Beutler and Gelbart (1985) have demonstrated lower plasma GSH levels that were not associated with chemotherapy or type of neoplasm in patients with a variety of malignant disorders. A diurnal variation of plasma GSH is well described (Akerboom and Sies 1981).

Interaction of GSH with antineoplastic therapy.

Two of the most important functions of GSH with respect to antineoplastic therapy are its role in xenobiotic biotransformation and in defence against oxygen toxicity (Russo et al 1986).

GSH is a strong nucleophile and as such can react with electrophilic metabolites of cytotoxic drugs. The conjugates formed are more water soluble and can be excreted in bile or metabolized further to mercaptopuric acids. Conjugation reactions are catalysed by glutathione-S-transferases which are widely distributed in hepatic and extra-hepatic tissues in man (Moldeus 1984, Jacoby 1985). GSH is involved in the metabolism of the following cytotoxic agents : melphalan, cyclophosphamide, the nitrosoureas, 6-thiopurine and amsacrine (Arrick and Nathan 1984).

GSH serves as a reducing agent in the metabolism of various peroxides and free

radicals. Antineoplastic agents that contain a quinone moiety (e.g. doxorubicin, daunorubicin and mitomycin-C) are believed to undergo metabolic activation by microsomal enzymes to free radicals, themselves toxic, which can interact with oxygen to generate free radical O_2 intermediates. These free radicals can be important determinants of drug toxicity because of their ability to oxidize membranes which results in cell damage. GSH has an important role in "scavenging" active radicals and thus preventing cellular damage (Kaplowitz et al 1985, Arrick and Nathan 1984).

GSH may play a role in the detoxification of radiation induced free radicals and/or the repair of critically damaged cell structures, but the precise mechanism by which GSH is involved in the cellular defence against radiotherapy has not been determined (Arrick and Nathan 1984).

GSH may also play a role in drug activation. Bleomycin is believed to affect its antitumour activity via an oxygen dependent mechanism of DNA cleavage. GSH binds to a ferric iron-bleomycin complex and reduces it to the active ferrous-iron complex (Arrick and Nathan 1984). Similarly, some of the new cisplatin analogues exist as complexes that appear to be activated following reduction by glutathione or other intracellular reducing agents (Eastman 1987).

The effect of changing GSH concentrations on antineoplastic therapy.

Because GSH is involved in the metabolism of many antineoplastic agents and in

the cellular damage induced by radiotherapy, the availability of GSH is theoretically an important determinant of the therapeutic efficacy and/or toxicity of anticancer therapy as discussed below.

i) Drug Resistance

The development of resistance to cytotoxic agents can be related to changes in GSH concentration. Human ovarian cancer cell lines selected for resistance have been shown to have GSH level two to four times higher than that of the parent line (Louie et al 1985, Hamilton et al 1985). Shrieve and Harris (1986) were able to sensitize cultured EMT6/SF tumour cells to cisplatin, phenylalanine mustard and nitrogen mustard following depletion of cellular GSH with buthionine sulphoximine (BSO) to less than 5% of control levels. Hamilton et al (1985) developed a series of human ovarian cancer cell lines with acquired resistance to melphalan, cisplatin and Adriamycin. This resistance could be reversed by lowering GSH levels. Similarly, both Ozols et al (1987) and Green et al (1984) demonstrated an increase in the cytotoxic action of melphalan in both in vitro and in vivo of human ovarian cancer following depletion of cellular GSH. Russo et al (1986) treated human lung fibroblast and lung adenocarcinoma cell lines with oxothiazolidine-4- carboxylate (an intracellular cysteine delivery system) to elevate intracellular GSH levels and were able to demonstrate an increased tolerance of these cells to both melphalan and cisplatin. These studies suggest that GSH depletion has potential value as an adjuvant in chemotherapy and radiotherapy with respect to increasing cytotoxicity or overcoming tumour resistance.

ii) Toxicity

Increasing GSH concentration may protect against unwanted toxicity (Meister 1983). This concept has been extensively studied with paracetamol, which is metabolized to a highly reactive intermediate and undergoes detoxification by conjugation with GSH (Miners et al 1984). Hepatotoxicity in mice following treatment with paracetamol is greatly increased if animals are pre-treated with BSO to deplete hepatic GSH, whereas thiol-containing nucleophiles (e.g. methionine and N- acetylcysteine (NAC)) are protective (Miners et al 1984). The hepatotoxicity in mice treated with paracetamol is exacerbated by concurrent treatment with Adriamycin which also depletes GSH (Wells et al 1980).

Olson et al (1980) demonstrated reduced levels of GSH in the heart, liver and red blood cells of mice following Adriamycin. Depletion of endogenous GSH by diethylmaleate (DEM) significantly potentiated the lethality of the drug whereas pre-treatment with thiol compounds provided dramatic protection.

The protective effect with respect to bone marrow toxicity of "priming" with low dose cyclophosphamide is thought to be related to the increase in GSH and glutathione transferase levels that has been demonstrated in mouse bone marrow following a priming dose (Carmichael et al 1986a, Adams et al 1985). This protective effect is lost following pre- treatment with BSO to block the rise in GSH (Carmichael et al 1986b).

iii) Radiation-induced toxicity

Irradiation leads to a decrease in the cellular thiols that protect cells against the effects

of irradiation. Tumours that are relatively resistant to irradiation and have high GSH concentrations would be expected to become more radiosensitive after treatment with inhibitors of GSH synthesis (Meister 1983). In support of this theory, Louie et al (1985) demonstrated a significant increase in radiation sensitivity in human ovarian cancer cell lines following depletion of GSH. Similarly, Chinese hamster ovary (CHO) cells under hypoxic conditions were sensitized to radiation following reduction of cellular GSH content to less that 5% of control (Bump et al 1982).

Misonidazole is a hypoxic cell radiosensitizer. GSH is thought to protect against misonidazole cytotoxicity by reducing the free radicals generated from the metabolism of misonidazole in hypoxic cells. Bump et al (1983) pretreated CHO cells with DEM to deplete intracellular GSH and demonstrated a substantial decrease in the shoulder of the survival curve of misonidazole treated hypoxic cells.

If the metabolism of amsacrine and CI-921 involves the detoxification of an active intermediate by GSH conjugation, changes in the concentration of cellular GSH might be expected to affect the therapeutic efficacy and /or toxicity of these drugs by interfering with their metabolism. The role of GSH in the metabolism and toxicity of amsacrine and CI-921 was investigated as described in Chapter 6.

CHAPTER 2

PHASE I CLINICAL TRIAL

CHAPTER 2. A PHASE I CLINICAL TRIAL OF CI-921.

INTRODUCTION

The first stage of testing a new anticancer drug in man (a phase I trial) involves the determination of the maximum dose that can safely be given and an appraisal of the spectrum of toxicities and their reversibility (see page 46). Three phase I studies of CI-921 were run simultaneously in Auckland, Ohio and Peurto-Rico, each centre using the drug according to a different treatment schedule.

In Auckland the phase I clinical trial of CI-921 using a three-day schedule was started in May 1986, following extensive pre-clinical testing in the Auckland Cancer Research Laboratory.

PATIENTS AND METHODS

Patients

All patients had advanced histologically documented carcinomas for which there was no conventional therapy or which had recurred following such treatment. Patients aged between 18 and 80 years were eligible. They were required to have a performance status of $\leq$ 2 on the ECOG scale, being ambulant, capable of self-care and spending less than 50% of day-time in bed (Appendix 1.1) and were expected to survive more than 6 weeks.

Patients were to have recovered from prior chemotherapy or radiotherapy induced toxicity. All patients had to have adequate pre-treatment bone marrow function (absolute granulocyte count (AGC) $\geq$ 1.5 x 10^9/l, platelets $\geq$ 100 x 10^9/l), liver function (alkaline phosphatase, aspartate transferase, bilirubin $\leq$ 1.5 x upper limit of normal) and renal function (serum creatinine $\leq$ 0.13 mmol/l or $\leq$ 0.16 if creatinine clearance $\geq$ 1.0 ml/sec). Because the cardiac toxicity of amsacrine has been associated with hypokalaemia, a serum potassium was measured within the 24 hours prior to treatment and corrected if necessary to within normal limits. Patients were ineligible if they had received radiotherapy in the 4 weeks prior to treatment with CI-921 (except small port radiotherapy to relieve symptoms), other chemotherapy in the 3 weeks prior to treatment (6 weeks for nitrosoureas or mitomycin-C), or major surgery within 14 days. Patients with more than one malignancy (except non-melanomatous skin cancer or uterine cervical carcinoma-in-situ), life-threatening illness unrelated to the tumour (including significant cardio-vascular disease), serious infection within the prior month, or who required concurrent chemotherapy, radiotherapy or surgery were excluded.

Assessment of Disease and Toxicity

Pre-treatment evaluation included a full history and physical examination, tumour measurement and performance status and the following laboratory studies: a complete biochemical profile (including serum electrolytes, liver function and renal function), full haematological profile with differential white blood count and urinalysis. Other baseline investigations included chest x-ray, electrocardiogram and echocardiogram. Additional x-ray, radionuclide and computerized tomographic studies were performed as appropriate for

assessment and measurement of disease status.

Prior to entry, all patients were informed of the experimental nature of the treatment and advised of the possible side-effects, risks and limitations of the drug. Patients were required to sign a consent form (Appendix 1.2) which together with the protocol, had been approved by the local institutional ethical committee. All patients were advised that they were able to leave the study at any time without prejudice to future care. During treatment, patients were evaluated weekly with physical examination and determination of weight and performance status. Biochemical and haematological screens were taken twice weekly. Chest x-ray, electrocardiogram and urinalysis were performed at the start of each cycle of therapy. Echocardiograms were repeated after every alternate cycle and at the end of the study period. Standard ECOG criteria were used for evaluation of toxicity (as defined in Appendix 1.3) with the exception of anaemia. Grade I haemoglobin toxicity was defined as a fall of 1.5 - 3 g/dl in haemoglobin, grade 2 as 3 - 3.9 g/dl, grade 3 as 4 - 4.9 g/dl and grade 4 as $\geq$ 5 g/dl. All patients were evaluated for toxicity. Standard WHO criteria were used for the evaluation of response (Miller et al 1981). Patients were evaluable for response if they completed one planned treatment cycle of three consecutive daily doses of CI-921.

Treatment.

CI-921 as the hydroxyethane sulfonate salt was reconstituted in sterile water and dissolved in 200ml 5% dextrose. Patients were hospitalized for drug administration. The total dose of CI-921 for each cycle of therapy was divided over three days. Each dose was given

intravenously into a peripheral arm vein via an intravenous cannula over 15 minutes, followed by a 50ml flush of 5% dextrose. Cycles of therapy were repeated three weekly. Vital signs were monitored during treatment and at 30 and 60 minutes following completion of the infusion. The starting dose was 39 mg/m^2 (13 mg/m^2 daily for three days). Doses were escalated according to an escalation schedule based on the modified Fibonacci search scheme (Table 2.1). At least three patients were treated at each dose level unless information was available from another trial centre documenting absence of toxicity allowing those levels to be omitted. Escalation within patients was permitted with each patient eligible to proceed to the next higher dose level providing they experienced no non-haematological toxicity $\geq$ grade 2, no AGC < 1.5 x 10^{9}l, or platelet count < 75 x 10^9/l and haematological recovery had occurred. Patients experiencing an AGC between 1.0 and 1.5 x 10^9/l were eligible for retreatment at the same dose level. Those having an AGC nadir <1.0 x 10^9/l were eligible for retreatment at one dose level below their last dose level.

Dose limiting toxicity was defined as AGC nadir < 0.5 x 10^9/l (or a platelet nadir of < 50 x 10^9/l), recovery to < 1.5 x 10^9/l AGC (or < 100 x 10^9/l platelets) by day 35 after the first day of treatment or $\geq$ grade 2 non-haematological toxicity (excluding alopecia). The maximum tolerated dose (MTD) was that dose which produced dose limiting toxicity in at least of 50% of patients initially treated at that dose level.

TABLE 2.1 Dose escalation in the phase I trial.

Total dose* (mg/m^2)	No. of patients	No. of courses
39	2	2
108	3	3
144	6	6
192	4	4
288	3	4
432	4	4
540 §	1	1
648	8	13
810	2	2

*total dose divided over three days

§ patient no. 005 who had received multiple doses and therefore a smaller dose increment at this level

RESULTS

Patient Characteristics (Table 2.2)

Sixteen patients (12 men and 4 women) were studied. The age range was 21 - 70 years (median 57). Tumour types were: non-small cell lung cancer (6 patients), malignant melanoma (4 patients), breast cancer (2 patients) and small cell lung, gastric, pancreatic and head and neck cancers (1 patient each). At the time of entry into the trial, three patients had a performance status of 0, twelve patients a performance status of 1, and one patient a performance status of 2. Previous therapy included surgery in six patients, radiotherapy in six patients and chemotherapy in five patients. Six patients had had no prior treatment. A total of 39 treatment courses were given. The median number of courses per patient was two (range 1 - 6).

TABLE 2.2 Patient characteristics - Phase I clinical trial.

No. patients	16
Sex ratio	12/4
Median age (range)	57 (21-70 years)
Tumour types	**No. patients**
Lung - NSCLC	6
- SCLC	1
Breast	2
Melanoma	4
Gastric	1
Pancreatic	1
Head and neck	1
Prior therapy	
Nil	6
Surgery (S)	1
Radiotherapy (RT)	3
Chemotherapy (CT)	1
S + RT	2
S, RT +CT	2
RT + S	1

Treatment

The starting dose was 39 mg/m^2 (13 mg/m^2 daily x 3). It was possible to start at this dose because there was information from another trial centre that lower doses were safe. Similarly, dose levels 60 mg/m^2 and 84 mg/m^2 (total dose) were omitted as safety information was available. Otherwise doses were escalated to 810 mg/m^2 as shown in Table 2.1.

Toxicity (Table 2.3)

-Haematological

Neutropenia was the dose limiting toxicity. No consistent myelosuppression was noted at doses $\leq$ 288 mg/m^2 (96 mg/m^2 daily x 3). Of four patients treated at 432 mg/m^2 (144 mg/m^2 daily x 3), two patients developed grade 1 neutropenia on days 10 and 11, with recovery by days 16 and 15 respectively and one heavily pretreated patient had grade 3 neutropenia with toxicity developing on day 8 and recovery by day 22. Neutropenia occurred in each of 13 courses at 648 mg/m^2 (216 mg/m^2 x 3), developing from day 7 to 13 (median day 8) with recovery by days 13 to 28 (median day 18). Two patients were treated at a dose of 810 mg/m^2 (270 mg/m^2 x 3). One patient had a grade 3 and the other a grade 4 nadir with neutropenia developing at days 7 and 8 with recovery by day 21. There was no treatment related death and no episode of severe sepsis. One patient developed a superficial skin infection at an implanted venous access site which responded to oral antibiotics. A grade 1 fall in haemoglobin was seen in one course at 288 mg/m^2, in three

TABLE 2.3 Frequency and grade of toxicity at dose levels ≥288mg/m^2 (total dose) - Phase I.

Dose* (mg/m^2)	288				432				648				810			
No. of courses	4				4				13				2			
Toxicity grade §	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
WBC	-	-	-	-	2	-	1	-	1	2	9	1	-	-	1	1
Nausea/vomiting	2	-	-	-	3	-	-	-	5	-	-	-	1	-	-	-
Phlebitis	1	-	-	-	2	1	-	-	5	1	-	-	1	1	-	-
Mouth ulcers	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Alkaline Phosphatase	-	1	-	-	1	-	-	-	5	1	-	-	-	-	-	-
Aspartate Transferase	-	1	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Renal	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-

*total dose over 3 days

§ ECOG toxicity criteria

courses at 432 mg/m², eight courses at 648 mg/m² and in one course at 810 mg/m². A grade 2 fall in haemoglobin was seen in single patients treated at 432, 648 and 810 mg/m². Thrombocytopenia did not occur.

-Non-haematological

i) Nausea and vomiting.

Mild nausea, vomiting and anorexia (Grade 1) was seen in eight of 18 patients treated at doses $\geq$ 192 mg/m². Nausea was generally well controlled with low dose oral metoclopramide. There was no episode of intractable vomiting.

ii) Venous irritation and phlebitis.

Most patients complained of mild irritation at the infusion site during drug infusion. Pain in the infusion arm occurred in six patients at $\geq$ 288 mg/m² necessitating an increase in the infusion volume to 250 ml followed by a 300 ml flush of 5% dextrose. Grade 2 thrombophlebitis with painful swelling and erythema in the infusion arm developed in single patients at 432 mg/m² (1 of 4 treatments), at 648 mg/m² (1 of 13 treatments) and at 810 mg/m² (1 of 2 treatments). There was no skin ulceration and the pain and swelling resolved within five days with local anti-inflammatory creams alone. In two patients inadvertent extravasation of CI-921 occurred during infusion, but this was not painful and did not result in soft tissue damage.

iii) Mouth ulcers.

Painful mouth ulcers (grade 2) occurred in one of two patients treated at 810 mg/m².

iv) Elevation of liver enzymes.

One patient developed a grade 1 increase in alkaline phosphatase (ALP) at 432 mg/m^2 without recovery to normal values and a grade 2 increase in ALP and aspartate transferase (AST) in a following cycle at 288 mg/m^2. This patient was subsequently shown to have liver metastases and the progressive deterioration in liver function which continued until her death two months later was probably related to this abnormality rather than to the treatment. Five of eight patients developed a transient grade 1 increase in ALP at 648 mg/m^2 on day 4 with recovery by days 7-10. One of these patients had developed a grade 2 elevation of ALP in a previous cycle, and a grade 1 increase in AST during each of three cycles at 648 mg/m^2.

v) Renal function.

One patient showed a transient grade 1 elevation of serum creatinine at 810 mg/m^2 with complete recovery within four days.

vi) Miscellaneous.

No alopecia, cardiac (see Chapter 5), CNS or pulmonary toxicity occurred. Post mortem examinations were performed on three patients only. No unexpected findings unrelated to malignancy were detected.

Response

No patient achieved an objective response. One patient with a previously rapidly growing small cell cancer of lung had very slow disease progression over three cycles of treatment

but did not meet the criteria of static disease.

DISCUSSION

CI-921 was found to be a relatively non-toxic drug, well tolerated by patients and easier to administer than amsacrine. While amsacrine must be dissolved in dimethylacetamide before dilution in a dextrose solution, CI-921 is more soluble and does not require organic solvents.

When given according to this protocol, neutropenia was a dose-limiting predictable toxicity. Myelosuppression developed at a median of eight days, but bone marrow recovery was generally rapid with recovery of pre-treatment counts within a median of ten days. The effect on haemoglobin was minor and there was no effect on platelet count. Venous irritation in the arm both during and following drug infusion was common. This was overcome to some extent by the use of larger infusion volumes, but in spite of this, at the higher doses, three patients (in 3 of 19 treatments) still developed grade 2 thrombophlebitis. Ulceration did not occur and resolution was rapid. In two patients there was inadvertent extravasation of drug during infusion without sequelae, suggesting that CI-921 is not a vesicant.

The maximum tolerated dose of 810 mg/m^2 (270 mg/m^2 x 3) was determined by the development of grade 4 neutropenia and grade 2 thrombophlebitis and oral toxicity. In contrast, in a parallel phase 1 study at another centre when CI-921 was given in a single dose repeated three weekly, the dose limiting toxicity was reversible renal impairment at 648 mg/m^2 although grade 3 and 4 myelosuppression was also seen (Robert et al 1987).

In a third phase 1 study CI-921 was given in a weekly schedule (for 3 consecutive weeks) and repeated every four weeks. In this trial the MTD was 340 mg/m^2/dose (rather than 270 mg/m^2/dose as originally anticipated) as determined by myelosuppression (Grove et al 1987) (Table 2.4).

The identification of renal toxicity in the single bolus study suggests that renal function needs to be followed closely in future studies.

Because of the widespread nature of the disease and obvious progression of malignancy in patients at time of death, autopsies were not routinely performed. No unexpected findings, unrelated to malignancy were detected in the few patients on whom post mortem examinations were done.

One of the difficulties in performing phase I studies is the need, for safety reasons, to start at extremely low doses with the consequence that patients entered early are usually submitted to subtherapeutic doses (see page 47). In an attempt to maximize the therapeutic potential for patients entered into the trial and reduce the number of patients entered at subtherapeutic dose levels, a number of modifications were undertaken in the design of this trial. These included the escalation of dose within patients and the "skipping" of dose levels already shown to be without toxicity in other centres. Close communication with the other trial centres and central co-ordination of trial results allowed omission of four treatment levels in this study (Table 2.4.3). If "within patient" dose escalation had not occurred and three new patients had been entered at each dose level using a standard modified Fibonacci dose escalation scheme, a total of 36 patients would have been required.

We entered 16 only, a reduction of 44%. There has been concern as to the safety of "within patient" dose escalation in phase I trials primarily because of the possibility of unexpected cumulative or late drug toxicity (see page 49), but this was not seen in any patient receiving CI-921 and was not expected following preclinical toxicity testing.

Tables 2.4.1 - 2.4.3 show the number of patients that were required to reach the MTD in each of the three trial centres. If three separate and independent phase 1 studies of CI-921 had been conducted using standard modified Fibonacci dose escalation schemes, a total of 126 patients would have been required. In the studies as performed and analysed by Grove et al (1987), predicting an MTD of 270 mg/m^2/dose in trial centre 2, the total number of patients entered in the three centres was 64, a reduction of 49%. This also served to reduce the time to completion of the phase I programme for CI-921.

Following the determination of a MTD for CI-921, phase II (efficacy) testing was able to proceed as described in the following chapter.

TABLE 2.4 Dose escalation methods used in each of the 3 trial centres for the phase I study of CI-921.*

TABLE 2.4.1 Trial centre 1 (Puerto Rico): single dose repeated every three weeks.

Level	Dose (mg/m^2)	Approx. % increase	No. new/total pts. at each level
1	4	-	3/3
2	8	100	2/3
3	12	50	2/3
4	24	100	1/4
5	40	67	1/3
6	60	50	2/3
7	84	40	2/3
8	108	30	3/4
9	144	33	2/4
10	192	33	2/4
11	288	50	2/4
12	432	50	4/4
13	648 §	50	4/6
			total = 30 new patients

* adapted from Grove *et al* 1987

§ = MTD

TABLE 2.4.2 **Trial centre 2 (Ohio) : weekly dose for three consecutive weeks, repeated every five weeks.**

Level	Dose (mg/m^2)	Approx. % increase.	No. new/total pts. at each dose level.
1	4	-	(skipped)
2	8	100	2/2
3	13	62	(skipped)
4	20	55	(skipped)
5	28	40	(skipped)
6	36	30	2/2
7	48	33	(skipped)
8	64	33	(skipped)
9	85	33	(skipped)
10	113	33	2/3
11	170	50	4/4
12	215	25	3/4
13	270*	25	5/5
			total = 18 new pts.

* = probable MTD.

TABLE 2.4.3 **Trial centre 3 (Auckland): Daily dose for three consecutive days, repeated every three weeks.**

Level	Dose (mg/m^2)	Approx. % increase	No. new/total pts. at each dose level
1	4	-	(skipped)
2	8	100	(skipped)
3	13	62	2/2
4	20	55	(skipped)
5	28	40	(skipped)
6	36	30	2/3
7	48	33	3/6
8	64	33	1/4
9	96	50	1/3
10	144	50	1/4
11	216	50	4/8
12	270*	25	2/2
			total = 16 new pts.

* = MTD.

CHAPTER 3

PHASE II CLINICAL TRIAL IN NON-SMALL CELL LUNG CANCER

CHAPTER 3. PHASE II CLINICAL TRIAL IN NON-SMALL CELL LUNG CANCER

INTRODUCTION

NSCLC was chosen as the tumour type to be studied in a limited phase II trial primarily because there is no conventional therapy which has been shown to be of benefit in this disease. Thus patients without prior therapy could be treated enabling assessment of efficacy and confirmation of toxicity in patients without bone marrow compromise. Furthermore, with serial chest x-rays and chest computerised tomography, the bulk of disease can usually be measured accurately. This is essential for following disease response or progression in a phase II trial.

PATIENTS AND METHODS

Patients

All patients had advanced histologically documented non-small cell lung cancer which was inoperable, or which had recurred following surgery or radiotherapy. All patients were required to have measurable disease, demonstrated to be progressive over the two months prior to study.

The eligibility and exclusion criteria and pre- and post- treatment evaluation were otherwise as documented for the phase I trial (see page 73).

A copy of the consent form signed by all patients in the phase II trial is shown in Appendix 1.4 along with the patient information sheet (Appendix 1.5).

Assessment of Disease and Toxicity

Pre-treatment assessment in all patients was similar to that in the phase I trial (Chapter 2) but included computerized tomography of the chest and upper abdomen for accurate assessment of tumour size. Patients were restaged with repeat computerized tomography of the chest after two courses of treatment unless there was obvious disease progression clinically or on chest x-ray after the first cycle. Standard WHO criteria were used for the evaluation of response (Miller et al 1981).

Treatment

From the phase I study, a CI-921 dose of 648mg/m^2 (216 mg/m^2 daily for 3 days) was recommended for the phase II study, this being one dose level below the maximum tolerated dose. If unacceptable toxicity occurred in the initial cycle, the dose in subsequent cycles was decreased by one dose level to 432mg/m^2 (144 mg/m^2 daily for 3 days). The drug was given in 250 ml 5% dextrose over 15 minutes on day 1, but in most patients pain at the site of infusion necessitated increasing the dilution to 500ml with infusion over 30 minutes for subsequent treatments. Patients shown to have progressive disease following two courses discontinued treatment, while those with static disease or disease response were to continue treatment.

RESULTS

Patient Characteristics (Table 3.1)

Nineteen patients (3 female and 16 males) were entered into the phase II trial. The median age was 62 years (range 44-73). Histological types and prior treatment are shown in Table 3.1. No patient had received prior chemotherapy. Of nine patients who had no prior treatment to the primary lesion, three had received cerebral irradiation for cerebral metastases and one local radiotherapy for painful bone metastases. At time of entry into the trial all patients had a performance status of 1 (ECOG scale).

Forty treatment courses were completed. The median number of treatment courses per patient was two (range 1 - 5).

Response

Sixteen patients were evaluable for response. Three patients failed to complete the three-day treatment course, two because of possible toxicity and one because of deteriorating general condition.

One patient with squamous cell lung cancer had a partial response after two treatment courses, with a decrease in size of a left upper lobe tumour mass from 6 x 5 cms to 3 x 3 cms. There was no further reduction in tumour size after four cycles. This response was maintained for five months before relapse and subsequent slow disease progression.

TABLE 3.1 Patient characteristics - Phase II clinical trial in NSCLC.

No. patients	19
Sex ratio (M/F)	16/3
Median age (range)	62 (44-73 years)
Histology	**No. patients**
Squamous cell	12
Adenocarcinoma	2
Mixed adenosquamous	2
Large cell undifferentiated	3
Prior therapy	
Nil	5
Surgery (S)	2
Radiotherapy (RT) - to primary lesion	6
- to metastases	4
Chemotherapy (CT)	0
S + RT	2

The median survival was seven months from original diagnosis (range 1 -34 months) and three months from first treatment (range 1/4 - 8 months).

Toxicity

Sixteen patients were evaluable for toxicity having completed at least one three-day treatment cycle. Of the two patients discontinuing treatment because of possible toxicity, one had intolerable discomfort in the back of the hand during drug infusion (with no extravasation or subsequent phlebitis) and one patient had intractable nausea and vomiting (almost certainly due to disease rather than the study drug) and depression.

Of the 13 patients who received a second cycle, four patients required dose reduction because of the degree of myelosuppression following the initial cycle or because of the development of infection in association with nadir counts.

Haematological toxicity (Table 3.2)

Haematological toxicity was assessed following 40 treatment courses. Grade 2 neutropenia was seen in eleven courses (27.5%), grade 3 in 14 courses (35%) and grade 4 in 11 courses (27.5%). The median onset of neutropenia ($\geq$ grade 1) was seen by day 8 (range 6 to 12) with recovery to < grade 1 by day 18 (range 15 to 29). Three patients developed chest infections in association with nadir counts but all responded to oral or parenteral antibiotics and there was no infective death related to such infection.

TABLE 3.2 Haematological toxicity

Toxicity Grade	Neutrophils	Haemoglobin	Platelets
	(% of 40 treatment courses (no. patients))		
0	10(3)	52.5(12)	97.5(15)
1	0(0)	42.5(12)	0(0)
2	27.5(7)	2.5(1)	2.5(1)
3	35(10)	2.5(1)	0(0)
4	27.5(8)	0(0)	0(0)

A grade 1 fall in haemoglobin was seen following 17 courses (42.5%). A $\geq$ grade 2 fall in haemoglobin was seen on two occasions in two patients in association with severe infective illnesses. One patient developed a grade 2 fall in platelet count on day 13 with recovery by day 22.

Non-haematological toxicity (Table 3.3)

Gastrointestinal toxicity was the most common non-haematological toxicity, but was generally mild. Treatment was associated with grade 1 nausea and vomiting in 22 courses (55%) in 13 patients and grade 2 nausea and vomiting in three courses (7.5%) in two patients. The nausea was mild, associated with loss of appetite and resolved by day 4.

TABLE 3.3 Non-Haematological Toxicity - Phase II

Toxicity	Grade §	No. courses	No. patients
Nausea/ vomiting	1	22 (55%)	13
	2	3 (7.5%)	2
Phlebitis	1	11 (27.5%)	9
	2	3 (7.5%)	2
Liver funtion ALP	1	4 (10%)	4
	2	3 (7.5%)	2
AST	1	5 (12%)	2
	2	3 (7.5%)	3
Renal function	1	1 (2.5%)	1
	4*	1 (2.5%)	1
Infection	1	1 (2.5%)	1
	2	1 (2.5%)	1
	4	1 (2.5%)	1
Seizures	3	3 (7.5%)	1

* probably related to study disease rather than study drug.

§ ECOG toxicity criteria

Despite an increased infusion volume and slower infusion rate, grade 1 phlebitis was still seen following eleven courses (27.5%) in nine patients and grade 2 phlebitis following three courses (7.5%) in two patients.

Mild abnormalities of liver function tests occurred infrequently. Grade 1 elevation of ALP was seen following four courses in four patients with a return to normal in four days (except for one patient who developed hepatic congestion). Two patients had grade 2 elevation of ALP but one of these patients was known to have bone and liver metastases and had mildly abnormal liver function tests prior to treatment. Four patients developed abnormalities of AST. One patient developed a grade 1 increase on day 4 following each of four treatment courses with return to normal levels by day 8. This was associated with a mild elevation of ALP (grade 1) on one occasion only. Three other patients developed transient increases in AST ($\leq$ grade 2) with return to normal values by day 20 in all cases.

Renal dysfunction was seen in two patients. One patient had mild impairment of renal function at the start of treatment (serum creatinine 0.16 mmol/l, serum urea 6.8 mmol/l) and developed severe renal dysfunction by day 10 of his first treatment course from which he subsequently died. Post-mortem examination revealed extensive intra-abdominal disease surrounding the ureters bilaterally. Although it is not possible to exclude a contribution from CI-921, the renal impairment was thought to have resulted primarily from disease rather than the treatment. One other patient had a transient deterioration of renal function (grade 1 toxicity) in association with an infective episode.

Possible neurological toxicity was seen in one patient. This 64 year old man had a grand mal seizure temporally associated with CI-921 infusion in three of four cycles. He had originally presented with dysphasia and grand mal seizures and was found to have cerebral metastases from a bronchoscopy proven lung carcinoma. He was commenced on dexamethasone and phenytoin and was referred for palliative whole brain irradiation. This was nearing completion at the time of the first course of CI-921. On day 3 of the first course he had a fit. The previous day he had been changed from phenytoin to carbamazepine following the development of a phenytoin-induced skin rash. His anti-epileptic medications were therefore likely to have been below the therapeutic range. Similarly, he had been on a decreasing dose of dexamethasone. There were no further fits until the third day of course 2. Carbamazepine levels were within the therapeutic range at this time but he was noted to be hyponatraemic (secondary to diuretic use) with a serum sodium of 124mmol/l. There were no seizures associated with course 3 at which time he had stable disease and the dexamethasone was discontinued. He had a further grand mal seizure, however, on day 2 of a fourth and final course of CI-921 at which time the serum sodium was again low at 126mmol/l. Seizures did not occur between courses. Subsequently, there was clinical evidence of disease progression both in the lung and the brain with worsening dysphasia and hemiplegia leading to the patient's death. A post-mortem examination was not performed.

There were no other unexpected side-effects noted. No cardiotoxicity was seen as investigated by echocardiography and ECG assessment (Chapter 5).

DISCUSSION

The aim of this limited phase II study was to confirm the dose identified in the phase I study in a group of patients without previous chemotherapy, to gain further information about the toxicity of the drug and to identify possible disease activity in patients prior to more broadly based phase II studies.

Unfortunately, only sixteen patients were entered into this phase II trial. The study was terminated at this point on the request of Parke-Davis Research Division, Warner-Lambert, who wished to review all the data from the phase I trials before making further decisions as to the running of phase II trials. Our study therefore provided only limited information as to the efficacy of the drug in NSCLC, but did provide further valuable assessment of its toxicity in a group of previously untreated patients.

The single response in this generally unresponsive group of patients with NSCLC suggests that further studies of the activity of CI-921 in this and other tumour types are warranted. If no responses had been documented in the first 14 patients, one could state that there was a 95% chance that CI-921 was less than 20% effective. The single response amongst 16 patients suggests only that CI-921 has activity against NSCLC unlikely to exceed 30% (at the 95% confidence level) but more patients would be required to define the activity accurately (Lee et al 1979). In view of the resistant nature of NSCLC to chemotherapy with no drug currently in use producing response rates greater than 30%, even a single response suggests that more testing is required.

The level of toxicity seen in this study was considered acceptable and suggested that the correct dose had been identified in the phase I study. Myelosuppression was the major toxicity with $\geq$ grade 3 neutropenia in 62.5% of treatment courses. Recovery was rapid however with return to normal blood counts within a median of 10 days of the initial onset of toxicity. Dose reduction for subsequent courses was required in four patients because of myelosuppression or the development of infection in association with neutrophil nadir counts.

Toxicity was otherwise as predicted from the phase I study, with mild nausea and vomiting, phlebitis and mild derangements of liver function. The latter was seen in six patients on day 4 following the three day treatment course.

The only unexpected toxicity was the association of grand mal seizures with CI-921 infusion in one patient in three of four cycles. The patient concerned had cerebral metastases and was completing a course of palliative cerebral irradiation. He had originally presented with grand mal seizures and had been stabilized on anti-epileptic medications and dexamethasone. There had been no further fits until the third day of the first course of CI-921. Subsequently, he fitted during both the second and fourth courses of treatment. The seizures only occurred on treatment days and never between courses. In all cases they occurred some hours following CI-921 infusion. On each occasion, however, there were additional factors that may have precipitated seizure activity, namely changing anti-epileptic medications, a reducing dose of steroids and hyponatraemia. It seems possible that CI-921 may have been sufficient to precipitate seizures in a patient already at risk. A similar pattern of neurotoxicity has been described for amsacrine, with several patients having had

seizures temporally associated with amsacrine infusion (see Chapter 1). Most of these patients also had some underlying metabolic disturbance that might have contributed to seizure activity. Perhaps the neurotoxicity can be likened to the cardiotoxicity of amsacrine, whereby an added factor or a "trigger" (e.g. hypokalaemia) is usually required for the toxicity to become manifest. Ideally, full investigation into the possible neurotoxicity of CI-921 would have included study of the cerebrospinal fluid plus electroencephalographic (EEG) studies and histological examination of the brain. Lumbar puncture was contraindicated however because of the raised intracranial pressure associated with the cerebral lesion. Similarly, EEG studies in a patient with cerebral metastases shortly after a fit were thought likely to be non-specifically abnormal and therefore unhelpful. Unfortunately consent was not obtained for a post-mortem examination.

In 1981, Wilson and Whitmore demonstrated an apparent enhancement of the radiosensitivity of chinese hamster ovary cells by amsacrine. This "cytokinetic co-operation" is thought to occur because of the cell cycle stage specificity of amsacrine and irradiation for different parts of the cell cycle (e.g. amsacrine shows more toxicity towards radioresistant S-phase cells). Thus the effect of the two agents when given together was greater than when each was given individually. There is no evidence to suggest however that amsacrine has a delayed radiation enhancement effect (as seen with doxorubicin and actinomycin-D) and there was no late radiation effect seen in those patients receiving CI-921 who had previously been treated with radiotherapy.

In a phase II study of amsacrine in NSCLC (Samson et al 1981) three partial responses amongst 76 patients were seen to give a predicted true response rate of $<1\%$ - 11% (95%

confidence limits). Further investigation in NSCLC was therefore not considered warranted. The single response reported in this study of CI-921 suggests that further studies are required both in NSCLC and in a variety of other tumours. Further world-wide phase II studies are planned.

CHAPTER 4

PHARMACOKINETIC STUDIES

CHAPTER 4. PHARMACOKINETIC STUDIES

INTRODUCTION

As part of the phase I and II clinical trials of CI-921 as described in Chapters 2 and 3, pharmacokinetic studies were undertaken to study the disposition of CI-921 in man.

CI-921 was measured in plasma by a high performance liquid chromatography (HPLC) method. HPLC is a separation technique based on differential rates of migration of components of a mixture along a chromatographic column. The mixture (or mobile phase), is pumped along a fixed column packed with solid particles (the stationary phase). Those components that are attracted to the column because of physical or chemical forces will show a favourable equilibrium distribution and will be "held to", and therefore move slowly along the column. Those components that are not strongly attracted to the stationary phase will move at a greater velocity along the column. Because of their different rates of flow the various components of a mixture can thus be separated and quantitated. (Fallon et al 1987, Brown 1984).

There are various types of HPLC based on the types of columns and solvents used. In normal phase HPLC, molecules are separated on the basis of their differential solubility in water. Hydrophilic binding groups on the column surface attract hydrophilic but not hydrophobic molecules. In reverse phase HPLC, molecules are separated on the basis of their solubility in organic solvents; hydrophobic binding groups attract hydrophobic and not

hydrophilic molecules. In ion exchange HPLC, the functional group attached to the column may express a positive or negative charge and attract counter-charged molecules. In size exclusion HPLC, the solid phase particles have pores in the surface that trap and retain small but not large sized molecules. The actual separation achieved will also be influenced by the choice of medium for the liquid phase which competes with the solid phase for sample molecules. For example, the polarity of the solvent will determine the degree to which molecules will adhere to the solid phase. Similarly, in ion exchange chromatography the affinity of charged molecules for the solid phase is determined by the pH of the buffer.

HPLC columns are usually silica based, but can be made of cellulose or organic resins. To maximize the time of exposure of the separating molecules with the column, the system is run under conditions of high back pressure (dependent on the column dimensions and size of the particles of the solid phase medium). The solvent is therefore delivered by a pump. Samples of constant volume can be delivered via an injection valve which is often automated. Close regulation of the flow rate of solvent through the column is achieved by microprocessor feed-back systems controlling the pumps. The various molecules emerging from the column can be detected by absorbency or fluorescent methods and charted continuously on an "in-line" chart recorder. The column retention time and time taken for the peak to emerge from the column can be measured from the chart recording. A substance can be quantitated by comparison with standard controls run through the same system.

HPLC provides an ideal system for the assay of drugs and metabolites in plasma or urine.

It allows for the identification and quantification of many components of a complex mixture simultaneously whereas a radioimmunoassay would require a number of different assays. It also allows for the detection of unanticipated components e.g. drug metabolites. The major disadvantages of HPLC are that it is costly with respect to time and equipment and is very sensitive to interference by solvent or sample contaminants resulting in artefacts. The selection of an appropriate combination of column, solvent and detector, however, will usually allow for the separation of most compounds (Bird 1989).

A reverse phase HPLC method was used for the assay of CI-921 concentrations in blood and urine in the phase I and II studies described here.

MATERIALS AND METHODS

The HPLC method for the measurement of total CI-921 concentrations in these studies was as previously described (Jurlina and Paxton 1985), with minor modifications to allow for the processing of a large number of samples.

Sample acquisition

i) Phase I

An indwelling plastic intravenous catheter flushed with normal saline was sited in a peripheral arm vein under local anaesthetic and was used to draw blood samples. The catheter was placed in the opposite arm to the delivery site and the first 2 ml drawn on each sampling occasion was discarded. Blood samples (5ml) were taken at 0, 7, 15, 20, 30,

45 minutes, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 hours following infusion on days 1 and 3, with pre- and end of infusion samples (time 0 and 15 minutes) only on day 2. Further samples were taken at 36, 48 and 72 and 96 hours after the third infusion. All blood samples were centrifuged immediately following collection and the plasma separated and frozen at -80°C until assay. All urine passed from the time of first drug administration was collected in 6 hour intervals for 72 hours and at 24 hour intervals thereafter for one week. Samples (20ml) from each collect were frozen at -80°C until assay.

ii) Phase II

Plasma was collected for pharmacokinetic analysis in the phase II study as described above for the phase I study but on day 1 only. Urine was collected throughout the three day treatment course. Pharmacokinetic studies were generally undertaken during cycle 1 only.

Sample measurement: High performance liquid chromatography (HPLC) method for the determination of CI-921 in plasma.

Materials

Millipore milli-Q water was used for the preparation of mobile phase and all aqueous reagents.

Pure CI-921 (as the isothionate salt) and the internal standard N-5-dimethyl-9-[(2-methoxy-4-ethylsulfonylamino) phenylamino]-4-acridine carboxamide, as the hydrochloride salt, were supplied by Dr B. Baguley of the Cancer Research Laboratory, Auckland School of Medicine, Auckland.

10% carbon tetrachloride (CCl_4) in hexane: 50 ml CCl_4 (May & Baker Ltd., Dagenham, England) plus 450 ml n-hexane (J.T. Baker, Phillipsburg, N.J., U.S.A.) prepared fresh for each assay.

Anhydrous diethyl-ether (Ajax Chemicals, Sydney, Australia) was re-distilled prior to each assay.

Saturated sodium tetraborate ($Na_2B_4O_7$). Approximately 50g $Na_2B_4O_7$ (Ajax Chemicals, Sydney Australia) was dissolved in 500 ml water.

1M triethylamine phosphate (TEAP). 34.8 ml triethylamine (BDH Chemicals, Poole, Great Britain) was added to 60 ml water on ice. Sufficient orthophosphoric acid (85%) (Ajax Chemicals, Sydney, Australia) was added to bring to pH 3.0 (approximately 15 ml). The solution was made up to 250 ml with water.

Mobile phase (acetonitrile/water [46:54] + 10mM TEAP). 2L mobile phase was prepared by mixing 920 ml acetonitrile (HPLC grade, J.T. Baker or Waters Assoc., Milford, MS, USA) with 1080 ml water. 20 ml was removed and replaced by 20 ml 1M TEAP. All mobile phase was filtered and thoroughly degassed before use.

Blank plasma was collected into heparinized tubes from normal healthy volunteers.

Standards and Quality Controls.

i) CI-921 standards.

A 0.5mM stock standard solution of CI-921 was made by dissolving the isothionate salt in methanol. From this stock solution, working standards of 100μM were prepared and stored at -20°C. Aliquots of the working standard were evaporated under oxygen-free nitrogen and re-dissolved in blank plasma to give five standard concentrations within the range 0.1-

20μM CI-921. Duplicate samples of each standard were included in every assay.

ii) Internal Standard.

The internal standard is structurally similar to CI-921 with the substitution of an ethyl for the methyl group on the sulfonanilide side chain. A 2mM stock internal standard solution was prepared in methanol from which 20μM working solutions were made by further dilution in methanol.

iii) Quality Controls.

Aliquots of CI-921 in methanol were diluted in blank plasma to give final concentrations of approximately 15, 1, 0.5 and 0.1μmol/l (referred to as SC15, SC1000, SC500 and SC100 respectively). Exact concentrations were determined in the pre-study evaluation. Each quality control was divided into 1.5 - 2.5 ml aliquots which were stored at -20°C. Duplicate samples of each of the three quality controls were included in each assay to ensure inter-assay reproducibility.

Sample extraction.

100μl of the 20μM internal standard (I.S.) solution and 200μl 0.5M HCI were simultaneously added to 0.5ml plasma samples in screw-cap glass culture tubes. After brief vortexing, 8ml 10% CCL_4 in hexane was added to extract less polar, long-retention time compounds. The tubes were capped and shaken for 20 minutes. After centrifugation for five minutes at 2200rpm, the upper hexane layer was removed. The pH of the remaining plasma was adjusted to pH 8.0 with 300μl saturated $Na_2B_40_7$ and 8ml diethyl ether added. After shaking

for 15 minutes, the samples were centrifuged as above and the ether layer transferred to a tapered glass tube and evaporated to dryness at 27°C under oxygen-free nitrogen. The residue was re-dissolved in 100μl mobile phase, vortexed vigorously and placed in limited volume inserts for chromatographic assay.

Chromatography.

Chromatographic conditions were as follows:

Apparatus:	Waters Association model 6000A pump.
	WISP MODEL 710B automatic injector.
	RCM-100 radial compression module.
	Waters model 440 u.v. detector
Column:	Waters "Resolve" Radial-pak C18 cartridge 10 x 0.8 cm, 10μ particle size.
Guard-Pak:	"Resolve" C18
Mobile Phase:	acetonitrile/water (46:54) + 10mM TEAP
Flow rate:	6.5 - 7 ml/min.
Pressure:	2,000 psi
Retention times:	4.0 $\pm$ 0.1 min CI-921 peak
	5.0 $\pm$ 0.1 min I.S. peak
WISP run time	6 min.
Injection : volumes	5 - 35μl
Detection:	UV absorbance at 254nm

Detector output signals were collected and processed by an Apple II computer equipped with a "Chromcard" chromatography data system (Analytical Computers, Copyright 1982). This recorded peak heights and areas and calculated the ratio of the CI-921 peak height to that of the I.S. (Fig.10).

Calculation of CI-921 concentrations.

Using an MKMODEL program (Holford 1985) and an IBM compatible personal computer, the peak height ratios of CI-921 to I.S. were plotted against corresponding CI-921 concentrations of the standards. The line of best fit was determined employing a weighting factor of 1/x. The concentration of CI-921 in unknown samples and quality controls was then determined by measuring the peak height ratio and using the standard curve to obtain a concentration.

Pharmacokinetic Analysis.

Model independent pharmacokinetic parameters were determined from the plasma concentration-time profiles using the same program. The slope (ß) of the terminal linear portion of the log concentration-time profile was estimated by unweighted least squares regression and the initial ($t_{1/2}$å) and terminal half-lives ($t_{1/2}$ß) were calculated from log 2 divided by the appropriate slope. The area under the plasma concentration-time curve (AUC) and the area under the first moment of the concentration time curve (AUMC) were determined using the trapezoidal rule. Both were extrapolated to infinity by addition of Ct/ß to the former and Ct/ß (Tt + 1/ß) to the latter, where Ct is the concentration at the last time point (Tt).

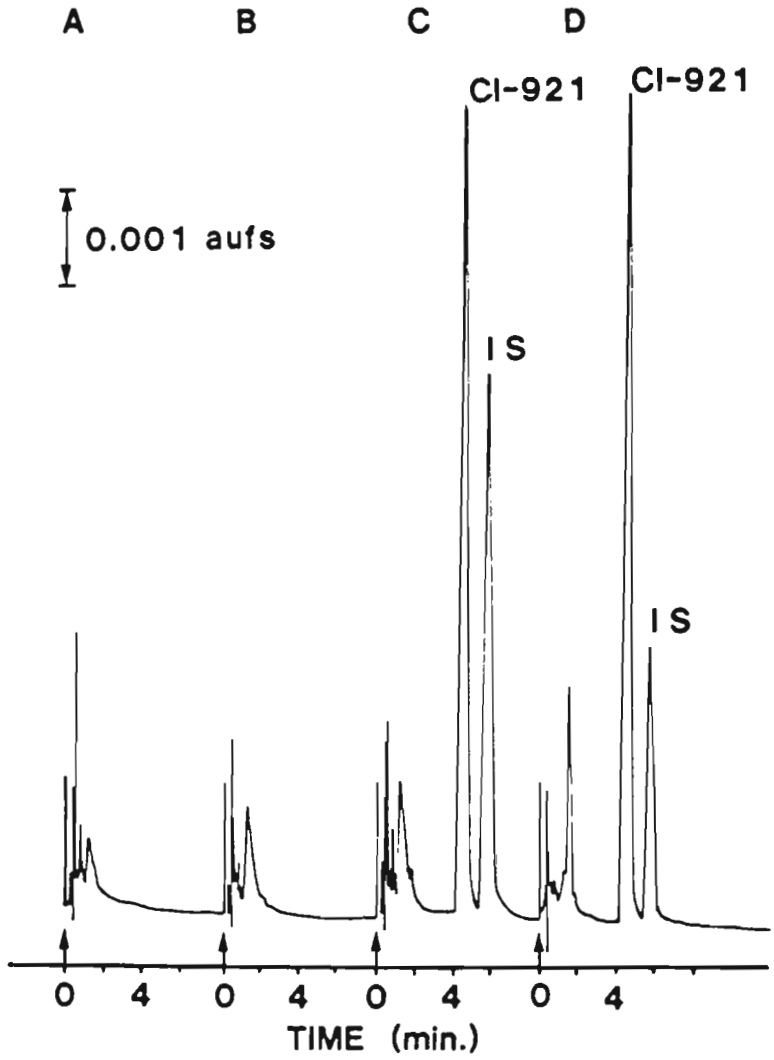


FIG. 10. Sample HPLC tracing for CI-921 and internal standard (IS)

A, B = blank plasma
C, D = post CI-921 infusion samples.

Total plasma clearance (C1) was calculated by dividing the administered dose by the AUC (Cl = DOSE/AUC). The mean residence time of drug elimination (MRT) and the apparent volume of distribution at steady-sate (V_{ss}) were calculated as follows:

$$MRT = [AUMC/AUC] - T/2$$

$$Vss = Cl \times MRT$$

where T is the time of infusion.

The plasma concentration-time profiles were also tested by MKMODEL to determine whether a one-or two-compartment open model would provide the better fit.

Pre-Study Validation.

On three consecutive days, standards at concentrations of 0, 50, 100, 200, 400, 600, 800, 1,000 and 2,000 nmol/l were prepared in plasma. Triplicate assays were performed with each standard and each quality control. Linear regression analyses of the standards from each day were obtained and various weightings considered. The concentrations of controls and standards were back calculated from the weighted standard curves (Fig 11).

Based on log likelihood and Schwartz criteria, the best fits and recoveries were obtained with a weighting of 1/x and a zero intercept. The percent co-efficient of variation (CV) of standards using this method ranged from 1.3 to 6.5%. The average back-calculated value for each standard was well within the 10% of theoretical value required (range: % difference from theoretical: 0 - 6.6%)(Appendix 2.1).

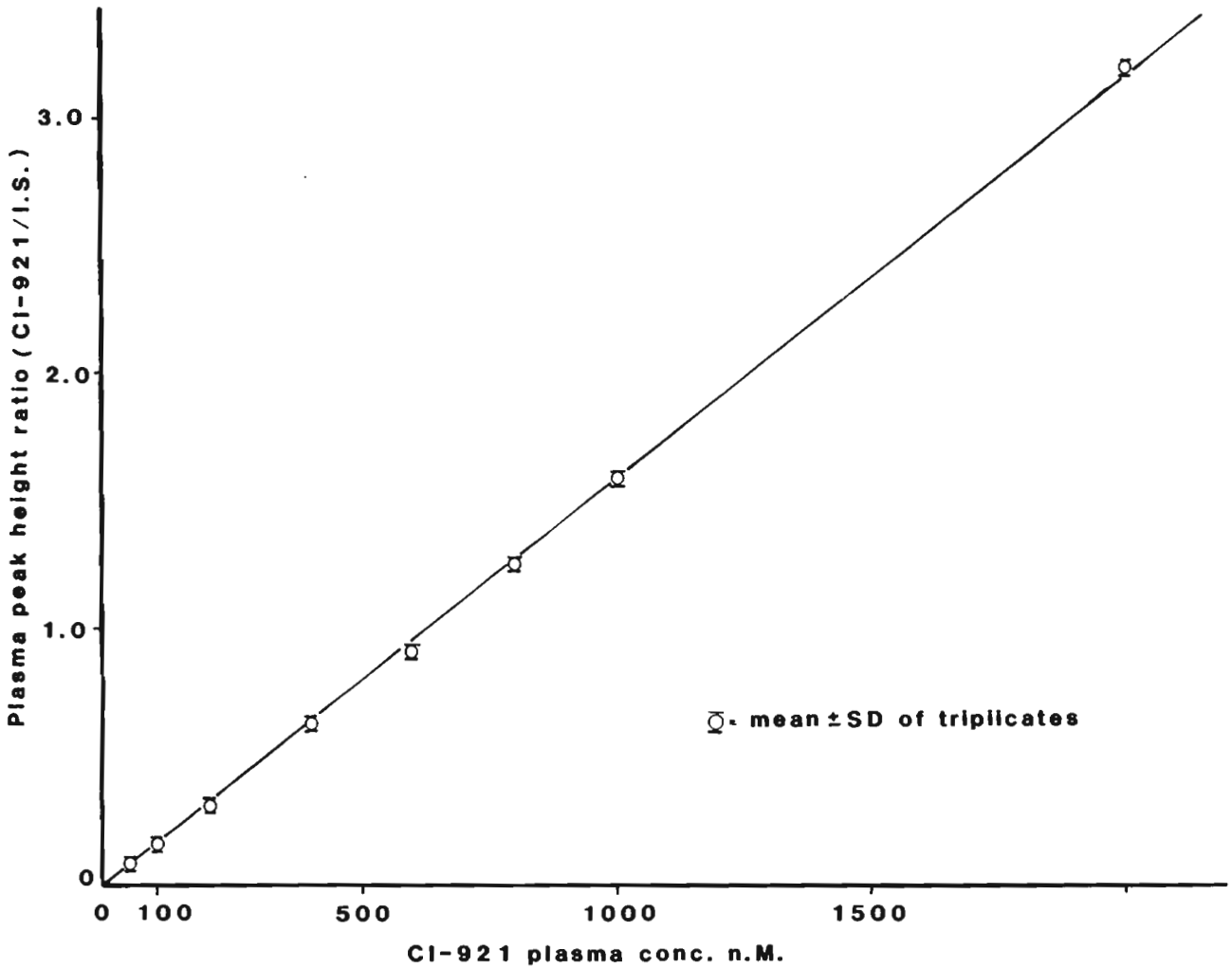


FIG. 11. Standard curve for back-calculation of CI-921 standards during assay validation.

The average value for each quality control was within 10% of its respective theoretical concentration (range: % difference from theoretical 1.5 - 6.9%) (Appendix 2.2).

The minimum plasma concentration that could be measured with acceptable precision (i.e. CV < 10%) was 0.05μmol/l. Lesser values were considered as zero. Concentrations greater than 20μmol/l were diluted to within the assay range with blank plasma.

The assay was validated in accordance with the criteria specified in the Parke-Davis Pharmaceutical Research Division, Warner Lambert Company (Ann Arbor, Michigan, USA) standard operating procedures for pharmacokinetic and drug metabolism assay validation. Each of the three trial centres underwent the same validation process to allow comparison of trial results between trial centres.

Urinary Assay

The assay to determine the concentration of CI-921 in urine was similar to the plasma assay except that the hexane wash was omitted and an alternate internal standard (N,4-ethyl-5-methyl-9-[2-methoxy-4-methylsulfonylamine) phenylamino]-4-acridine carboxamide) was used to avoid interference from other urinary compounds. This assay was less precise and less sensitive with 0.1 μmol/l being the lowest concentration measurable with a CV of < 10%. The assay was validated as above (Appendices 2.3, 2.4).

Statistics.

Pharmacokinetic parameters from the first and third infusions were compared by the two-tailed Student's paired t-test. Relationships between dose and pharmacokinetic parameters and urinary excretion were assessed by Pearsons's correlation co-efficient (r) and differences in daily excretion of unchanged drug by one-way analysis of variance within subjects (Winer 1971).

Quality Control

After 65 consecutive assays in the phase I study the mean concentration for each quality control was well within the theoretical value +/- 10% with a CV < 8% (Table 4.1). A greater number of samples was analysed per assay in the phase II trial. After 9 assays the mean concentration for each quality control was well within the allowable limits as above, with a CV of < 5% (Table 4.2).

RESULTS

Phase I

The pharmacokinetics of CI-921 were studied following 65 infusions on day 1 and day 3 in 16 patients over a dose range of 13 to 270 mg/m^2 (Appendix 3.1). Plasma disposition curves appeared biexponential and the modelling program (Holford 1985) indicated that a 2-compartment open model was the best fit for most patients (Fig 12). The peak plasma concentration (Cmax) ranged from 3.36 to 85.6μmol/l. The area under the concentration

time curve (AUC) range from 2.44 to 99.04μmol.h/l. The mean model-independent pharmacokinetic parameters were as shown in Table 4.3.

There was no significant difference between any of the kinetic parameters on day 1 and day 3. The mean ratios of the day 3/day 1 pharmacokinetic parameters are shown in Table 4.4. Mean kinetic parameters at increasing i.v. dose are shown in Table 4.5.
There was a highly significant linear correlation between dose and AUC (rs = 0.953, $P<0.001$) and dose and Cmax (rs = 0.949, $P<0.001$). There was no strong correlation ($r>0.5$) between Cl, Vss, $t_{½}\alpha$, $t_{½}\beta$ or MRT dose (Table 4.6, Figs 13.1 -13.6).

Over the 20-fold dose range, 3-fold variations were seen in Cl (94-290 ml/h/kg), Vss (219 - 614 ml/kg) and MRT (1.1 - 3.5 h) with even greater variations in $t_{½}\beta$ (1.1-5.0 h). Large variations in these parameters were also observed within a single dose level (e.g. 216 mg/m^2) as well as within individual patients at different dose levels (Fig 20).

Less than 1% (0.18 - 0.92%) of the total dose of CI-921 was excreted unchanged in the urine (Appendix 3.1). Most of this appeared in the sample collected over the 6 hours immediately following treatment. By 18 - 24 hour, CI-921 was usually undetectable. There was a weak linear correlation between dose and percent of drug excreted unchanged in the urine ($r = 0.393$, $p = 0.043$, 25df) (Fig 14) and a small but significant increase in the proportion of unchanged drug excreted per day over the three treatment days ($p = 0.0109$). The overall mean (SD) percentage of drug excreted in the three treatment days were 0.45% (0.03%), 0.53% (0.05%) and 0.62% (0.05%) respectively.

Toxicity of treatment as defined by nadir AGC following treatment gave a significant linear correlation with parameters of exposure of tissue to drug as measured by drug dose, AUC and Cmax, but was best correlated with dose in mg/m^2 (Table 4.7, Figs 15.1 - 15.3).

Table 4.1 Quality control of CI-921 plasma assay - Phase I. Variation over 65 assays.

Quality Controls*	SC 100	SC 500§	SC 1000	SC 15000§
Mean	95	456	1010	14543
SD	6.9	8.5	40.2	574.5
% CV	7.3	4.5	4.0	4.0
Min.	77	424	880	13250
Max.	113	491	1095	15868
N	67	12	67	55

* Cl-921 concentration of approximately 0.1, 0.5, 1.0 and 15μmol/l (see text)

§ the 15μmol/l quality control was substituted for the 0.5μmol/l quality control following assay 11 as higher concentrations of CI-921 were being measured, with validation as documented.

Table 4.2 Quality control of CI-921 plasma assay - Phase II. Variation over 9 assays.

Quality Control*	SC 100	SC 1000	SC 15000
Mean	95.1	926.6	16061.6
SD	4.04	37.1	755
% CV	4.3	4.0	4.7
Min	87	879	14859
Max	100	985	17529
No.	9	9	8

*CI-921 concentrations of approximately 0.1, 1.0 and 15μmol/l.

FIG. 12.

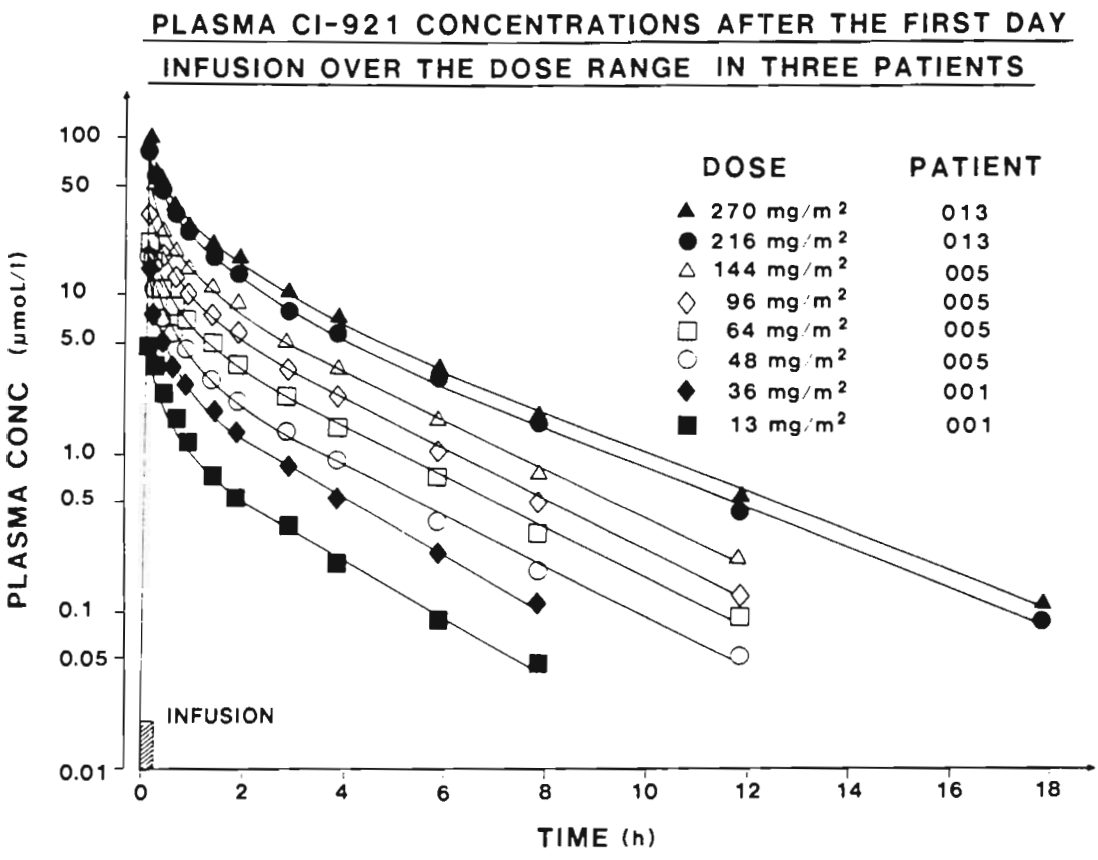

PLASMA CI-921 CONCENTRATIONS AFTER THE FIRST DAY
INFUSION OVER THE DOSE RANGE IN THREE PATIENTS
DOSE
PATIENT
270 mg/m²
013
216 mg/m²
013
144 mg/m²
005
96 mg/m²
005
64 mg/m²
005
48 mg/m²
005
36 mg/m²
001
13 mg/m²
001
INFUSION
PLASMA CONC (μmol/l)
100
50
10
5.0
1.0
0.5
0.1
0.05
0.01
0
2
4
6
8
10
12
14
16
18
TIME (h)

Table 4.3. Mean model-independent pharmacokinetic parameters in the phase I trial.

Parameter	Mean value	Range
$t_{½}$å	0.6 h	0.24 - 1.08 h
$t_{½}$ß	2.63 h	1.08 - 4.98 h
MRT	2.0 h	1.05 - 3.35 h
Cl	158 ml/h/kg	95 - 290 ml/h/kg
Vss	319 ml/kg	219 - 614 ml/kg

TABLE 4.4 Mean ratios of day 3/ day 1 pharmacokinetic parameters after the first and third infusion of a three -day course with increasing dose.

Dose	Courses/ Patients	Cmax	AUC	CL	Vss	MRT	$t_{1/2}$å	t1/2ß
13	2/2	1.19	0.96	1.06	0.92	0.89	0.76	0.84
36	3/3	1.03	0.97	1.05	0.84	0.81	0.85	0.75
48	4/4	1.06	1.10	0.92	0.97	1.07	1.15	1.18
64	3/3	1.13	1.01	0.99	0.99	1.01	0.96	1.10
96	3/3	1.00	0.89	1.16	1.21	1.05	0.92	1.19
144	4/4	1.14	0.98	1.04	1.04	1.01	0.89	1.11
216	10/7	1.00	1.02	0.98	1.01	1.04	1.18	1.27
270	2/2	0.97	1.02	1.15	1.00	0.87	0.87	0.74
Mean		1.06	0.99	1.04	1.00	0.97	0.95	1.02
% CV		7.5	6.1	7.9	10.6	10.1	15.4	20.8

TABLE 4.5 Mean (SD) kinetic parameters at increasing i.v. dose.

Dose (mg/m^2)	Infusions/ patients	AUC (µmol.h/l)	CL (ml/h/kg) Cmax (µmol/l)		Vss (ml/kg)	MRT (h)	$t_{1/2}$å (h)	$t_{1/2}$ß (h)
13	4/2	3.2 (0.9)	4.2 (1.1)	225 (76)	286 (54)	1.3 (0.1)	0.3 (0.0)	1.4 (0.1)
36	6/3	11.8 (2.7)	9.9 (0.4)	185 (18)	376 (124)	2.1 (0.7)	0.5 (0.1)	2.2 (0.8)
48	8/4	17.6 (4.4)	17.1 (2.2)	153 (23)	334 (29)	2.3 (0.6)	0.4 (0.1)	2.8 (0.7)
64	6/3	22.7 (4.4)	22.4 (4.3)	150 (32)	308 (13)	2.1 (0.4)	0.4 (0.0)	2.4 (0.6)
96	6/3	35.2 (6.6)	34.3 (4.2)	152 (22)	299 (40)	1.9 (0)	0.4 (0)	2.2 (0.2)
144	7/4	52.5 (3.3)	53.3 (10.3)	156 (17)	314 (74)	2.0 (0.2)	0.4 (0.1)	2.5 (0.4)
216	21/11	73.8 (14.1)	64.3 (10.2)	156 (38)	318 (72)	2.1 (0.4)	0.6 (0.2)	3.0 (0.8)
270	4/2	88.1 (9.7)	82.2 (1.2)	126 (14)	282 (35)	2.1 (0.1)	0.5 (0.0)	3.2 (0.9)

TABLE 4.6 Relatonship between dose (mg/m2) and pharmacokinetic parameters - phase I.

Parameter	Correlation* coefficient	Significance
AUC	0.953	p< 0.0001
Cmax	0.949	p< 0.0001
CL	-0.357	p=0.0035
Vss	-0.103	NS
t1/2å	0.455	p= 0.0001
t1/2ß	0.456	p= 0.0001
MRT	0.223	NS

* Pearson's correlation coefficient (63 degrees of freedom).

FIG. 13.1.

RELATIONSHIP BETWEEN DOSE & AUC OF CI-921 AFTER 65 INFUSIONS IN 16 PATIENTS DURING A PHASE 1 TRIAL

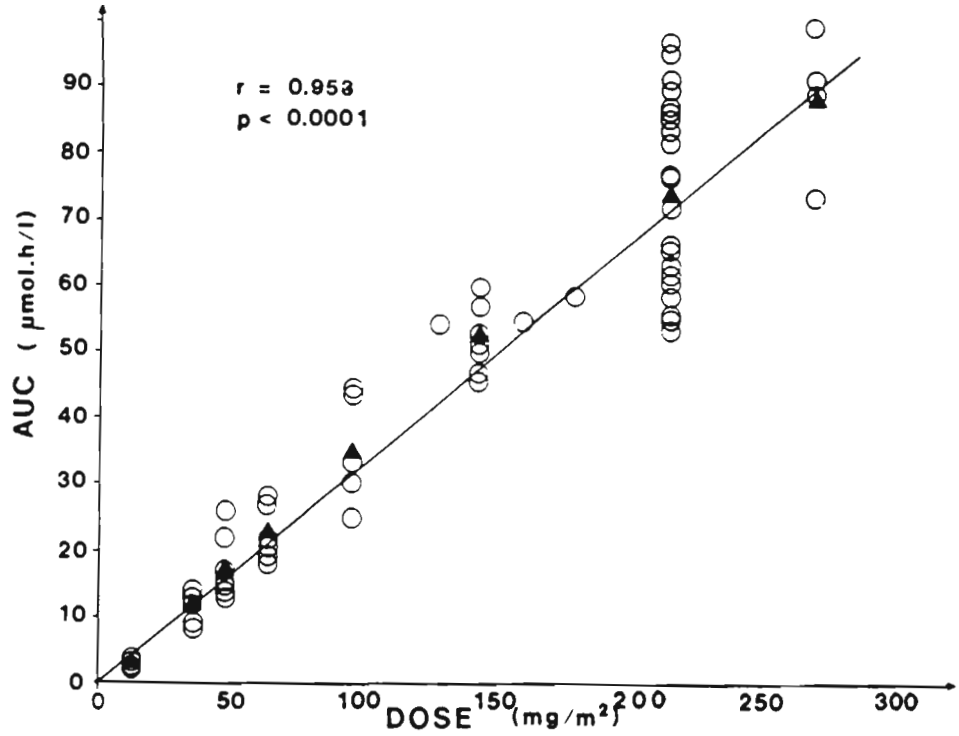
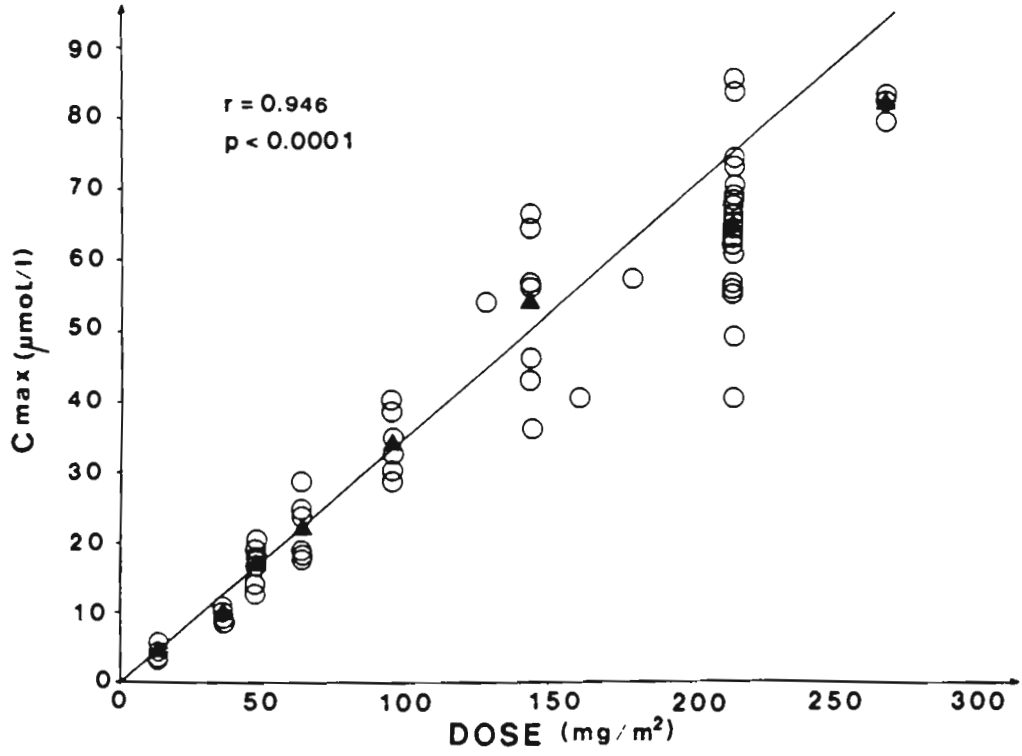


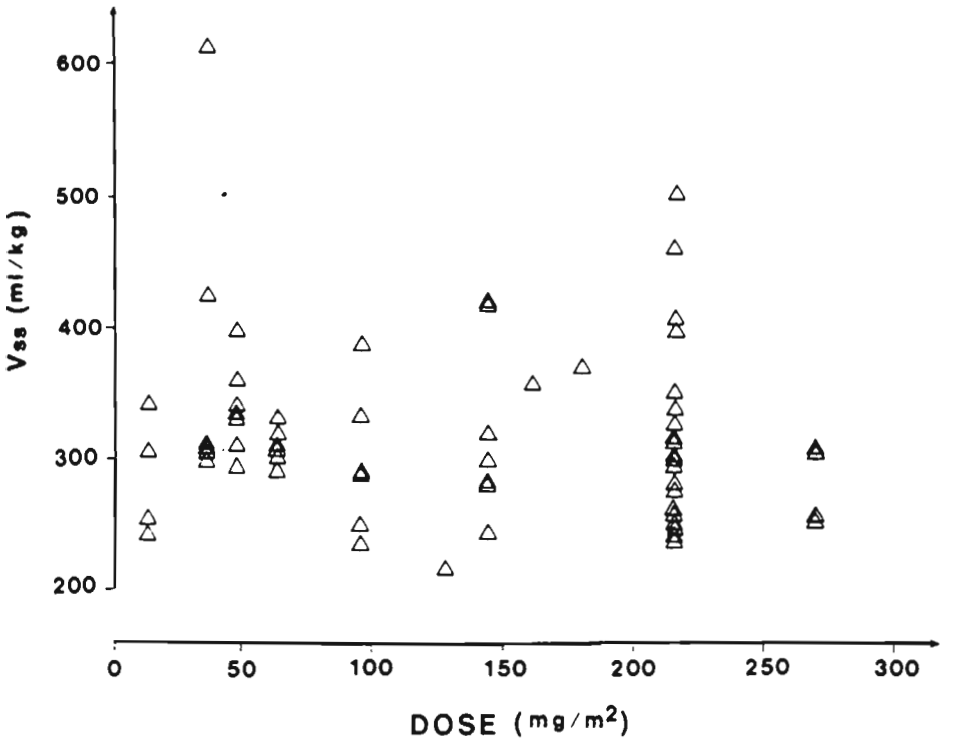
FIG. 13.2.

RELATIONSHIP BETWEEN DOSE & Cmax OF CI-921 AFTER 65 INFUSIONS IN 16 PATIENTS DURING A PHASE 1 TRIAL



RELATIONSHIP BETWEEN DOSE AND USS (FIG.13.3) AND DOSE AND CI (FIG.13.4) AFTER 65 INFUSIONS IN 16 PATIENTS DURING A PHASE I TRIAL

Fig.13.3



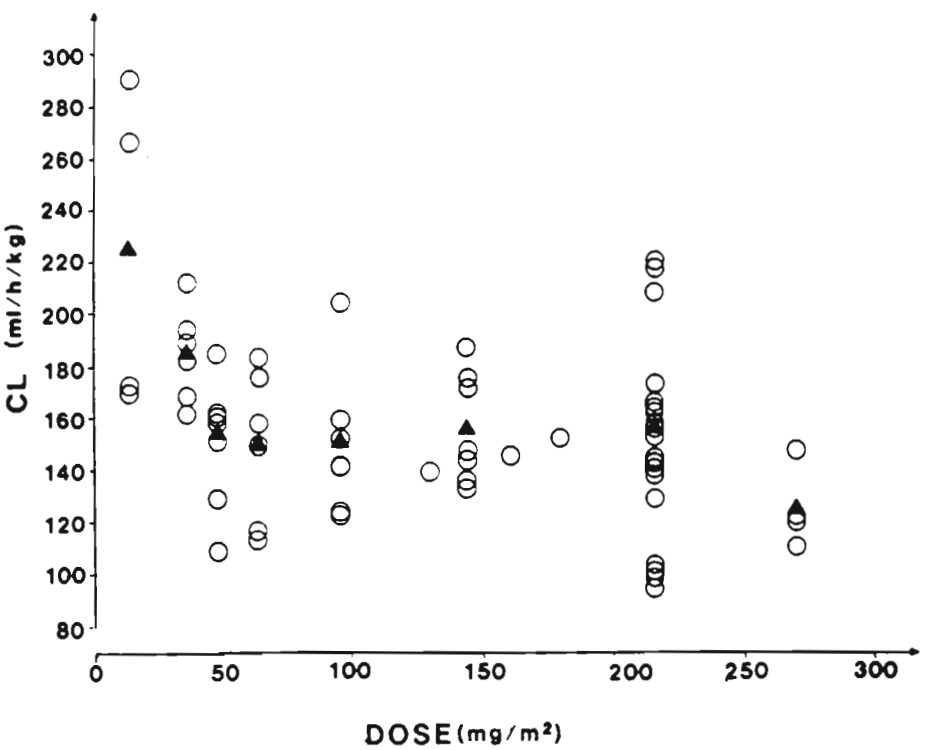
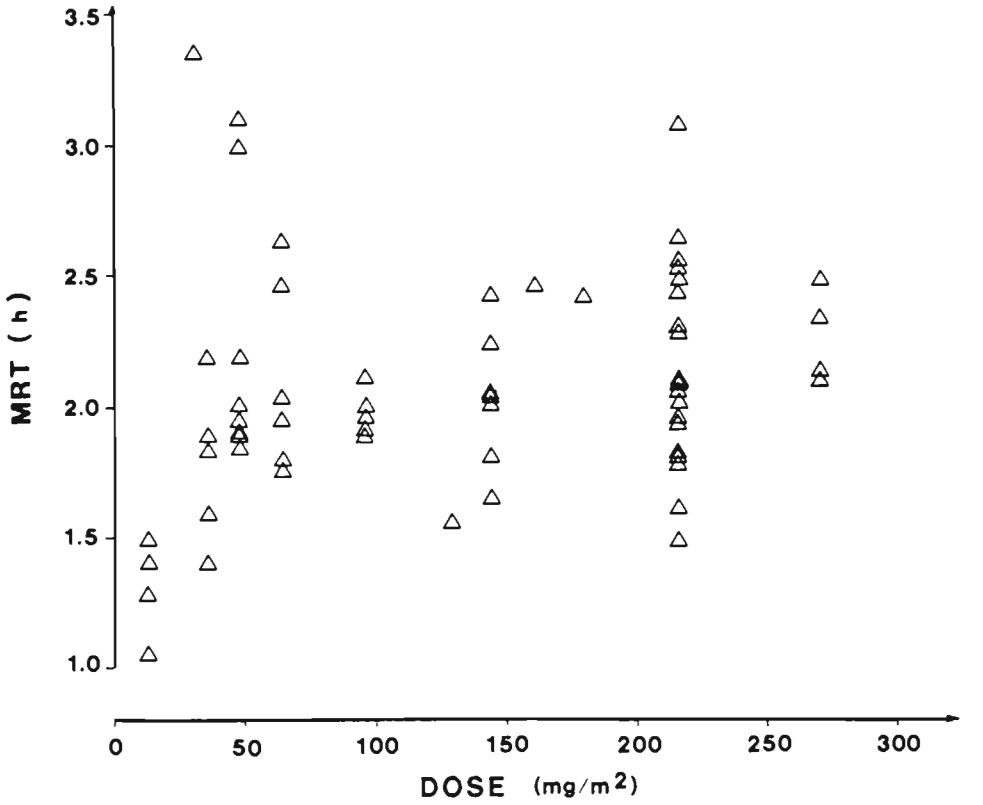


Fig.13.4

RELATIONSHIP BETWEEN DOSE AND MRT (FIG.13.5) AND DOSE AND +½ß (FIG.13.6) AFTER 65 INFUSIONS IN 16 PATIENTS DURING A PHASE I TRIAL

Fig.13.5



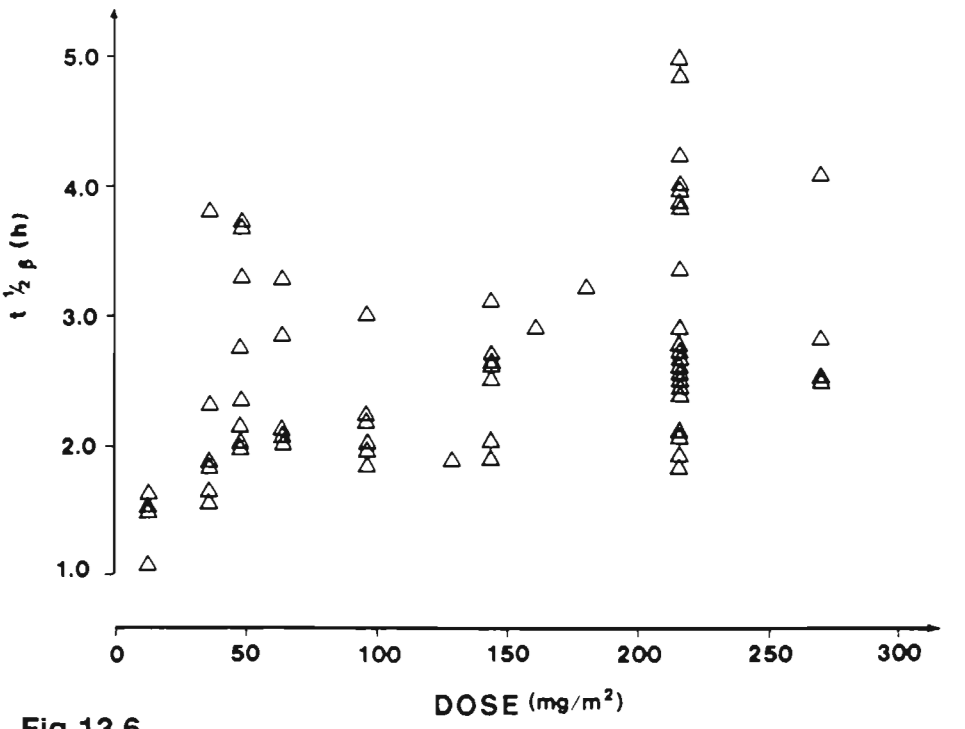


Fig.13.6

FIG. 14.

RELATIONSHIP BETWEEN DOSE OF CI-921 AND % UNCHANGED DRUG EXCRETED IN URINE

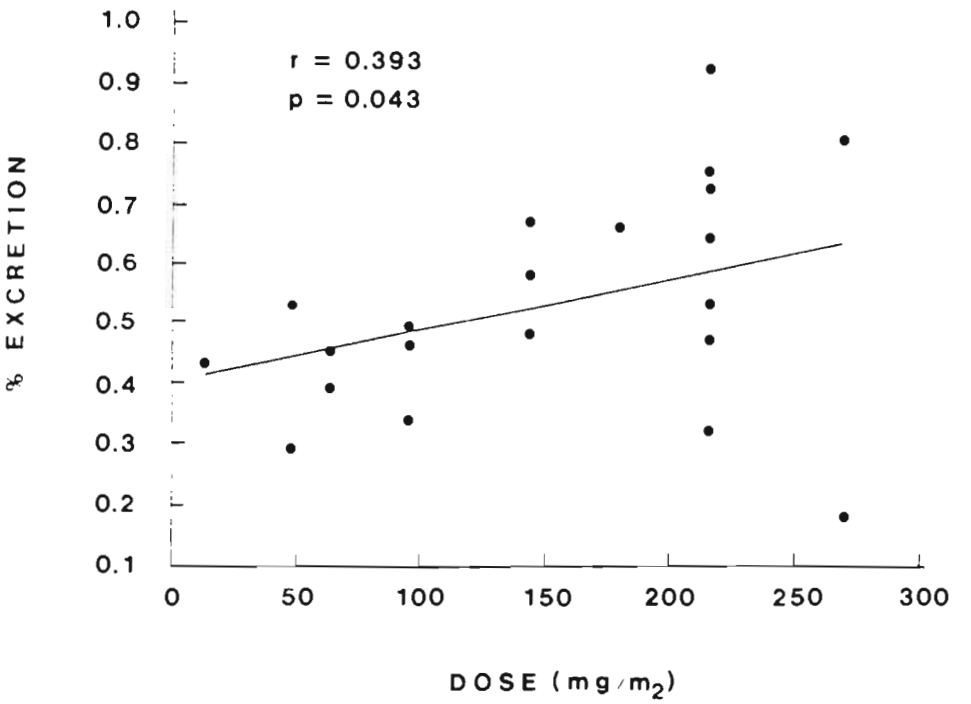


TABLE 4.7 The relationship between toxicity (nadir absolute granulocyte count) and pharmacokinetic parameters - Phase I

Parameter	Correlation*	Significance
AUC	-0.8377	1×10^{-6}
Cmax	-0.8579	1×10^{-6}
dose (μmol/kg)	-0.7678	1×10^{-6}
dose (mg/m^2)	-0.8653	1×10^{-6}

*Pearson's correlation co-efficient.

FIGS.15.1-15.3. Relationship between toxicity (nadir absolute granulocyte count) and pharmacokinetic parameters.

FIG. 15.1. RELATIONSHIP BETWEEN TOXICITY OF CI-921 AND AREA UNDER CURVE

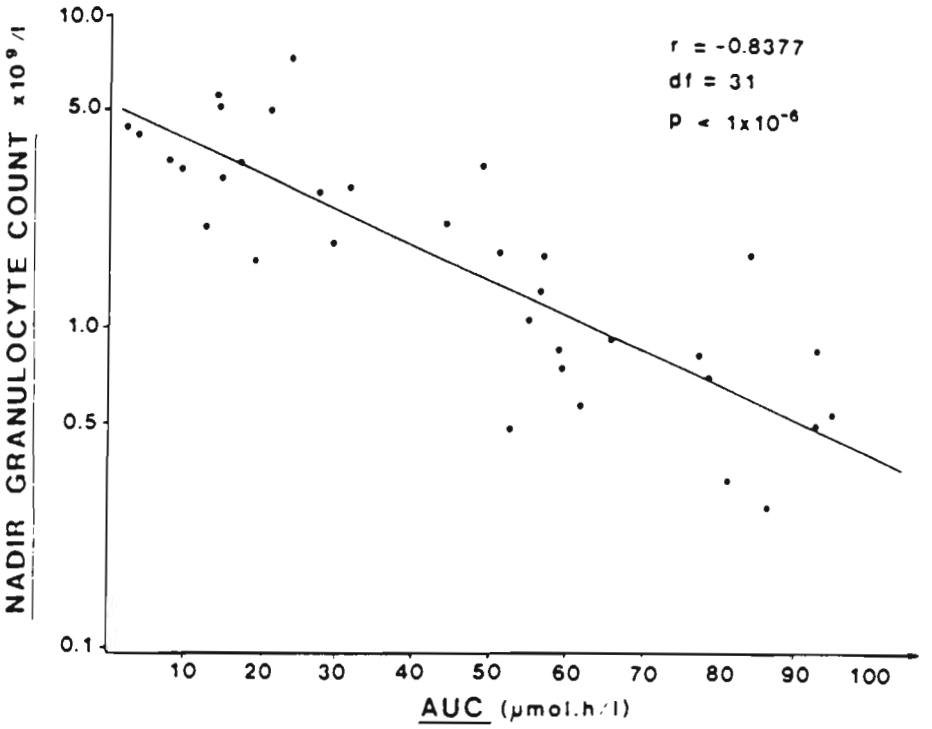


FIG. 15.2.

RELATIONSHIP BETWEEN TOXICITY OF CI-921 AND MAXIMUM PLASMA CONCENTRATION

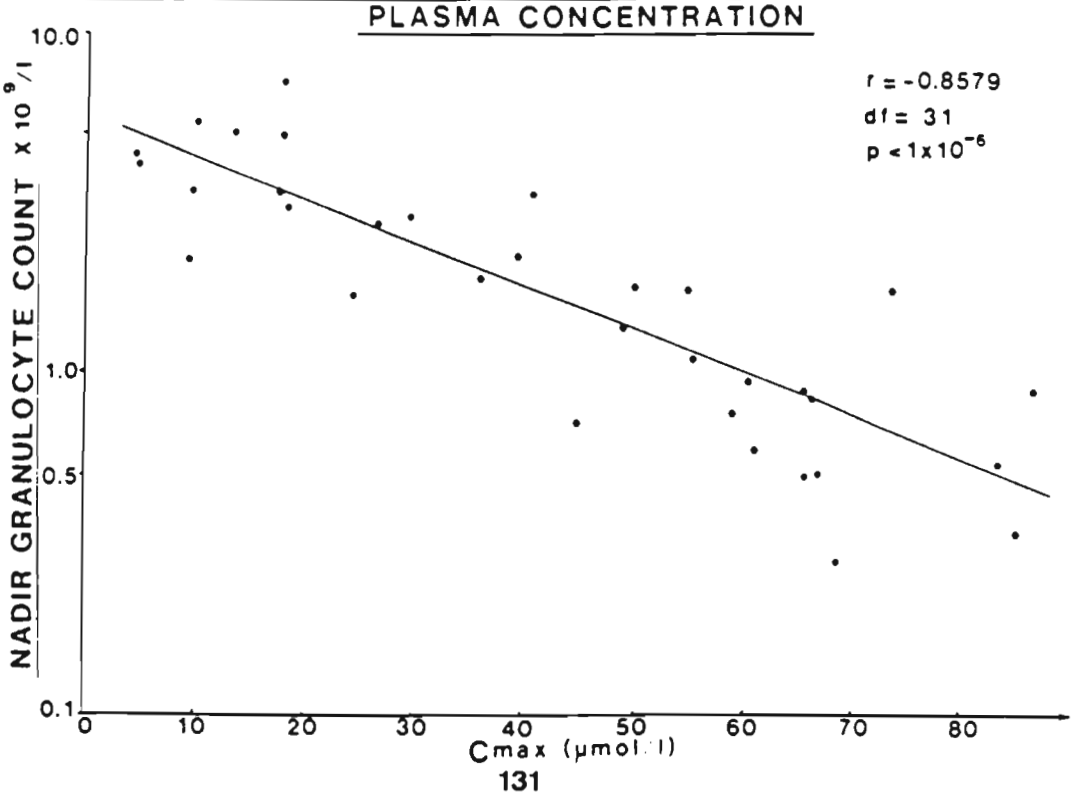
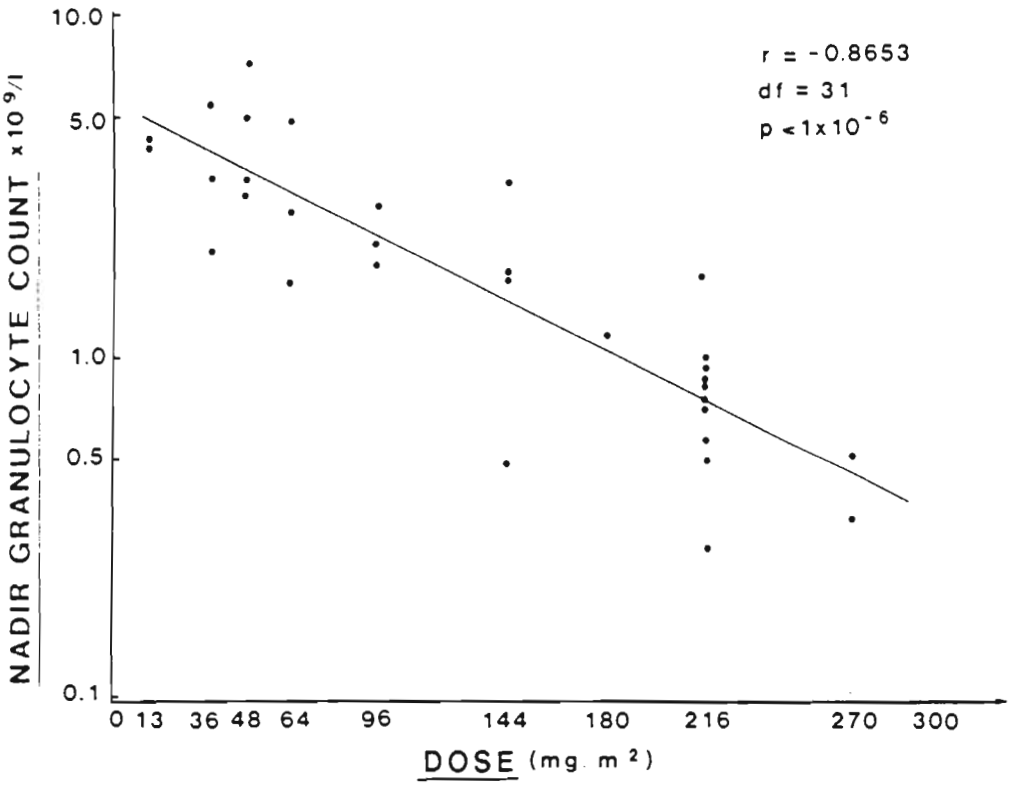


FIG. 15.3.

RELATIONSHIP BETWEEN TOXICITY OF CI-921 AND DOSE



Phase II

Plasma was collected after 18 infusions in 15 patients in the phase II trial (Appendix 3.2). Samples were taken on day 1, cycle 1 only, except for three patients who had kinetic studies on day 1 of a second cycle. The mean model-independent pharmacokinetic parameters were as shown in Table 4.8.

These results were compared with the pharmacokinetic parameters of eight patients in the phase 1 trial who received the same dose (Appendix 3.3). There was no significant difference between the means of the two groups for any parameter except Cmax and AUC. The mean Cmax of the phase II study (78.1μmol/l) was significantly greater than the mean Cmax of this dose in the phase I study (64.3μmol/l, $p = 0.012$). Similarly, the mean AUC of the phase II study was significantly greater (91.8μmol.h/l cf. 73.8μmol.h/l, $p = 0.018$).

Toxicity, as defined by nadir absolute granulocyte count, was well correlated with AUC and Cl (Table 4.9, Fig 16). There was no correlation with other pharmacokinetic parameters.

Table 4.8 Mean model-independent pharmacokinetic parameters in the phase II trial.

Parameter	Mean value	Range
Cmax	78.1 μmol/l	56.3 - 106.5 μmol/l
AUC	91.8 μmol.h/l	43 - 121 μmol.h/l
$t_{½}$å	0.54 h	0.33 - 0.83 h
$t_{½}$ß	3.2 h	1.86 - 4.39 h
MRT	2.37 h	1.21 - 3.21 h
CL	135 ml/h/kg	95 - 253 ml/h/kg
Vss	313 ml/kg	205 - 499 ml/kg

DISCUSSION

Phase I

Over the dose range studied in this centre (13 - 810 mg/m^2), a biexponential disposition of CI-921 was observed in plasma following a 15 minute i.v. infusion. A similar pattern of distribution and elimination was seen with amsacrine (Jurlina et al 1985).

Despite appearing more potent than amsacrine in pre-clinical studies (Baguley et al 1984),

Table 4.9 The relationship between toxicity (nadir absolute granulocyte count) and pharmacokinetic parameters - Phase II.

Parameter	Correlation* co-efficient	Significance
CL	0.8356	2×10^{-5}
AUC	-0.8346	2×10^{-5}
Cmax	-0.4913	0.038
Vss	0.505	0.032
dose (μmol/kg)	-0.2625	NS
MRT	-0.2824	NS
$t_{1/2}\beta$	-0.1971	NS

*Pearson's correlation co-efficient.

FIGS.16.1-16.3. Relationship between toxicity (nadir absolute granulocyte count) and pharmacokinetic parameters. Phase II.

FIG. 16.1.

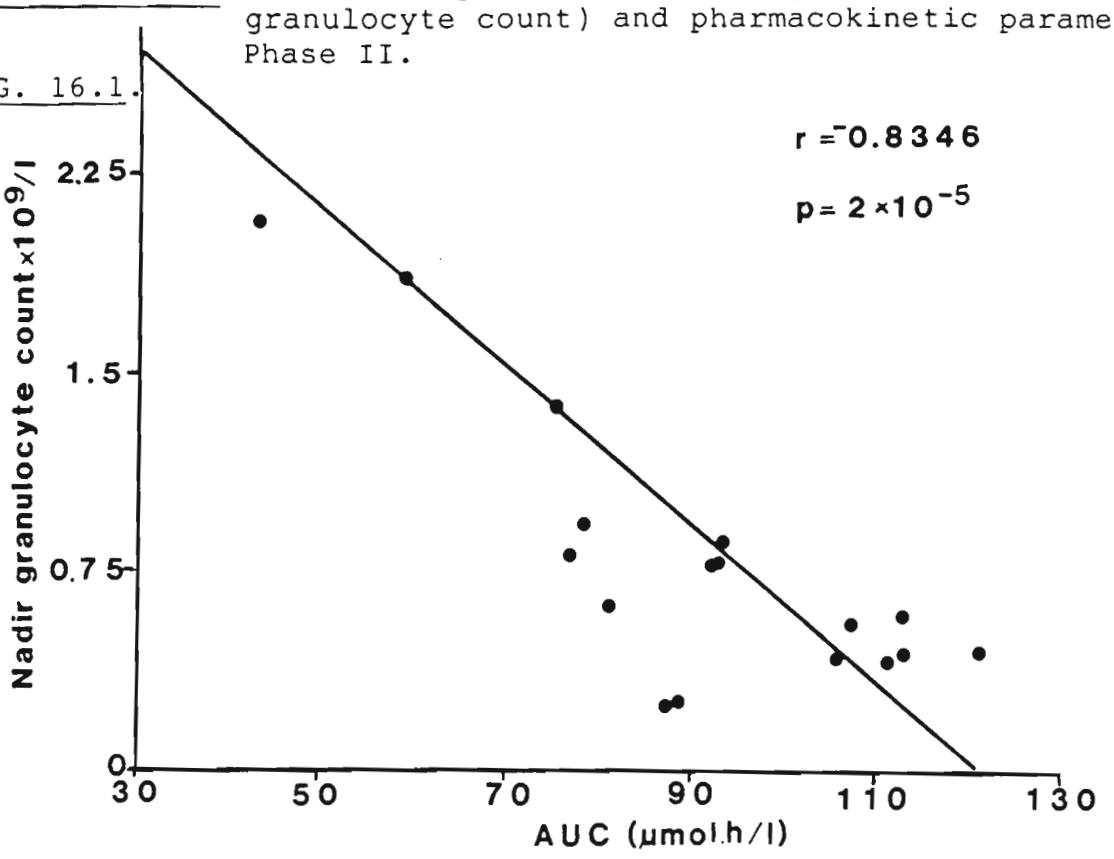


FIG. 16.2

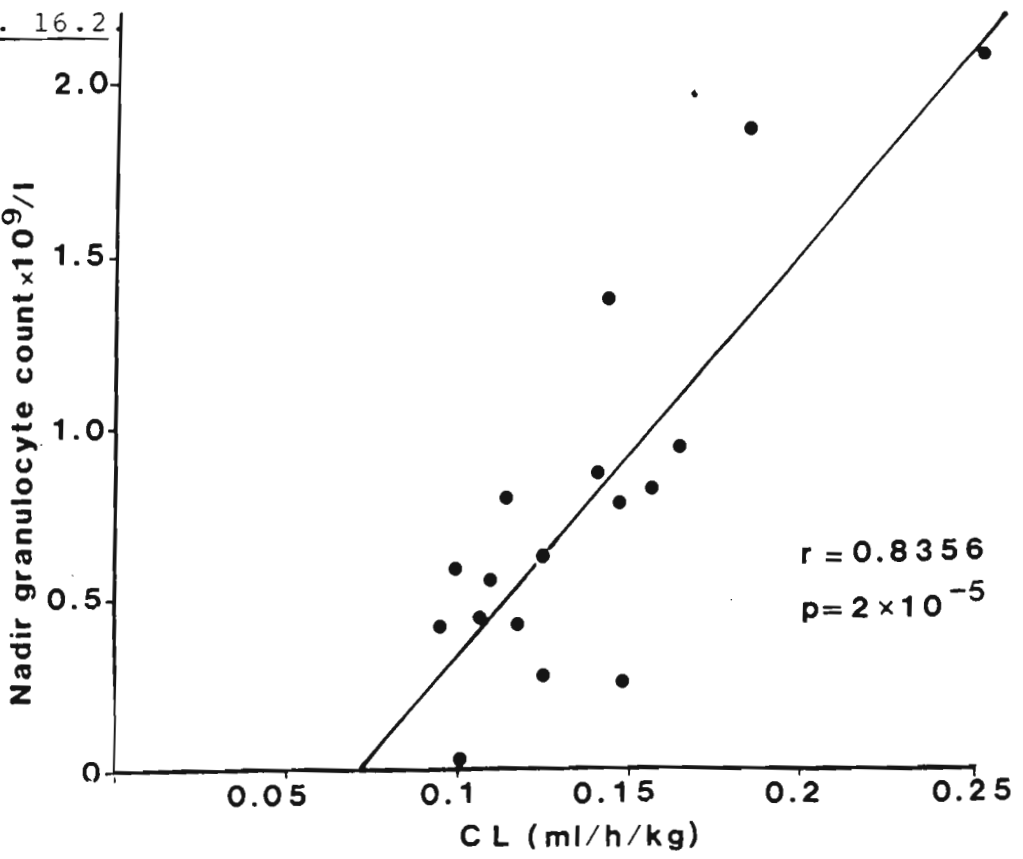
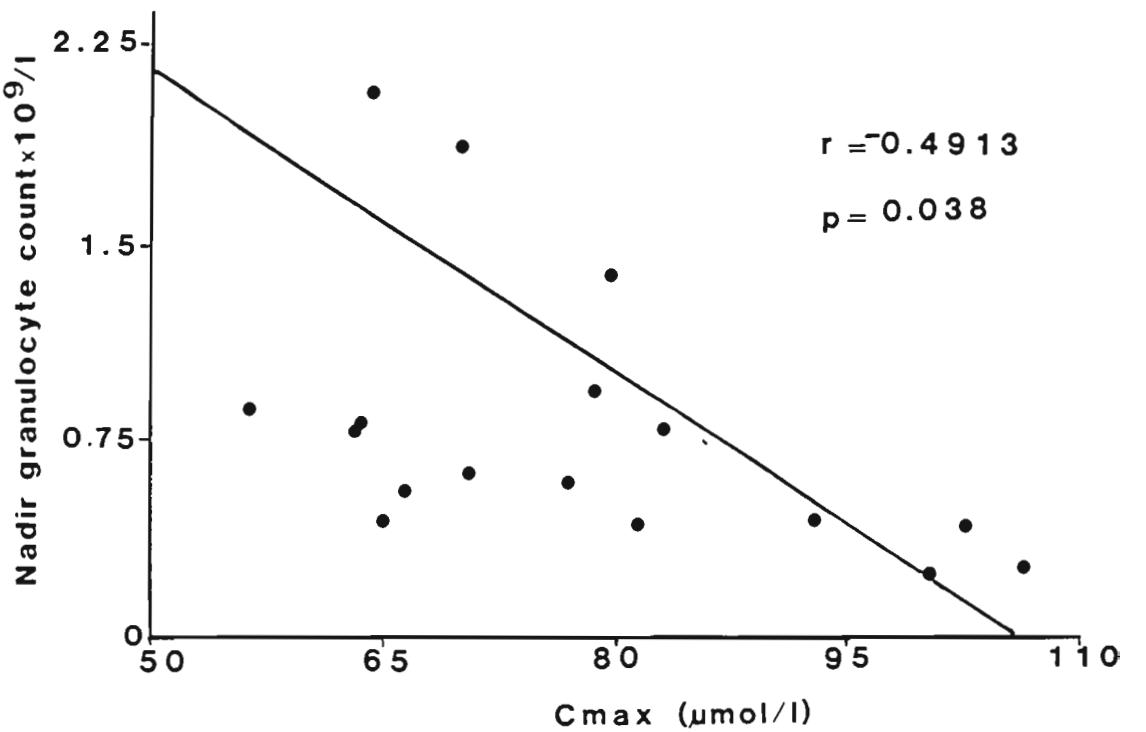


FIG. 16.3.


Nadir granulocyte count x 10^9/l
2.25
1.5
0.75
0
50
65
80
95
110
Cmax (µmol/l)
r = -0.4913
p = 0.038

the maximum tolerated dose of CI-921 was higher than that of amsacrine given in the same schedule in phase I studies against solid tumours (Legha et al 1978). This may be related to differences in physicochemical and pharmacokinetic properties. Both amsacrine and CI-921 are highly protein bound in plasma, the unbound fraction being 0.3% for CI-921(Paxton and Jurlina 1986) and approximately 3.0% for amsacrine (Jurlina et al 1985). This 10-fold variation in plasma unbound fraction makes direct comparison of the pharmacokinetics of CI-921 and amsacrine difficult when total plasma concentrations are measured as in this assay. When Paxton and Jurlina (1986) studied the plasma pharmacokinetics of total and unbound amsacrine and CI-921 in rabbits, following equimolar dose intravenous infusion, three-fold higher total plasma concentrations were achieved with CI-921 than with amsacrine whereas the unbound fraction was significantly less for CI-921 (0.33%) than for amsacrine (2.78%). There was no significant difference between distribution and elimination half-lives and MRT, but the Vss and Cl of unbound CI-921 was 3 - 4 times greater than the corresponding parameters for unbound amsacrine. They suggest that the greater distribution or tissue uptake of CI-921, despite higher protein binding in plasma, might be responsible for the greater solid tumour activity of the drug in pre-clinical studies. In a pharmacokinetic study by Jurlina et al (1985), where patients with acute myeloid leukaemia were given amsacrine in a similar schedule to this phase I study, the initial distribution half-life ($t_{½}$å) was similar to that of CI-921 (0.61 cf 0.46h) but the elimination half-life ($t_{½}$ß) was almost twice that of CI-921 (4.7 cf 2.6 h). The differences in $t_{½}$ß can be related to the difference in CL and Vss of the two drugs. Half-life is proportional to Vss and inversely proportional to Cl. Although the Vss of unbound CI-921 is 2 - 3 times greater than that of amsacrine, the clearance of unbound CI-921 is almost 6 times greater. This accounts for the difference in the elimination half-lives of the two drugs (Paxton et al

1988).

The highly significant linear correlation between dose and AUC shown in this study is characteristic of linear kinetics. This implies that over the dose range studied in this trial there was no saturation of the enzyme systems involved in the metabolism of CI-921 such that the clearance of drug was constant and not affected by increasing dose.

In another trial centre, CI-921 was given as a single dose on day 1 (rather than divided over 3 days as in this study) so that much higher individual doses were given. The pharmacokinetic data from this trial was obviously non-linear, especially at doses >500 mg/m^2 (Figs 17, 18), with a disproportionate increase in AUC and a decrease in clearance with increasing dose, resulting in a longer $t_{1/2}$ at higher dose. Therefore, although the data from this study was suggestive of linear kinetics over the dose range 13 to 270 mg/m^2, the pharmacokinetic parameters of CI-921 over the entire phase I range (4 to 648 mg/m^2) appears non-linear or dose dependent (Fig.19). This is important because a small increase in dose at high levels will result in a large increase in AUC and therefore might result in greater toxicity than might be expected for a small dose increment.

One disadvantage in having such a relatively small number of patients in a phase I trial is the limitation this imposes on the interpretation of pharmacokinetic studies where there is considerable interpatient variability in some parameters, e.g. Cl, Vss, MRT, $t_{1/2}ß$.

FIG. 17.

PROTOCOL 921–1: (single dose study)

RELATIONSHIP BETWEEN DOSE AND MEAN AREA UNDER THE CURVE

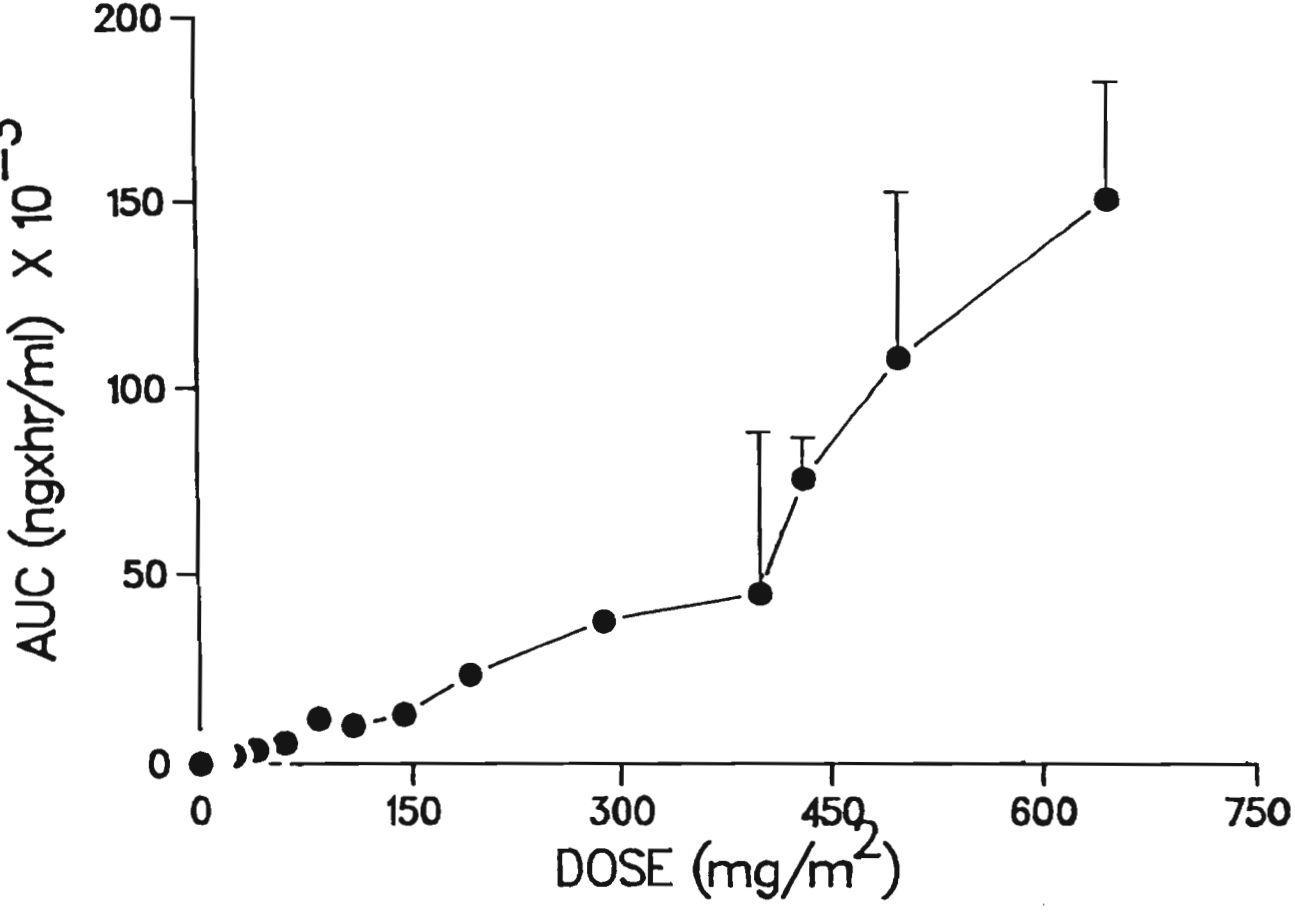
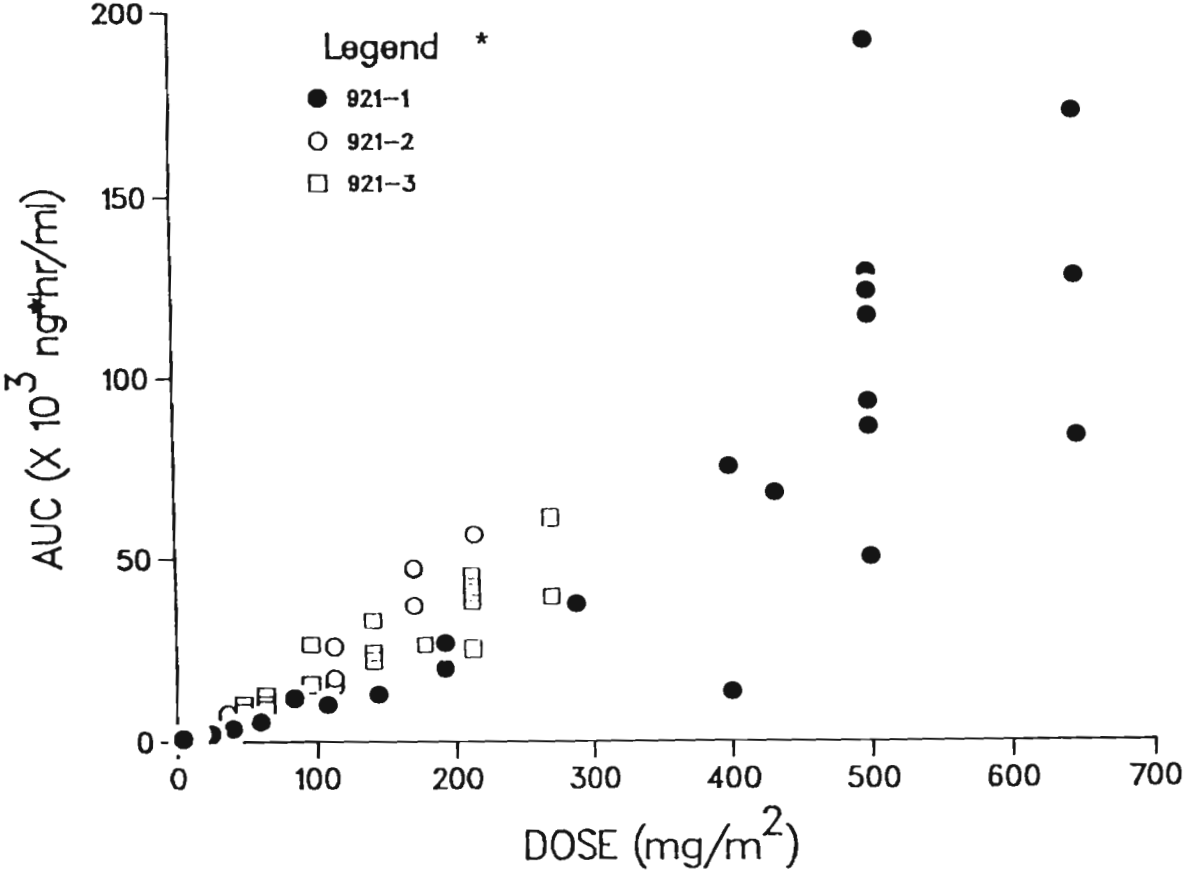


FIG. 18.

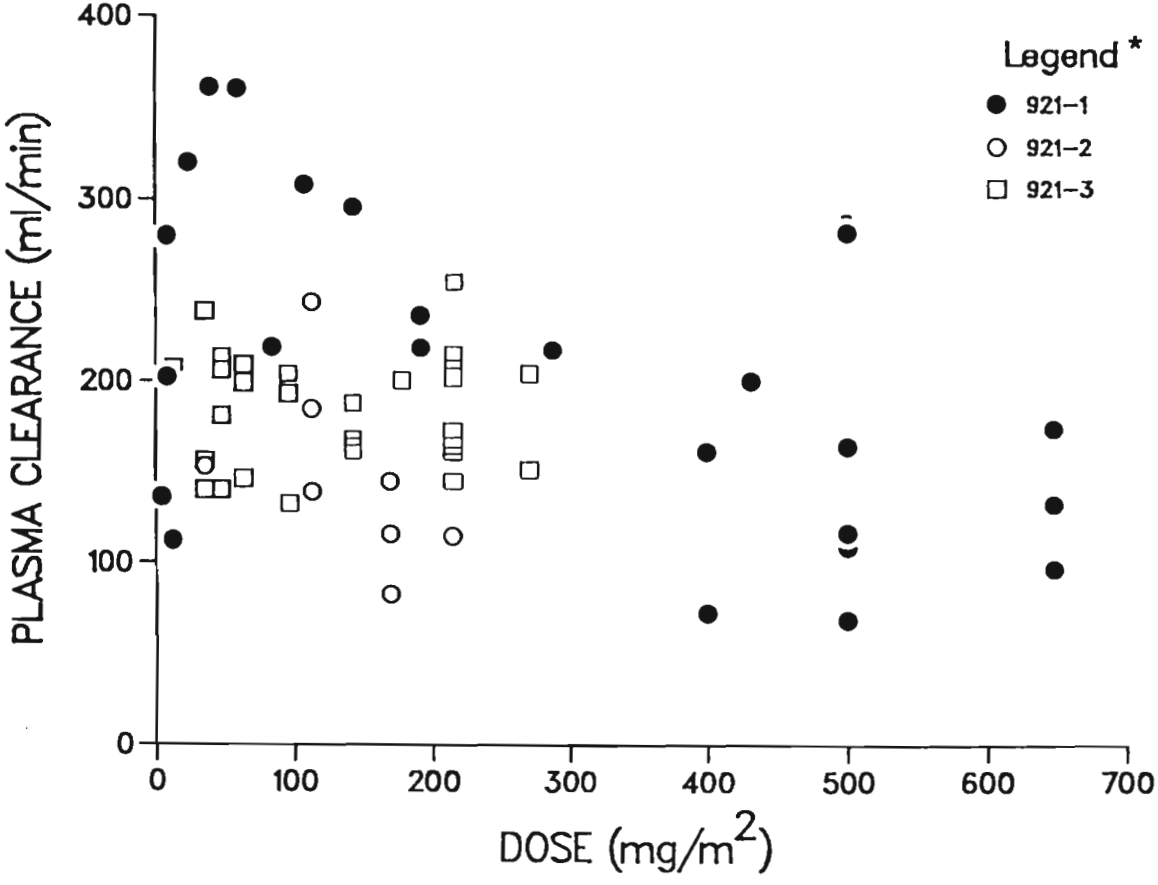
RELATIONSHIP BETWEEN AREA UNDER THE CURVE AND DOSE



* Combined data from the 3 phase I study centres.
921-1 single dose, 3 weekly (Puerto-Rico)
921-2 weekly dose x 3, repeated every 5 weeks (Ohio)
921-3 daily dose x 3, 3 weekly (Auckland).

FIG. 19.

RELATIONSHIP BETWEEN CLEARANCE AND DOSE



* Combined data from 3 phase I study centres.
921-1 single dose, 3 weekly (Puerto-Rico)
921-2 weekly dose x 3, repeated every 5 weeks (Ohio)
921-3 daily dose x 3, 3 weekly (Auckland).

Variations in pharmacokinetic patterns was also seen between patients receiving multiple courses of CI-921 at increasing dose (Fig. 20). For example, the pharmacokinetic studies of patient 005 who received six courses over a dose range 48 to 216 mg/m^2 shows a definite linear correlation between dose and AUC. In contrast, both patient 001 and 008 show a non-linear kinetic pattern with increasing dose. Patient no. 005 was fit and well with a high performance status throughout and survived some six months following completion of treatment. On the other hand, both patients 001 and 008 had end-stage breast cancer and their general condition deteriorated whilst on study with both dying within two months of completing treatment. It is possible that dose dependent kinetics in these patients were a reflection of deteriorating health and/or subclinical hepatic impairment. No obvious relationship was seen between pharmacokinetic parameters and age, sex or concurrent medications. One patient on morphine (patient no. 010) was found to have a relatively long elimination half-life at 3.83 h on day 1 and 4.83 h on day 3, with a mean of 4.3 h. However, the mean $t_{1/2}\beta$ (2.87 h +/- 0.93) following 14 infusions (day 1 and day 3) of the 5 patients receiving oral morphine in 7 courses was not significantly different from the overall mean in all patients (2.5 h $\pm$ 0.7) (Table 4.10).

In pre-clinical studies, 95% of ^{14}C-labelled CI-921 administered i.v. to dogs was recovered in the faeces and only 4% in the urine (Investigators Brochure, 1985). Similarly in mice, over 95% of ^{14}C-labelled CI-921 administered i.p. was excreted in faeces, with only about 3% detected in urine (P. Kestrell, personal communication). Less than 1% of unchanged CI-921 was excreted in the urine of patients in the phase I trial. This suggests that the metabolism of CI-921 is similar to that of amsacrine, where renal elimination plays a minor role. As no patient had significant renal impairment at the time of drug infusion the

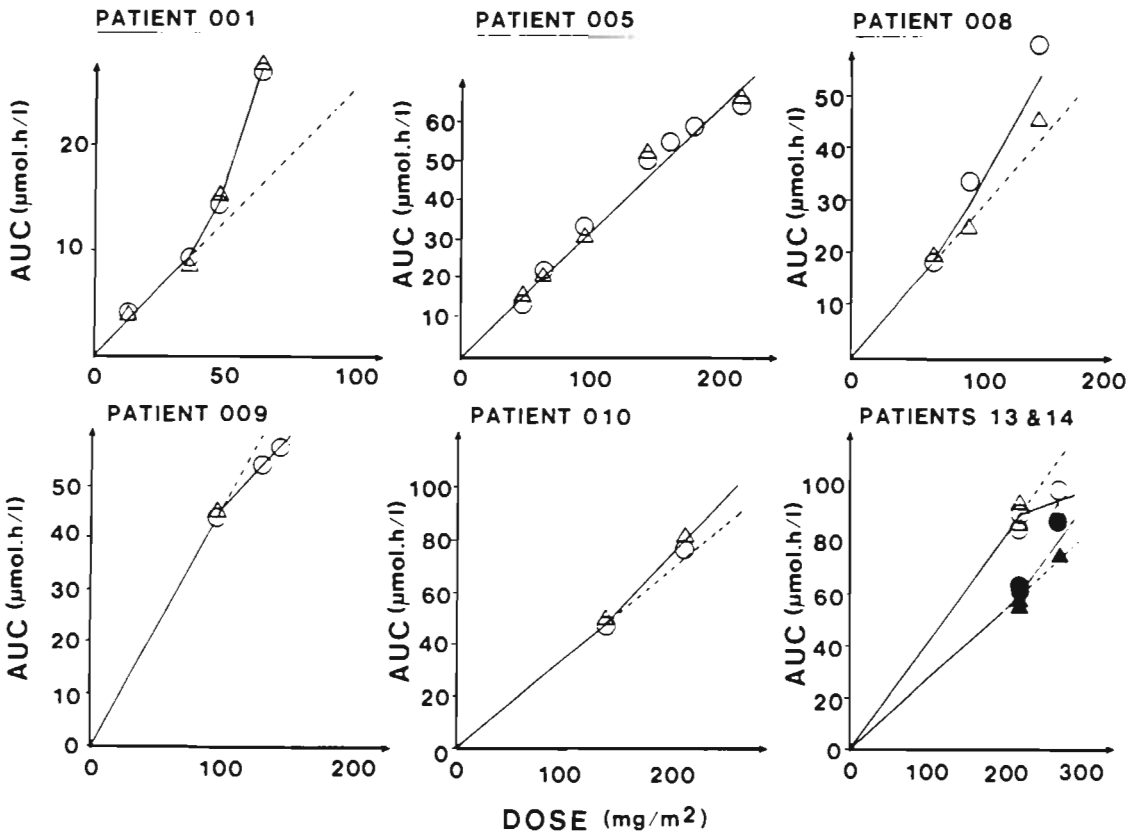


FIG. 20. Relationship between pharmacokinetic parameters and dose in individual patients.

TABLE 4.10 Mean terminal half-life following day 1 and day 3 infusions.

Patient no.	Course no. 1	2	3	4	5	6
001	1.5	1.7	3.2*	3.1*		
002	1.3*					
003	1.7					
004	3.1					
005	2.1	2.0	2.0	2.4	3.1*	2.9
006	3.5					
007	2.2*					
008	2.1	2.1	2.6			
009	2.5	1.9	1.9			
010	2.9*	4.3*				
011	1.8					
012	2.6					
013	2.5	2.7	3.4			
014	3.8	2.2	2.1			
015	3.1	3.8				
016	4.1					

* courses in which patients received morphine

mean $t_{1/2}\beta$ for patients on morphine = 2.87h

mean $t_{1/2}\beta$ for patients not on morphine = 2.52h

importance of adequate renal function in the elimination of CI-921 has not been addressed in this study.

As the AUC is a measure of concentration and persistence of drug in plasma (i.e. drug exposure), the size and duration of biological response and/or toxicity might be expected to be closely related to AUC. AUC has in fact been found to be a better predictor of toxicity than absolute concentration for many cytotoxic drugs (Collins et al 1986). Although a highly significant correlation between toxicity and AUC has been documented in this study, the correlation of toxicity with maximum concentration in plasma (Cmax) and total dose was just as strong, at least with respect to myelosuppression, because of the linear pattern of kinetics. One of the main reasons for undertaking pharmacokinetic studies in phase I trials is to determine the relation between dose and effect, i.e. to determine whether the pharmacokinetic parameters follow a linear pattern as shown above (where the degree of toxicity could be just as well predicted by increasing dose), or whether the kinetics are non-linear. The latter implies a disproportionate increase in AUC with increasing dose and therefore unpredictable toxicity with increasing dose. This is especially important because of the narrow therapeutic index for many of these agents, with only small margins between therapeutically effective and unacceptably toxic levels.

It has been proposed (as discussed in Chapter 1) that pharmacokinetic data be used to predict starting doses and subsequent dose escalations of cytotoxic drugs in man as a further attempt to reduce the number of patients treated at clearly sub-therapeutic doses. The dose of CI-921 in mg/m^2, which was found to be toxic in man could not be predicted from murine studies as there was a 5 to 6-fold difference between the LD_{10} in mice and MTD

doses in man.

Unfortunately pharmacokinetic parameters were no better guide in this study as there was a 7-fold difference between the AUC of the mouse LD_{10} (determined in this laboratory to be about 40 μmol.h/l) and the AUC of the MTD of CI-921 in man (90 μmol.h/l x 3). Conversely both the LD_{10} mouse/MTD human AUC ratios and the dose ratios in mg/m^2 for amsacrine are reported to be close to unity (1.3 and 0.8 respectively) (Collins et al 1986). The toxicity studies with amsacrine described in Chapter 6 support this dose ratio in that the mouse LD_{10} of about 55mg/kg (approximately 165 mg/m^2) approximates the single dose MTD of 160 mg/m^2 reported by Bodey et al (1983). In this laboratory there was a 3-fold difference between the AUC of the mouse LD_{10} (approximately 14 μmol.h/l) and the AUC of the MTD of amsacrine against solid tumours in man (46 μmol.h/l).

If pharmacokinetics had been used to monitor dose escalation in the phase I trial of CI-921 one might have predicted a dose of 144 mg/m^2 as being the MTD (as the AUC x 3 at this level approximates 40 μmol.h/l) and subsequent dose escalation might have been more cautious than those employed. Such a scheme would have been even more inappropriate in the case of CI-921 where the MTD was determined to be 810 mg/m^2. These findings are not totally unexpected. The EORTC pharmacokinetic and metabolism group (1987) have concluded that pharmacokinetically guided dose escalation is only practical if: the toxicity of the drug is AUC related, the pharmacokinetics are linear, the active species of the drug is being measured, the intrinsic sensitivities of critical tissues such as bone marrow are similar in mouse and man and the extent of protein binding in mice and man is similar. A potential problem with CI-921 in this scheme is that although the pharmacokinetics

appeared to follow a linear pattern over the dose range studied in this phase I trial, the pharmacokinetics of CI-921 are not linear at higher doses (personal communication, Parke-Davis Research Division, Warner-Lambert Co.). It is likely that CI-921 is just as highly protein bound in mice as in man but there is controversy as to which is the active species in the anilino-acridine drugs. As discussed in Chapter 1, Shoemaker et al (1984) suggest that the microsomal oxidation products of amsacrine are considerably more cytotoxic than amsacrine itself while Robbie et al (1988) have shown unchanged amsacrine to be active. Whether unchanged CI-921 or its metabolites have the greatest cytotoxic activity in mice and man has yet to be determined. The fact that the parent compound, which may not be the active cytotoxic agent, is being measured in this assay might explain the discrepancies in pharmacokinetic prediction in this case. Of greater significance however is the large variation seen in AUC both between patients treated at the same dose (Appendix 3.1) and in individual patients treated at the same dose in multiple courses (Fig 20). This obviously makes any predictions based on AUC estimates very difficult and has been quoted as a reason for this method of dose escalation to fail in other phase I studies (Graham et al 1988).

Thus pharmacokinetic studies would have been valuable in guiding the dose escalation of amsacrine in a phase I study but have not been helpful in guiding the dose escalation of CI-921 in the current phase I trial. This may be related to the difference in protein binding of the two drugs. Both CI-921 and amsacrine are highly bound in plasma, but the active unbound fraction is significantly less (10-fold) for CI-921 than for amsacrine (Paxton and Jurlina 1986).

Phase II

The pharmacokinetic parameters from the phase II trial are consistent with those of the high dose treatment in phase I although there appeared to be a tendency towards a tri-phasic pattern of disposition in several patients.

The reason for the significant difference between the Cmax and AUC of the phase II study and phase I study group treated at the same dose, is unknown. There does appear to be a trend towards a reduced clearance in the phase II group (135 compared to 155ml/h/kg), but this difference is not significant.

There was no significant difference between the age or the performance status of the two groups and their general fitness was similar. It seems unlikely that the tumour type is important as four of eight patients in the phase I group treated at the same dose also had NSCLC. Perhaps the difference can be related to prior treatment. Three of eight patients (37.5%) in the phase I group had had no prior treatment and three of eight had had prior chemotherapy (37.5%), whereas four of the 15 (26.5%) patients in the phase II study group were previously untreated and no patient had had chemotherapy. The most likely reason for the difference between the two groups however is the small number of patients studied, especially in the phase I group. These issues could only be clarified by the study of pharmacokinetic parameters in a much larger group of patients, ideally previously untreated and receiving the same dose of CI-921 in an extended phase II trial.

When individual patient results are considered (Appendix 3.2), the one patient who attained a partial response (no. 019) received the highest dose on a μmol/kg basis, but had the lowest Cmax and an AUC, similar to the mean value of the whole group. The terminal half-life and MRT values are both at the upper end of the range. Therefore, in this single patient there was no pharmacokinetic parameter that "stands out" as being particularly important with respect to predicting for response.

In patients with pre-existing hepatic dysfunction a significant reduction in the clearance of amsacrine and prolongation of terminal half-life has been reported (Hall et al 1983, Staubus et al 1980). Furthermore, these patients experienced severe clinical toxicity and it has been recommended that the dose of amsacrine be reduced when treating patients with impaired liver function (Mahal et al 1981). Patient no. 023 had liver metastases and mildly abnormal liver function, without elevation of serum bilirubin. This patient had the maximum MRT (3.21 h), the second lowest Cl (100 ml/h/kg) and one of the highest AUC's (112 μmol.h/l). The $t_{1/2}\beta$ in this patient was within one standard deviation of the mean value. He did not suffer excess toxicity however and dose reduction was not required.

Patient no. 027 showed minimal suppression of bone marrow function despite standard doses of CI-921. Of interest, he was found to have the lowest AUC (43 μmol.h/l), $t_{1/2}\beta$ (1.86 h) and MRT (1.21 h) and the greatest Cl (253 ml/h/kg) in his initial course. As discussed previously, AUC is a reflection of total drug exposure and is often the best indicator of drug toxicity.

This same patient had three grand mal seizures temporally associated with CI-921 infusion

in three of four cycles (see Chapter 3). The high Vss, especially in the second course, raises the possibility of greater CNS distribution of drug in this patient and subsequent neurotoxicity. One of those patients described by Stewart et al (1984) who developed grand mal seizures during amsacrine infusion, was found to have a high cerebral concentration of amsacrine at autopsy compared to patients without this complication. A diagnosis of drug induced neurotoxicity cannot be made until structural or metabolic causes have been excluded however. As discussed in Chapter 3, this man had cerebral metastases treated with cranial radiotherapy, a recent change in anti-epileptic medication and hyponatremia, all factors which may precipitate epileptiform activity.

Many drugs have been shown to have reduced Cl in the elderly (Woodhouse et al 1984). Conversely one might expect to see more rapid Cl in young patients. However, the youngest patient in our study (no. 035, aged 44 years) had the lowest Cl (95 ml/h/kg), $t_{1/2}$å (0.33 h) and the lowest Vss (250 ml/kg). The oldest patient (no. 017, aged 73 years) was found to have the longest $t_{1/2}$ß of 3.49 hours but no obvious difference in any of the other parameters and a Cl close to the mean of the whole group. This patient had borderline renal function at the time of treatment and subsequently died of disease-related renal failure.

The pharmacokinetic parameters of the only female in the group (no. 022) were unremarkable.

The effect of concomitant medication has been examined. The drug plasma clearance of the three patients on beta-blockers (nos. 020, 024 and 025) was at the lower end of the

range in all cases but there was no obvious trend in any of the other kinetic parameters. Beta-blockers can interfere with hepatic blood flow, but as both CI-921 and amsacrine have been shown to have low initial hepatic extraction (Paxton and Jurlina 1986), concurrent treatment with beta-blockers would not be expected to interfere with plasma clearance by this mechanism. However, beta-blockers, especially those with high lipid solubility, (e.g. propranolol) have also been shown to inhibit microsomal enzymes, which may account for the low Cl seen in the current study (Park 1984). No patient in the phase II trial was taking morphine at the time the pharmacokinetic studies were performed.

The pharmacokinetic parameters in most of the patients who had studies performed on two occasions (nos. 013, 014, 015, 018, 019) were similar, but patient 027 showed marked variability in Cl, MRT $t_{1/2}\beta$ Vss and the AUC of courses 1 and 2 (Appendix 3.**2**). It is possible that this may be related to the difference in anti-epileptic medications taken in the two courses. This patient had previously been stabilised on phenytoin but was changed to carbamazepine after the first course. Both drugs are known to stimulate hepatic microsomal enzymes, but a comparative study of the relative enzyme inducing properties of anti-convulsant drugs by Perucca et al (1984) has shown phenytoin to have greater potency with respect to enzyme induction than carbamazepine. This could account for the difference in kinetic parameters in the two courses. The other patients (nos. 013, 014, 015, 018, 019) were all stabilised on their medications with no change between courses and no major change in their general fitness or condition.

With the significant intra-patient variability in pharmacokinetic parameters it is likely that a formal study would be required to detect the effect of concomitant medication on pharmacokinetic profiles.

CHAPTER 5

THE ASSESSMENT OF CI-921 FOR CARDIOTOXICITY

CHAPTER 5. THE ASSESSMENT OF CI-921 FOR CARDIO-TOXICITY

INTRODUCTION

Because of the cardiotoxicity which has been shown in association with amsacrine, (Weiss et al 1986) (Chapter 1) patients receiving CI-921 were evaluated prior to and following treatment in an attempt to exclude drug-related cardiotoxicity.

MATERIALS AND METHODS

Patients

All patients receiving CI-921 as part of the phase I and II clinical trials were studied. Because the cardiotoxicity associated with amsacrine has often been associated with hypokalaemia, all patients had blood taken for assessment of serum potassium within the 24 hours prior to each infusion, with correction to within the normal range if required.

Phase I

Of the 16 patients in the phase I trial, five had received prior chemotherapy, but only two of these had received anthracyclines. Four patients had been treated with radiotherapy to the chest or mediastinum. This is a known contributory factor in the cardiotoxicity associated with anthracyclines. Six patients had a history of cardiovascular disease; five patients had hypertension and one patient ischaemic heart disease. Cardiac medications

included pindolol, nifedipine, methyldopa and prazosin (in single patients) and diuretics (in three patients).

The dose of CI-921 was escalated from 39 mg/m2 to 810 mg/m^2 (total dose over three days) within the 16 patients.

Phase II

No patient in the phase II trial had received prior chemotherapy, but eight of the 19 patients had had prior radiotherapy to the chest and/or mediastinum. Nine patients had a history of cardio-vascular disease; myocardial infarction (two patients), recurrent tachyarrhythmias (two patients), hypertension (two patients) and myocarditis, ischaemic heart disease and peripheral vascular disease in single patients. Cardiac medications included digoxin (two patients), ß- blockers (three patients) and amiodarone, diuretics, flecainide and a calcium antagonist (diltiazem) (one patient each). All patients received a total dose of 648 mg/m^2 (216 mg/m^2 daily for three days). The median number of courses given was two (range 1- 5).

Cardiac evaluation.

Echocardiograms were performed with an ATL Ultramark 8 ultrasound system (Advanced Technology Laboratories). One of two technicians performed and analysed all echocardiograms. The assessment of left ventricular function by two-dimensional (2-D) echocardiography was by the method described by Wahr et al (1983) as modifed by Sharpe et al (1988).

M-mode and 2-D echocardiography was undertaken prior to treatment (baseline study), following every alternate course and following completion of treatment ("off- study"). Standard 12-lead electrocardiograms (ECGs) were taken prior to every course of treatment and with each echocardiogram.

The cardiac parameters estimated were:

i) ECG: heart rate, rhythm, Q-T interval and QRS voltage.

ii) M-mode echocardiography: fractional shortening (FS), systolic time interval (STI) and velocity of circumferential fibre shortening (VCF).

iii) 2-D echocardiography: left ventricular ejection fraction (LVEF).

$$FS = \frac{\text{average LVEDD - av. LVESD}}{\text{av. LVEDD}}$$

$$STI = \frac{\text{av.PEP}}{\text{av.LVET}}$$

$$VCF = \frac{\text{F.S.}}{\text{av.LVET}}$$

LVEDD = left ventricular end-diastolic dimension

LVESD = left ventricular end-systolic dimension

PEP = pre-ejection period.

LVET = left ventricular ejection time.

Toxicity

Cardiac toxicity was defined by the following parameters:

i) clinical - the development of congestive heart failure

ii) ECG - abnormal rate, rhythm or wave-form

- decrease in sum of QRS (ΣQRS) voltages by >30% baseline.
- QT interval: prolongation of the corrected Q-T (QTc) interval to >0.46 sec.

$$QTc = \frac{\text{QT interval (msec)}}{\text{R - R interval (msec)}}$$

iii) M-mode echocardiography

- decrease in FS < 25% baseline.
- increase in STI to > 0.45
- fall in VCF to < 0.7

iv) 2-D echocardiography

- decrease in LVEF by > 10% from baseline.

Statistics

The paired students' t-test (two-tailed) was used to compare the difference between the means of the pre- and post-treatment parameters.

RESULTS

Phase I

Thirteen patients were evaluable for cardiotoxicity having had ECGs and echocardiograms performed prior to and following at least one course of treatment. Three patients were too ill for post-treatment cardiac assessment because of rapid progression of disease.

All 13 patients were assessed by ECG but complete M-mode echocardiography was possible in only nine patients and only five patients could be assessed for change in LVEF by 2-D echocardiography, primarily because of deteriorating patient health and technical difficulties in obtaining adequate scans. Only three of 13 patients (23%) underwent pre- and post-treatment evaluation of all parameters specified (Table 5.1).

No patient developed clinical evidence of congestive heart failure or cardiac arrhythmias. Three patients developed sinus tachycardia during treatment but this was thought to be due to advancing disease and deteriorating health rather than a reflection of cardiac toxicity.

There was marked variation between patients, but no significant difference between the means of pre-treatment and post-treatment assessment of any of the cardiac parameters (Fig 21).

TABLE 5.1 Pre- and post-treatment cardiac parameters - Phase I

Patient no.	Cardiac parameter											
	ΣQRS (mm)		QTc (sec)		F.S.(%)		V.C.F.		S.T.I.		E.F.(%)	
	*	§	*	§	*	§	*	§	*	§	*	§
001	25	26	0.45	0.41	27	27	0.96	0.98	0.19	0.21	-	-
002	45	44.5	0.45	0.45	30	31	1.3	1.35	1.35	0.35	60	60
003	41	44.5	0.37	0.40	34	28	1.2	1.1	0.32	0.27	-	-
004	45	40.5	0.41	0.41	39	39	1.26	1.36	0.26	0.24	-	-
005	45	35.5	0.36	0.41	30	31	1.5	1.1	0.20	0.31	55	54
008	37.5	31.5	0.39	0.41	-	-	-	-	-	-	-	-
009	37	35	0.41	0.41	36	31	1.6	1.3	0.39	0.38	-	-
010	29	32	0.42	0.42	41	41	1.64	1.52	0.34	0.3	-	-
011	50.5	45	0.41	0.41	36	36	1.2	1.4	0.33	0.36	-	-
013	50.5	44	0.4	0.39	-	-	-	-	-	-	-	-
014	37.0	29.5	0.39	0.47	-	-	-	-	-	-	48	49
015	39	47	0.37	0.38	42	32	1.43	1.06	0.23	0.3	57	54
016	37.0	35	0.39	0.43	-	-	-	-	-	-	48	49
Mean	39.8	37.7	0.4	0.42	35	33	1.34	1.24	0.29	0.3	54	53

* pre-treatment § post-treatment

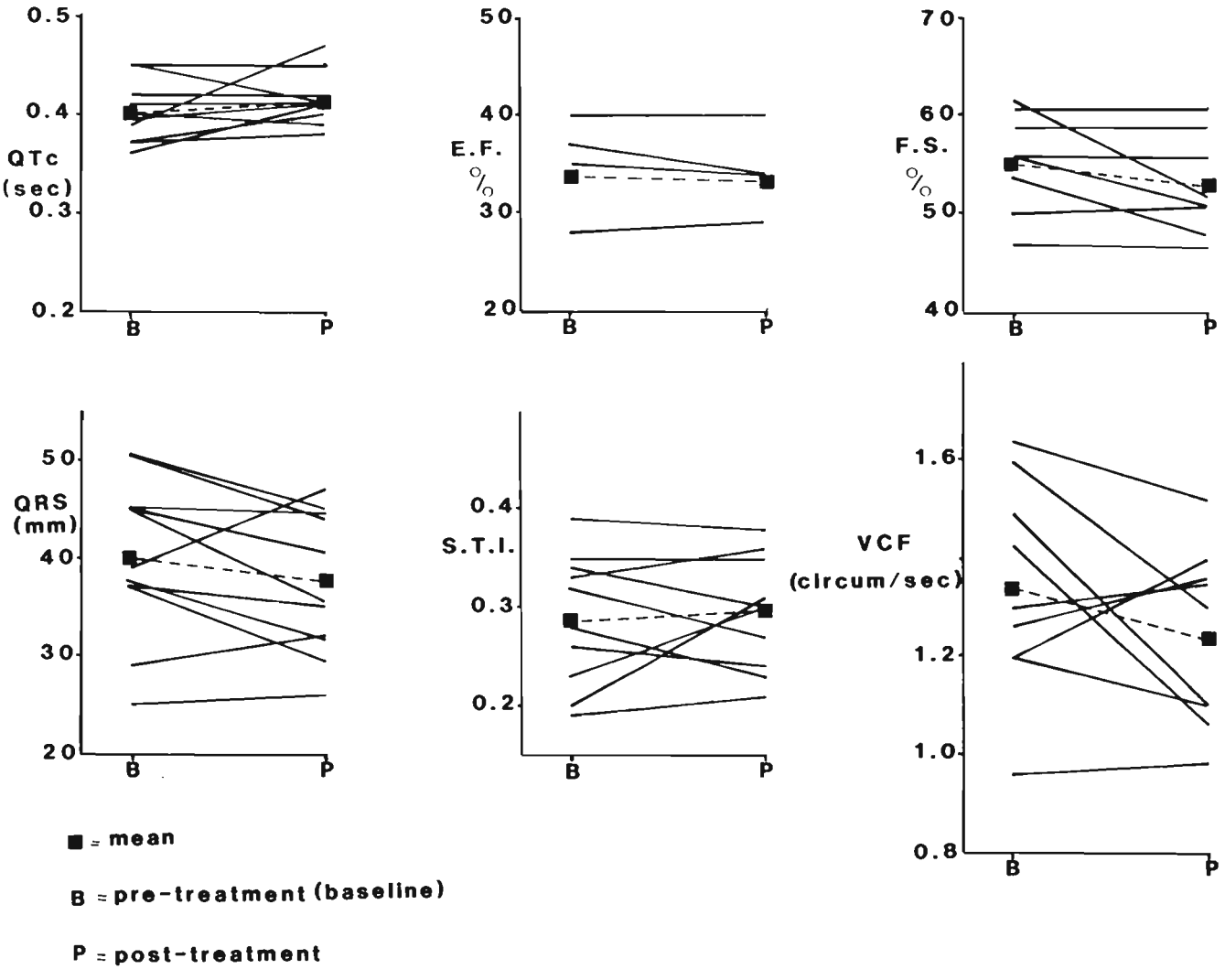


FIG. 21. Baseline and post-treatment cardiac parameters following treatment with CI-921 - Phase I clinical trial.

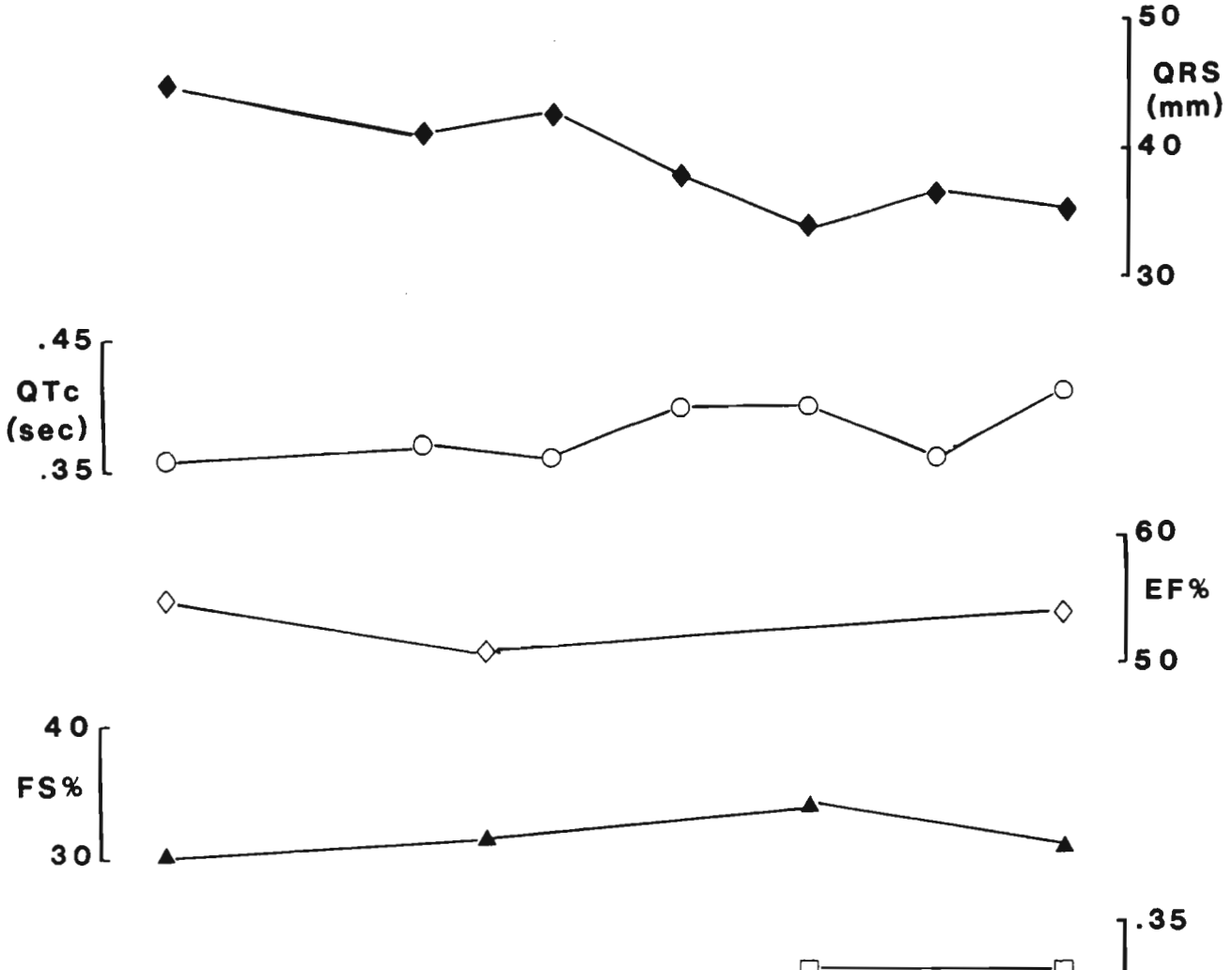
In no patient did the sum of the QRS complexes (ΣQRS) fall by more than 30% of baseline. The maximum decrease of 20% was seen in patients 005 and 014. Patient 005 received the greatest cumulative dose of CI-921 and patient 014 was a diabetic. This latter patient also developed slight prolongation of QTc at 0.47 sec (upper limit of normal = 0.46) but with no sequelae.

There was no significant fall in FS (to less than 25%) or in VCF (to less than 0.7 circum/sec). The maximum decrease in FS (24%) was seen in patient 015 who had had prior treatment with anthracyclines and was associated with a fall in VCF from 1.43 to 1.06 circum/sec.

Although no patient showed an increase in STI to toxic levels (> 0.45), two patients (nos. 005 and 015) did show a marked increase in post-treatment values as compared to pre-treatment levels (Table 5.1).

Patient 005 received six cycles of CI-921 (the maximum number in the phase I trial) over the dose range 144 mg/m2 to 648 mg/m2 (total dose over 3 days), with a total cumulative dose of 4142 mg. The cardiac parameters of this patient are presented separately in Fig 22. Although there appears to be a downward trend in ΣQRS and VCF and an increase in STI with increasing dose, toxic limits were not reached for any parameter.

FIG. 22. Cardiac parameters of patient 005 over 6 cycles of CI-921.



Phase II

Fourteen patients were evaluable for cardiotoxicity. Pre- and post-treatment M-mode echocardiograms were completed in seven patients and LVEF by 2-D echocardiography in 11 patients. Full cardiac evaluation of all parameters pre- and post-treatment was only possible in seven patients (50%) (Table 5.2). There was no significant difference between the pre-treatment and post-treatment mean values for any of the cardiac parameters (Fig. 23).

One patient (no. 035) known to have a malignant pericardial effusion, had some impairment of left ventricular function prior to treatment, with an abnormal ECG, low FS, VCF and EF and STI well above that of any other patient. Repeat echocardiography following two courses of treatment showed some further deterioration, with a decrease in FS from 29% to 21% and reduction in LVEF from 49% to 40%. There was mild ventricular dilatation and localized impairment of left ventricular wall activity suggestive of some localized pathology, e.g. ischaemia or tumour infiltration rather than global impairment as might be expected from drug toxicity. There was no significant increase in the amount of pericardial fluid following treatment and no clinical evidence of heart failure.

Two other patients (nos. 025 and 027) developed non-specific ECG ST- wave changes during treatment, in association with normal echocardiograms and subsequent return to normal. Two patients (nos. 019 and 035) developed mild prolongation of QTc to 0.48s and 0.49s respectively, but with no sequelae. The ΣQRS of patient no. 025 fell by 27%

TABLE 5.2 Pre- and post-treatment cardiac parameters - Phase II.

Patient no.	Cardiac parameter											
	ΣQRS(mm)		QTc(sec)		F.S. (%)		V.C.F.		S.T.I.		E.F.(%)	
	*	§	*	§	*	§	*	§	*	§	*	§
018	20.5	17.0	0.45	0.36	30	36	1.36	1.63	0.41	0.41	50	59
019	66.5	72.0	0.42	0.48	33	38	1.32	1.46	0.32	0.31	52	55
020	27.0	29.0	0.41	0.42	25	28	0.89	1.03	0.38	0.41	36	35
022	38.5	41.5	0.34	0.36	31	36	0.84	-	0.37	-	58	-
023	36.5	36.5	0.43	0.43	33	-	1.19	-	0.28	0.38	53	-
024	22.0	19.5	0.35	0.39	-	-	-	-	-	-	54	61
025	68.0	49.5	0.42	0.43	32	33	1.03	1.14	0.31	0.29	50	52
026	32.0	24.0	0.45	0.41	38	39	-	1.39	-	0.28	-	-
027	26.5	35.0	0.38	0.38	40	37	0.87	0.95	0.39	0.39	49	49
028	42.0	43.5	0.42	0.44	-	-	-	-	0.32	0.41	53	51
030	20.0	27.5	0.38	0.39	39	-	-	-	0.27	0.27	52	49
031	43.0	43.5	0.38	0.40	32	30	1.45	1.25	0.22	0.24	61	58
033	43.5	41.0	0.39	0.43	-	-	-	-	0.33	0.30	51	51
035	35.5	36.0	0.42	0.49	29	21	0.48	0.35	0.61	0.59	49	40
Mean	37.4	36.8	0.40	0.42	32	33	1.06	1.12	0.35	0.36	51	51

* pre-treatment § post-treatment

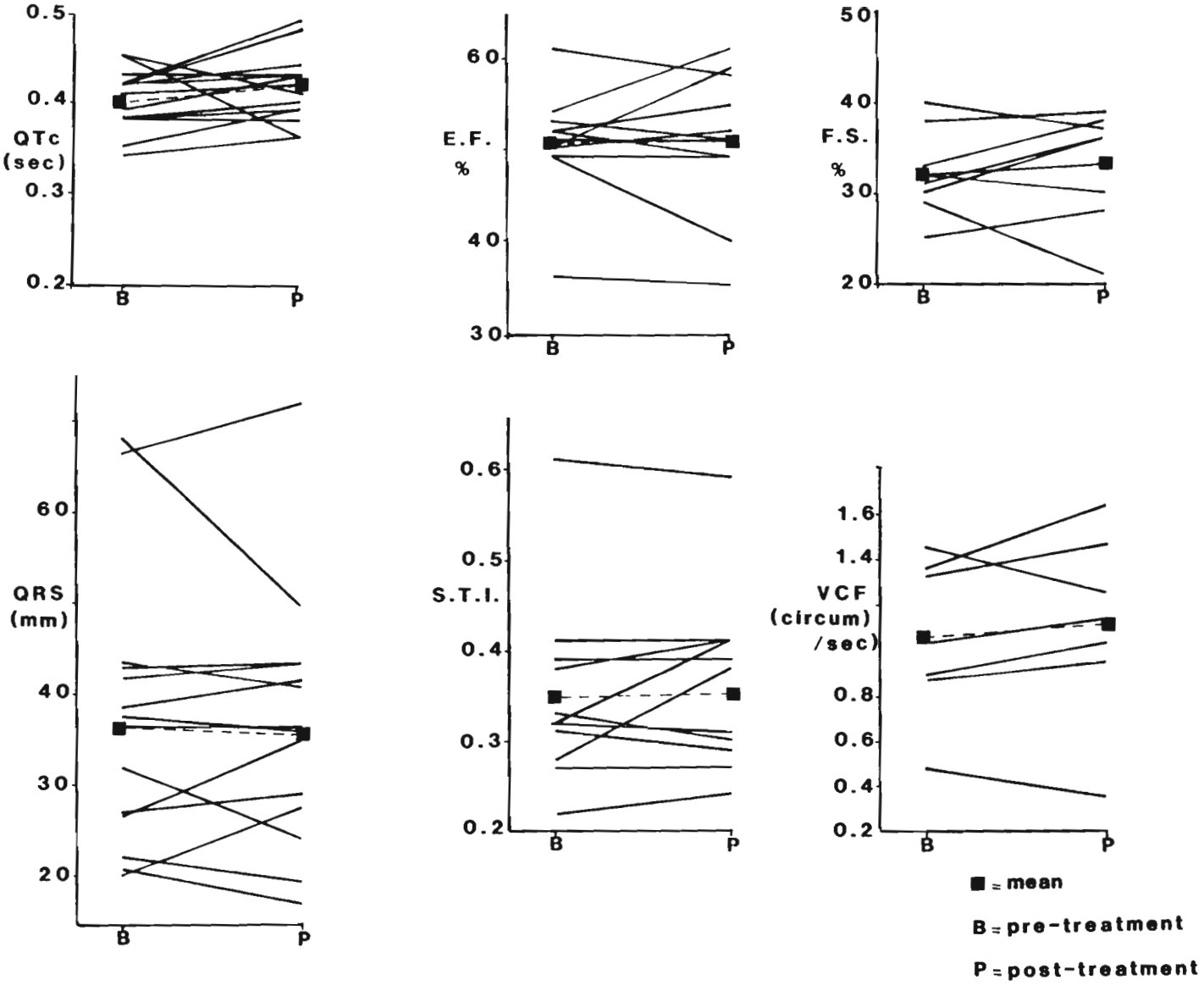


FIG. 23. Baseline and post-treatment cardiac parameters following treatment with CI-921 - Phase II clinical trial.

over five treatment courses. There were no significant changes in any of the other parameters however and left ventricular function was normal on repeat echocardiography.

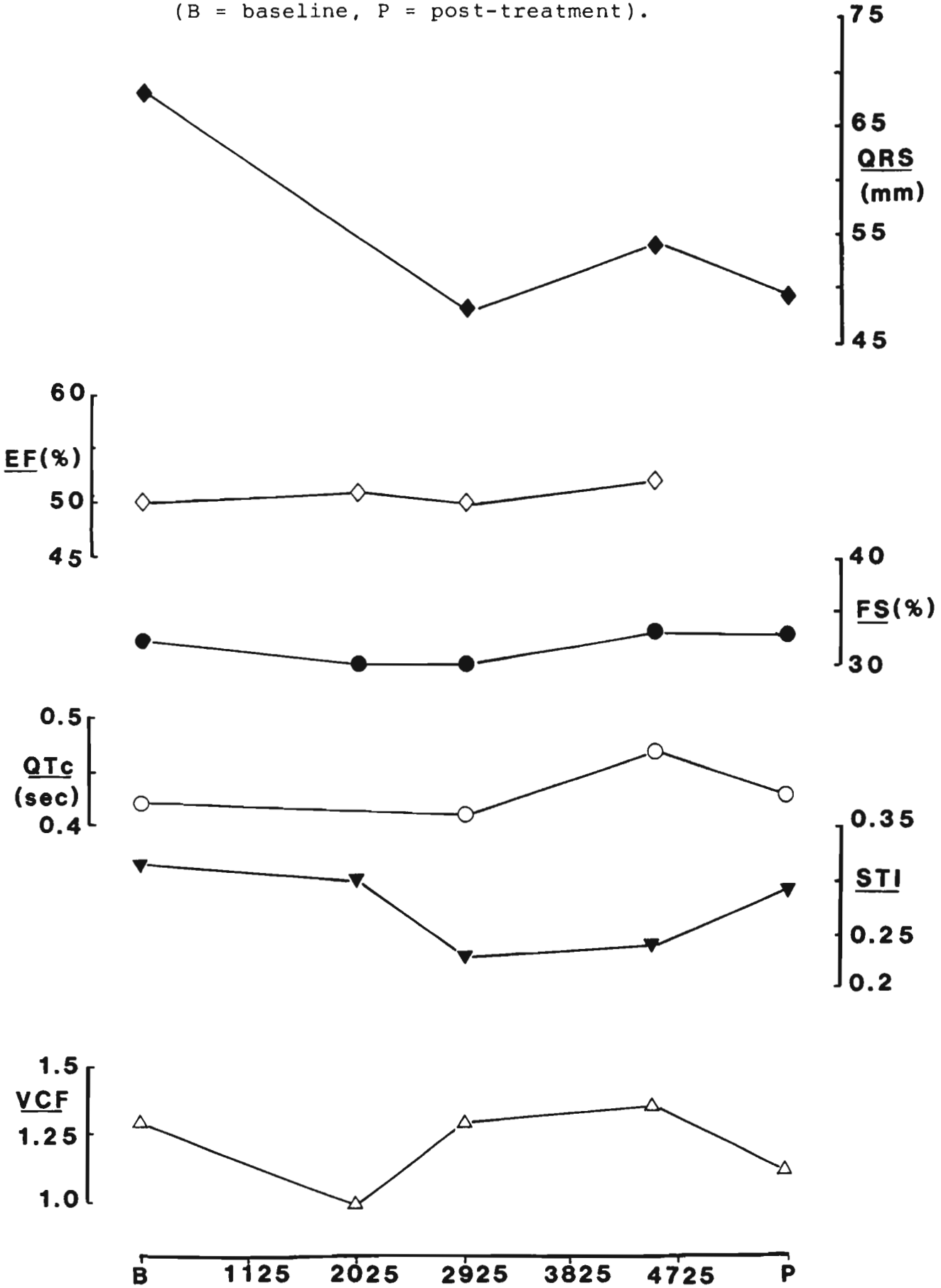
Patient no.020 had a history of ischaemic heart disease and had a low pre-treatment LVEF that did not change significantly following two treatments. Baseline echocardiography in this patient showed mild dilatation of the left ventricle with regional wall abnormalities consistent with his previous anterior myocardial infarction. This was associated with an STI at the upper limit of normal and a low VCF.

Patient no.025 had had the greatest cumulative dose of CI-921 in the phase II trial, 4725 mg over five courses. The cardiac parameters of this patient are plotted separately in Fig 24. Apart from the fall in ΣQRS as previously discussed, there was no significant change in any of the parameters over this dose range.

With the exception of patient no. 035, there was no significant fall in FS or VCF. Two patients (nos. 028 and 023) had an increase in STI in post-treatment scans, but in no case was the STI > 0.45.

The myocardium was examined post-mortem in five patients (three from phase I and two from the phase II trial). No unexpected histological changes unrelated to malignancy were detected.

FIG. 24. Cardiac parameters of patient no. 025 over 5 cycles of CI-921.
(B = baseline, P = post-treatment).



DISCUSSION

It was previously accepted that cardiac catheterization and radionuclide angiography provided the most accurate assessment of cardiac function. Over the last few years however, echocardiography has been increasingly used and has been found to compare favourably with angiography in assessing ventricular performance (Starling et al 1981, Feigenbaum 1986, Quinones et al 1976). The combination of electrocardiography and echocardiography as used in this study provides a non-invasive means of following several parameters that allow recognition of the development of cardiotoxicity following treatment with cytotoxic agents (Myers 1982). Some investigators have found that a 30% decrease in QRS voltage from baseline correlates with the development of congestive heart failure (Unverferth et al 1982). This is not universally accepted however as many factors can affect QRS voltage, e.g. pericardial and pleural effusions, chronic airways disease, body build and thyroid status (Bloom et al 1978). Also, a fall in ΣQRS is coincidental with the onset of cardiomyopathy and is not a sensitive index of early cardiac damage (Myers 1982). The efficiency of contraction can be measured by FS and VCF (Feigenbaum 1986, Cooper et al 1972). Both can be assessed by M-mode echocardiography. VCF has the advantage in being the parameter that is least dependent on changes in preload (Quinones and Gaasch 1976). The LVEF obtained from 2-D echocardiography is comparable to that obtained by cardiac catheterization and cineangiography (Steinherz et al 1982). Some workers have found VCF and LVEF to be the most reliable and useful parameters when assessing cardiotoxicity (Bloom et al 1978).

Although none of these parameters consistently predict early cardiotoxicity and there are

no definite guidelines for termination of treatment, it was hoped that the combination of the various parameters as described above might provide early detection of the development of any cardiotoxicity. The major disadvantage of this method was the difficulty in performing adequate echocardiograms. Other authors have reported that technically satisfactory echocardiograms can only be performed in about 60% of patients (Unverferth et al 1982) and in our experience the success rate was even less. Complete assessment of all cardiac parameters was only possible in 23% of patients in phase 1 and in 50% of patients in phase II. The particular characteristics of this study group resulted in technical problems. All patients studied had a terminal illness and were often unwell and unable to lie in certain positions for any length of time. Many had chest wall and/or mediastinal disease making proper assessment impossible. Many patients in this study had pre-existing heart disease, a rapid heart rate, malignant involvement of the mediastinum and/or pericardium or had been treated with mediastinal irradiation. This makes interpretation of changing cardiac function difficult.

The parameters of the two patients known to have major cardiac compromise, i.e. patients no. 020 and 035, are well outside the limits of the other patients. This suggested that this method of assessment of cardiac function is reliable in detecting impaired ventricular performance, but does not provide information as to whether early disease could be detected.

Apart from the single patient in the phase II study with compromised cardiac function (almost certainly due to disease rather than drug toxicity), no change in cardiac parameter to within definite toxic levels was seen and there was no significant difference between pre-

and post-treatment results. It must be noted that all patients in this study had very limited drug exposure, and that any cardiotoxicity might only be expected after prolonged exposure at high dose. Of interest, both patients who received the greatest number of courses and the greatest cumulative dose of CI- 921 in the phase I and phase II trials (nos. 005 and 025) showed a fall of ΣQRS in post-treatment compared to pre- treatment scans. This was associated with a slight increase in STI, and fall in VCF in patient no. 005, but neither patient developed a significant abnormality of any other cardiac parameter. As previously discussed a fall in ΣQRS is unlikely to be a sensitive index of early cardiotoxicity in the absence of any change in any of the other parameters (Bloom et al 1978).

Both patients no.025 and 027 had transient non-specific abnormalities on ECG which may have been drug related, but may just as easily have reflected ischaemia, as both patients had a history of cardiovascular disease. Minor changes in STI, FS and VCF seen in patients 005, 015, 028 and 023 did not reach toxic limits and occurred without significant change in any other parameter.

In the few patients on whom autopsy data were available, no unexpected histological changes were detected in the myocardium.

In summary our results have failed to show any definite evidence of a toxic effect of CI-921 on the myocardium. Unfortunately however, this study does not definitely exclude the possibility that CI-921 might be cardiotoxic. Although as previously discussed, the combination of electrocardiography and echocardiography is probably as good as radionuclide angiography in assessing ventricular function, a full assessment using these

modalities could only be done in a proportion of patients. This was primarily because of the type of patient under study, many of whom were in poor general condition and had chest wall and/or mediastinal disease such that echocardiography was technically impossible. Cardiac scintigraphy may have been appropriate in these patients, but this technique was not available for this study. Furthermore, many of the patients studied received a very low cumulative dose of CI-921 (well below the MTD), that would not be expected to produce a deleterious effect even if the drug was cardiotoxic. The myocardium was examined after death in too few patients to exclude the possibility of sub-clinical histological damage.
As discussed in Chapter 1 the echocardiagraphic changes following treatment with amsacrine were seen most frequently in those patients who had received high cumulative doses of drug especially if combined with an anthracycline (Steinherz et al 1982). Similarly, hypokalaemia has been identified as a major risk factor in the development of the cardiotoxicity associated with amsacrine (Riela et al 1981, McLaughlin et al 1983, Foldes et al 1982). As serum potassium was carefully monitored in this study, and corrected to within normal limits if necessary, the possibility of cardiotoxicity being associated with hypokalaemia in the case of CI-921 has not been addressed.

Further studies, ideally utilising cardiac scintigraphy, of more patients receiving high cumulative doses of CI-921 over multiple courses will be necessary before the possibility of cardiotoxicity can be fully excluded, especially as it has been so well described for the structurally similar parent drug amsacrine.

CHAPTER 6

METABOLIC STUDIES

CHAPTER 6. METABOLIC STUDIES

INTRODUCTION

As has been discussed in Chapter 1, GSH is thought to be involved in the metabolism of several cytotoxic agents including amsacrine. The aim of the metabolic studies described in this chapter was to define the importance of GSH in the metabolism and toxicity of CI-921 and to compare the metabolic pathway with that of amsacrine. To this end the concentration of GSH in the blood of patients treated with CI-921 was measured and compared to control levels in healthy volunteers. Similarly, GSH concentrations in whole blood of mice was measured following i.v. drug administration. It was postulated that falling levels might reflect utilization of GSH in drug conjugation.

As decreased concentrations of GSH have been demonstrated in the livers of mice following treatment with amsacrine (Cysyk 1977), mouse hepatic GSH levels were measured following treatment with CI-921, as a reduction in GSH concentration might suggest a similar route of metabolism for the two drugs.

It is possible that differing concentrations of GSH may effect the toxicity of CI-921. Attempts were therefore made to alter the toxicity of CI-921 by the experimental

manipulation of GSH with various agents. Buthionine sulphoximine (BSO) is a specific inhibitor of δ-glutamyl cysteine synthetase. It has been shown to lower tissue GSH levels markedly and is non-toxic in animals at doses used to deplete GSH maximally (Drew and Miners 1984). BSO does not affect other enzymes involved in the formation and/or removal of reactive metabolites. Diethylmaleate (DEM) was previously used experimentally to deplete cellular GSH. It acts as a competitive substrate for GSH-transferases but has the disadvantage of reacting with other reduced thiol groups in addition to that of GSH and interfering with mixed function oxidase activity (Russo et al 1986). BSO was used in this study to deplete mouse hepatic GSH.

The narcotic agents morphine, propoxophene and L-å-acetyl methadol all significantly lower hepatic GSH in mice (James et al 1982). The morphine-induced depletion is thought to be due to the conversion of morphine to morphinone which then binds to GSH (Nagamatsu et al 1986). This has particular clinical interest in that many patients receiving cytotoxic drugs which have been shown to deplete GSH require narcotic analgesia and morphine is often the drug of choice. The possibility of increased toxicity resulting from the additive depletion of drug and morphine was investigated.

Glutathione synthesis can be stimulated by the addition of cysteine, or by methionine or N-acetyl-cysteine (NAC), both of which are rapidly metabolized to cysteine in vivo (Miners et al 1984). NAC appears to be the drug of choice as it is non-toxic when given in high dose (Orrenius and Moldeus 1984). Its use in the treatment of paracetamol poisoning is well described (Prescott et al 1979). NAC was used as a protective agent in these studies to see if stimulation of cell GSH synthesis would protect against the toxicity of CI-921.

Any effect on the toxicity of CI-921 following the experimental manipulation of GSH has theoretical importance in the treatment of tumours in man. If the primary role of GSH is one of detoxification, a "protective effect" might be seen with the concomitant use of agents like N-acetyl-cysteine that have been shown to increase tissue GSH levels. This is of particular relevance to those cytotoxic agents that show steep dose-response curves, in that higher drug doses could theoretically be given without unacceptable toxicity. Similarly, toxicity might theoretically be avoided if agents known to reduce GSH levels were not used concurrently with CI-921.

MATERIALS AND METHODS.

Materials

The following chemicals were used:

Millipore milli-Q water was used for the preparation of all aqueous reagents.

Deproteinizing solution: 20% trichloroacetic acid (May and Baker Ltd, Dagenham, England) containing 0.02M HCL and 2mM ethylene diamine-tetra-acetic acid (EDTA) (Sigma Chemical Co., St. Louis, MO, USA).

Anhydrous diethyl ether (Ajax Chemicals, Sydney, Australia) was re-distilled prior to each assay.

Tietze buffer: 0.1M sodium phosphate (J.T. Baker Chem. Co., Phillipsburg, N.J.) pH 7.5 containing 1mM EDTA.

EDTA buffer: 1mM EDTA containing 0.01M HCL.

GSH reference solution: 5.0μM, prepared in EDTA buffer. Oxidation was prevented by

flushing the solution with N_2 and storing at -20°C.

Ellman's Reagent: 5, 5'dithiobic (2-nitrobenzoic acid) (DTNB) (Sigma Chemical Co., St. Louis, MO, USA), 1mM in Tietze buffer.

Nicotinamide adenine dinucleotide phosphate : reduced form, tetra-sodium salt, type 1, 95-97% (NADPH) (Sigma Chemical Co., St. Louis, MO, USA), 1mM in EDTA buffer.

Glutathione reductase (GR): NAD(P)H oxidized glutathione reductase, type IV, from baker's yeast (Sigma Chemical Co., St. Louis, MO, USA), 200u/ml.

Assay reagent: prepared immediately prior to assay by combining 2.81 mls 1mM DTNB, 3.75mls 1mM NADPH, 93.75 µl GR and 5.85 ml Tietze buffer on ice.

1.15% KCL: 5.75 mls 1mM KCL (E.Merck, Darmstadt, Germany) dissolved in 500 mls H_20.

12.5% TCA solution: 12.5% trichloroacetic acid containing 0.0125M HCL and 1.25M EDTA.

CI-921 as the isothionate salt (from Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, Michigan), prepared for infusion as in the phase I trial, i.e. reconstituted in sterile water or 5% dextrose to 4 or 5mg free base/ml.

Amsacrine as the free base ("Amsidyl" from Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, Michigan), 50mg/ml in N-N, dimethyl acetamide, diluted in 0.0353M L-lactic acid.

L- buthionine-S-R-sulfoximine (BSO) (Chemical Dynamics Co., South Plainfield, New Jersey, USA), dissolved in normal saline and adjusted to pH 8.5 with 0.1M NaOH to give a final solution of 80mg/ml.

Morphine sulphate: (David Bull Laboratory, Mulgrave, Victoria, Australia), 30mg/ml in water for injection.

N-acetyl cysteine (NAC) as a 20% solution of the sodium salt (Meed Johnson Ltd, N.S.W,

Australia), diluted in normal saline to 40mg/ml.

Animals

BDF_1 hybrid mice of either sex were bred in the Auckland Cancer Research Laboratory, University of Auckland School of Medicine under standard conditions and maintained on diet 86 and water ad libitum.

Ethics

Local institution animal ethics committee approval was gained prior to these studies.

Methods

The measurement of GSH in blood and in murine hepatic tissue.

GSH concentrations were measured in the whole blood of patients and mice before and after i.v. CI-921 and in normal healthy controls. GSH concentrations in hepatic tissue of mice were measured following treatment with CI-921 and amsacrine and following depletion of GSH by BSO and morphine sulphate.

Sample acquisition.

i) Whole blood of patients receiving CI-921.

Blood samples were collected by venepuncture from patients receiving CI-921 i.v. at a dose of 216 mg/m^2 daily for three days; prior to therapy (sample 1), 8 hours following the day 1 infusion (sample 2) and 24 hours following the day 1, 2 and 3 infusions (samples 3, 4 and 5). For assay of whole blood GSH, 100μl samples were diluted immediately in 900μl deproteinizing solution on ice and spun at 3,000 rpm for 5 minutes. 300μl of the supernatant was removed and stored at -80°C until assayed. The packed cell volume (PCV) of each blood sample was also determined.

ii) Whole blood of mice receiving CI-921.

Blood was collected from the orbital vessels following enucleation of the eye in anaesthetized mice that were sacrificed immediately following collection. Samples were taken from mice prior to treatment with CI-921 (34 mg/kg i.v.) and at 1, 2, 3, 4 and 5 hours following injection. 100μl aliquots were prepared for assay as above.

iii) Whole blood of healthy controls.

Blood samples from normal healthy volunteers were taken at similar time intervals as the patients receiving CI-921 and prepared for assay as above.

iv) Hepatic tissue of mice following CI-921 and amsacrine.

Mice were given a single i.v. injection of CI-921 (50mg/kg) or amsacrine (60mg/kg) at time 0 and sacrificed by cervical dislocation at 1, 2, 3, 4, 6, 15 or 24 hours thereafter for assay of hepatic GSH. The livers were removed, rinsed in ice-cold 1.15% KCI, blotted dry, weighed and homogenized in four volumes (weight:volume) 12.5% TCA solution. The precipitated protein was pelleted by centrifugation at 10,000 rpm for two minutes. 300μl

samples of the supernatant were removed in duplicate and stored at -20°C until assayed.

v) Hepatic tissue of mice following BSO.

Mice were given BSO (1.5g/kg) by i.p. injection (20ml/kg) and sacrificed after 1, 2, 4, 6, 10, 15 or 20 hours. Liver tissue was prepared for assay of GSH as above. Control animals were given the vehicle alone (20ml/kg normal saline adjusted to pH 8.5 with 0.1M NaOH) and sacrificed at 1, 5 and 20 hours.

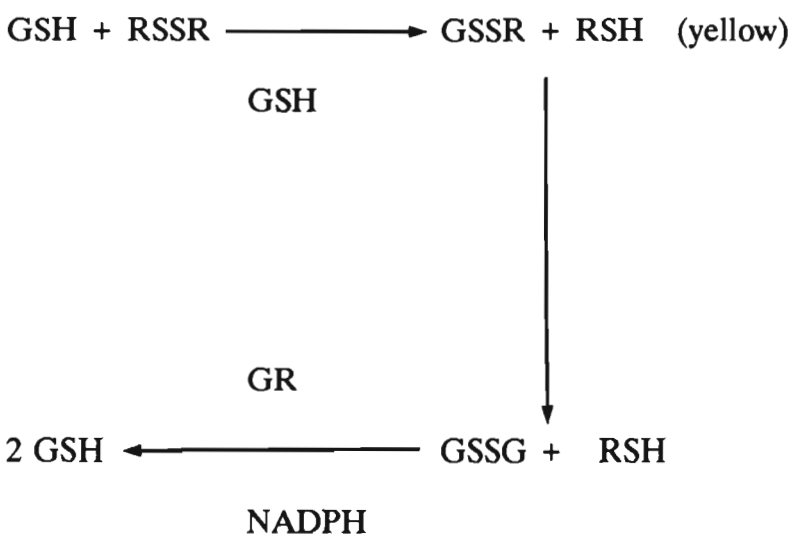
vi) Hepatic tissue of mice following morphine.

Mice were given a s.c. injection of morphine sulphate (250mg/kg) and sacrificed at 2, 4, 6, 8 and 24 hours for assay of hepatic GSH. Control mice were given an equivalent volume of normal saline s.c. (10ml/kg).

GSH assay.

Glutathione (GSH) was measured by the spectrophotometric method described by Tietze (1969) and modified by Wilson et al (Auckland Cancer Research Laboratory). In the presence of GSH (in the reduced (GSH) or oxidised (GSSG) form), glutathione reductase (GR) and NADPH, Ellman's reagent (DTNB:5, 5'-dithiobis (2-nitrobenzoic acid)) is reduced to a coloured compound that absorbs maximally at 412 nm. The reaction can be depicted as follows:

(RSSR = DTNB)



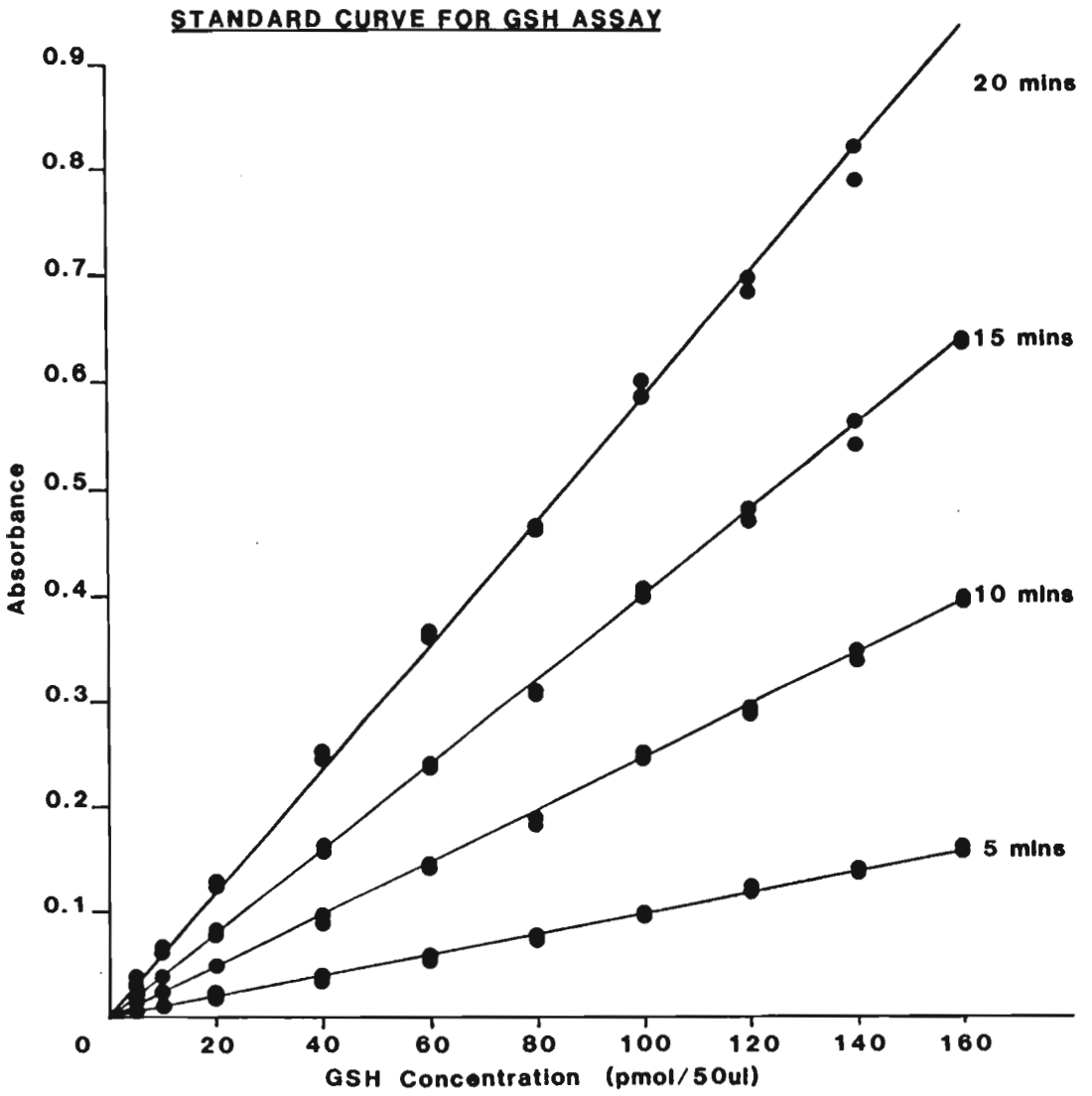
Overall reaction:

GSH (GSSG) is involved catalytically resulting in a highly sensitive assay, and has the advantage of measuring both the reduced (sulphydryl) and oxidized (disulphide) forms of GSH. This method was used to measure GSH levels in human blood and mouse liver samples as follows:

Samples for assay were thawed, washed twice in two volumes (600μl) freshly distilled ether on ice, placed in a desiccator at room temperature and evacuated for 10-15 minutes to remove residual ether. Samples were diluted in EDTA buffer to give a final GSH concentration within the range of the GSH standards (1:1,500 human whole blood, 1:4,000 mouse liver homogenates). Ten standard GSH solutions within the range 5-160 pmol/50μl were prepared from the GSH reference solution by diluting with EDTA buffer. 50μl aliquots of standards and unknown samples were placed in duplicate in wells of a 96- well spectrophotometric plate. 100μl of the assay reagent solution was added to each well at timed intervals. Absorbances were read at 5, 10 and 15 minutes at A410nm-A630nm with EDTA buffer as a blank using a MR600 microplate reader (Dynatech Instruments Inc., Torrance, California). The concentration of GSH in the unknown samples was determined relative to the standards read at the same time (Fig 25).

All samples were taken in duplicate and assayed in duplicate so that the values given represent the mean and standard deviation of four measurements. The mean co-efficient of variation across all samples was 8.95% for human whole blood samples and 7.9% for the mouse hepatic samples.

FIG. 25.


STANDARD CURVE FOR GSH ASSAY
Absorbance
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
120
140
160
GSH Concentration (pmol/50ul)
20 mins
15 mins
10 mins
5 mins

Toxicity Studies

The MTD of CI-921 and amsacrine was determined to define a level of toxicity. Mice were then pre-treated with agents known to deplete or protect hepatic GSH. The toxicity of CI-921 and amsacrine in mice following the experimental manipulation of GSH was investigated to determine the importance of GSH in the detoxification of these agents.

The maximum tolerated dose of CI-921 and amsacrine.

Groups of ten mice were given a single i.v. tail vein injection (10ml/kg) of CI-921 at doses 60, 50 or 40mg/kg or amsacrine at doses 30, 40, 50 or 60 mg/kg. Control mice were given 5% dextrose, or dimethyl acetamide/L-lactic acid (10ml/kg i.v.), the respective vehicles for CI- 921 and amsacrine. Mice were weighed twice weekly for the first two weeks and weekly thereafter. Observations for toxicity, time and cause of death were made twice daily for the first ten days and daily thereafter. Surviving mice were sacrificed at 28 days.

The effect of BSO pre-treatment on toxicity.

Ten mice were given BSO (1.5g/kg) by i.p. injection (20ml/kg) three hours prior to a single i.v. injection of CI- 921 (50mg/kg). Two mice were given BSO alone. All animals were weighed and observed for toxicity as above for 28 days.

The effect of morphine pre-treatment on toxicity.

Ten mice were given a s.c. injection of morphine sulphate (250mg/kg) three hours prior to a single i.v. injection of CI-921 (50mg/kg) and observed for toxicity as above. Two mice received morphine alone (30ml/kg s.c.).

The effect of NAC pre-treatment on toxicity.

i) Mice were given an i.p. injection of NAC (500mg/kg) one hour prior to CI-921 (50mg/kg i.v.). Two animals received NAC alone.

ii) In an attempt to confirm a protective effect for NAC, a higher dose of CI-921 (60mg/kg i.v.) was given to mice pre-treated with NAC (500mg/kg) as above. Control mice were given CI-921 (60mg/kg i.v.) alone.

iii) To maximise any protective effect of NAC at the higher dose of CI-921, NAC (500mg/kg) was given i.p. one hour prior and three hours post CI-921 (60mg/kg i.v.). Control mice received CI-921 (60mg/kg i.v.) alone and two mice received NAC alone.

Histological Studies

Post-mortem studies were performed on mice following lethal doses of CI-921 and amsacrine to determine the cause of death. Mice in extremis following CI-921 or amsacrine treatment were sacrificed for post-mortem histological study. Tissues of interest

were preserved in formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Bone sections were decalcified.

Statistics.

The difference in whole blood GSH between the five sample groups in all patients was determined by one-way analysis of variance within subjects (Winer 1971). The difference between control and study groups was determined by the Students t-test (two tailed).

RESULTS

Whole blood GSH in patients receiving CI-921.

(Table 6.1, Fig 26).

There was no significant change in serial GSH concentrations over the three treatment days or at 24 hours after the last infusion. There was no significant difference within patients between any of the post-infusion concentrations on any of the three study days or between pre-treatment and post-treatment concentrations. GSH concentrations ranged from 276μg/ml to 779μg/ml with an overall mean of 507μg/ml for all samples.

Whole blood GSH in healthy subjects.

(Table 6.2, Fig 27)

There was no significant difference in the whole blood GSH concentrations at any of the five sample times. GSH concentrations ranged from 268μg/ml to 716μg/ml with an overall

mean of 498μg/ml for all samples. There was no significant difference (at the 0.05 level) between the mean GSH concentrations of healthy subjects and patient groups.

Mouse whole blood GSH following CI-921.

(Table 6.3)

There was no fall in whole blood GSH concentration in mice up to five hours following i.v. CI-921. GSH levels ranged from 219 to 546μg/ml with an overall mean of 400 ± 91μg/ml.

Mouse hepatic GSH following amsacrine and CI-921.

(Table 6.4, 6.5, Figs 28, 29, Appendices 4.1.1 - 4.2.2)

Amsacrine, given i.v. at a dose of 60mg/kg resulted in a fall of hepatic GSH to 62% of control levels at four hours ($p<0.001$) with recovery to control levels by 24 hours. CI-921 (50mg/kg i.v.) resulted in a less marked fall in hepatic GSH with 86% and 83% of control levels being measured at two and four hours ($p<0.05$), with an "over-shoot" to 128% of control levels at 6 hours that was maintained to 24 hours.

Mouse hepatic GSH following treatment with BSO.

(Table 6.6, Fig 30, Appendices 4.3.1, 4.3.2)

A single i.p injection of BSO resulted in a marked depletion of hepatic GSH to 14% of control levels ($p<0.001$). Maximum depletion occurred by four to six hours with recovery to control levels by 20 hours.

Mouse hepatic GSH following morphine.

(Table 6.7, Fig 31, Appendix 4.4)

Treatment of mice with a s.c. injection of morphine sulphate resulted in depletion of hepatic GSH to 39% of control levels ($p<0.01$) with recovery within 24 hours to 94% of control.

Toxicity Studies

The maximum tolerated dose (MTD) of CI-921.

Mice were initially given CI-921 at 60mg/kg i.v. Toxicity was evident by day 5. Treated mice grouped together, were inactive, failed to eat or drink and assumed a hunched posture with spiked fur, pointed nose and ears and bilateral ptosis. There was a 30% weight loss from the mean pretreatment weight in surviving mice by day 8 (Fig 32). Nine out of the ten mice (90%) died or were sacrificed in extremis from day 6 to day 8 (Fig 33). There were no deaths amongst control mice and only minimal weight loss (<5%) in the first few days following injection (Fig 32).

A dose of 50mg/kg resulted in obvious toxicity as above from day 4 in all animals. Two of nine mice (22%) died on days 6 and 7, with recovery of the remaining mice, giving a survival rate of 78%. Weight loss (20%) was seen at day 4, with subsequent recovery to pre-treatment weight by day 14.

At 40mg/kg i.v., no deaths occurred and no toxicity was evident. Weight loss (5%) was minimal at day 4 with recovery by day 8 to approximate controls. A MTD of 50mg/kg was established for further studies.

The maximum tolerated dose of amsacrine.

There was no obvious toxicity, minimal weight loss and no deaths in mice treated at 30, 40 or 50mg/kg amsacrine i.v. (Fig 32). At 60mg/kg, toxicity was obvious at day 3. One out of nine mice died on day 2 and four died between day 5 and 9 (total deaths 56%) with recovery of surviving mice by days 9-10 (Fig 34) giving a survival rate of 44%. The maximum weight loss (22%) was seen at day 7, with recovery to mean pre-treatment weight by day 20. There were no deaths amongst control mice receiving vehicle alone. The clinical toxicity observed for mice treated with amsacrine was similar to that observed with CI-921. A maximum tolerated dose of 60mg/kg was identified.

The effect of BSO pretreatment on toxicity of CI-921.

Pretreatment of mice with BSO prior to CI-921 (50mg/kg i.v.) resulted in a marked increase in morbidity and mortality (Fig 35). Toxicity was seen from day 3, followed by death in nine out of ten mice (90%) from day 5 to day 7. There was marked weight loss (40%) by day 6 (Fig. 36). Mice receiving BSO alone showed no toxicity and no deaths to 28 days.

The effect of morphine pretreatment on toxicity of CI-921.

Pretreatment of mice with morphine (250mg/kg s.c.) did not increase the toxicity of CI-921 (50mg/kg i.v.). Mice appeared mildly unwell from day 3 to day 6 but there were no deaths (Fig 35). Weight loss (7% at day 5) was minimal with recovery by day 15 (Fig 36). The "narcotic" effect of morphine was obvious within half an hour following injection, with

decreased mobility, staggering gait, hyper-extension of tail and limbs and curling back of ears in treated mice. Recovery was seen within 6 hours and there were no deaths amongst mice receiving morphine alone to 28 days.

The effect of NAC pretreatment on toxicity of CI-921.

i) NAC (500mg/kg i.p.) 1 hour prior to CI-921 (50mg/kg i.v.)

All mice showed signs of toxicity by day 5 with recovery of surviving mice by day 9 (Fig 35). One of ten mice (10%) died on day 8, giving a survival rate of 90%. A 10% weight loss was seen on day 7, with recovery to mean pretreatment weight by day 16 (Fig 36). There was no toxicity in mice receiving NAC alone to 28 days.

ii) NAC (500mg/kg i.p.) one hour prior to CI-921 (60mg/kg i.v.)

NAC did not protect against the toxicity of CI-921 at this dose. All treated mice were unwell from day 4, with death of all mice on days 6 and 7. All control mice treated with CI-921 alone died on day 6 (Fig 37).

iii) NAC (500 mg/kg i.p.) 1 hour prior and 3 hours post CI-921 (60mg/kg i.v.)

All mice showed signs of toxicity by day 3. Six of eight mice (75%) died on days 5 and 6. The two surviving mice recovered by day 10. Weight loss to 68% of pretreatment levels was seen at day 5. All control mice treated with CI-921 died on days 5, 6 and 7, but there was no obvious toxicity and no deaths amongst mice receiving NAC alone (Fig 37).

TABLE 6.1 GSH levels in whole blood of patients receiving CI-921 §

Patient no. sex/age	PCV	Sample no.* 1	2	3	4	5
005	0.37	418	452	397	461	403
M/47		(655)	(574)	(504)	(586)	(512)
013	0.41	408±23	433±16	378±4	397±13	496±65
M/57		(468)	(496)	(433)	(455)	(569)
014	0.33	469±16	433±28	547±26	452±35	464±41
M/54		(688)	(618)	(779)	(644)	(661)
015	0.41	470±27	569±90	473±59	516±74	412±36
M/57		(539)	(652)	(542)	(592)	(472)
016	0.35	334±59	328±45	321±16	346±3	398±64
M/57		(448)	(440)	(431)	(465)	(534)
018	0.30	432±48	367±16	309±13	315±50	357
M/63		(677)	(575)	(484)	(494)	(559)
019	0.33	203±18	292±14	289±107	204±25	314
M/61		(289)	(416)	(412)	(291)	(447)
022	0.38	327±63	287±24	223±20	274±32	286±51
F/59		(404)	(355)	(276)	(339)	(354)
024	0.48	409±4	463±26	378±15	498±31	565±48
M/67		(400)	(453)	(468)	(488)	(553)
025	0.37	487±52	439±35	490±26	417±19	461±43
M/63		(619)	(558)	(622)	(530)	(568)

TABLE 6.1 (cont)

Mean	517	514	495	488	523
Range - min	289	355	276	291	354
- max	677	652	779	644	661

---

§ µg/ml ± SD, n =4 (corrected for PCV = 0.47)

* sample no. 1 = baseline

2 = 8h post CI-921 infusion

3,4,5 = 24h post day 1 to 3 CI-921 infusions

FIG. 26. Whole blood GSH concentrations in patients receiving CI-921.

Sample 1 = baseline.
2 = 8h. post day-1 infusion.
3,4,5 = 24h post days 1-3 infusion.

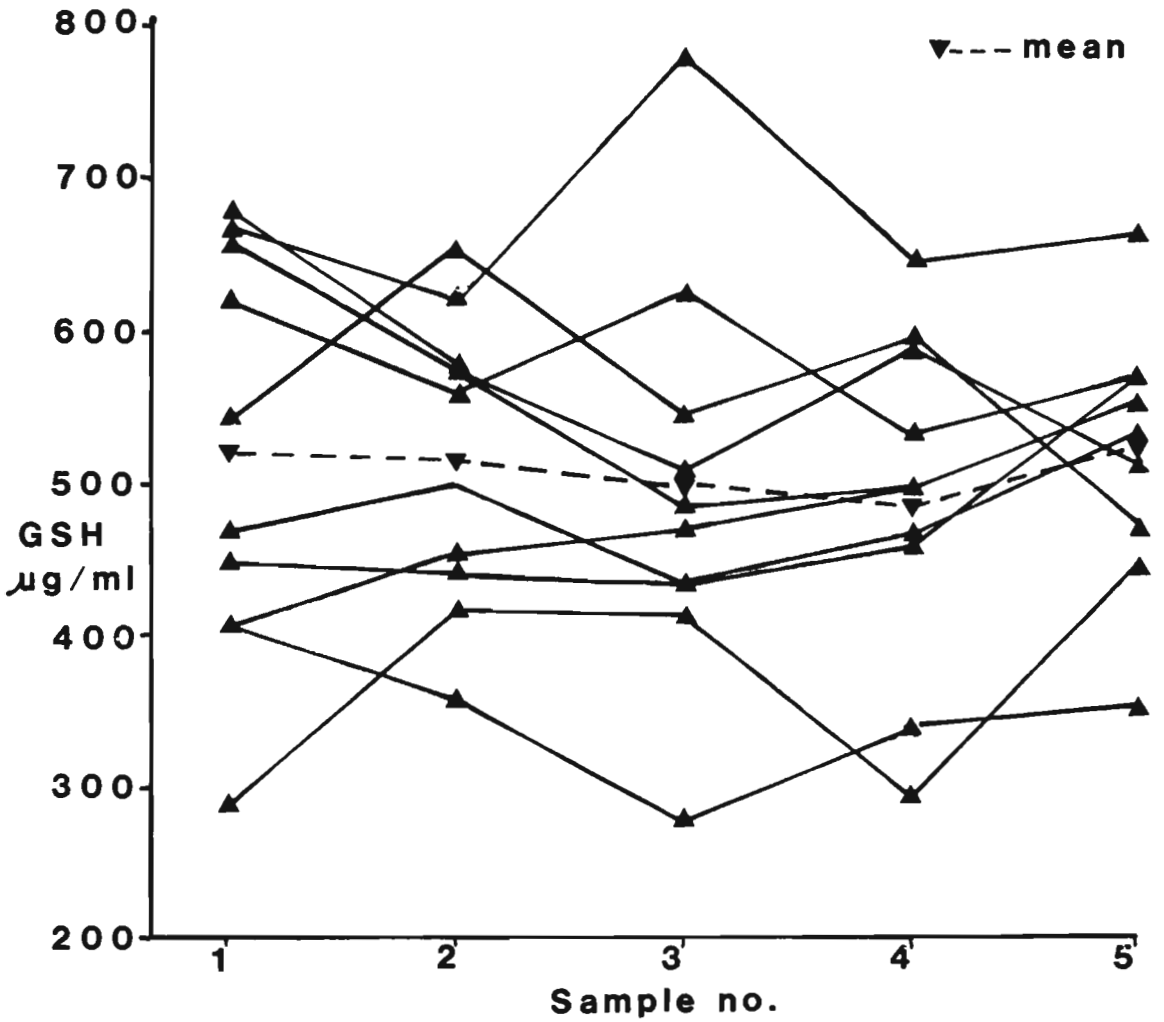


TABLE 6.2 GSH levels in whole blood of healthy subjects §

Controls sex/age	PCV	Sample no.* 1	2	3	4	5
A.D.	0.47	500±68	532±22	381±17	501±64	668±62
M/29		(500)	(532)	(381)	(501)	(668)
D.M.	0.33	421±4	459±36	377±16	486±55	421±33
F/35		(600)	(654)	(537)	(692)	(600)
P.T.	0.45	589±56	496±49	411±12	492±44	537±52
M/29		(615)	(518)	(429)	(514)	(561)
P.E.	0.34	288±64	235±18	423±12	272±38	375±57
F/39		(398)	(324)	(584)	(375)	(410)
J.H.	0.39	378±39	328	363±32	320±54	334±28
F/31		(456)	(395)	(437)	(386)	(402)
L.B.	0.49	279±34	331	332	432±15	291±19
M/25		(268)	(317)	(318)	(414)	(279)
L.L	0.44	694±25	342±41	485±12	389	301±20
M/29		(741)	(365)	(518)	(416)	(321)
R.D.	0.47	627±19	602±25	506±19	545±92	642±7
M/23		(627)	(602)	(506)	(545)	(642)
C.F.	0.43	655±14	412±44	440±115	645±135	623±16
F/22		(716)	(450)	(481)	(705)	(681)

TABLE 6.2 (cont)

Mean	547	462	462	505	513
Range - min	268	317	318	375	279
- max	741	654	584	692	681

---

§ µg/ml ± SD, n = 4 (corrected for PCV = 0.47)

* samples taken at equivalent times as patient samples

FIG. 27. Whole blood GSH concentrations in healthy controls.

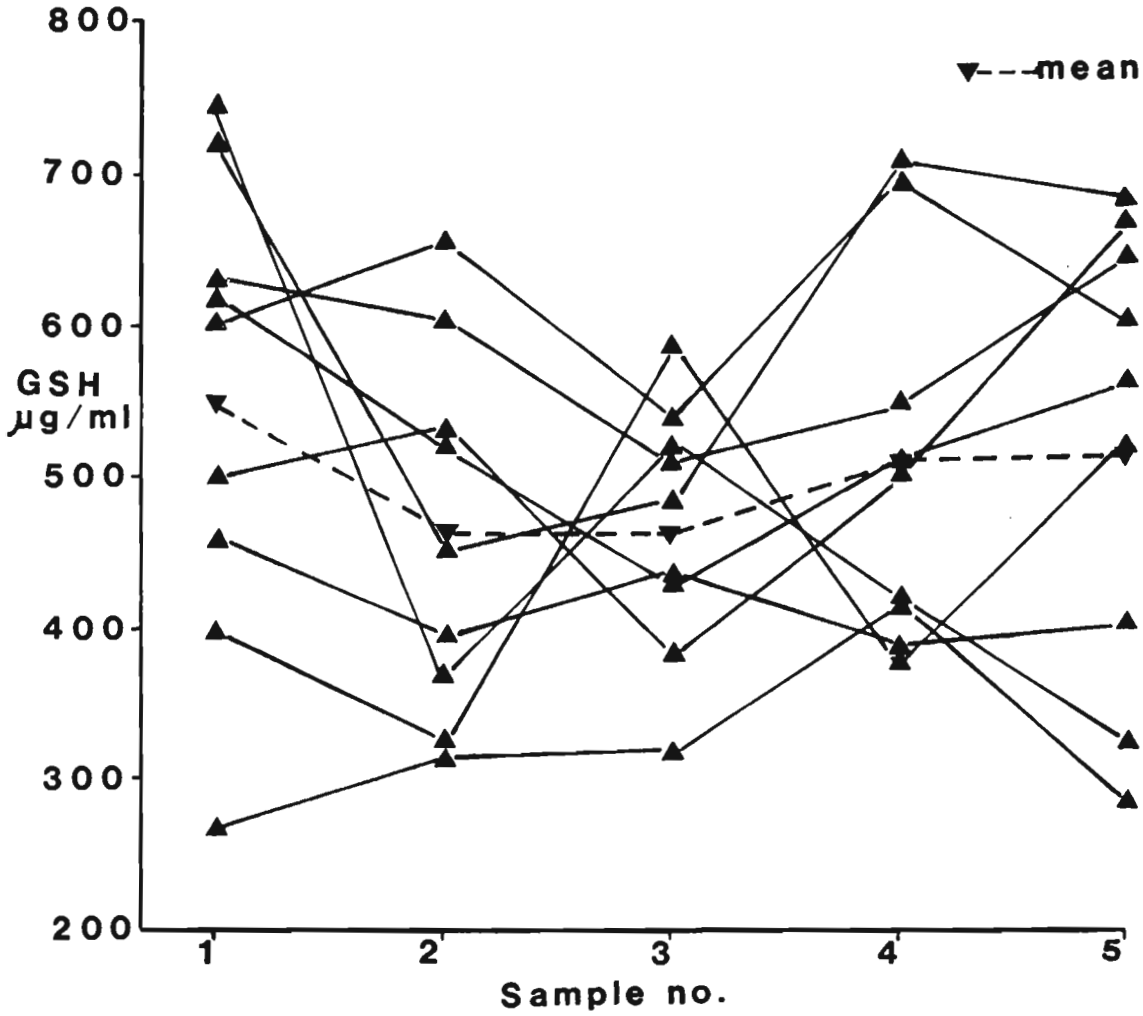


TABLE 6.3 GSH levels in whole blood of mice following CI-921.

Time post injection(h)	Mouse no.	GSH ± SD (μg/ml)	Mean
0 (base-line)	1	345 ± 71	
	2	219 ± 38	282
1	3	427 ± 12	
	4	360 ± 4	
	5	319 ± 32	368
2	6	481 ± 29	
	7	540 ± 22	
	8	445 ± 20	488
3	9	508 ± 9	
	10	546 ± 13	
	11	436 ± 61	497
4	12	417 ± 2	
	13	369 ± 9	
	14	332	372
5	15	413 ± 11	
	16	267 ± 9	
	17	379 ± 13	353

TABLE 6.4 Mouse hepatic GSH following amsacrine

Time post injection(h)	No. of mice	% of untreated control (mean ± SD)
0 (control)	3	100±2.0
1	4	83± 3.6
2	3	79 ± 3.8
4	3	* 62 ± 6.3
6	5	68 ± 13.4
15	3	83 ± 2.1
24	3	97 ± 7.6

(see Appendix 4.1 for raw data)

* $p < 0.001$

TABLE 6.5 Mouse hepatic GSH following CI-921.

Time post injection(h)	No. of mice	% of untreated controls (mean ± SD)
0 (control)	4	100 ± 5.9
1	2	101 ± 4.2
2	2	86 ± 3.5
3	1	87
4	2	*83 ± 4.9
6	3	128 ± 5.5
15	3	112 ± 15.3
24	3	122 ± 10.0

(see Appendix 4.2 for raw data)

* $p < 0.05$

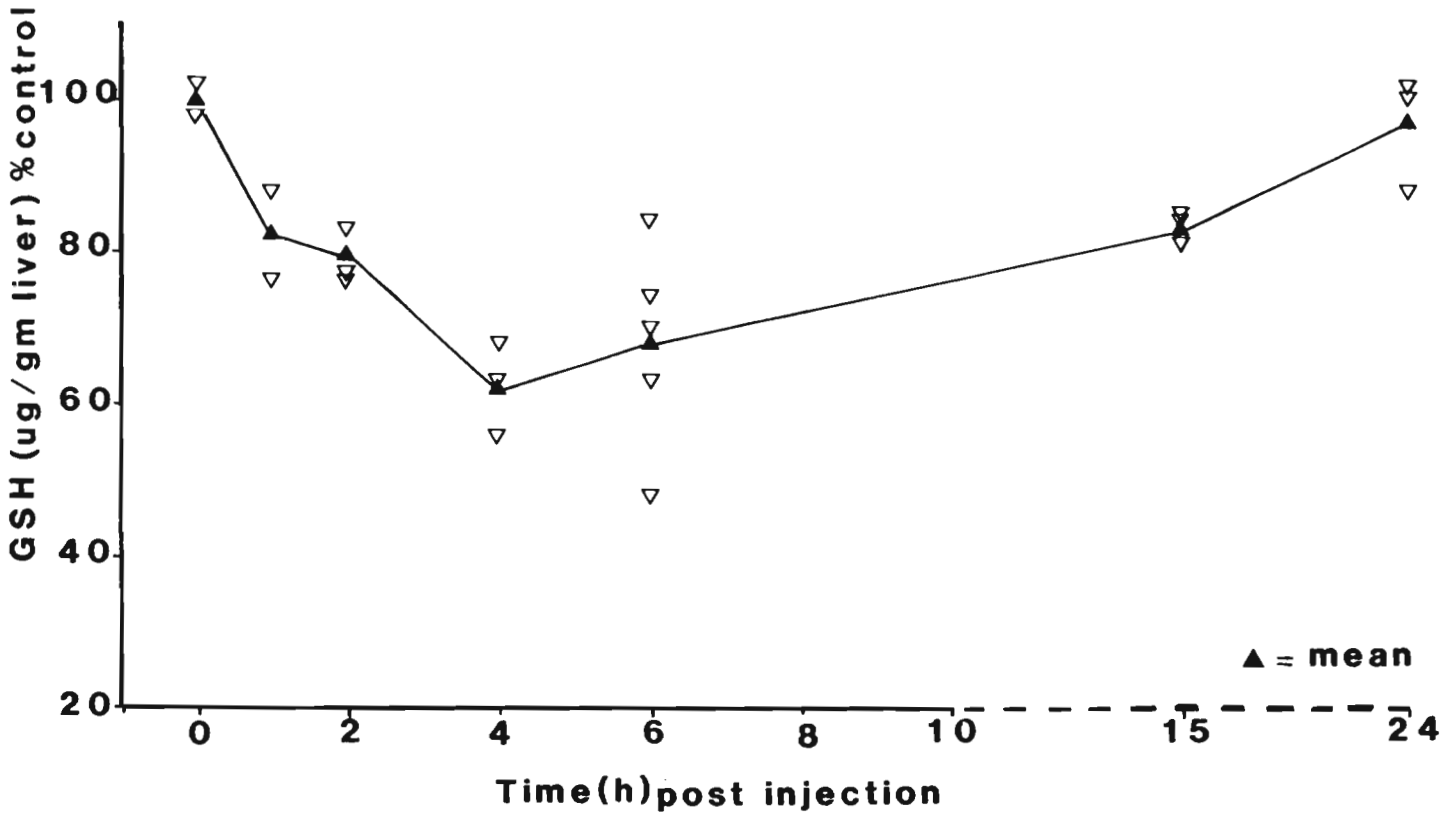


FIG. 28. Mouse hepatic GSH following amsacrine.

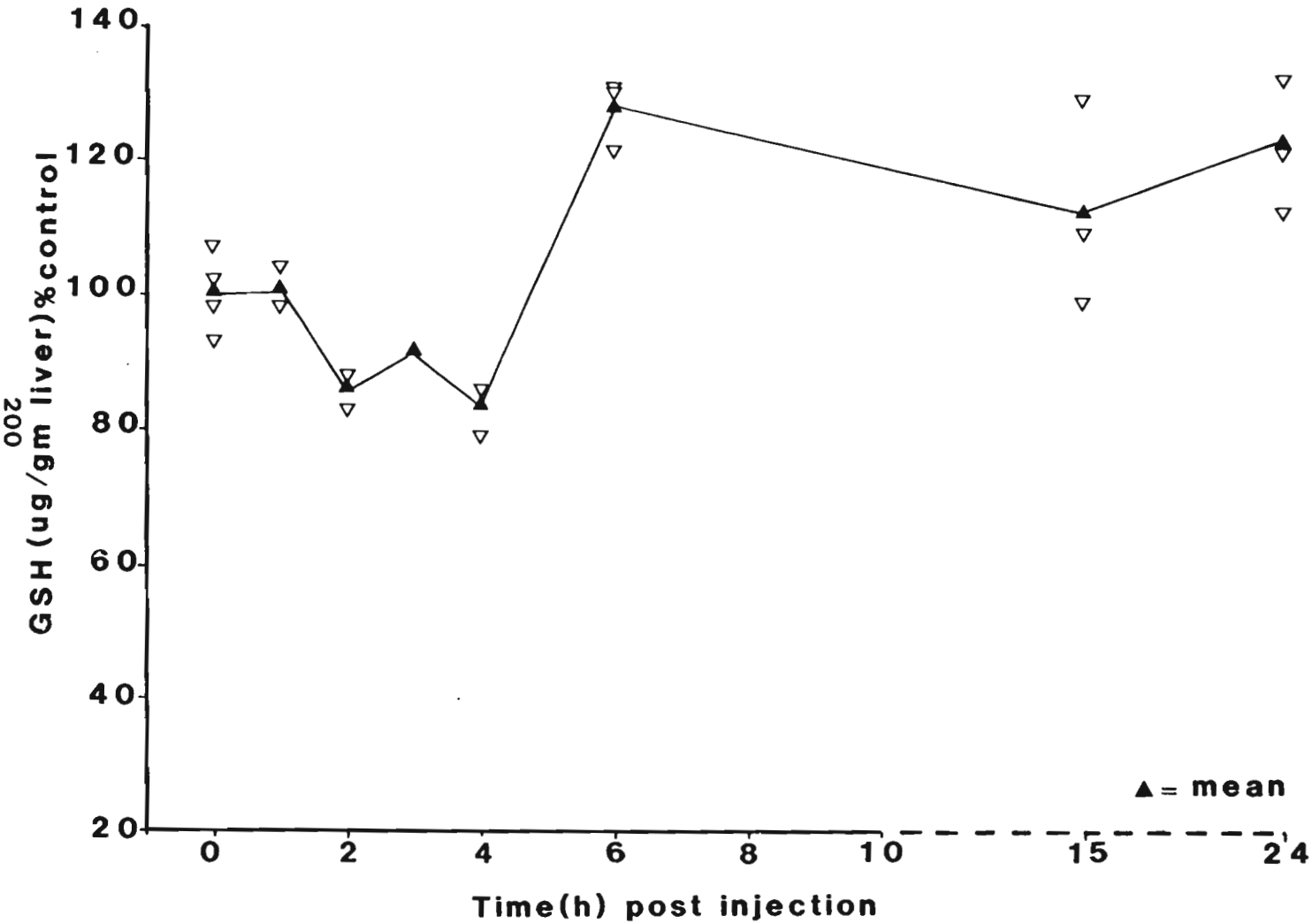


FIG. 29. Mouse hepatic GSH following CI-921.

TABLE 6.6 Mouse hepatic GSH following BSO.

Time post injection (h)	No. of mice.	% of untreated controls (mean ± SD)
0 (control)	6	100 ± 3.5
5 (control)	2	124 ± 26
20 (control)	2	115 ± 4.9
1	2	64 ± 4.2
2	2	30 ± 0
4	3	16 ± 3
6	3	* 14 ± 2.5
10	2	69 ± 12.7
15	2	84 ± 1.4
20	2	96 ± 11.3

(see Appendix 4.3 for raw data)
* $p < 0.001$

FIG. 30. Mouse hepatic GSH following BSO.

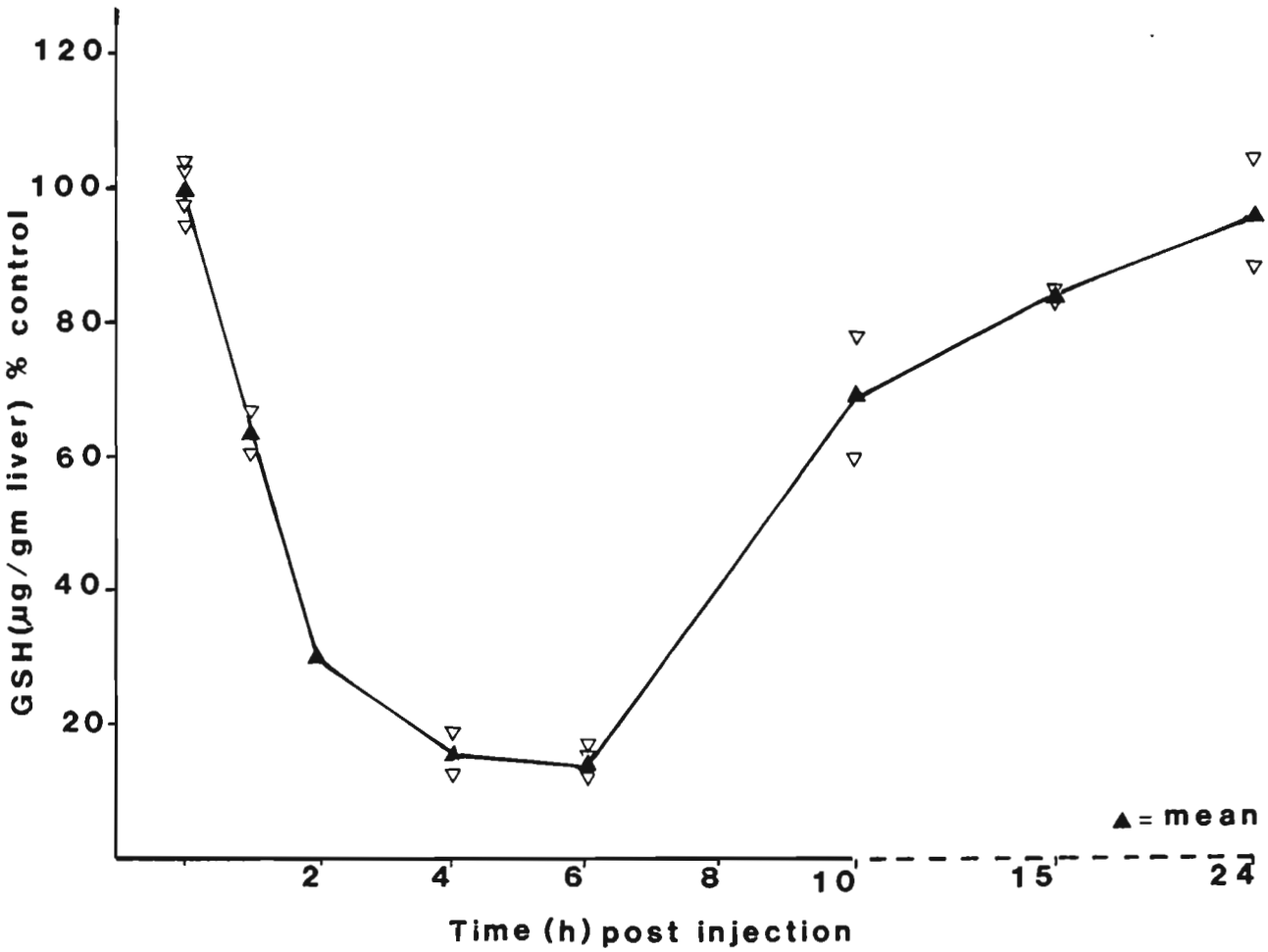


TABLE 6.7 Mouse hepatic GSH following morphine.

Time post injection (h)	No. of mice	% of untreated controls (mean± SD)
0 (control)	3	100 ± 12.2
2	3	80 ± 2.5
4	3	* 39 ± 16.9
6	3	67 ± 4.6
8	2	59 ± 11.3
24	1	94

(see Appendix 4.4 for raw data)

*$p < 0.01$

FIG. 31. Mouse hepatic GSH following morphine.

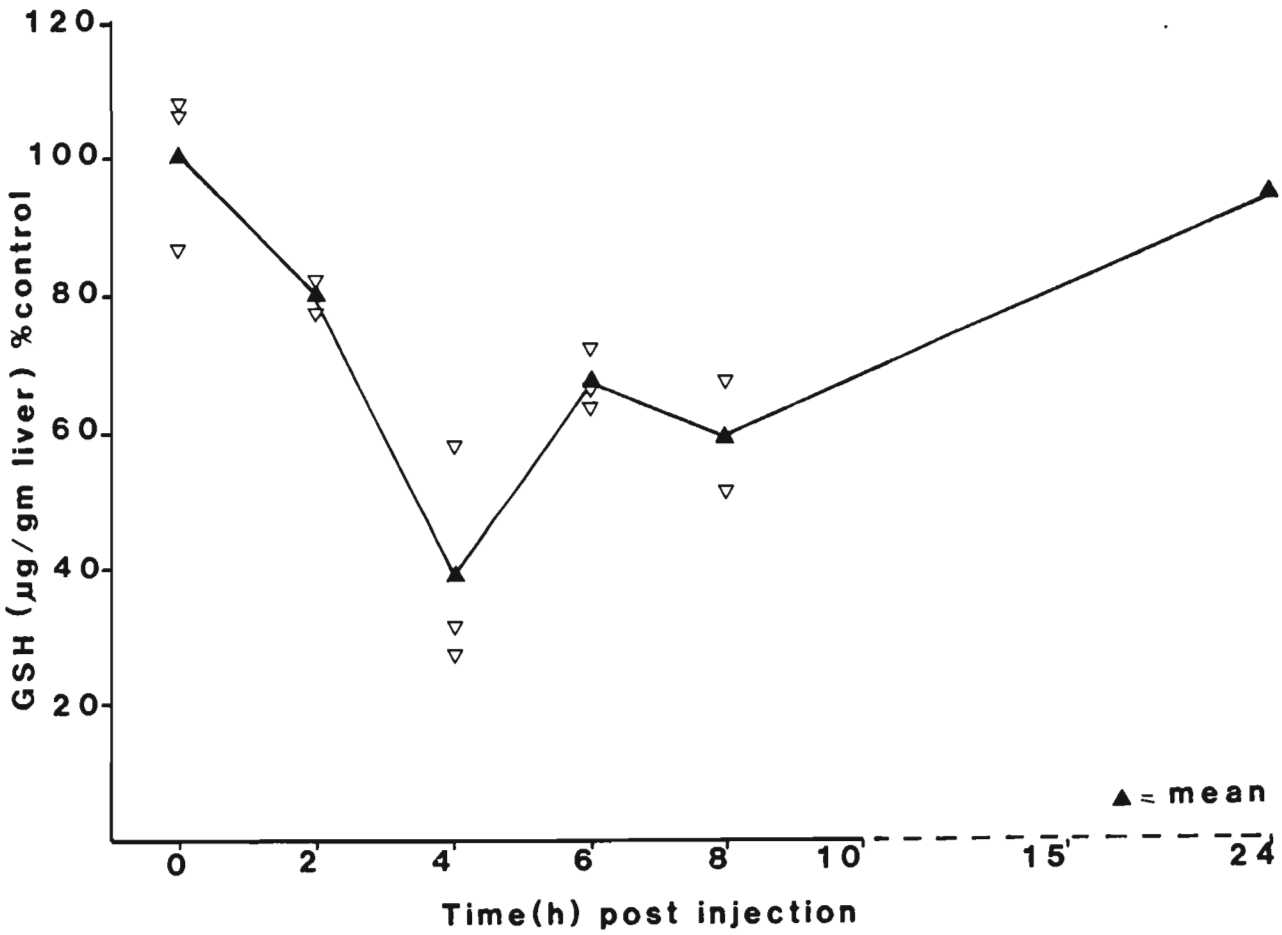
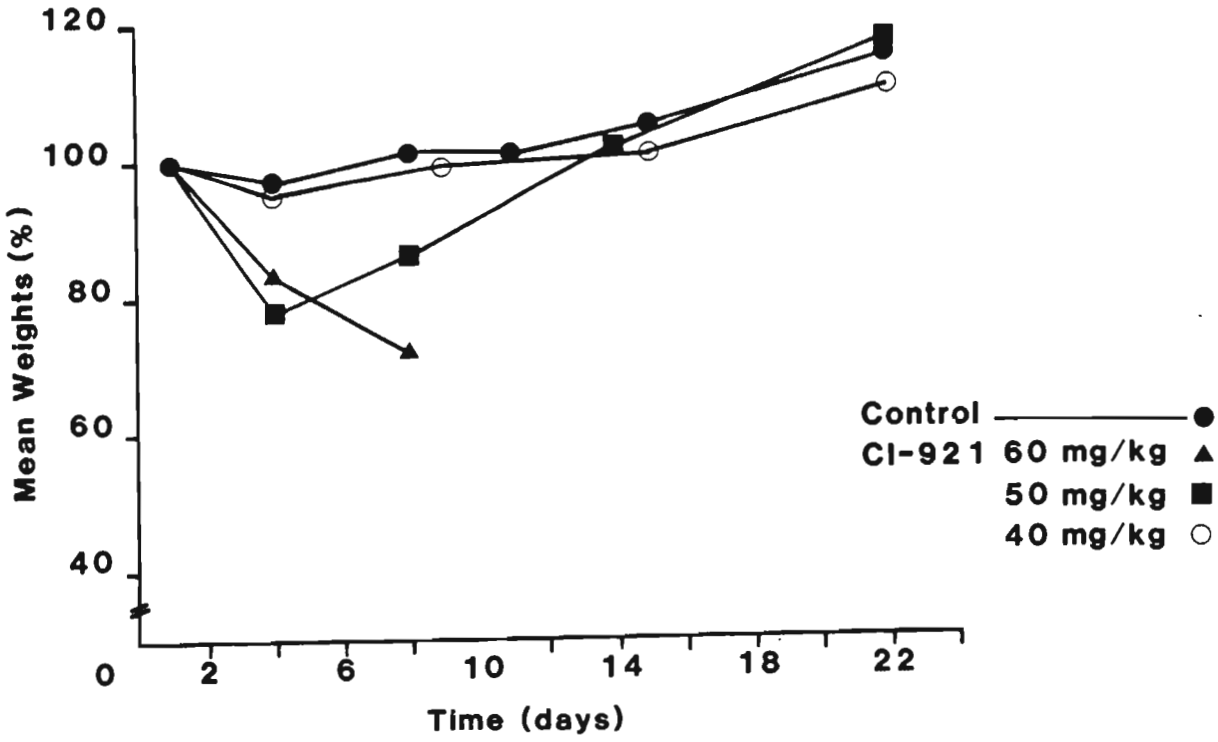
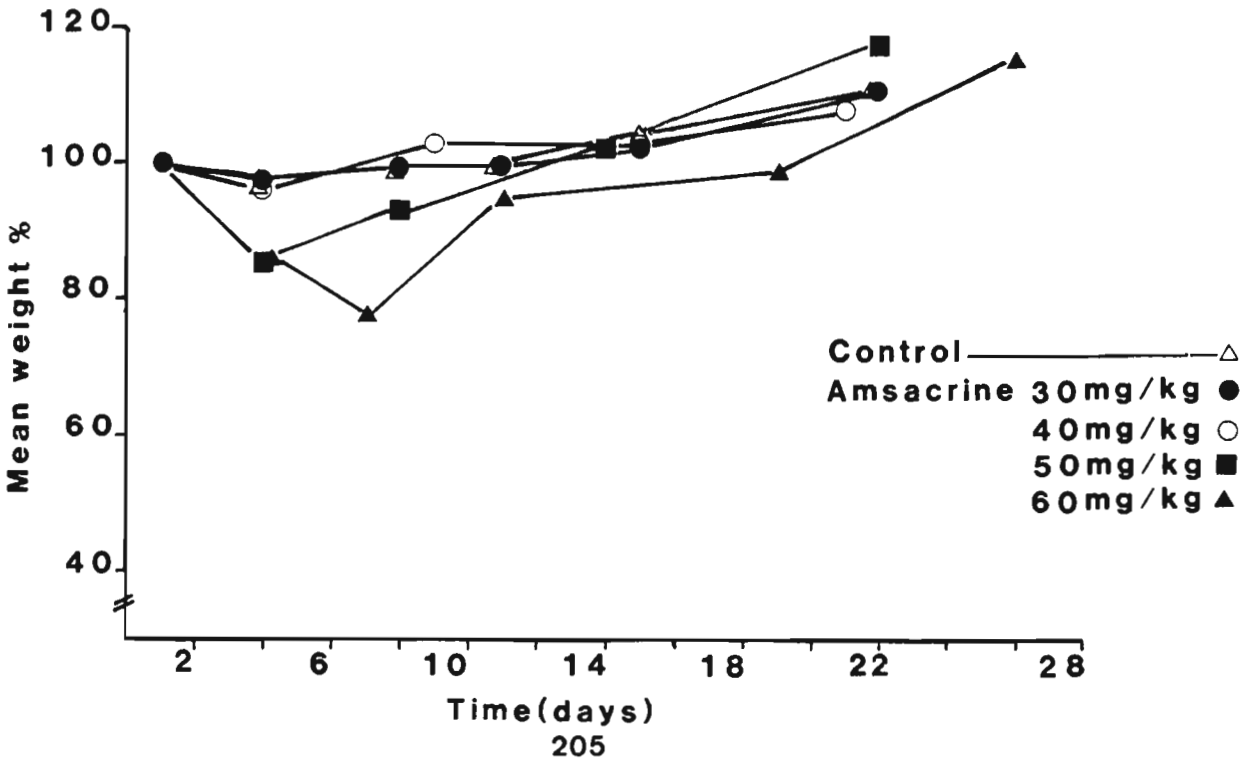
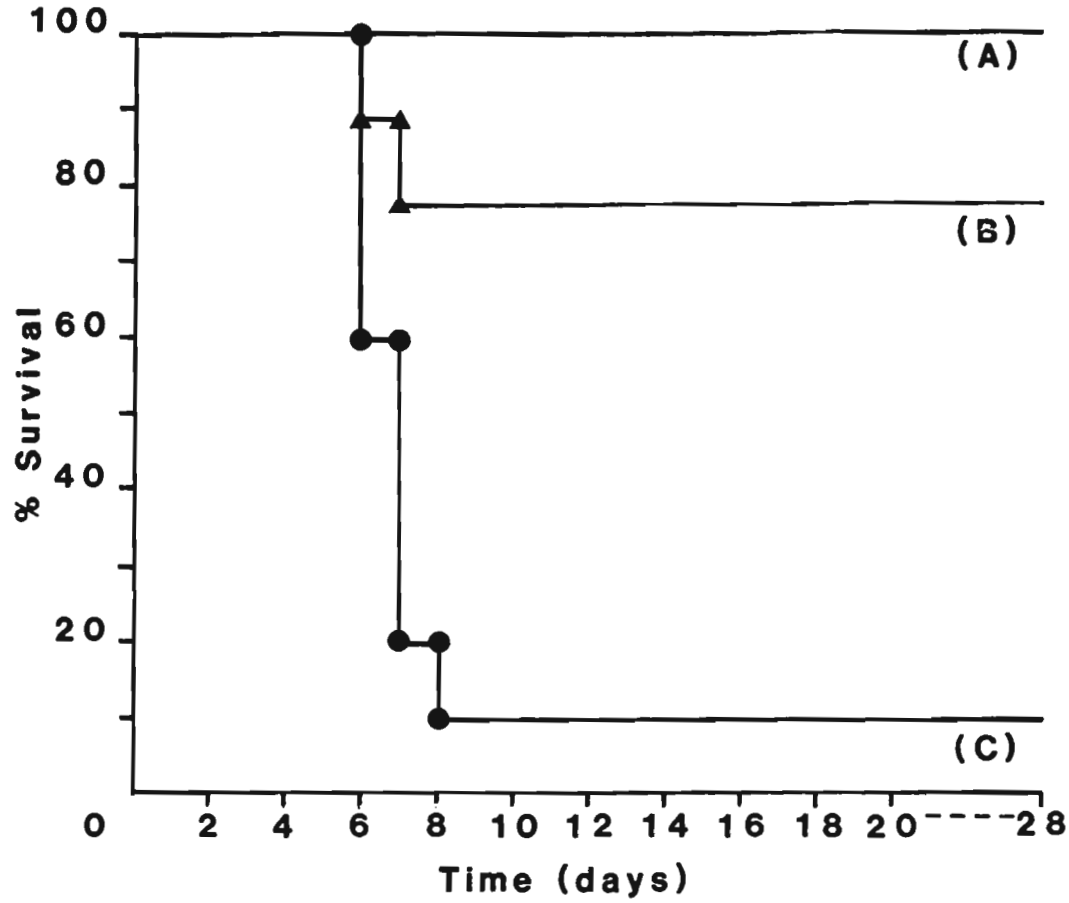


FIG. 32. Mean weight of surviving mice following treatment with CI-921 and amsacrine.





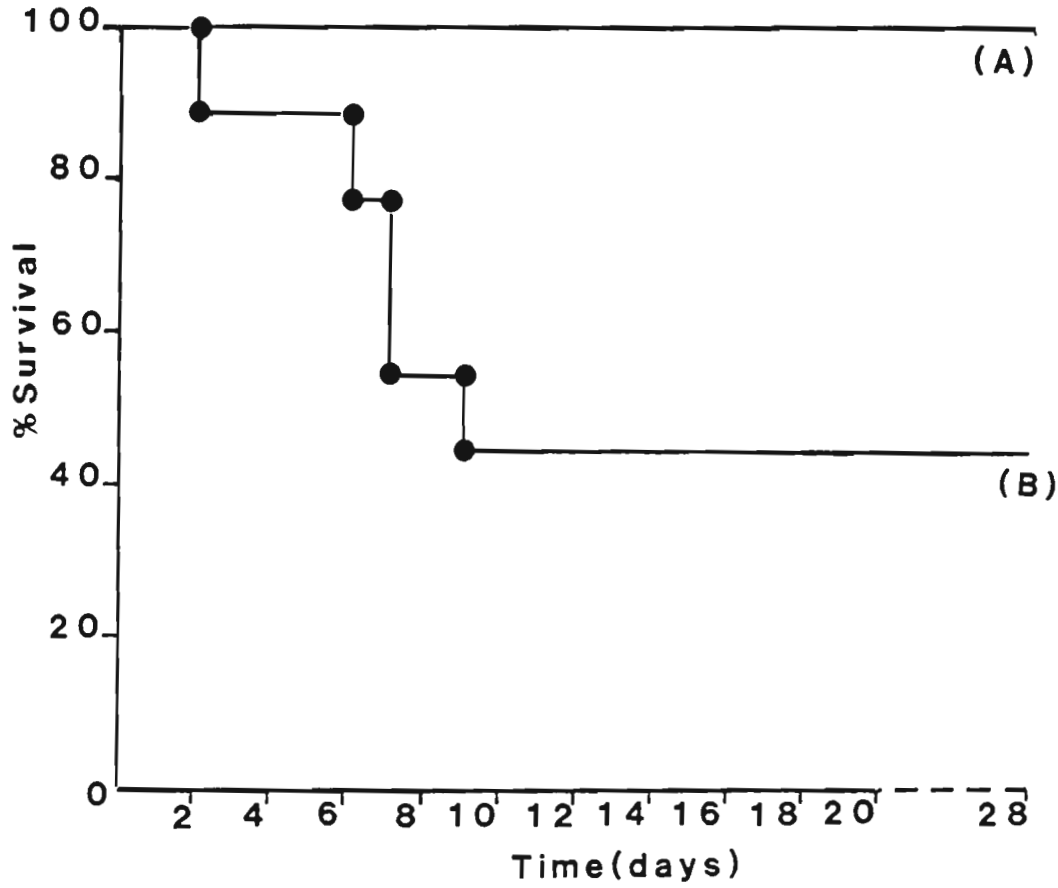


DOSE CI-921:

(A) 40 mg/kg
(B) 50 mg/kg
(C) 60 mg/kg

FIG. 33. Survival curves of mice following treatment with CI-921 at doses 40-60mg/kg iv.

FIG. 34. Survival curves of mice following treatment with amsacrine at 30-60mg/kg iv.



Dose amsacrine
(A) 30,40,50mg/kg
(B) 60mg/kg

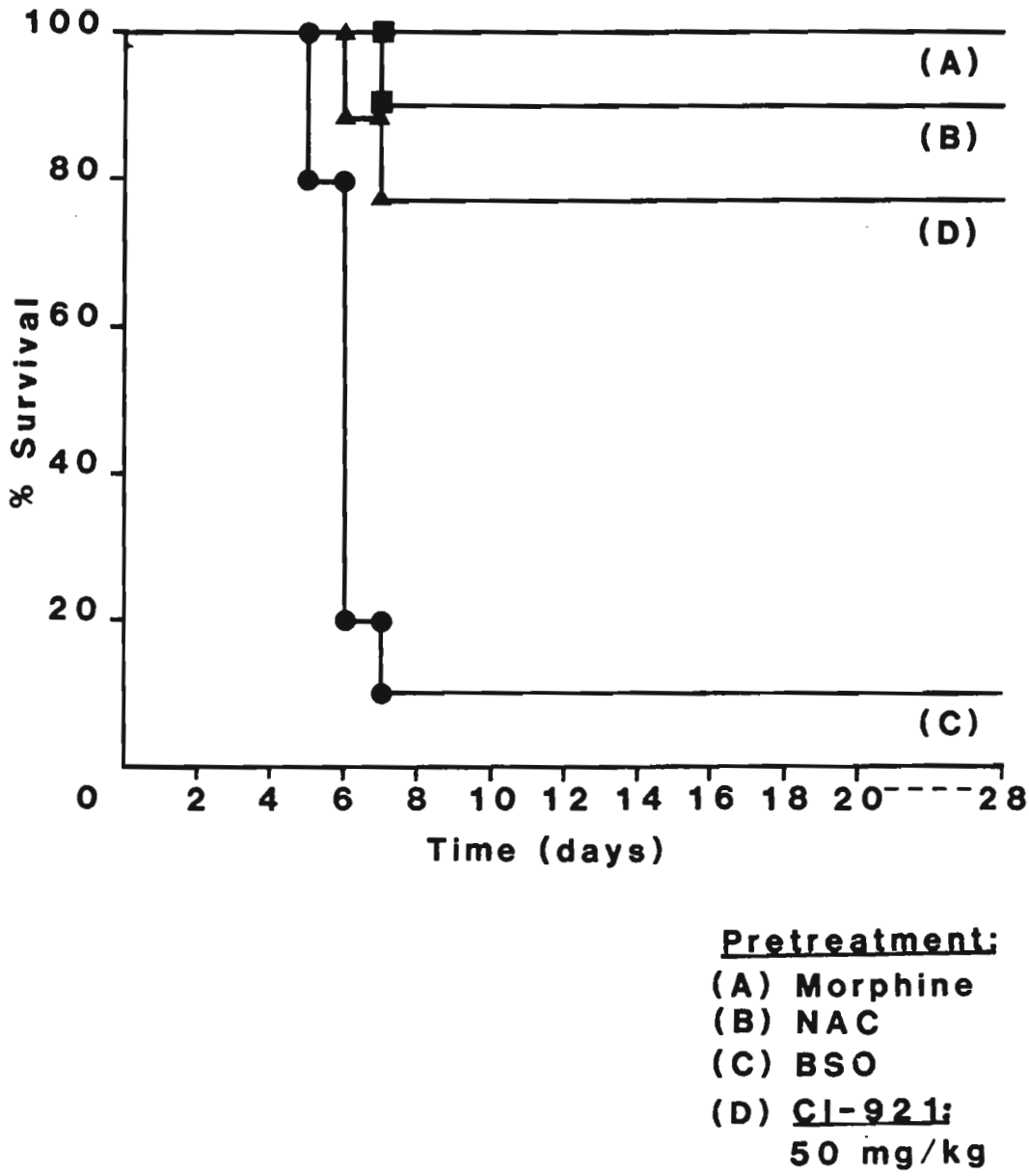
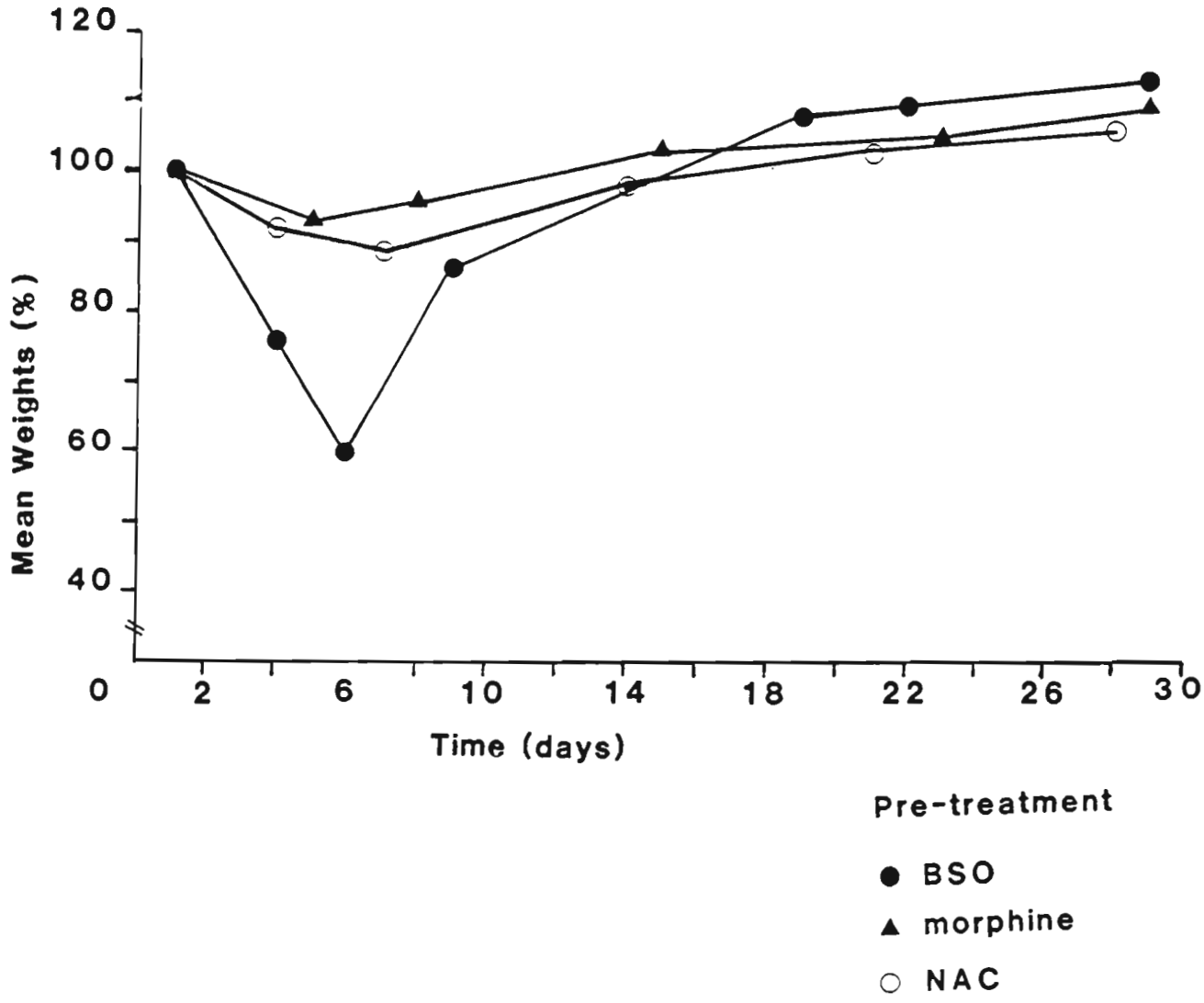


FIG. 35. Survival curves of mice pre-treated with morphine, NAC and BSO compared to the survival of mice treated with CI-921 alone.

FIG. 36. Mean weight of surviving mice pre-treated with BSO, morphine sulphate and NAC prior to CI-921.



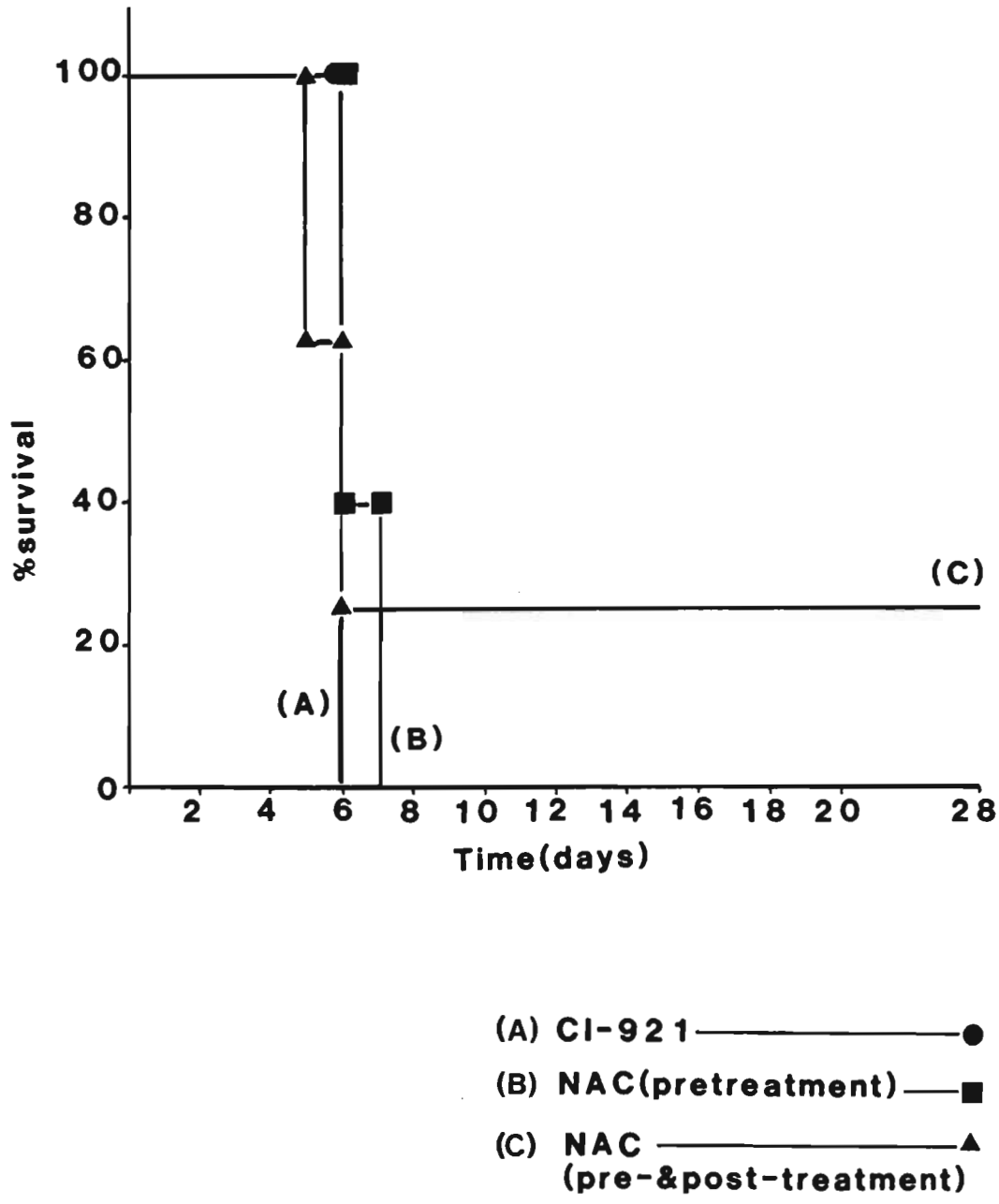


FIG. 37. Survival curves of mice following treatment with CI-921 and NAC compared to CI-921 alone.

Histological Studies

There was no significant histological difference between hepatic tissue of controls and those of mice treated with lethal doses of CI-921 or amsacrine. Minor patchy changes and vacuolation in some samples was thought to have resulted from post-mortem autolysis rather than hepatic toxicity. Bone marrow sections showed a complete loss of differentiated haematopoietic tissue and congestion of marrow spaces with red blood cells in those mice treated with CI-921 or amsacrine. Sections of stomach and gastrointestine from mice treated with CI-921 appeared normal. Sections of lung and kidney were normal. The primary cause of death therefore appeared to be bone marrow failure.

DISCUSSION

Measurement of GSH levels in whole blood.

The mean GSH concentration of 502.5μg/ml in whole blood of healthy subjects and patients in this study is slightly higher than the 396μg/ml found by Tietze (1969) or the average, 328 μg/ml, of literature values reported in the same paper. The 2.5 to 3-fold range in GSH levels within this study population is similar to the variation reported by others (Tietze 1969).

Beutler and Gelbart (1985) were unable to demonstrate a fall in plasma GSH in patients following treatment with a range of cytotoxic agents, but did observe markedly lowered plasma GSH concentrations in many patients with malignancies. In this study, the mean

GSH concentration in whole blood of the cancer patients was not significantly different than that measured in the control group of fit healthy volunteers. The control and patient groups were not age or sex matched but there was no obvious difference in levels between the sexes and no correlation with age.

A fall in whole blood GSH following CI-921 administration was not observed but was perhaps not unexpected. If the metabolism of CI-921 involves conjugation with glutathione (as is the case with amsacrine), one might expect a fall in hepatic GSH following treatment with this drug. However, liver GSH levels cannot be measured easily in man and whole blood GSH may not reflect changes in hepatic GSH (Pyke et al 1986, Kaplowitz et al 1985). While the liver is the major source of plasma GSH (Kaplowitz et al 1985) the quantity of GSH in plasma is very small ($< 0.1\%$) compared to the concentration in red blood cells (Beutler and Gelbart 1985). Whole blood GSH is therefore primarily a measure of red blood cell GSH and may not reflect hepatic GSH. Similarly, erythrocytes are not a major site of cytochrome P450-dependent oxidative metabolism. Therefore, whole blood GSH depletion might not be expected even in the presence of extensive hepatic GSH depletion as they are not sites of drug metabolism. Further studies in this area are therefore probably limited to animal studies where assessment of hepatic tissue GSH is possible.

There was no fall in the GSH concentration of mouse whole blood following the dose of CI-921 chosen to approximate a "treatment dose" in man. However, this dose (34 mg/kg i.v.) was well below that dose of CI-921 (50 mg/kg i.v.) which was demonstrated to result in decreased hepatic levels of GSH in mice.

Mouse hepatic GSH following amsacrine and CI-921

Cysyk et al (1977) demonstrated a 40% decline in liver GSH levels in mice following a single i.p. injection of amsacrine (100mg/kg) with a 20% decrease in liver GSH-transferase activity persisting 24 hours after treatment. Shoemaker et al (1982) subsequently identified a GSH conjugation product as being the principle biliary metabolite of amsacrine. Similar results were seen in this study with GSH concentrations falling by almost 40% of control levels following i.v. amsacrine (60mg/kg) with recovery to pre-treatment levels within 24 hours. Treatment with CI-921 at an approximately equitoxic dose also resulted in a fall in hepatic GSH levels suggesting that CI-921 may follow a similar pathway of metabolism. The fall in GSH measured at 2-4 hours of approximately 15% from control levels was less than that seen with amsacrine.

A finding of particular interest was the "over-shoot" of hepatic GSH concentrations to almost 130% of control after five hours. Adams et al (1985) have shown a 2-3 fold "over-shoot" of both GSH and GSH-transferase in the bone marrow and liver of mice following a low "priming" dose of cyclophosphamide. This is thought to explain the known protective effect of low dose cyclophosphamide "priming" prior to a subsequent lethal dose. Treatment with 1-ß-D-arabinofuranosylcytosine and irradiation also resulted in a similar elevation of bone marrow GSH levels (following an initial fall), raising the possibility that increased levels of intracellular GSH and GSH dependent enzymes represent part of a general response of tissues to cytotoxic insult. Similar GSH elevation in vivo has also been demonstrated in rats following exposure to hyperoxia (Kimball et al 1976) and after intoxication with carbon tetrachloride and thioacetamide (Simplicio 1982). This may

explain the "over-shoot" phenomena noted here, although the time course is shorter and the elevation of GSH not as great as that reported by Adams et al (1985).

Mouse hepatic GSH following the experimental manipulation with BSO and morphine.

The marked reduction in hepatic GSH that can be induced following treatment with BSO was well illustrated in this study. Drew and Miners (1984) reported a reduction in mouse hepatic GSH to approximately 35% of control levels after BSO (1.6 g/kg i.p.) with the maximum depletion occurring at 4-6 hours post injection. No further reduction was observed with higher doses of BSO. An even greater fall in GSH concentration was seen in this study with approximately 15% of control concentrations measured at 4 hours with recovery by 20 hours. With this information, treatment with CI-921 could be timed to co-incide with the maximum depletion of GSH following BSO to investigate any relationship between GSH concentrations and the toxicity of CI-921.

The study with morphine was initiated because this commonly used drug has been shown to lower GSH concentrations in animals (James et al 1982, Nagamatsu et al 1986). The 60% fall in hepatic GSH levels at 4 hours following morphine with recovery to >90% of control levels by 24 hours shown in this study is similar to that found by Nagamatsu et al (1982). They demonstrated a dose-dependent decrease in mouse liver non-protein sulphydryl content following s.c. morphine that was maximal at a dose of 250 mg/kg. This may be of considerable clinical relevance as many patients receiving cytotoxic agents require

narcotic analgesia with morphine for pain control. Thus when morphine is given with agents such as amsacrine or CI-921 which require GSH for their detoxification, drug interaction may occur. This was investigated in the toxicity studies.

Toxicity studies

The toxicity studies in mice were performed to investigate the importance of GSH in the metabolism of CI-921, assuming that the GSH conjugation step results in detoxification of the drug. It was first necessary to define a MTD of CI-921 on which to base the other studies. The dose of CI-921 (50 mg/kg i.v.) found in our studies to be lethal in approximately 20% of mice is in agreement with the preclinical toxicity studies of CI-921 in mice (Investigators Brochure 1985) which identified an LD_{10} of 42mg/kg and an LD_{50} of 60mg/kg (Table 6.8). The toxicity profile in the present study was steeper however in that 90-100% mortality was seen at 60mg/kg i.v. In contrast, the preclinical toxicity profile for amsacrine as reported by Henry et al (1980) was very narrow, with only 7mg/kg between the LD_{10} (30.4mg/kg) and the LD_{90} (37.3mg/kg) (Table 6.8). All deaths in this study were said to occur within 4 hours of injection. In the current study, acute toxicity in mice in the form of hypoactivity, prostration, laboured respirations and convulsions immediately following the injection of drug was seen in some cases. Issell (1980) has suggested that this acute toxicity is primarily neurological and related to the amsacrine vehicle, dimethyl acetamide (DMA). Henry et al (1980) however, documented a 70-fold difference between the LD values of amsacrine plus vehicle and vehicle alone, with the dose of DMA necessary to produce toxicity being much greater than that given with amsacrine to produce equivalent

toxicity. In the current study, this acute toxicity was minimized by restricting the total i.v. fluid load to <11ml/kg and slowing the rate of infusion, but occasional acute deaths were still seen. If mice survived the immediate post infusion period, no further toxicity was seen to 28 days with doses up to 50mg/kg. At 60mg/kg the pattern of toxicity for amsacrine was similar to that of CI-921 with clinical toxicity obvious by day 3-4 and death of animals from days 5-9.

On a mg/kg basis, CI-921 was more potent than amsacrine with respect to delayed toxicity. A dose of 60mg/kg i.v. CI-921 resulted in 90-100% mortality whereas this dose of amsacrine was lethal in only about 50% of mice. Statistical analysis of this difference is not possible however because of the relatively small number of animals used. Because of the problems encountered with the acute toxicity of amsacrine injections as discussed above and difficulties in reproducing results (possibly because of the steepness of the amsacrine toxicity curve) complete toxicity studies were performed with CI-921 only.

Once a MTD had been defined, mice were pretreated with known GSH depletors and protectors to see if this had any influence on the toxicity of CI-921. Treatment with BSO led to a marked increase in the toxicity and mortality of CI-921. Mice developed clinical toxicity earlier (day 3 rather than day 4 or 5 when treated with CI-921 alone) and 90% of mice were dead by day 7 following a dose of CI-921 that had been lethal in only 22% of control mice receiving CI-921 alone at the same dose. This suggests that a depletion of hepatic GSH to 15% of control levels as shown in the previous experiments, may well be sufficient to interfere with the conjugation and detoxification of CI-921.

Treatment with morphine did not increase toxicity however. In fact, there were no deaths and clinical toxicity was not marked. The fall in GSH to 40% of control levels following treatment with morphine shown in this study was less marked than that following BSO pre-treatment. Perhaps this decrease in GSH concentration was not sufficient to interfere with the metabolism of CI-921.

TABLE 6.8. Preclinical toxicology studies (single dose i.v.)

	CI-921*	Amsacrine §
LD_{10}mg/kg	42	30.4
mg/m^2	126	91.2
LD_{50}mg/kg	60	33.7
mg/m^2	180	101
LD_{90}mg/kg	86	37.3
mg/m^2	258	111.9

* Investigator's brochure 1985

§ Henry et al 1980

NAC was used as pretreatment in an attempt to stimulate GSH production and thus to protect against the toxicity of unconjugated CI-921. The "protective effect" of NAC shown in the initial experiments using CI-921 at a dose of 50mg/kg may simply reflect experimental variation in the mortality rate of this dose of CI-921. The number of mice used in these experiments was not sufficient for statistical analysis. In an attempt to demonstrate a true protective effect, a higher dose of CI-921 was used. A single dose of NAC did not protect against the toxicity of this increased dose, while pre- and post-treatment with NAC resulted in a 25% survival rate. Whether this represents a true protective effect is once again not clear. In addition the exact timing of the NAC administration relative to the CI-921 may be crucial or an even higher dose of NAC may be required to prevent toxicity. Hepatic GSH following NAC was not measured in this study and it was not shown whether any protective effect is dependent on increased GSH levels. Further studies with larger numbers of animals would be required to answer these questions.

Histological studies

Histological studies were performed in an attempt to define the cause of death in mice following lethal doses of CI-921 and amsacrine. In pre-clinical toxicity studies in mice treated with amsacrine, bone marrow depression was seen following i.v., p.o., and i.p. drug administration, but gut toxicity was thought to be the cause of death following p.o. and i.p. administered drug (Baguley 1983). A dose-related appearance of cytoplasmic vacuoles in the liver has been described, but this was not thought to be lethal (Baguley 1983). The mice in this study appeared to die from bone marrow failure, with no definite evidence of

hepatic toxicity. There were no histological changes to suggest gastrointestinal toxicity in mice treated with CI-921. The recovery of mice following obvious clinical toxicity suggests that the bone marrow suppression was reversible.

In summary, the pattern of toxicity of CI-921 is similar to that of amsacrine with bone marrow suppression appearing to be the major toxicity of both drugs. GSH may well have an important role in the detoxification of CI-921 as shown by the increased toxicity following the profound depletion of hepatic GSH by BSO. On the other hand, we were unable to demonstrate a deleterious effect by the reduction of GSH to a lesser extent with morphine or a protective effect following the addition of exogenous thiols.

CHAPTER 7

CONCLUSIONS

CHAPTER 7. CONCLUSIONS.

While the development of anti-cancer drugs over the last forty years has led to the cure of some patients with advanced malignancies and the palliation of many more (De Vita 1985), there is vast potential for improvement. The majority of the common cancers are either unresponsive (e.g. lung and gastrointestinal cancers) or only temporarily responsive (e.g. breast) to systemic therapy. Even in those cancers where cures are possible, a proportion of patients will not respond or will relapse and would be candidates for more effective therapy. In this setting numerous groups are striving to produce better drugs to enable more effective therapy.

CI-921, an analogue of the antileukaemic agent amsacrine, was developed to clinical trial on the basis of its exceptional activity in pre-clinical studies (Baguley 1984). It was hoped that the in vivo and in vitro solid tumour systems utilised would be better predictors of activity in solid tumours in man than the previously used test systems (Finlay and Baguley 1981).

The phase I study of CI-921 described in this thesis was a standard study required to define the maximum safe dose of the drug in man when given in a three-day intravenous schedule. It was the first phase I study of a cytotoxic agent to be undertaken in New Zealand and will hopefully set a precedent for the performance of further such studies in the future.

Sixteen patients with cancer for which there was no conventional treatment, or who had relapsed following treatment, were entered into the phase I trial. CI-921 was found to be a relatively non-toxic drug, well tolerated by patients. It was easier to administer than amsacrine because of its greater solubility and does not require organic solvents for reconstitution.

The toxicity of CI-921 was similar to that seen in the phase I trials of amsacrine (Legha et al 1978). Predictable, reversible myelosuppression was the dose limiting toxicity and defined a maximum tolerated dose of 810mg/m^2 (270 mg/m^2 daily x 3). Nausea and vomiting was mild and easily controlled with standard anti-emetics. Alopecia did not occur. Other major toxicities seen were thrombophlebitis at the site of drug infusion and mucositis at the maximum tolerated dose.

No objective tumour response was seen but this was not unexpected. All patients in the phase I trial were either heavily pretreated or had tumours generally unresponsive to chemotherapy.

The pharmacokinetics of CI-921 were studied using a high performance liquid chromatography system. Serial measurements of CI-921 in plasma following a 15 minute i.v. infusion revealed a biexponential pattern of drug disposition with an initial distribution half-life of 0.5 hours and an elimination half-life of about 2.6 hours. The kinetics appeared linear over the dose range tested in this centre, but were non-linear, or dose-dependent at the higher dose levels tested by another trial centre where the drug was given in a single i.v. dose rather than divided over three days as in this study. This finding is important as

non-linear pharmacokinetics result in disproportionately large increases in AUC with small increases in dose, which can result in unexpected toxicity.

The toxicity of most drugs is closely related to total drug exposure (as expressed by AUC). In this study, over the dose range used, toxicity as defined by nadir absolute granulocyte count correlated well with AUC, but was equally well correlated with maximum drug concentration and total dose (both in mg/m^2 and $\mu g/kg$). As might be expected with linear kinetics, the pharmacokinetic parameters did not help in the prediction of maximum dose and toxicity in this study but may be very important at those dose levels shown to follow non-linear kinetics in the other phase I studies. Even so, important information regarding the pattern of elimination was provided. No unexpected drug accumulation or evidence of enzyme saturation was noted using the three day drug schedule with the kinetic parameters on day three being equivalent to those on day one.

Renal elimination played a minor role in the elimination of CI-921 with less than 1% of the total dose of drug being excreted unchanged in the urine. The elimination of CI-921 is therefore unlikely to be affected by renal dysfunction, but may be affected by impaired liver function if it follows a similar route of hepatic metabolism as amsacrine.

The starting dose of a cytotoxic drug in a phase I trial in man is based on pre-clinical animal studies and is usually 1/10 the LD_{10} in mice in mg/m^2. Future phase I studies may rely more on pre-clinical pharmacokinetic studies to guide dose escalation assuming drug exposure (AUC) to be a better predictor of the MTD than dose (Calvert and Balmanno 1987). In the present situation pharmacokinetic studies in mice would have been of little

value because they would have under-estimated the MTD of CI-921, there being a seven-fold difference between the AUC of the mouse LD_{10} and the AUC of the MTD of CI-921 in man. Similarly the dose in mg/m² in mice was not a good predictor of toxicity in man.

The use of a central data collection system to co-ordinate the results of the three phase I trials of CI-921 allowed for more rapid dose escalation than would otherwise have occurred. Dose levels that had already shown to be without toxicity in other centres could be "skipped", allowing the omission of four treatment levels and "within patient" dose escalation. Sixteen patients were entered in total, whereas 36 patients would have been required if three new patients had been entered at each dose level as per a standard modified Fibonacci dose escalation scheme.

Although CI-921 appeared more potent than amsacrine in pre-clinical studies, the MTD of CI-921 (810 mg/m²) was much higher than that of amsacrine (150 mg/m²) when given according to a similar three day i.v. schedule (Legha et al 1978). This is probably related to differences in physico-chemical and pharmacokinetic properties of the two drugs. Both drugs are highly protein bound, but the active unbound fraction is significantly less for CI-921 (0.3%) than for amsacrine (3.0%) (Jurlina et al 1985). Although CI-921 has a greater volume of distribution than amsacrine (related to its greater tissue distribution), its clearance is proportionally greater. Therefore the elimination of CI-921 is about half that of amsacrine.

Following the identification of the MTD in the phase I trial a limited phase II study of CI-921 in NSCLC was undertaken using a total dose of 648 mg/m² in the same three-day

schedule. Of sixteen evaluable patients, one partial response was seen in a patient with squamous cell lung cancer that was maintained for 5 months. This patient received the highest dose of CI-921 on a μmol/kg basis, but had an AUC similar to that of the whole group and the lowest Cmax. There is no current standard treatment for NSCLC, with no current drug regimen providing response rates consistently greater than 20%. Therefore even a single response in this relatively common, notoriously chemo-resistant tumour is encouraging and suggests that further trials of CI-921 in NSCLC are warranted.

The level of toxicity seen in the phase II study was acceptable and indicated that the correct dose for further phase II studies had been identified in the phase I study. The patients studied in each trial were similar in that the majority of patients in the phase I study (11 of 16) and no patient in the phase II trial had been previously treated with chemotherapy. The dose may well require modification in pre-treated patients however.

As has been described with amsacrine, a possible neurotoxic drug effect was noted in a patient who had three grand mal seizures in 3 of 4 courses temporally related to CI-921 infusion. There were no other unexpected side effects. Cardiac evaluation by ECG and echocardiographic assessment prior to and following treatment with CI-921 failed to show any evidence of cardiotoxicity to this dose level but the number of patients studied was relatively small and the cumulative dose of CI-921 was low, with a median of only two courses per patient.

The pharmacokinetic parameters from the phase II trial were consistent with those seen in the phase I study except for a tendency towards a tri-phasic pattern of distribution in several

patients. There was a significant difference between the Cmax and AUC of the phase II study and the phase I study group treated at the same dose. This probably relates to the small number of patients in the trial rather than to any difference in extent of prior treatment, age or performance status between the patient groups. When individual patient results are considered, the pharmacokinetic parameters could not be directly related to response, abnormality of liver function, sex, age or concomitant medication.

Studies were undertaken to define the pattern of metabolism of CI-921. Glutathione is probably involved in the metabolism of CI-921 as has been shown with amsacrine (Shoemaker et al 1980). Although no decrease in GSH concentration was demonstrated in whole blood of patients in this study or mice following CI-921 infusion, a decrease in mouse hepatic GSH was found following treatment with both amsacrine and CI-921. The failure to show lower levels of GSH in man during CI-921 administration was probably due to the inability to measure GSH in liver, peripheral blood concentrations not reflecting total levels to any degree. The toxicity of CI-921 in mice was greatly increased following the experimental depletion of hepatic GSH with BSO, suggesting that the metabolic process involving GSH is one of detoxification. However, toxicity was not increased following pre-treatment with morphine which was shown to be less effective in lowering mouse hepatic GSH concentrations. This is an important finding as morphine is commonly used as an analgesic in patients with cancer and is unlikely to enhance the toxicity of chemotherapy. Similarly, we were unable to demonstrate any lessening of toxicity following pre-treatment with N-acetyl cysteine, an agent known to stimulate GSH production.

Post-mortem histological studies of mice given CI-921 or amsacrine suggested that the

primary cause of death following lethal doses was bone-marrow failure. There was no gross evidence of hepatic or gastrointestinal toxicity, thus supporting the clinical evidence of the toxicity found in man.

The clinical studies of CI-921 have allowed the definition of a maximum tolerated dose in man and in a preliminary study, have suggested possible activity in NSCLC. Concomitant pharmacological studies of CI-921 have defined various parameters of distribution, metabolism and elimination. The presumed hepatic metabolism and the lack of renal elimination accords with amsacrine. The studies of metabolism of CI-921 and amsacrine in mice have shown that GSH depletion is an important determinant of drug metabolism. Although various drugs given concomitantly with chemotherapy can effect GSH concentrations, studies in mice following morphine and NAC suggested that any alteration in GSH concentration with these agents was insufficient to alter the toxicity profile. This will require further investigation in man. Following the three phase I studies and two limited phase II studies that have been completed, further world-wide phase II studies are being considered to define the activity of CI-921 in various tumour types. If areas of activity can be defined then comparison with standard therapies (phase III clinical studies) either alone or in combination with other agents will be required to establish the place of CI-921 in the cytotoxic armamentarium.

APPENDICES

APPENDIX 1.1 Performance status (ECOG)*

Grade	
0	Normal activity
1	Symptoms, but nearly fully ambulatory
2	Some bed time, but needs to be in bed less than 50% of normal day time
3	Needs to be in bed more than 50% of normal day time
4	Unable to get out of bed

* Eastern co-operative oncology grade

APPENDIX 1.2

INFORMED CONSENT FORM:

PHASE I TRIAL OF CI-921 IN ADVANCED MALIGNANCY

I.....have agreed to receive the drug CI-921. Doctor.....has explained to me that I have advanced..... cancer for which there is no known curative treatment.

I understand that CI-921 is an experimental drug which has been extensively tested in the laboratory and in animals but not yet in patients. It has not yet been approved for general use by the Department of Health in New Zealand or in any other country. I understand that I shall be receiving this drug to determine the correct dose, to discover the side-effects and perhaps its effect against cancer, but I appreciate that it is not yet possible to predict the effect of CI-921 on my cancer. I understand that other physicians might choose to treat my disease at this time without chemotherapy or with a different drug or combination of drugs. I have been given the opportunity to ask questions about my participation in this study and have received clear and precise answers in return, which are satisfactory to me. I retain the right to ask questions throughout the study.

I understand that CI-921 will be given by injection into a vein (via a "drip") on three successive days and it will be necessary for me to remain in hospital under observation for the duration of this time. I appreciate that drug administration will continue at 3 week intervals provided my blood count permits, but will be discontinued if my cancer progresses, my medical advisers think that it is in my best interests, or I wish to withdraw from the study.

I have been told, and understand, that the toxicities likely to occur include nausea, vomiting, hair loss, mouth ulcers, diarrhoea, and that my blood count may become lowered leading to a danger of severe infections. I have agreed to obtain periodic blood counts so that the need for treatment of low blood counts by transfusion or antibiotics may be determined. I also understand that it is possible that other toxicities may occur but that my medical attendants will be paying particular attention to the occurrence of these in an attempt to prevent them or detect them at an early age.

I appreciate that if I suffer side-effects from CI-921 all necessary medical treatment will be provided but financial compensation cannot be given for any injuries suffered during this programme.

I am aware that I may withdraw from this study at any time and that this will not jeopardise any possible additional and future care for my disease.

I agree to participate in this experimental drug trial.

SIGNED.....WITNESS.....

DOCTOR.....DATE.....

APPENDIX 1.3 Toxicity grading.

	GRADE	0 NONE	1 MILD	2 MODERATE	3 SEVERE	4 MAXIMAL OR LIFE THREATENING
HEMATOLOGY	Hgb gm%	No Drop	<3 gm drop	3-3.9 gm drop	4-4.9 gm drop	≥5 gm drop
	WBC x 10^3	≥4.5	3.0-4.4	2.0-2.9	1.0-1.9	<1.0
	Neut. x 10^3	>1.9	1.5-1.8	1.0-1.4	0.5-0.9	<0.5
	Platelets x 10^3	>130	90-129	50-89	25-49	<25
	Hemorrhage (2° to thrombocytopenia)	None	Mild: petechiae, microscopic hematuria, epistaxis	Gross bleeding, may require transfusion	Bleeding causing some functional disability requiring <4U blood	Massive hemorrhage w/ profound funct. disability and/or >4U blood
	Infection (2° to granulocytopenia)	None	Infection controlled by oral antibiotics	Infection w/ fever >38° C (>100.4° F) requiring parenteral AB	Severe infection w/chills and/or fever >40° C (104° F)	Fulminant infection w/sepsis, shock or system failure
GU	BUN	≤ 7 mmol/L	7.5-14 mmol/L	14.5-21.5 mmol/L	> 22 mmol/L	Symptomatic uremia
	Creatinine	≤ 110 μmol/L	115-180 μmol/L	185-265 μmol/L	> 265 μmol/L	
	Creat. Cl (% change)	100 baseline	<25% drop	25-49.9% drop	50-74 9% drop	>75% drop
	Hematuria unrel to thrombocytopenia	Neg	Dysuria only	Microscopic pos; WBC and/or RBC or hemocc positive	Gross hematuria and/or infection	W/obst. uropathy or exsanguinat. hemorrhage
	Proteinuria	Neg	1+	2+ - 3+	4+	
HEPATIC	SGOT	<1.5 x nl	1.5-2 x normal	2.1-5 x normal	>5 x normal	
	Alk. Phosphatase	<1.5 x nl	1.5-2 x normal	2.1-5 x normal	>5 x normal	
	Bilirubin	< 20 μmol/L	21-50 μmol/L	51-120 μmol/L	>120 μmol/L	
	Clinical				Precoma	Hepatic coma
GI	Nausea & Vomiting	None	Nausea & Vomiting	Controllable N&V	Intractable N&V	Hospitalization req.
	Oral	None	Soreness &/or erythema	Mucositis &/or ulcers Can still eat	Mucositis &/or ulcers Cannot eat	Hospitalization for alimentation (PO, IV)
	Diarrhea	None	Frequent & loose BM up to 4/day	Frequent & loose BM >4/day	Bloody diarrhea	Hospitalization req.
ALLER	Skin	None	Transient rash	Urticaria	Serum sickness	Anaphylaxis
	Pulmonary	None	None	Mild bronchospasm requires oral meds.	Bronchospasm requires parenteral medications	Severe bronchospasm
PUL	PFT's; X-ray, Clinical	WNL	25-50% ↓ in PFTs, no sx No X-ray changes	>50% ↓ in PFTs, mod. sx X-ray changes	Significant X-ray findings, dyspnea at rest	Ass't vent. or conc. O_2 needed.
CARDIAC	EKG & Clinical	WNL	ST-T changes	Atrial arrhythmias	Mild CHF &/or lg heart	Severe or refract CHF
	Rate	WNL	Sinus Tachy>110 at rest	Unifocal PVCs	Multifocal PVCs	Ventric tachy or equiv.
	QRS Voltage Decrease	None	≤40%	>40%	Pericarditis &/or pericardial effusion	Tamponade
NERVOUS SYSTEM	PERIPH. NEURO.	No deficit	±DTRs &/or mild paresthesias; mild constipation	Absent DTRs &/or mod. paresth; can hold objects, mild weakness, severe constipation	Disabling; cannot hold objects; severe neuritic pain &/or weakness; needs assistance	Paralysis, severe constipation requiring surgery
	CNS					
	State of Consciousness	Alert	Transient lethargy	Somnolent < 50% waking hours	Somnolent >50% waking hours	Comatose
	Mood	Unaffected	Mild anxiety or depression	Moderate anxiety or depression	Mania or severe depression	Suicidal
	Ideation	Unaffected	Intermittent confusion	Continuous confusion	Hallucination, psychosis	
	Headache	None	Mild	Requires analgesics	Unrelenting	
	Motor activity	Unaffected	Mild hyperactive	Tremor	Convulsions	Status Epilepticus
	Fundi	Normal			Papilledema &/or hemorrhage	Blindness
	Cerebellar	Unaffected	Mild dyskinesia	Limb ataxia (finger to nose to finger)	Gait ataxia (Romberg)	
LOCAL	Skin (including phlebitis)	WNL	Transient erythema, rash, pigmentation, nail changes	Vesiculation, dry desquamation, phlebitis (w/pain)	Ulceration, moist desquamation, severe pain	Exfoliation, necrosis requiring surgical intervention
OTHER	Hair	No loss	Mild hair loss	Moderate hair loss	Pronounced hair loss	
	Fever	≤37.5° C ≤99.5° F	≤38° C ≤100.4° F	38° C - 40° C 100.4° F - 104° F	>40° C >104° F	Any fever w/hypotension
	Weight	Normal gain	No gain	Loss < 10% initial wt.	Loss >10% initial wt.	
	Performance Status	Normal activity	Symptoms but ambulatory	In bed ≤50% of time	In bed >50% of time	Bedridden 100% of time

APPENDIX 1.4

INFORMED CONSENT

PHASE II TRIAL OF CI-921 IN ADVANCED NON SMALL CELL LUNG CANCER

I ..... have agreed to receive

the drug CI-921. Doctor ..... has

explained to me that I have advanced non small cell lung cancer for which there is no known curative treatment.

I understand that CI-921 is an experimental drug which has been extensively tested in the laboratory and in animals but to date has only been used in a limited number of patients. It has not yet been approved for general use by the Department of Health in New Zealand or in any other country. I understand that I shall be receiving the drug to determine its effect against my cancer and to discover possible side effects but I appreciate that it is not yet possible to predict the effect of CI-921 on my cancer. I understand that other physicians might choose to treat my disease at this time without chemotherapy or with a different drug or combination of drugs. I have been given the opportunity to ask questions about my participation in this study and I have received clear and precise answers in return which are satisfactory to me. I retain the right to ask questions throughout the study.

I understand that CI-921 will be given by injection into a vein (via a "drip") on 3 successive days and it will be necessary for me to remain in hospital under observation for the duration of this time. I appreciate that the drug administration will continue at 3 weekly intervals provided my blood count permits but will be discontinued if my cancer progresses, my medical advisors think that it is in my best interests or I wish to withdraw from the study. I have been told and understand that the side effects of the drug may include nausea, vomiting, mouth ulcers, hair loss and that my blood count may become lowered leading to a danger of severe infection. I have agreed to obtain periodic blood counts so that the need for treatment of low blood counts by transfusion or antibiotics may be determined. I also understand that it is possible that other toxicities may occur but that my medical attendants will be paying particular attention to the occurrence of these in an attempt to prevent them or detect them at an early stage.

I appreciate that if I suffer side effects from CI-921 all necessary medical treatment will be provided but financial compensation cannot be given for any injuries suffered during this programme.

I am aware that I may withdraw from this study at any time and that this will not jeopardise any possible additional or future care for my disease. I agree to participate in this experimental drug trial.

Signed .....

Witness .....

Doctor .....

Date .....

APPENDIX 1.5

CI-921 ("Amsalog") Experimental Chemotherapy Trial for Lung Cancer

Information for Patients

You have a type of lung cancer (non-small cell lung cancer) which is not suitable for surgery (operation) or radiotherapy (x-ray treatment). The drugs (chemotherapy) presently available for lung cancer do not usually help.

The Auckland Cancer Research Group have developed a new drug (CI-921, also known as "Amsalog") in conjunction with the pharmaceutical company Warner Lambert. It is hoped that this drug may be active against lung cancer. It has shown great promise in the laboratory but its activity in patients is not yet known.

Those patients suitable for the trial must have a cancer which can be accurately assessed (by examination or an x-ray) and which is known to be growing. They must also have normal function of liver and kidneys as shown by blood tests and be relatively fit (out of bed most of the day).

If you meet these requirements and wish to try the treatment your doctors will arrange for you to see one of the doctors concerned.

One of the disadvantages of the trial is that patients need to be admitted to hospital for 3 - 4 days every 3 weeks or so to receive the drug. They are also required to attend the hospital regularly following treatment for observation and blood tests. You may prefer to spend more time with your family and friends at this stage of your illness. It is possible that the drug may have side effects but no serious ones have yet been found. No patient has lost any hair. Some patients have felt off their food for a few days following treatment but nausea and vomiting has not been a problem so far. Your blood count will fall following treatment but will be checked regularly so that antibiotics or transfusions could be given if necessary.

The advantage of entering the trial is that the growth of your cancer may be arrested or slowed for a while but this is not likely to be a cure.

Your doctor will discuss this further with you if you wish.

APPENDIX 2.1 Assay validation - backcalculated concentrations of plasma standards *

Standards in plasma (nmol/l)	Day 1			Day 2			Day 3		
0	0	0	0	0	0	0	0	0	0
50	53.8	51.0	50.5	45.1	45.1	-	52.8	51.0	51.0
100	97.7	99.9	97.1	98.1	94.2	96.2	104.8	98.5.	-
200	200.6	200.6	195.9	187.4	186.2	186.9	196.7	192.2	198.1
400	408.9	420.1	407.2	405.3	404.4	403.1	389.8	402.9	402.4
600	588.5	593.6	605.5	591.4	615.1	589.3	575.1	579.5	584.1
800	788.5	783.8	793.2	828.3	794.5	-	805.3	813.5	801.7
1000	999.4	1015	-	1018	1035	1033	1005	1028	1008
2000	2191	2178	2206	2090	2126	2084	2043	-	-
Slope		1.47×10^{-3}			1.54×10^{-3}			1.57×10^{-3}	
Intercept		-2.5×10^{-12}			-1.77×10^{-12}			-1.41×10^{-12}	
Log likelihood		135.4			95.4			109.4	
Schwartz criterior		130.8			90.9			104.2	

* data was obtained through linear regression analysis of peak height ratios vs. concentration of added CI-921 employing 1/x as the weighting factor

APPENDIX 2.1 (cont.) Assay validation - backcalculated concentrations of plasma standards

	Standards in plasma (nmol/l)							
	50	100	200	400	600	800	1000	2000
No. determinations	8	8	9	9	9	8	8	7
Mean	50.0	98.3	193.8	405.9	591.3	801.1	1018	2131
(SD)	3.24	3.1	5.8	6.1	12.4	14.5	13.2	62.0
Coefficient of variation (%)	6.5	3.2	3.0	1.5	2.1	1.8	1.3	2.9
Difference (%) from theoretical	0	-1.7	-3.1	+1.5	-1.4	+0.9	+1.8	+6.6

APPENDIX 2.2 Assay validation - backcalculated concentrations of seeded quality controls (SC) in plasma

	Quality controls (nmol/l)		
	SC 1000	SC 500	SC 100
Day 1	1057	459	96.7
	1033	459	94.2
	1030	518	94.3
Day 2	967	467	96.9
	948	464	93.0
	958	460	95.4
Day 3	952	439	90.6
	980	458	90.8
	943	-	89.4
No. determinations	9	8	9
Mean	985	465.5	93.5
Standard deviation	43	22.8	2.6
Coefficient of variation(%)	4.4	4.9	2.8
% difference from theoretical	-1.5	-6.9	-6.5

APPENDIX 2.3 Assay validation - backcalculated concentrations of urine standards*

Concentration (µmol/l)	Day 1			Day 2			Day 3		
0.1	0.107	0.095	0.993	0.101	0.103	-	0.105	0.10	0.098
0.5	0.457	0.472	0.475	0.469	0.491	0.471	0.551	0.544	0.470
1.0	0.977	0.994	0.961	0.989	1.025	1.066	1.053	1.024	0.990
5.0	4.742	5.025	4.706	4.627	5.300	4.669	4.380	4.357	4.569
10	10.6	9.73	9.59	9.747	10.92	-	10.19	10.13	10.29
20	20.37	20.05	19.81	20.28	18.48	-	19.03	20.54	20.62
Slope		0.388			0.415			0.381	
Log likeli-hood		-14.44			43.98			-43.78	
Schwartz criteria		-17.44			41.09			-46.82	

* data was obtained through linear regression analysis of peak height ratios vs concentration of CI-921, employing 1/x as the weighting factor and a zero intercept.

APPENDIX 2.3 (cont) **Assay validation - backcalculated concentrations of urine standards**

	Concentration of standards (μmol/l)					
	0.1	0.5	1.0	5.0	10.0	20.0
No. determinations	8	9	9	9	8	8
Mean	0.101	0.489	1.009	4.708	10.15	19.9
SD	0.006	0.034	0.035	0.298	0.46	0.077
%CV	5.5	7.0	3.5	6.3	4.5	3.8
% difference from theoretical	+1.0	-2.2	1.0	-5.8	+1.5	-0.5

APPENDIX 2.4 Assay validation - backcalculated concentrations of seeded quality controls (SCU) in urine.

	Quality controls (μmols/l)			
	SCU 0.1	SCU 1.0	SCU 5.0	SCU 15
Day 1	0.098	0.879	4.84	13.66
	0.114	0.890	4.46	14.42
	-	0.902	4.68	14.74
Day 2	0.113	0.825	4.115	12.95
	0.081	0.848	4.243	14.93
	-	0.910	4.764	14.22
Day 3	0.097	1.014	4.86	14.15
	0.092	0.900	4.913	14.15
	0.100	0.910	4.856	14.44
No. determinations	7	9	9	9
Mean	0.099	0.898	4.633	14.18
SD	0.012	0.05	0.293	0.59
% CV	11.6	5.8	6.3	4.2
% difference from theoretical	-1.0	-10	-7.3	-5.5

APPENDIX 3.1 Mean CI-921 pharmacokinetic parameters calculated from the first and third infusions after different doses in all patients in the phase I trial.

Patient	Dose (mg/m2)	Cmax (µmol/l)	AUC (µmol.h/l)	CL (ml/h/kg)	Vss (ml/kg)	MRT (h)	$t_{1/2}$å (h)	$t_{1/2}$ß (h)	UE § (%)
001	13	4.93	3.85	171	248	1.4	0.3	1.5	0.43
"	36	9.88	8.78	201	299	1.5	0.4	1.7	0.17
"	48	18.56	14.91	157	320	2.0	0.4	3.2	0.29
"	64	26.90	27.63	116	295	2.6	0.4	3.1	0.46
002	13	3.40	2.54	279	324	1.2	0.3	1.3	-
003	36	10.26	13.94	166	309	1.9	0.5	1.7	-
004	36	9.46	12.79	189	520	2.8	0.5	3.1	-
005	48	13.78	14.30	174	348	2.0	0.4	2.1	0.53
"	64	18.35	21.14	154	308	2.0	0.4	2.0	0.45
"	96	29.44	31.88	152	311	2.0	0.4	2.0	0.49
"	144	50.00	51.26	141	289	2.1	0.4	2.4	0.57
"	180	49.00	56.50	150	365	2.4	0.5	3.1	0.66
"	216	60.67	65.88	165	317	1.9	0.55	2.9	0.53
006	48	18.17	23.94	120	367	3.1	0.5	3.5	-
007	48	17.93	17.15	161	302	1.9	0.4	2.2	-
008	64	22.00	19.19	180	320	1.8	0.4	2.1	0.39
"	96	33.77	29.32	179	340	1.9	0.4	2.1	0.46
"	144	65.58	52.68	155	302	1.9	0.3	2.6	0.67
009	96	39.57	44.35	126	245	1.9	0.5	2.5	0.34
"	129*	53.97	54.10	140	219	1.6	0.5	1.9	} 0.48
"	144*	56.24	57.08	149	246	1.7	0.4	1.9	

010	144	41.26	49.15	180	420	2.3	0.5	2.9	0.58
"	216	44.95	78.88	169	483	2.9	1.0	4.3	0.70
011	216*	55.43	55.14	220	328	1.5	0.5	1.8	0.54
012	216	61.59	61.70	214	404	1.9	0.5	2.6	0.64
013	270	81.33	95.02	116	257	2.2	0.5	2.5	0.80
"	216	68.72	86.53	104	253	2.4	0.5	2.7	0.92
"	216	66.80	92.84	97	236	2.5	0.6	3.4	0.75
014	270	83.07	81.27	136	307	2.0	0.5	3.8	0.18
"	216	59.12	59.61	149	280	1.9	0.4	2.2	0.32
"	216	65.62	59.21	151	255	1.7	0.4	2.1	0.47
015	216	73.78	84.15	160	315	2.0	0.6	3.1	0.54
"	216	84.66	93.08	150	307	2.1	0.6	3.8	0.72
016	216	66.37	74.23	135	318	2.6	0.6	4.1	0.53
Mean				158	319	2.1	0.5	2.6	0.52
n				34	34	34	34	34	28
SD				36	68	0.4	0.1	0.9	0.18
%CV				23	21	21	29	33	33

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* single dose

§ percent of drug excreted as unchanged drug in the urine (mean of 3 days)

APPENDIX 3.2 CI-921 pharmacokinetic parameters calculated from the first infusion (216 mg/m$^{2)}$ - Phase II trial

Patient	Age (yr)	Dose (μmol/kg)	Cmax (μmol/l)	AUC (μmol.h/l)	CL (ml/h/kg)	Vss (ml/kg)	MRT (h)	$t_{1/2}$å (h)	$t_{1/2}$ß (h)
017	73	10.24	70.5	81	125	344	2.52	0.56	4.39
018 #1	63	11.91	66.3	107.2	111	303	2.73	0.76	3.48
#2		12.1	65	113.1	107	308	2.88	0.83	3.76
019 #1	61	13.54	63.2	91.9	147	449	3.05	0.68	4.37
#2		13.15	56.3	93.1	141	426	3.02	0.77	4.01
020	59	11.08	106.5	88.5	125	234	1.87	0.38	2.64
022	59	12.92	100.3	87.4	148	24.5	1.66	0.47	3.08
023	52	11.3	76.8	112.8	100	322	3.21	0.56	3.89
024	67	11.6	85.4	115.4	101	259	2.58	0.65	3.73
025	63	12.48	81.4	105.5	118	258	2.18	0.59	2.7
027 #1	64	10.9	64.1	43	253	307	1.21	0.34	1.86
#2		10.9	69.8	58.9	185	499	2.69	0.35	3.82
028	56	10.82	79.5	75	144	255	1.77	0.46	2.54
029	69	10.59	83	92.6	114	289	2.53	0.53	3.88
030	66	12.92	92.9	121	107	268	2.51	0.50	2.78
031	69	12.95	78.5	78.3	165	329	1.99	0.41	2.36
033	62	12	63.6	76.6	157	336	2.14	0.48	2.36
035	44	10.58	102.7	111.1	95	205	2.16	0.33	2.09
Mean	62	11.78	78.1	91.8	135	313	2.37	0.54	3.2
SD		1.03	14.8	20.9	38.5	77.6	0.54	0.15	0.81
%CV		8.7	19.0	22.7	28.4	24.8	22.7	28.6	25.2

APPENDIX 3.3 CI-921 pharmacokinetic parameters calculated from those patients in the phase I trial treated at 216 mg/m^2

Patient	Age (yr)	Cmax (μmol/l)	AUC (μmol.h/l)	CL (ml/kg)	Vss (ml/kg)	MRT (h)	$t_{1/2}$å (h)	$t_{1/2}$ß (h)
005	47	60.7	65.9	165	317	1.9	0.6	2.9
010	70	45.0	78.9	169	483	2.9	1.0	4.3
011	21	55.4	55.1	220	328	1.5	0.5	1.8
012	65	61.6	61.7	214	404	1.9	0.5	2.6
013 #1	57	68.7	86.5	104	253	2.4	0.5	2.7
#2		66.8	92.8	97	236	2.5	0.6	3.4
014 #1	54	59.1	59.6	149	280	1.9	0.4	2.2
#2		65.6	59.2	151	255	1.7	0.4	2.1
015 #1	57	73.8	84.2	160	315	2.0	0.6	3.1
#2		84.7	93.1	150	307	2.1	0.6	3.8
016	57	66.4	74.2	135	318	2.6	0.6	4.1
Mean	54	64.3	73.8	156	318	2.13	0.57	3.0
SD		10.2	14.1	38.0	71.6	0.42	0.16	0.83
%CV		15.8	19.1	24.4	22.5	19.8	28.3	27.6

APPENDIX 4.1.1 Mouse hepatic GSH concentration following amsacrine. Assay 1. (raw data)

Time post injection (h)	Mouse no.	GSH± SD (μg/gm liver)	Mean	% control	Mean (%)
0 (control)	1	2822 ± 224		98	
	2	2966 ± 216	2894	102	100
1	3	2545 ± 113		88	
	4	2207 ± 289	2376	76	82
2	5	2410 ± 116		83	
	6	2238		77	
	7	2207	2285	76	79
4	8	1976 ± 382	1976	68	68
6	9	2155 ± 225		74	
	10	1395 ± 134	1775	48	61

APPENDIX 4.1.2 Mouse hepatic GSH concentration following amsacrine. Assay 2. (raw data)

Time post injection (h)	Mouse no.	GSH ± SD (µg/gm liver)	Mean	% control	Mean (%)
0 (control)	1	4558		100	
1	2	3734 ± 117		82	
	3	3716 ± 174	3725	82	82
4	4	2254 ± 270		56	
	5	2843 ± 250	2699	63	59
6	6	3169 ± 200		70	
	7	2870 ± 158		63	
	8	3802 ± 197	3020	84	72
15	9	3802 ± 197		84	
	10	3697 ± 330		81	
	11	3663 ± 130	3785	85	83
24	12	4656 ± 205		102	
	13	4558 ± 178		100	
	14	4002 ± 91	4405	88	97

APPENDIX 4.2.1 Mouse hepatic concentration following CI-921. Assay 3. (raw data)

Time post injection (h)	Mouse no.	GSH ± SD (μg/gm liver)	Mean	% control	Mean (%)
0 (control)	1	3886 ± 213		98	
	2	4020 ± 502	3953	102	100
1	3	3863 ± 169		98	
	4	4112 ± 212	3992	104	101
2	5	3273 ± 291		83	
	6	3467 ± 520	3370	88	86
4	7	3393 ± 395		86	
	8	3140 ± 151	3267	79	83
6	9	4790 ± 508		121	
	10	5145 ± 465		130	
	11	5191 ± 299	5042	131	128

APPENDIX 4.2.2 Mouse hepatic GSH concentration following CI-921 Assay 4. (raw data)

Time post injection (h)	Mouse no.	GSH ± SD (µg/gm liver)	Mean	% control	Mean (%)
0 (control)	1	2632 ± 260		93	
	2	3024 ± 373	2828	107	100
3	3	2471 ± 482	2471	87	87
15	4	3070 ± 283		109	
	5	3661 ± 179		129	
	6	2794 ± 479	3175	99	112
24	7	3730 ± 355		132	
	8	3412 ± 244		121	
	9	3176 ± 133	3439	112	122

APPENDIX 4.3.1 Mouse hepatic GSH concentration following BSO. Assay 5. (raw data)

Time post injection(h)	Mouse no.	GSH ± SD (μg/gm liver)	Mean	% control	Mean (%)
0	1	2080 ± 163		98	
	2	2014 ± 202		95	
	3	2194 ± 309		103	
	4	2209 ± 114	2124	104	100
1	5	1428 ± 35		67	
	6	1292 ± 68	1360	61	64
2	7	645 ± 57		30	
	8	629 ± 81	637	30	30
4	9	393 ± 31		19	
	10	350 ± 35		16	
	11	280 ± 14	341	13	16
6	12	251 ± 22		12	
	13	355 ± 21		17	
	14	311 ± 34	306	15	14

APPENDIX 4.3.2 Mouse hepatic GSH concentration following BSO. Assay 6. (raw data)

Time post injection(h)	Mouse no.	GSH ± SD (μg/gm liver)	Mean	% control	Mean (%)
0 (control)	1	2594		98	
	2	2680	2637	102	100
5	3	2785 ± 323		106	
	4	3774 ± 229	3280	143	124
20 (control)	5	2938 ± 177		111	
	6	3110 ± 351	3024	118	115
10	7	1574 ± 120		60	
	8	2059 ± 117	1817	78	69
15	9	2245 ± 150		85	
	10	2176 ± 162	2211	83	84
20	11	2748 ± 265		104	
	12	2311 ± 49	2530	88	96

APPENDIX 4.4 Mouse hepatic GSH concentration following morphine. Assay 7. (raw data)

Time post injection(h)	Mouse no.	GSH ± SD (µg/gm liver)	Mean	% control	Mean (%)
0 (control)	1	3239 ± 130		108	
	2	2603 ± 21		86	
	3	3193 ± 115	3012	106	100
2	4	2410 ± 313		80	
	5	2324 ± 14		77	
	6	2483 ± 232	2406	82	80
4	7	1737 ± 136		58	
	8	820 ± 90		27	
	9	940 ± 83	1166	31	39
6	10	1976 ± 98		66	
	11	1896 ± 74		63	
	12	2167 ± 104	2013	72	67
8	13	1546		51	
	14	2004 ± 254	1777	67	59
24	15	2821 ± 294	2821	94	94

REFERENCES

REFERENCES

Adams DJ, Carmichael J, Wolf CR

Altered mouse bone marrow glutathione and glutathione transferase levels in response to cytotoxins. Cancer Res , 1985; 45:1669-1673

Aisner J

Identification of new drugs in small cell lung cancer: Phase II agents first? Cancer Treat Reports, 1987; 71:1131-1133

Aisner J, Hansen HH

Commentary: Current status of chemotherapy for non-small cell lung cancer. Cancer Treat Rep, 1981; 65:979-985

Akerboom TPM, Sies H

Assays of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. Methods Enzymol, 1981; 77:373-388

Al-Turk W, Stohs SJ, El-Rashidy FH, Othman S

Changes in glutathione and its metabolizing enzymes in human erythrocytes and lymphocytes with age. J Pharm Pharmacol, 1987; 39:13-16

Appelbaum RR, Shulman HM

Fatal hepatoxicity associated with AMSA therapy. Cancer Treat Rep, 1982; 66:1863-1865

Arlin ZA

Current status of amsacrine (AMSA) combination chemotherapy programs in acute leukaemia. Cancer Treat Rep, 1983; 67:967-970

Arlin ZA, Sklaroff RB, Gee TS, et al

Phase I and II trial of 4'-(9-acridinylamino)methanesulfon-m- anisidide in patients with acute leukaemia. Cancer Res, 1980; 40:3304-3306

Arrick BA, Nathan CF

Glutathione metabolism as a determinent of therapeutic efficacy: a review. Cancer Res, 1984; 44:4224-4232

Atwell GJ, Cain BF, Seelye RN

Potential antitumor agents 12. 9-anilinoacridines. J Med Chem, 1972; 15:611-615

Atwell GJ, Rewcastle GW, Denny WA, Cain BF, Baguley BC

Potential antitumor agents 41. Analogues of amsacrine with electron-donor substituents in the anilino ring. J Med Chem, 1984; 27:367-372

Baguley BC

Preclinical profile of amsacrine. In: Amsacrine: Current perspectives and clinical results with a new anticancer agent. Communications Media for education. 1983 :3-13

Baguley BC

Amsacrine; A new antileukamic agent. Drugs of Today, 1984; 20:237-245

Baguley BC

Resistance to DNA-binding antitumour drugs: implications for drug design. Drugs of the Future, 1987; 12:375-383

Baguley BC, Atwell GJ, Denny WA

CI-921. Drugs of the Future, 1984; 9:575-576

Baguley BC, Cain BF

Comparison of the in vivo and in vitro antileukaemic activity of monosubstituted derivatives of 4'-(9- acridinylamino)methanesulfon-m-anisidide. Mol Pharmacol, 1982; 22:486-492

Baguley BC, Calveley SB, Harvey VJ

Effects of CI-921, an analogue of amsacrine, on advanced Lewis lung tumours in mice: relevance to clinical trials. Eur J Cancer Clin Oncol, 1988; 24: 211-218

Baguley BC, Denny WA, Atwell GJ, et al

Synthesis, antitumor activity, and DNA binding properties of a new derivative of amsacrine, N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridine carboxamide. Cancer Res, 1984; 44:3245-3251

Baguley B, Denny WA, Atwell GJ, Cain BF

Potential antitumour agents 34. Quantitative relationship between DNA binding and molecular structure for 9-anilino acridines substituted in the anilino ring. J Med Chem, 1981(a); 24:170-177

Baguley BC, Denny WA, Atwell GJ, Cain BF

Potential antitumor agents 35. Quantitative relationships between antitumour (L1210) potency and DNA binding for 4'- (9-acridinylamino)methanesulfon-m-anisidide analogues. J Med Chem, 1981(b); 24:520-525

Baguley BC, Grimwade CD, Kernohan AR

Schedule dependence of activity of the amsacrine analogue CI-921 towards P388 leukaemia and Lewis lung carcinoma. Eur J Cancer Clin Oncol, 1985; 20:1337-1341

Baguley BC, Kernohan AR, Wilson WR

Divergent activity of derivatives of amsacrine (m-AMSA) towards Lewis lung carcinoma and P388 leukaemia in mice. Eur J Cancer Clin Oncol, 1983; 19:1607-1613

Baguley BC, Nash R.

Antitumour activity of substituted 9-anilinoacridines - comparison of in vivo and in vitro testing systems. Eur J Cancer, 1981; 17:671-679

Bakowski MT, Crouch JC

Chemotherapy of non-small cell lung cancer: a reappraisal and look to the future. Cancer Treat Rev, 1983; 10:159-172

Beutler E, Gelbart T

Plasma glutathione in health and in patients with malignant disease.J Lab Clin Med, 1985; 105:581-584

Bidlack WR, Brown RC, Mohan C

Nutritional parameters that alter hepatic drug metabolism,conjugation and toxicity. Fed Proc, 1986; 45:142-148

Bird IM

High performance liquid chromatography : principles and clinical applications. Br Med J, 1989; 299: 783-787

Bloom KR, Bini RM, Williams CM, Sonly MJ, Gribbin MA

Echocardiography in adriamycin cardiotoxicity. Cancer, 1978; 41:1265-1269

Bodey GP, Legha SS, Valdivieso M

Phase I clinical trials: results on amsacrine. In: Amsacrine: current perspectives and clinical results with a new anti-cancer agent. Communications Media for Education: Princeton Junction, 1983: 25-32

Bonomi P

Brief over-view of combination chemotherapy in non-small-cell lung cancer. Semin Oncol, 1986; 13:89-91

Brown PR

Chromatography. In HPLC in nucleic acid research. Brown PR ed. Chromatographic science series. Vol 28. Marcel Dekker Inc, 1984; 49-79

Burchenal JH, Pancoast T, Elslager E.

Anthrapyrrazole and amsacrine analogs in mouse and human leukemia in vitro and in vivo (Abstract). Proc Am Assoc Cancer Res, 1985; 26:224

Bump EA, Taylor YC, Brown JM

Role of glutathione in the hypoxic cell cytotoxicity of misonidazole. Cancer Res, 1983; 43:997-1002

Bump EA, Yu NY, Brown JM

The use of drugs which deplete intracellular glutathione in hypoxic cell radiosensitization. Int J Radiat Oncol Bio Phys, 1982; 8:439-442

Cabanillas F

Amsacrine in the treatment of recurrent lymphoma.In: Amsacrine: current perspectives and clinical results with a new anti-cancer agent. Communications Media for Education: Princeton Junction, 1983: 55-62

Cabanillas F, Legha SS, Bodey GP, Freireich EJ

Initial experience with AMSA as single agent treatment against malignant lymphoproliferative disorders. Blood, 1981; 57:614-616

Cain BF, Atwell GJ

The experimental antitumour properties of three cogeners of the acridylmethane sulphonanilide (AMSA) series. Eur J Cancer, 1974; 10:539-549

Cain BF, Atwell GJ

Potential antitumor agents 20. Structure-activity site relationships for the 4'-(9-acridinylamino) alkane sulfonanilides. J Med Chem, 1976; 19:1409-1416

Cain BF, Atwell GJ, Denny WA

Potential antitumor agents 16. 4'-(Acridin-9-ylamino) methane sulfonanilides. J Med Chem, 1975; 18:1110-1117

Cain BF, Seelye RN, Atwell GJ

Potential antitumor agents. 14. Acridylmethane sulfonanilides. J Med Chem, 1974; 17:966-930

Calvert AH, Balmanno K

Anti-cancer drugs phase I trials. Cancer Topics, 1987; 6:51-52

Carmichael J, Friedman N, Tochner Z, et al

Inhibition of the protective effect of cyclophosphamide by pre-treatment with buthionine sulphoximine.Int J Radiat Oncol Biol Phys, 1986; 12:1191-1193

Carmichael J, Adams DJ, Ansell J, Wolf CR

Glutathione and glutathione transferase levels in mouse granulocytes following cyclophosphamide administration. Cancer Res, 1986; 46:735-739

Carter SK, Goldin A

Experimental models and their clinical correlations. NCI monograph: USA - USSR, 1977 : 45; 63-74

Carter SK, Selawry O, Slavik M

Phase I clinical trials. NCI monograph, 1977; 45:75-80

Carter SK, Selawry O

Phase II clinical trials. NCI monograph, 1977; 45:81-92

Collins JM, Grieshaber CK

Anti-cancer drugs: the role of toxicology in drug development. Cancer Topics, 1987; 6:38-39

Collins JM, Zaharko DS, Dedrick RL, Chabner BA

Potential roles for preclinical pharmacology in phase I clinical trials. Cancer Treat Rep, 1986; 70:73-80

Cooper RH, O'Rourke RA, Karliner JS, Peterson KL, Leopold GR
Comparison of ultrasound and cineangiographic measurements of circumferential fiber shortening in man. Circulation, 1972; 46:914-923

Covey JM, Pommier YG, Kohn KW
DNA damage produced by amsacrine and related acridines in L1210 cells and isolated nuclei (Abstract). Clin. Res, 1986; 34:561A

Cozzarelli NR
DNA gyrase and the supercoiling of DNA. Science, 1981; 207:935-960

Crivellari D, Tirelli U, Frustaci S, et al
A phase II study of AMSA in head and neck cancer.Am J Clin Oncol, 1987; 10:23-25

Cysyk RL, Shoemaker D, Adamson RH
The pharmacologic disposition of 4'-(9-acridinylamino) methanesulfon-m-anisidide in mice and rats. Drug Metab Dispos, 1977; 5:579-591

D'Alessandro N, Gebbia N, Crescimanno M, et al
Effects of amsacrine (m-AMSA) a new aminoacridine antitumor drug on the rabbit heart.Cancer Treat Rep, 1983; 67:467-474

Denny WA

Beyond amsacrine : a New Zealand contribution to the development of better anticancer drugs. Chemistry in New Zealand, 1987; 51:89-93

Denny WA, Atwell GJ, Baguley BC

Potential antitumor agents. 38. 3-substituted 5-carboxamido derivatives of amsacrine.J Med Chem, 1983(a); 26:1619-1625

Denny WA, Atwell GJ, Baguley BC

Potential antitumor agents. 39. Anilino ring geometry of amsacrine and derivatives: relationship to DNA binding and antitumor activity. J Med Chem, 1983(b); 26:1625-1630

Denny WA, Atwell GJ, Baguley BC

Potential antitumor agents. 40. Orally active 4, 5- disubstituted derivates of amsacrine.J Med Chem, 1984; 27:363-367

Denny WA, Atwell GJ, Baguley BC, Rewcastle GW

QSAR in the design of potential antitumor agents : the example of the 9-anilinoacridines.In: QSAR in the design of bioactive compounds. ed: Kuchar M, JR Prous, Barcelona, Spain 1984:97-116

Denny WA, Atwell GJ, Cain BF

Potential antitumor agents 32. Role of agent base strength in the quantitative structure-antitumor relationships for 4'-(- 9-acridinylamino)methane sulfonanilide analogues. J Med Chem, 1979; 22:1453-1460

Denny WA, Cain BF, Atwell GJ, Hansch C, Panthananickal A, Leo A

Potential antitumor agents. 36. Quantitative relationships between experimental antitumor activity, toxicity and structure for the general class of 9-anilinoacridine antitumor agents. J Med Chem, 1982; 25:276-315

Denny WA, Twigden SJ, Baguley BC

Steric constraints of DNA binding and biological activity in the amsacrine series. Anti-cancer drug design, 1986; 1: 125-132

Denny WA, Wakelin LPG

Kinetic and equilibrium studies of the interaction of amsacrine and anilino ring-substituted analogues with DNA. Cancer Res, 1986; 46:1717-1721

De Vita VT

Principles of chemotherapy. In: Cancer, Principles and Practice of Oncology. De Vita VT, Hellman S, Rosenberg SA eds. 2nd edition. JB Lippincott Co. Philadelphia, 1985: 278-282

De Vita VT

Cancer Drug development. In: Cancer, Principles and Practice of Oncology. De Vita VT, Hellman S, Rosenberg SA eds. 3rd edition. JB Lippincott Co. Philadelphia, 1989: 290-296

Dion S

The ethical approach to phase II trials in oncology. Drugs Exptl Clin Res, 1986;12: 41-42

Dodion P

Clinical pharmacology in oncology. Recent advances. Eur J Cancer Clin Oncol, 1983; 19:1499-1507

Dodion P, Kenis Y, Staquet M

Phase I trials of single agents in adult solid tumours: preclinical and clinical aspects. Drugs Exptl Clin Res, 1986;12:23-30

Double JA, Bibby MC

Therapeutic index : a vital component in selection of anticancer agents for clinical trial. J Natl Cancer Inst, 1989; 81:988-994

Drew R, Miners JO

The effects of buthionine sulphoximine (BSO) on glutathione depletion and

Drewinko B, Yang LY, Barlogie B

Lethal activity and kinetic response of cultured human cells to 4'-(9-acridinylamino)methanesulfon-m-anisidide. Cancer Res, 1982; 42:107-111

Durry G, Dion S

The ethical approach to phase I clinical trials in oncology.

Drugs Exptl Clin Res, 1986;12:21-22

Earnshaw WC, Halligan B, Cooke CA, Heck MMS, Liu LF

Topoisomerase II is a structural component of mitotic chromosome scaffolds. J Cell Biol, 1985; 100:1706-1715

Earnshaw WC, Heck MMS

Localization of topoisomerase II in mitotic chromosomes. J Cell Biol, 1985; 100:1716-1725

Eastman A

Glutathione - mediated activation of anti-cancer platinum (IV) complexes.

Biochem Pharmacol, 1987;36:4177-4178

Elliot JA

Is there standard chemotherapy for non-small cell lung cancer? Eur J Cancer Clin Oncol, 1986; 22:369-371

EORTC new drug development committee

EORTC guidelines for phase I trials with single agents in adults.Eur J Cancer Clin Oncol 1985;21:1005-1007

EORTC pharmacokinetics and metabolism group

Pharmacokinetically guided dose escalation in phase I clinical trials. Commentary and proposed guidelines. Eur J Cancer Clin Oncol, 1987; 23:1083-1087

Erlichman C, Donehower RC, Chabner BA

The practical benefits of pharmacokinetics in the use of antineoplastic agents. Cancer Chemother Pharmacol, 1980; 4:139-145

Estey E, Hoth D, Simon R, Marsoni S, Leyland-Jones B, Wittes R

Therapeutic response in phase I trials of antineoplastic agents. Cancer Treat Rep, 1986; 70:1105-1115

Falkson G

Multiple ventricular extrasystoles following administration of 4'-(9-acridinylamino)-methane sulfon-m-anisidide (AMSA). Cancer Treat Rep, 1979; 64:358

Fallon A, Booth RFG, Bell LD

Applications of HPLC in biochemistry. Laboratory techniques in

biochemistry and molecular biology. Vol 17. Burdon RH, van Knippenberg PH eds. Elsevier Amsterdam 1987.

Feigenbaum H

Echocardiographic evaluation of cardiac chambers In: Echocardiography. 4th edition. Lea and Febiger, Philadelphia 1986 :127-133

Ferguson LR, Baguley BC

Relationship between the induction of chromosome damage and cytotoxicity for amsacrine and cogeners. Cancer Treat Rep, 1984; 68:625-630

Finlay GJ, Baguley BC

The use of human cancer cell lines as a primary screening system for antineoplastic compounds. Eur J Cancer Clin Oncol, 1981; 20: 947-951

Foldes JA, Yagil Y, Kornberg A

Ventricular fibrillation, hypokalemia, and amsacrine therapy. Ann Intern Med, 1982; 96:121-122

Glisson B, Gupta R, Smellwood-Kentro S, Ross W

Characterization of acquired epipodophyllotoxin resistance in a chinese hamster ovary cell line: loss of drug-stimulated DNA cleavage activity. Cancer Res, 1986(a); 46:1934-1938

Glisson B, Gupta R, Hodges P, Ross W

Cross-resistance to intercalating agents in an epidophyllotoxin-resistant chinese hamster ovary cell line: evidence for a common intracellular target. Cancer Res, 1986(b); 46:1939-1942

Goldin A, Venditti JM, MacDonald JS, Muggia FM, Henney JE, DeVita VT

Current results of the screening program at the division of cancer treatment, National Cancer Institute. Eur J Cancer, 1981; 17:129-142

Goldsmith MA, Slavik M, Carter SK

Quantitative prediction of drug toxicity in humans from toxicology in small and large animals. Cancer Res, 1975;35:1354-1364

Gralla RJ, Casper ES, Kelsen DP

Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung : a randomized trial investigating two dosage schedules. Ann Intern Med, 1981;95:414-420

Graham MA, Foster BJ, Newell DR, Gumbrell LA, Calvert AH

Phase I study of the anthrapyrazole CI-921 with pharmacokinetically guided dose escalation (Abstract). Br J Cancer,1988;58:258

Greco FA

Rationale for chemotherapy for patients with advanced non-small-cell lung cancer.Semin Oncol, 1986; 13:92-96

Green JA, Vistica DT, Young RC, Hamilton TC, Rogan AM, Ozols RF
Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion. Cancer Res, 1984; 44:5427-5431

Grieshaber CK, Marsoni S
Relation of preclinical toxicology to findings in early clinical trials.
Cancer Treat Rep, 1986; 70:65-72

Grillo-Lopez AJ, Hess J
Cardiotoxicity associated with amsacrine (Abstract). Proc Am Assoc Soc Clin Oncol, 1983; 24:183

Grove WR, Grillo-Lopex AJ, Robert F, et al
Optimizing therapeutic potential of phase I studies - co-ordination of three worldwide studies of CI-921 (Abstract). Proc Europ Conf Clin Oncol, 1987; 4:287

Hall SW, Friedman J, Legha SS, Benjamin RS, Gutterman JU, Loo TL
Human pharmacokinetics of a new acridine derivative, 4'-(9-acridinylamino)methanesulfon-m-anisidide (NSC 249992) Cancer Res, 1983; 43:3422-3426

Hansen M, Hansen HH, Dombernowsky P
Long-term survival in small cell carcinoma of the lung.
J Am Med Assoc, 1980; 244:247-250

Hamilton TC, Winker MA, Louie KG, et al

Augmentation of Adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. Biochem Pharmacol, 1985; 34:2583-2586

Harrison AC

The management of non-small cell lung cancer.NZ Med J, 1985; 98:142-144

Harvey VJ, Kolbe J

The management of small cell lung cancer. NZ Med J, 1986; 99:104-106

Hazelton GA, Lang CA

Glutathione contents of tissues in the aging mouse. Biochem J, 1980; 188:25-30

Henry MC, Port CD, Levine BS

Preclinical toxicologic evaluation of 4'-(9-acridinylamino)methanesulfon-m-anisidide (AMSA) in mice, dogs and monkeys. Cancer Treat Rep, 1980; 64:855-860

Hoffman PC, Bitran JD, Golomb HM

Chemotherapy of metastatic non-small cell bronchogenic carcinoma. Semin Oncol, 1983; 10:111-122

Hofmann GA, Bartus JO, Mong S-M, et al

Characterization of the mechanism of resistance of a subline of P388 leukaemia resistant to amsacrine.Proc Am Assoc Cancer Res, 1986; 27:269

Holford NGH

MKMODEL, a modelling tool for microcomputers - a pharmacokinetic evaluation and comparison with standard computer programs.Clin Exp Pharmacol Physiol, 1986; 9:95

Investigator's Brochure, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan USA, 1985

Issell BF

Amsacrine (AMSA). Cancer Treat Rev, 1980; 7:73-83

Jacoby WB

Glutathione transferases: an overview.Methods Enzymol, 1985; 113:495-499

James RC, Goodman DR, Harbison RD

Hepatic glutathione and hepatotoxicity: changes induced by selected narcotics. J Pharmacol Exp Ther, 1982; 221:708-714

Johnson RK, Howard WS

Development and cross-resistance characteristics of a subline of P388 leukaemia resistant to 4'-(9-acridinylamino)- methanesulfon-m-anisidide. Eur J Cancer Clin Oncol, 1982; 18:479-487

Jurlina JL, Varcoe AR, Paxton JW

Pharmacokinetics of amsacrine in patients receiving combined chemotherapy for treatment of acute myelogenous leukaemia. Cancer Chemother Pharmacol, 1985; 14:21-25

Jurlina JL, Paxton JW

Determination of N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide in plasma by high-performance liquid chromatography. J Chromatogr, 1985; 342:431-435

Kaplowitz N, Aw TY, Ookhtens M

The regulation of hepatic glutathione. Annu Rev Pharmacol Toxicol, 1985; 25:715-744

Keating MJ, Gehan EA, Smith TL, et al

A strategy for evaluation of new treatments in untreated patients: application to a clinical trial of AMSA for acute leukaemia.J Clin Oncol, 1987; 5:710-721

Kimball RE, Reddy K, Peirce TH, Schwartz LW, Mustafa MG, Cross CE
Oxygen toxicity: augmentation of antioxidant defence mechanisms in rat lung.Am J Physiol, 1976; 230:1425-1431

Klastersky J
Therapy with cisplatin and etoposide for non-small-cell lung cancer. Semin Oncol, 1986; 13:104-114

Krook JE, Jett JR, Little C - North Central Cancer Treatment Group.
A phase I - II study of sequential infusion of VP-16 and cisplatin therapy in advanced lung cancer. Am J Clin Oncol, 1989; 12:114-117

Ladish H, Darnell J, Baltimore D
Superhelicity in DNA; topoisomerases.In: Molecular cell biology.
Scientific American Books New York, W.H. Freeman & Co, N.Y. 1986: 517-568

Lee YJ, Catane R, Rozencweig M, et al
Analysis and interpretation of response rates for anticancer drugs.
Cancer Treat Rep, 1979; 63:1713-1720

Legha SS
Cardiac toxicity of amsacrine - results and perspective. In: Amsacrine.
Current perspectives and clinical results with a new anticancer agent.
Communications Media for Education, Princeton Junction, NJ 1983: 33-39

Legha SS, Blumenschein GR, Buzdar AU, Hortobagyi GN, Bodey GP
Phase II study of 4'-(9-acridinylamino)methanesulfon-m- anisidide (AMSA) in metastatic breast cancer. Cancer Treat Rep, 1979; 63:1961-1964

Legha SS, Gutterman JU, Hall SW, et al
Phase I clinical investigation of 4'-(9-acridinylamino) methanesulfon-m-anisidide (NSC 249992), a new acridine derivative. Cancer Res, 1978; 38:3712-3716

Legha SS, Keating MJ, Zander AR, McCredie KM, Bodey GP, Freireich EJ
4'-(9-acridinylamino)methanesulfon-m-anisidide (AMSA); a new drug effective in the treatment of adult acute leukaemia. Ann Intern Med, 1980; 93:17-21

Legha SS, Latreille J, McCredie KB, Bodey GP
Neurologic and cardiac rhythm abnormalities associated with 4'-(9-acridinylamino)methanesulfon-m-anisidide (AMSA) therapy. Cancer Treat Rep, 1979; 63:2001-2003

Leopold WR, Corbett TH, Griswold DP, Plowman J, Baguley BC
Experimental antitumor activity of the amsacrine analogue CI-921. J Natl Cancer Inst, 1987; 79:343-349

Lerman LS

Structural considerations in the interaction of DNA and acridines. J Mol Biol, 1961; 3:18-30

Lewin B

The topology of nucleic acids. In: Genes. 2nd edition. John Wiley & sons.1983: 61-62

Lewin B

Recombination and other topological manipulations of DNA. In: Genes. 2nd edition. John Wiley & sons.1983: 574-577.

Lindpaintner K, Lindpaintner LS, Wentworth M, Burns CP

Acute myocardial necrosis during administration of amsacrine.

Cancer, 1986; 57:1284-1286

Liu LF, Rowe TC, Yang L, Tewey KM, Chen GL

Cleavage of DNA by mammalian DNA topoisomerase II. J Biol Chem, 1983; 258:15365-15370

Long BH, Musial ST, Brattain MG

Comparison of cytotoxicity and DNA breakage activity of congeners of podophyllotoxin including VP16-213 and VM26: a quantitative structure-activity relationship. Biochemistry, 1984; 23:1183-1188

Louie KG, Behrens BC, Kinsella TJ, et al
Radiation survival parameters of antineoplastic drug-sensitive and resistant human ovarian cancer cell lines and their modification by buthionine sulfoximine. Cancer Res, 1985; 45:2110-2115

Mahal PS, Legha SS, Valdivieso M, Luna M, Benjamin RS, Bodey GP
AMSA toxicity in patients with abnormal liver function.
Eur J Cancer Clin Oncol, 1981; 17:1343-1348

Markovits J, Pommier Y, Kerrigan D, Covey JM, Tilchen EJ, Kohn KW
Topoisomerase II-mediated DNA breaks and cytotoxicity in relation to cell cycle in NIH 3T3 fibroblasts and L1210 leukemia cells.
Cancer Res, 1987; 47: 2050-2055

Marsoni S, Hoth D, Simon R, Leyland-Jones B, DeRosa M, Wittes RE
Clinical drug development: an analysis of phase II trials, 1970-1985. Cancer Treat Rep, 1987; 71:71-80

Marsoni S, Wittes RE
Clinical development of anti-cancer agents - A National Cancer Institute perspective. Cancer Treat Rep, 1984; 68:77-85

McLaughlin P, Salvador PG, Cabanillas F, Legha SS
Ventricular fibrillation following AMSA. Uncomplicated retreatment

following correction of hypokalaemia. Cancer, 1983; 52:557-558

Meister A

Selective modification of glutathione metabolism. Science, 1983; 220: 471-477

Meister A, Anderson ME

Glutathione. Annu Rev Biochem, 1983; 52:711-760

Miller AB, Hoogstraten B, Staquet M, Winkler A

Reporting results of cancer treatment. Cancer, 1981; 47:207-214

Miller CF, Rajdev N

Acute ECG changes associated with AMSA treatment. Cancer Treat Rep, 1982; 66:1678-1680

Miners JO, Drew R, Birkett DJ

Mechanism of action of paracetamol protective agents in mice in vivo. Biochem Pharmacol, 1984; 33:2995-3000

Moldeus P

Role of glutathione in the metabolism and toxicity of drugs and carcinogens. In: Drug metabolism: molecular approaches and pharmacology implications. Siest G. ed. Pergamon Press, 1984; 69-73

Muggia FM

Closing the loop : providing feedback on drug development. Cancer Treat Rep, 1987; 71:1-2

Mulshine JL, Glatstein E, Ruckdeschel JC

Treatment of non-small cell lung cancer. J Clin Oncol, 1986; 4:1704-1715

Myers CE

Cardiac toxicology. In: Cancer, Principles and Practice of Oncology. DeVita VT, Hellman S, Rosenberg SA. eds. J.B. Lippincott Co., Philadelphia, 1982:1707-1712

Nagamatsu K, Kido Y, Terao T, Ishida T, Toki S

Protective effect of sulfhydryl compounds on acute toxicity of morphinone. Life Sci, 1982; 30:1121-1127

Nagamatsu K, Ohna Y, Ikebuchi H, Takahashi A, Terao T,Takanaka A

Morphine metabolism in isolated rat hepatocytes and its implications for hepatotoxicity. Biochem Pharmacol, 1986; 35:3543-3548

Natale RB, Yagoda A, Blumenreich MS, Watson RC

Phase II trial of amsacrine (AMSA) in urinary bladder cancer. Cancer Treat Rep, 1983; 67:391-392

Nelson EM, Tewey KM, Liu LF

Mechanism of antitumour drug action: poisoning of mammalian DNA topoisomerase II on DNA by 4'-(9- acridinylamino)methanesulfon-m-anisidide. Proc Natl Acad Sci, 1984; 81:1361-1365

O'Connell JP, Kris MG, Gralla RJ, et al

Frequency and prognostic importance of pre-treatment clinical characteristics of patients with advanced non-small cell lung cancer treated with combination chemotherapy. J Clin Oncol, 1986; 4:1604-1614

Olson RD, MacDonald JS, vanBoxtel CJ, et al

Regulatory role of glutathione and soluble sulphydryl groups in the toxicity of Adriamycin. J Pharmacol Exp Ther, 1980; 215:450-454

Orrenius S, Moldeus P

The multiple roles of glutathione in drug metabolism. TIPS Oct, 1984; 433-435

Ozols RF, Louie KG, Plowman J, et al

Enhanced melphalan cytotoxicity in human ovarian cancer in vitro and in tumour-bearing nude mice by buthionine sulphoximine depletion of glutathione. Biochem Pharmacol, 1987; 36:147-153

Park BK

Prediction of metabolic drug interactions involving ß- adrenoceptor blocking drugs.Br J Clin Pharmacol, 1984; 17:3S-10S

Paxton JW, Jurlina JL, Foote SE

The binding of amsacrine to human plasma proteins.J Pharm Pharmacol, 1986; 38:432-438

Paxton JW, Jurlina JL

Comparison of the pharmacokinetics and protein binding of the anticancer drug amsacrine and a new analogue N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4- acridinecarboxamide in rabbits. Cancer Chemother Pharmacol, 1986; 16:253-257

Paxton JW, Hardy JR, Evans PC, Harvey VJ, Baguley BC

The clinical pharmacokinetics of N-5-dimethyl-9-[(2-methoxy- 4-methyl-sulphonylamino)phenylamino]-4-acridinecarboxamide (CI-921) in a phase I trial. Cancer Chem Pharmacol, 1988; 22:235-240

Perucca E, Hedges A, Makki KA, Ruprah M, Wilson JF, Richens A

A comparative study of the relative enzyme inducing properties of anticonvulsant drugs in epileptic patients. Br J Clin Pharmacol, 1984; 18:401-410

Pommier Y, Covey JM, Kerrigan D, Markovits J, Pham R

DNA unwinding and inhibition of mouse leukaemia L1210 DNA topoisomerase I by intercalators. Nucleic Acids Res, 1987; 15:6713-6731

Pommier Y, Kerrigan D, Schwartz RE, Swack JA, McCurdy A
Altered DNA topoisomerase II activity in chinese hamster cells resistant to topoisomerase II inhibitors. Cancer Res, 1986; 46:3075-3081

Pommier Y, Minford JK, Schwartz RE, Zwelling LA, Kohn KW
Effects of the DNA intercalators 4'-(-9-acridinylamino)methanesulfon-m-anisidide and 2-methyl-9- hydroxyellipticinium on topoisomerase II mediated DNA strand cleavage and strand passage.Biochem, 1985; 24:6410-6416

Pommier Y, Zwelling LA, Kao-Shan C-S, Whang-Peng J, Bradley MO
Correlations between intercalator-induced DNA strand breaks and sister chromatid exchanges, mutations and cytotoxicity in chinese hamster cells. Cancer Res, 1985; 45:3143-3149

Powis G
Effect of human renal and hepatic disease on the pharmacokinetics of anticancer drugs. Cancer Treat Rep, 1982; 9:85-124

Powis G.
Anticancer drug pharmacodynamics. Cancer Chemother Pharmacol, 1985; 14:177-183

Prescott LF, Illingworth RN, Critchley JA, Stewart MJ, Adam RD, Proudfoot AT
Intravenous N-acetyl cysteine: treatment of choice for paracetamol poisoning. Brit Med J, 1979; 2:1097-1100

Pyke S, Lew H, Quintanilha A

Severe depletion in liver glutathione during physical exercise. Biochem Biophys Res Commun, 1986; 139:926-931

Quinones MA, Gaasch WH, Alexander JK

Influence of acute changes in preload, afterload, contractile state and heart rate on ejection and isovolumic indices of myocardial contractility in man. Circulation, 1976; 53:293-301

Quinones MA, Pickering E, Alexander JK

Percentage of shortening of the echocardiographic left ventricular dimension. Chest, 1979; 74:59-65

Riela AR, Kimball JC, Patterson RB, Land VJ

Cardiac arrhythmia associated with AMSA in a child: a Southwest Oncology Group Study.Cancer Treat Rep, 1981; 65:1121-1123

Robert F, Mignucci M, Grove W, Javier J, Asmar A, Grillo- Lopez A

Phase I study of CI-921: single dose schedule (Abstract). Proc Am Assoc Clin Oncol, 1987; 6:26

Robbie M

Studies of the mechanism of resistance of non-cycling cells to amsacrine and related drugs.(Ph.D. Thesis) University of Auckland, Auckland, N.Z.1988.

Ross WE

DNA topoisomerases as targets for cancer therapy. Biochem Pharm, 1985; 34:4191-4195

Ross WE, Glaubiger D, Kohn KW

Qualitative and quantitative aspects of intercalator-induced DNA strand breaks. Biochim Biophys Acta, 1979; 562:41-50

Ross W, Rowe T, Glisson B, Yalowich J, Liu L

Role of topoisomerase II in mediating epipodophyllotoxin-induced DNA cleavage. Cancer Res, 1984; 44:5857-5860

Rowe TC, Chen GL, Hsiang Y-H, Liu LF

DNA damage by antitumor acridines mediated by mammalian DNA topoisomerase II. Cancer Res, 1986; 46:2021-2026

Rowe T, Kupfer G, Ross W

Inhibition of epipodophyllotoxin cytotoxicity by interference with topoisomerase-mediated DNA cleavage.Biochem Pharmacol, 1985; 34:2483-2487

Russo A, Carmichael J, Friedman N, et al

The roles of intracellular glutathione in antineoplastic chemotherapy. Int J Radiat Oncol Biol Phys, 1986; 12:1347-1354

Russo A, Tochner Z, Phillips T et al

In vivo modulation of glutathione by buthionine sulfoximine: effect on marrow response to melphalan. Int J Radiat Oncol Biol Phys, 1986; 12:1187-1189

Rutty CJ, Newell DR, Muindi JRF, Harrap KR

The comparative pharmacokinetics of pentamethylmelamine in man, rat, and mouse. Cancer Chemother Pharmacol, 1982; 8:105-111

Sahyoun N, Wolf M, Besterman J, et al

Protein kinase C phosphorylates topoisomerase II. Topoisomerase II activation and its possible role in phorbol ester-induced differentiation of HL-60 cells. Proc Natl Acad Sci, 1986; 83:1603-1607

Salmon SE

Human tumour colony assay and chemosensitivity testing. Cancer Treat Rep,1984; 68:117-125

Salmon SE, Hamburger AW, Soehnlen B, et al.

Quantitation of differential sensitivity of human tumour stem cells to anticancer drugs. N Eng J Med, 1978; 298:1321-1327

Salmon SE, Meyskens FL, Alberts DS, Soehnlen B, Young L

New drugs in ovarian cancer and malignant melanoma : In vitro phase II screening with the human tumour stem cell assay. Cancer Treat Rep,1981;65:1-12

Samson MK, Fraile RJ, Baker LH, Cummings G, Tally RW

Phase II study of AMSA in lung cancer. Cancer Treat Rep, 1981; 65:655-658

Sculier JP, Klastersky J

Progress in chemotherapy of non-smell cell lung cancer. Eur J Cancer Clin Oncol, 1984; 20:1329-1333

Selby P, Buick RN, Tannock I

A critical appraisal of the "human tumour stem-cell assay". N Eng J Med, 1983; 308:129-134

Sharpe N, Murphy J, Smith H, Hannan S

Treatment of left ventricular dysfunction in asymptomatic patients. Lancet, 1988; 1:255-259

Shiner E, Hasin Y

Acute electrocardiographic changes induced by amsacrine. Cancer Treat Rep, 1984; 68:1169-1172

Shoemaker DD, Cysyk RL, Gormley PE, DeSouza JJV, Malspeis L

Metabolism of 4'-(9-acridinylamino)methanesulfon-m-anisidide by rat liver microsomes. Cancer Res, 1984; 44:1939-1945

Shoemaker DD, Cysyk RL, Padmanabhan S, Phat HB, Malspeis L
Identification of the principal biliary metabolite of 4'-(9-acridinylamino)methanesulfon-m-anisidide in rats. Drug Metab Dispos, 1982; 10:35-39

Shoemaker DD, Gormley PE, Cysyk RL
Biliary excretion of 4'-(9-acridinylamino)methanesulfon-m- anisidide (AMSA) in rats: effect of pre-treatment with diethyl maleate, phenobarbitol and metyrapone. Drug Metab Dispos, 1980; 8:467-468

Shrieve DC, Harris JW
Effects of glutathione depletion by buthionine sulfoximine on the sensitivity of EMT6/SF cells to chemotherapy agents or X radiation. Int J Radiat Oncol Biol Phys, 1986; 12:1171-1174

Silverberg E, Lubera J
Cancer Statistics. CA, 1987; 37:2-19

Simon R, Wittes RE, Ellenberg SS
Randomized phase II clinical trials. Cancer Treat Rep 1985; 69: 1375-1381

Simplicio PD
Glutathione and glutathione S-transferases in rat liver and in plasma after carbon tetrachloride and thioacetamide intoxication. Pharmacol Res Commun, 1982; 14:909-920

Starling MR, Crawford MH, Sorensen SG, Levi B, Richards KL, O'Rourke RA
Comparative accuracy of apical biplane cross-sectional echocardiography and gated equilibrium radionuclide angiography for estimating left ventricular size and performance. Circulation, 1981; 63:1075-1084

Staquet MJ, Byar DP, Green SB, Rozencweig M
Clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumours : rationale for a three-stage strategy.
Cancer Treat Rep. 1983; 67:753-765.

Staubus A, Neidhart J, Young D, Malspeis L
Pharmacokinetics of m-amsacrine (NSC 249992) in humans (Abstract).
Proc Am Assoc Cancer Res, 1980; 21:198

Steinherz LJ, Steinherz PG, Mangiacasale D, Tan C, Miller DR
Cardiac abnormalities after AMSA administration. Cancer Treat Rep, 1982; 66:483-488

Stewart DJ, Zhengang G, Lu K, et al
Human tissue distribution of 4'-(9-acridinylamino)- methanesulfon-m-anisidide (NSC 141549, AMSA). Cancer Chemother Pharmacol, 1984; 12:116-119

Sullivan DM, Glisson BS, Hodges PK, Smallwood-Kentro S, Ross WE
Proliferative dependence of topoisomerase II mediated drug action. Biochem, 1986; 25:2248-2256

Sullivan DM, Latham MD, Ross WE

Proliferation-dependent topoisomerase II content as a determinant of antineoplastic drug action in human, mouse and chinese hamster ovary cells. Cancer Res, 1987; 47:3973-3979

Takita H, Marabella PC, Edgerton F, Rizzo D

Cis-dichlorodiammineplatinum (II), Adriamycin, cyclophosphamide, CCNU and vincristine in non-small cell lung carcinoma: a preliminary report. Cancer Treat Rep, 1979; 63:29-33

Tewey KM, Chen GL, Nelson EM, Liu LF

Intercalative antitumour drugs interfere with the breakage- reunion reaction of mammalian DNA topoisomerase II.J Biol Chem, 1984; 259:9182-9187

Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF

Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science, 1984; 226:466-468

Tietze F

Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione :applications to mammalian blood and other tissues. Anal Biochem, 1969; 27:502-522

Tognoni G, Bellantuono C, Bonati M, et al

Clinical relevance of pharmacokinetics.Clin Pharmacokinet, 1980; 5:105-136

Tozer TN

Pharmacokinetic concepts basic to cancer chemotherapy. In : Pharmacokinetics of anticancer agents in humans. Ames MM, Powis G, Kovach JS eds. Elsevier 1983; 1-27

Traganos F, Bueti C, Darzynkiewicz Z, Melamed MR

Effects of a new amsacrine derivative, N,-5-dimethyl-9-(2-methoxy-4-methylsulfonylamino)phenylamino-4-acridine carboxamide, on cultured mammalian cells. Cancer Res, 1987; 47:424-432

Twentyman PR

Predictive chemosensitivity testing. Br J Cancer, 1985; 51 : 295-299

Unverferth DV, Magorien RD, Leier CV, Balcerzak SP

Doxorubicin cardiotoxicity. Cancer Treat Rev, 1982; 9:149-164

Van Echo DA, Chiuten DF, Gormley PE, Lichtenfeld JL, Scotock M, Wiernik PH

Phase I clinical and pharmacological study of 4'-(9- acridinylamino)-methanesulfon-m-anisidide using an intermittent biweekly schedule. Cancer Res, 1979; 39:3881-3884

von Hoff DD, Clark GM, Stogdill BJ, et al

Prospective clinical trial of a human tumor cloning system.Cancer Res,1983; 43:1926-1931

von Hoff DD, Elson D, Polk G, Coltman C

Acute ventricular fibrillation and death during infusion of 4'-(9-acridinylamino)methanesulfon-m-anisidide (AMSA). Cancer Treat Rep, 1980; 64:356-357

Vorobiof DA, Iturralde M, Falkson G

Amsacrine cardiotoxicity: assessment of ventricular function by radionuclide angiography.Cancer Treat Rep, 1983; 67:1115-1117

Wahr DW, Wang YS, Schiller NB

Left ventricular volumes determined by two-dimensional echocardiography in a normal adult population. J Am Coll Cardiol, 1983; 1:863-868

Wang JC

DNA topoisomerases. Sci Am, 1982; 247:84-95

Wang JC

DNA topoisomerases. Annu Rev Biochem, 1985; 54:665-697

Waring MJ

DNA-binding characteristics of acridinylmethanesulphonilide drugs: comparison with antitumour properties. Eur J Cancer, 1976; 12:995-1011

Waring MJ

DNA modification and cancer. Annu Rev Biochem, 1981; 50:159-192

Weick JK, Jones SE, Ryan DH

Phase II study of amsacrine (m-AMSA) in advanced lymphomas: a Southwest Oncology Group Study.Cancer Treat Rep, 1983; 67:489-492

Weiss RB, Grillo-Lopez AJ, Marsoni S, Posada JG, Hess F, Ross BJ

Amsacrine-associated cardiotoxicity : an analysis of 82 cases. J Clin Oncol,1986;4:918-928

Weiss RB, Moquin D, Adams JD, Griffin JD, Zimbler H

Electrocardiogram abnormalities induced by amsacrine. Cancer Chemother Pharmacol, 1983; 10:133-134

Wells PG, Boerth RC, Oates J, Harbison R

Toxicologic enhancement by a combination of drugs which deplete hepatic glutathione :acetaminophen and doxorubicin (Adriamycin). Toxicol Appl Pharmacol, 1980; 54:197-209

Whitehead R, Crowley J, Carbone, PP

Cis-dichlorodiammineplatinum (II),Adriamycin, cyclophosphamide, CCNU and vincristine (PACCO) combination chemotherapy in advanced non-small cell bronchogenic carcinoma. Proc Am Assoc Clin Oncol, 1980; 21:458

Wilson WR, Baguley BC, Wakelin LPG, Waring MJ

Interaction of the antitumour drug 4'-(9-acridinylamino)methanesulfon-m-anisidide and related acridines with nucleic acids. Mol Pharmacol, 1981; 20:404-414

Wilson WR, Giesbreicht JL, Hill RP, Whitmore GF

Toxicity of 4'-(9-acridinylamino)methane-sulfon-m-anisidide in exponential and plateau phase Chinese hamster cell cultures. Cancer Res,1981; 41:2809-2816

Wilson WR, Harris NM, Ferguson LR

Comparison of the mutagenic and clastogenic activity of amsacrine and other DNA-intercalating drugs in cultured V79 chinese hamster cells. Cancer Res, 1984; 44:4420-4431

Wilson WR, Whitmore GF

Cell-cycle-stage specificity of 4'-(9-acridinylamino)methane sulfon-m-anisidide (m-AMSA) and interaction with ionizing radiation in mammalian cell cultures. Rad Research, 1981; 87:121-136.

Winer BJ

Single-factor experiments having repeated measures on the same elements. In: Statistical principles in experimental design, 2nd edition. McGraw-Hill Inc, New York. 1971: 261-272

Wittes RE, Marsoni S, Simon R, Leyland-Jones B
The phase II trial. Cancer Treat Rep, 1985; 69:1235-1239

Woodhouse KW, Mutch E, Williams FM, Rawlins MD, James OFW
The effect of age on pathways of drug metabolism in human liver.
Age and Ageing, 1984; 13:328-334

Woods RL, Levi JA, Page J
Non small cell cancer: a randomised comparison of chemotherapy with
no chemotherapy (Abstract). Proc Am Assoc Clin Oncol, 1985; 4:177

Yang L, Rowe TC, Nelson EM, Liu LF
In vivo mapping of DNA topoisomerase II-specific cleavage sites on SV40
chromatin. Cell, 1985; 41:127-132

Zinreich ES, Baker RR, Ettinger DS, Order SE
New frontiers in the treatment of lung cancer. CCR Crit Rev Oncol Haematol,
1985; 3:279-308

Zittoun R
m-AMSA: a review of clinical data. Eur J Cancer Clin Oncol, 1985; 21:649-653

Zwelling L, Estey E, Silberman L, Hittelman WN
m-AMSA induced DNA strand breaks:a potential measure of the malignant
phenotype (Abstract).Proc Am Assoc Cancer Res, 1985; 26:227

Zwelling LA, Kerrigan D, Lippman ME

Protein-associated intercalator-induced DNA scission is enhanced by estrogen stimulation in human breast cancer cells. Proc Natl Acad Sci, 1983; 80:6182-6186

Zwelling LA, Kerrigan D, Michaels S

Cytotoxicity and DNA strand breaks by 5-iminodaunorubicin in mouse leukaemia L1210 cells: comparison with adriamycin and 4'-(9-acridinylamino)methanesulfon-m-anisidide. Cancer Res, 1982; 42:2687-2691

Zwelling LA, Michaels S, Erickson LC, Ungerleider RS, Nichols M, Kohn KW

Protein-associated deoxyribonucleic acid strand breaks in L1210 cells treated with the deoxyribonucleic acid intercalating agents 4'-(9-acridinylamino) methanesulfon-m- anisidide and adriamycin. Biochemistry, 1981; 20:6553-6563

ASSOCIATED PUBLICATIONS

Harvey VJ, Hardy JR, Evans P, Paxton J, Grove W, Grillo-Lopez A, Baguley B

A phase I clinical and pharmacokinetic study of CI-921 (Abstract). Proc Am Soc Clin Oncol, 1987; 6:23

Harvey VJ, Hardy JR, Evans P, Paxton J, Grove W, Grillo-Lopez A, Baguley B

A phase I clinical and pharmacokinetic study of CI-921 (Abstract). Proc Europ Conf Clin Oncol, 1987; 4:77

Grove WR, Grillo-Lopez AJ, Robert F, Harvey VJ, Leiby J, Javier J, Hardy JR, Grever M

Optimizing therapeutic potential of phase I studies; co-ordinating of three world-wide studies of CI-921 (Abstract). Proc Europ Conf Clin Oncol, 1987; 4:77

Hardy JR, Harvey VJ, Paxton JW, Evans P, Smith S, Grove W,
Grillo-Lopez AJ, Baguley BC
A phase I trial of the amsacrine analog 9-[[2- methoxy-4-
[(methylsulfonyl)amino]-phenyl]amino]- N,5-dimethyl-4-acridine
carboxamide (CI-921). Cancer Res, 1988; 48:6593-6596

Harvey V, Hardy J, Evans P, Paxton J, Grove W, Grillo-Lopez A,
Baguley B
A phase II study of CI-921 in patients with non-small cell
cancer of the lung (NSCLC) (Abstract). Proc Am Soc Clin Oncol, 1988; 7 : 789

Paxton JW, Hardy JR, Evans PC, Harvey VJ, Baguley BC
The clincal pharmacokinetics of N-5-dimethyl-9- [(2-methoxy-
4-methyl sulphonylamino)phenylamino]-4-acridine carboxamide (CI-921) in
a phase I trial. Cancer Chemother Pharmacol, 1988; 22 :235-240

Paxton JW, Evans P, Hardy JR
The effect of cimetidine, phenobarbitone and buthionine
sulphoximine on the disposition of N-5-dimethyl-9-[(2-
methoxy-4-methyl-sulphonylamino) phenylamino]-4-acridine carboxamide (CI-921).Cancer
Chemother Pharmacol, 1988; 22 : 235-240.