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The pharmacokinetics and toxicity of the anthrapyrazole anti-cancer drug CI-941 in the mouse: a guide for rational dose escalation in patients

Martin A. Graham*, David R. Newell, Brenda J. Foster, and A. Hilary Calvert

Clinical Pharmacology Team, Drug Development Section, The Institute of Cancer Research, Belmont, Sutton, Surrey, SM2 5PX, U.K

Summary. CI-941 is a new synthetic DNA-binding agent selected for phase I clinical evaluation. The drug has broad-spectrum antitumour activity against a number of murine tumours and, in contrast to doxorubicin, is unlikely to induce cardiotoxicity by a free-radical-mediated mechanism. In this study the toxicity and pharmacokinetics of CI-941 were studied in the mouse to enable the implementation of a pharmacokinetically guided dose-escalation strategy in patients. Following a single i.v. bolus injection in mice, CI-941 induced dose-dependent leukopenia. The white blood cell counts were suppressed on day 3 by 18%, 50% and 65% of control, at doses of 10, 15 and 20 mg/kg CI-941, respectively. Other toxicities such as weight loss, alopecia, diarrhoea and convulsions were observed at doses >20 mg/kg. Lethality studies in female Balb-c mice resulted in an LD_{10} value of 20 mg/kg (95% confidence limits; range, 19–21 mg/kg) and an LD_{50} value of 22 mg/kg (95% confidence limits; range, 21–23 mg/kg). The pharmacokinetics of CI-941 were studied at four dose levels from 1/10 of the LD_{10} to the LD_{10} (20 mg/kg). The drug was rapidly cleared from the plasma (250–400 ml/min per kg) at a rate approaching the cardiac output of mice, displaying triphasic plasma pharmacokinetics. The area under the plasma CI-941 concentration vs time curve (AUC) was linear with respect to the dose, up to and including 15 mg/kg (AUC = 110 $\mu M \times$ min at 15 mg/kg), but became non-linear at 20 mg/kg (AUC = 277 $\mu M \times$ min). Despite 80%–84% plasma protein binding, CI-941 was rapidly and extensively distributed into tissues, especially the kidney. Following i.v. bolus injections at doses of 1.5 and 15 mg/kg, elimination of the parent compound by urinary excretion accounted for 12%–18% of the delivered dose. A phase-I starting dose (based on that equivalent to 1/10 of the LD_{10} in the mouse) of 5 mg/m^2 CI-941 is recommended for single administration schedules. In addition, a pharmacokinetically guided dose-escalation strategy, based on achieving a target AUC of 110 $\mu M \times$ min, is proposed.

Introduction

The anthrapyrazoles were developed in the search for a non-cardiotoxic DNA-complexing drug with anti-tumour activity equivalent or superior to that of doxorubicin [19]. A large number of synthetic DNA-intercalating agents, exemplified by the anthraquinones, have been developed in recent years [4, 6]. Of these drugs, mitozantrone is the only anthraquinone that has extensively been evaluated in patients. Although mitozantrone appears to be less cardiotoxic than the anthracyclines, it remains to be seen whether it will supercede doxorubicin in terms of improved therapeutic index [29]. Experimental anti-tumour tests in mice have demonstrated that the anthrapyrazoles have high-level, broad-spectrum activity [19, 32]. A number of lead compounds (CI-937, CI-942 and CI-941) (Fig. 1) have displayed marked activity, similar to that of doxorubicin and mitozantrone, against rapidly growing murine tumours, resulting in long increases in life span and a high proportion of cures [19, 32]. Against a number of slower-growing tumours, which arguably give a more representative prediction of clinical activity, the anthrapyrazoles have been proven to be clearly superior to mitozantrone, and displaying either equivalent or superior activity to that of doxorubicin [19].

In addition to their activity in experimental anti-tumour tests, the anthrapyrazoles do not undergo reductive metabolism to form reactive free radicals [14]. Although other mechanisms may be involved [26], reactive free-radical formation has been implicated in doxorubicin cardiotoxicity [1, 17, 25, 27]. Hence, the rationale behind the synthesis of the anthrapyrazoles [30, 31] stems from the observation that the quinoneimine anthracycline, 5-iminodaunorubicin, has a reduced tendency for free-radical forma tion [20] and has proved to be less cardiotoxic than doxorubicin in experimental systems [33]. By analogy to 5-iminodaunorubicin, CI-941 as a modified quinoneimine is theoretically a poor candidate for free-radical formation. In agreement with this prediction, it has recently been demonstrated that CI-941 does not undergo metabolic activation to a drug free-radical or generate a significant amount of superoxide anion formation [14]. Consequently, CI-941 is unlikely to induce cardiotoxicity by a free-radical-mediated mechanism [10, 14].

In general, pre-clinical toxicity studies are carried out to establish the maximally tolerated dose (MTD) of a drug and to determine the nature of the dose-limiting side effect. A retrospective analysis of toxicity data accrued from various animal species with numerous anti-cancer drugs shows that there is a good correlation of the MTD between mouse and man when doses are expressed on the basis of

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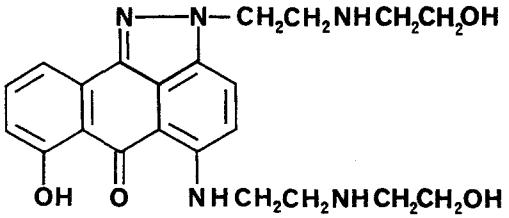

Fig. 1. The molecular structure of the anthrapyrazole CI-941

surface area [11]. Accordingly, the phase-I trial starting dose is based on that equivalent to 1/10 of the LD_{10} in the mouse (quantitated in relation to surface area), which affords a sufficient safety margin for most anti-cancer drugs [11].

Traditionally, this dose has been escalated by applying an arithmetical scheme such as a modified Fibonacci escalation, an approach that can be lengthy, with large numbers of patients treated at sub-therapeutic doses. However, a pharmacokinetically guided dose-escalation strategy that may reduce the empiricism of current procedures has recently been proposed [7, 9]. The present study examines the toxicity and pharmacokinetics of CI-941 in mice as a prelude to phase-I clinical evaluation. In turn, this information will assist in evaluating the MTD of CI-941 in patients by determining the phase-I starting dose and guiding subsequent dose escalations.

Methods

Chemicals and reagents

All chemicals and solvents used were either analytical reagent grade or HPLC grade. CI-941 was generously donated by Warner Lambert Pharmaceutical Research (Ann Arbor, Mich., USA) as a freeze-dried solid containing CI-941 and mannitol (1:2 w/w) and reconstituted in 0.9% NaCl. Doses were given as a single i.v. bolus injection (10 ml/kg) via the tail vein.

Toxicity studies

Animal studies were carried out in either male or female Balb-c mice weighing 18–22 g (National Institute for Medical Research, The Ridgeway, Millhill, London). The study was designed in four parts. The first was a dose-ranging experiment carried out in female Balb-c mice (10 mice/group) at dose intervals of 10 mg/kg (range, 10–40 mg/kg). The animals were weighed and observed daily for 21 days and given food and water ad libitum. LD_{50} and LD_{10} values were derived from probit analysis of the data.

The second experiment examined whether there were sex differences in the toxicity of CI-941. Male Balb-c mice (5 mice/group) were treated at 5 mg/kg dose intervals (range, 15–30 mg/kg) and assessed daily as above.

The third experiment investigated the haematological toxicity of CI-941. Female Balb-c mice (5 mice/group) were treated at 5 mg/kg dose intervals (range, 10–30 mg/kg). Blood counts were determined weekly or twice weekly by venepuncture of the tail vein and the samples were collected in heparinised tubes. White blood cell (WBC) counts were done by diluting 20 µl blood in 10 ml Isoton counting fluid (Coulter Electronics Ltd., Northwell Drive, Luton, Beds., UK). The 1:500 blood dilution was lysed by the addition of three drops of Zaponin (Coulter Electronics Ltd.) prior to counting (Model Z-F Coulter counter, Coulter Electronics Ltd.). The red blood cell (RBC) counts were done by preparing a 1:50,000 dilution of the blood in Isoton. Platelet counts were determined by diluting 20 µl blood in 1 ml Isoton containing 10 IU/ml heparin. The samples were centrifuged for approximately 2 min using a Thrombofuge (Coulter Electronics Ltd.) and 50 µl supernatant was diluted with 10 ml Isoton prior to counting (Thrombocounter-C, Coulter Electronics Ltd.).

Pharmacokinetic studies

Animal treatment. Female Balb-c mice (18–22 g) were randomised into two groups, one of which was used for the pharmacokinetic study and the other, for a parallel toxicity study representing the fourth component of the toxicological evaluation. For the pharmacokinetic experiments, the mice were treated at 20, 15, 10 and 1.5 mg/kg CI-941 (four mice/time point) by single i.v. bolus injection via the tail vein. At 5, 10, 15 and 30 mins and 1, 4, 8 and 24 h post-administration, the animals were anaesthetised with diethyl ether and blood was collected in heparinised tubes by cardiac puncture. The toxicity group was treated within 4 h on the same day with a dose equivalent to that of the pharmacokinetic group. The animals (5–10 mice/group) were treated at 5 mg/kg CI-941 dose intervals (range, 15–30 mg/kg) and monitored as described above.

Sample analysis. Plasma was prepared by centrifugation (1,000 *g* for 2 min) and the four plasma samples were pooled and assayed for CI-941 by solid-phase extraction and HPLC as previously described [15]. Aliquots (0.5 ml) of plasma (or plasma standards at 50 ng/ml, 500 ng/ml and 5 µg/ml CI-941) were passed through 200 mg/3 ml C_2 Bond Elut columns (Jones Chromatography Ltd., Colliery Road, Llanbradach, Mid. Glamorgan, UK). Prior to their use, the columns were solvated with approximately 3 ml methanol followed by 10 ml deionised water. Plasma contaminants were eluted in 10 ml deionised water prior to elution of the analyte in a mixture of 2 ml methanol/hydrochloric acid 10.2 *M* (19:1 v/v). The plasma samples or standards were evaporated to dryness under a stream of nitrogen at 45° C, reconstituted in 200 µl HPLC mobile phase, vortexed and centrifuged at 1,000 *g* for 5 min prior to analysis of the supernatant by HPLC. The recovery of CI-941 from mouse plasma was determined by comparing the peak area of the plasma standards to that of CI-941 standards, which had been prepared by diluting the same amount of drug directly into 200 µl HPLC mobile phase [15]. The percentage of recovery by this procedure over the concentration range of 50 ng/ml–5 µg/ml was found to be 85% ± 4%.

Pharmacokinetic analyses. The pharmacokinetic data were analysed by a computer-generated non-linear least-squares regression analysis with a weighting of $1/(y+\hat{y})^2$ [16, 28]. The best estimates were obtained by fitting a triexponential model to the data points using the equation

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ze^{-\zeta t},$$

where C is the plasma CI-941 concentration at time t; A, B and Z are the concentration constants for the 1st, 2nd and 3rd phases, respectively; and α, β and ζ are the 1st-order

rate constants for the respective phases [35]. The area under the plasma CI-941 concentration vs time curve (AUC) was determined using the equation

$$AUC = A/\alpha + B/\beta + Z/\zeta,$$

and the plasma clearance [35] was determined by

$$Cl = Dose/AUC.$$

The 1st-, 2nd- and 3rd-phase half-lives ($t_{1/2}$) were calculated from the 1st-order rate constants [35].

Tissue distribution. At 1, 8 and 24 h, the heart, lungs, liver, kidneys, pancreas, spleen and brain were excised from the animals treated at 20, 15, 10 and 1.5 mg/kg CI-941. The tissues were rinsed in deionised water, blotted dry and frozen at −40° C until analysed. Tissue homogenates (10% w/v) were prepared in 0.1 *M* TRIS/HCl buffer (pH 7.4) using a Teflon glass homogeniser. Aliquots (0.1–0.5 ml) of the homogenates were precipitated with an equal volume of methanol/acetonitrile (1:1 v/v). The samples were vortexed and centrifuged at 4° C for 15 min at 1,000 *g*, and 20 µl aliquots of the supernatant were assayed directly by HPLC as previously described [15]. Standards were prepared from the injection solutions at 5 and 0.5 µg/ml CI-941 by diluting the drug in a mixture of methanol/acetonitrile (1:1 v/v) and assaying 20 µl aliquots by HPLC.

Urinary and faecal excretion. The urinary and faecal excretion of CI-941 was determined in female Balb-c mice. The animals (four mice/group) were treated at 1.5 and 15 mg/kg CI-941 by a bolus i.v. injection into the tail vein. The mice were placed in Metabowls (Jencons Scientific Ltd., Cherrycourt Way Industrial Estate, Leighton Buzzard, Beds., UK) and provided with food and water ad libitum. Urine and faeces were collected at 24 h periods up to 96 h post-administration. The urine samples were diluted with 0.1 *M* TRIS/HCl (pH 7.4) (1:1 v/v) and vortexed, and the entire sample was passed through a C_2 Bond Elut column (solvated prior to use, as above). The drug was isolated as described above, reconstituted in 500 µl HPLC mobile phase and 25 µl aliquots of the reconstituted analyte were assayed by HPLC. The percentage of recovery of CI-941 (500 ng/ml) from mouse urine by this procedure was 85%±4%. The faecal excretion of CI-941 could not be determined due to the low recovery of the drug after its addition to control mouse homogenates (< 10%).

In vitro plasma protein binding. Plasma protein binding was measured in both mouse and human plasma. CI-941 was diluted to 25, 50, 75 and 100 µg/ml in 1 ml aliquots of fresh Balb-c mouse plasma. CI-941 standards were prepared at the same concentrations in 0.1 *M* TRIS/HCl buffer (pH 7.4) to determine the degree of non-specific binding to the filter. Plasma protein binding was measured using Amicon Centrifree micropartition filters (Amicon Corp. Upper Mill, Stonehouse, Glous., UK). The plasma samples and standards were centrifuged using a fixed-angle rotor;for 15 min at 3,000 *g* (21° C), and 10 µl aliquots of the ultrafiltrate were directly assayed without extraction by the previously described HPLC method. The percentage of CI-941 bound to plasma protein was determined by comparing the peak area of the plasma ultrafiltrate to that of the unfiltered standard. Correction for the non-specific binding of CI-941 to the filter was made by determining the peak area of the CI-941 standard before and after ultrafiltration.

The degree of plasma protein binding to fresh human plasma was measured by taking blood samples from six healthy volunteers (three male, three female). Plasma was prepared by centrifugation (1,000 *g*; 10 min) and protein binding was measured at 10 and 40 µg/ml CI-941.

Results

Toxicity experiments

The acute toxicity of CI-941 was studied in Balb-c mice following a single i.v. bolus injection. In female Balb-c mice, probit analysis of the combined mortality data resulted in an LD_{10} value of 20 mg/kg (95% confidence limits; range 19–21 mg/kg) and an LD_{50} value of 22 mg/kg (range 21–23 mg/kg). In male Balb-c mice, the LD_{50} value was 21 mg/kg (95% confidence limits; range 20–22 mg/kg) indicating no sex difference in lethality. Insufficient intermediate doses precluded the definition of an LD_{10} value in male mice.

The results of the haematological toxicity experiment demonstrated that CI-941 induces leukopenia, suppressing the WBC count (nadir, day 3) by 18%, 50% and 65% of control at doses of 10, 15 and 20 mg/kg, respectively (Fig. 2). No effect was observed on the RBC or platelet

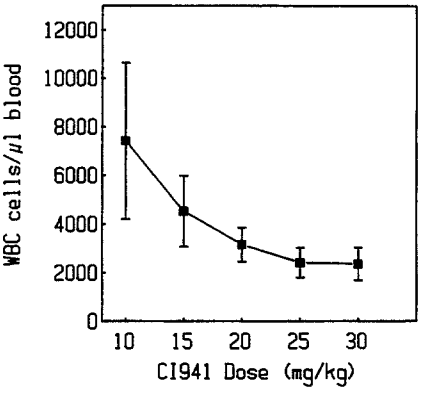

Fig. 2. The effect of CI-941 on the WBC count 3 days following an i.v. bolus injection (10–30 mg/kg) in female Balb-c mice. Control WBC count: 10,000 cells/µl blood. The error bars show the SD of five observations

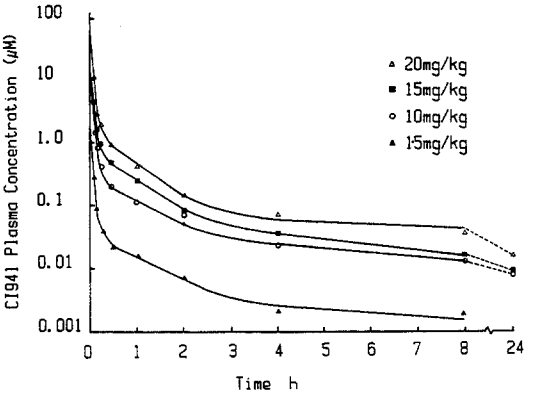

Fig. 3. The pharmacokinetics of CI-941 in the plasma of Balb-c mice following an i.v. bolus injection. Each point shows the CI-941 concentration in plasma pooled from four mice. The lines represent the computer-generated triexponential fit

Table 1. Relationship of CI-941 AUC and clearance to dose

CI-941 Dose mg/kg	AUC μMx mins	Ratio*	Clearance ml/min/kg
1.5	8	0.07	371
10.0	78	0.71	256
15.0	110	1.00	271
20.0	277	2.50	144

* Ratio of the observed AUC, to the AUC at 15 mg/kg

Table 2. The pharmacokinetics of CI-941 in Balb-c mice

CI-941 Dose (mg/kg)	A (μM)	$t_{1/2}\alpha$ (min)	B (nM)	$t_{1/2}\beta$ (min)	Z (nM)	$t_{1/2}\zeta$ (Hrs)
1.5	1.3± 0.7*	2.2±0.5	35±1	38±10	3.2± 1	8±1
10.0	8.9± 3.4	2.5±0.4	290±70	37± 7	19± 4	19±7
15.0	10.5± 1.2	2.8±0.1	760±51	34± 2	23± 2	15±1
20.0	45.8±27.2	2.1±0.5	1760±490	28± 5	71±16	11±3

* Values calculated from the computer generated triexponential fit (as described in materials and methods) ± the asymptotic standard error

counts. The mice treated at 25 and 30 mg/kg received universally lethal doses, with deaths occurring on days 10–12 and 8–9 post-treatment, respectively. At 40 mg/kg CI-941, an assynchronous, clonic, generalised seizure that proved to be lethal was observed immediately after injection. Non-lethal seizures were also observed at 25 and 30 mg/kg. At 25 mg/kg, a weight loss of 24%±8% (nadir, days 8–9) was observed, and at a dose of 30 mg/kg there was progressive weight loss until death (days 8–9). Other toxicities, such as alopecia and diarrhoea (days 2–6), were noted in the groups receiving doses >20 mg/kg. In addition, conjunctivitis in either one or both eyes was observed 12–14 days post-treatment at 20 mg/kg CI-941.

Pharmacokinetic experiments

Following an i.v. bolus injection of CI-941, the drug was rapidly cleared from the plasma (Fig. 3, Table 1). A three-compartmental, triexponential open model could be fitted to the CI-941 plasma concentration vs time curves at all four dose levels (Fig. 3, Table 2). As shown in Table 1, the AUC was linear with respect to the dose, up to and including 15 mg/kg; however, at 20 mg/kg the AUC was 2.5-fold higher than that at 15 mg/kg, thereby indicating non-linear pharmacokinetics.

CI-941 was extensively bound to plasma proteins in mouse plasma (80%–84%; Table 3). Similar results were obtained using human plasma (96%±2% at 10 μg/ml and 92%±2% at 40 μg/ml). Despite this high degree of protein binding, the drug was rapidly and extensively distributed into tissues (Figs. 4, 5). Comparable drug levels were found in the pancreas, heart and lungs (7–10 μg/g tissue wet weight at 15 mg/kg CI-941). The liver and spleen attained levels <5 μg/g tissue (Fig. 4), and CI-941 was undetectable in the brain (<0.1 μg/g tissue). Of particular interest were the high levels of CI-941 in the kidneys (Fig. 5). The kidney/plasma CI-941 concentration ratios at 15 mg/kg

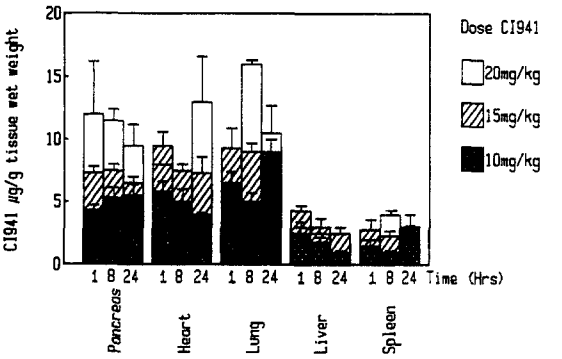

Fig. 4. The tissue distribution of CI-941 (following an i.v. bolus injection) 1, 8 and 24 h post-administration. The error bars show the SD of four observations

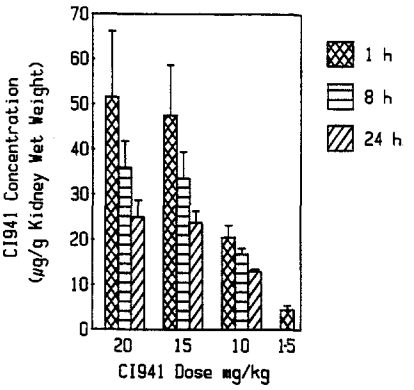

Fig. 5. The saturable renal uptake of CI-941 following i.v. bolus injections at doses of 1.5, 10, 15 and 20 mg/kg. Error bars show the SD of four observations

were 400, 4200 and 6060, which represented 4.2%, 2.9% and 2.1% of the dose at 1, 8 and 24 h respectively. The high level of CI-941 in the kidneys was prolonged, with drug levels of >20 μg/g tissue at 24 h. Interestingly, the renal uptake of CI-941 appeared to be saturable, with levels following 20 mg/kg being no greater than those at 15 mg/kg (Fig. 5), despite a 2.5-fold increase in AUC (Table 1).

Following i.v. administration at doses of 1.5 and 15 mg/kg CI-941, 18% and 12% of the dose was excreted in the urine, respectively, the majority being excreted within the first 24 h (Fig. 6). Faecal excretion could not be accurately determined due to poor recovery of the drug from faecal homogenates (<10%).

Table 3. CI-941 plasma protein binding to fresh Balb-c mouse plasma

CI-941 concentration μg/ml*	% bound±SD
25	80±7
50	81±2
75	77±2
100	84±4

* Determined in triplicate at each concentration

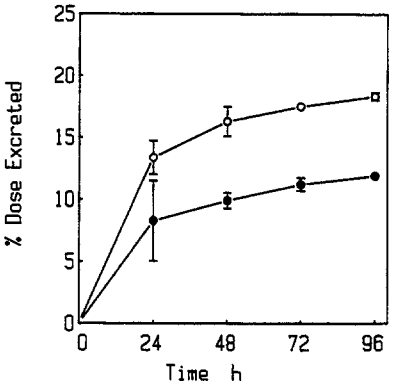

Fig. 6. The urinary excretion of CI-941 at 15 mg/kg (● ——— ●) and 1.5 mg/kg (○ ——— ○) following an i.v. bolus injection in Balb-c mice. Error bars show the SD of 4 observations

Discussion

The toxicity and pharmacokinetics of CI-941 were investigated as a prelude to studies designed to implement a pharmacokinetically guided dose-escalation scheme for patients. In general, pre-clinical toxicology is undertaken to determine the MTD and the dose-limiting toxicity and to derive a safe starting dose for clinical studies. Freireich et al. [11] have retrospectively shown that a good correlation exists between the toxicity of a number of anti-cancer drugs in the mouse and in patients with respect to the MTD, when the dose is normalised to the surface area. These authors also showed that a phase-I trial starting dose of 1/10 of the LD_{10} in the mouse affords a sufficient safety margin for most drugs [11]. Hence, most phase-I starting doses are now calculated from the mouse LD_{10} data. In addition to its quantitative value, toxicity data accrued from these pre-clinical studies in mice can be used to predict the nature of the toxicities likely to be encountered during the clinical trial.

In this study, the toxicity of CI-941 was investigated in the mouse to enable the recommendation of a safe starting dose for phase-I evaluation. Analysis of the mortality data from the studies carried out on female Balb-c mice resulted in an LD_{10} value of 20 mg/kg, which is equivalent to a dose of 52 mg/m^2 [5]. An independent study on male MF1 mice gave LD_{10} and LD_{50} values of 20 mg/kg (range, 17–23 mg/kg) and 26 mg/kg (range 22–30 mg/kg), respectively (CRC unpublished results). The recommended phase-I starting dose (equivalent to 1/10 of the LD_{10}), 5 mg/m^2, is derived from these data. The major dose-limiting toxicity was myelosuppression. Doses of 25 and 30 mg/kg resulted in pronounced leukopenia and, ultimately, death (Fig. 2). No other haematological toxicities were observed. By analogy, clinical studies with the structurally similar anthraquinone mitozantrone have also shown that the major dose-limiting toxicity is leukopenia [29]. Although doxorubicin does induce myelosuppression [2], the cumulative dose-limiting toxicity in both experimental animals and patients is cardiotoxicity [3, 24]. Comparative clinical studies with mitozantrone and doxorubicin indicate that the incidence of cardiotoxicity is lower with mitozantrone [29]. Although cardiotoxicity was not determined in the present experiments, it is unlikely to have been observed following a single i.v. dose. Hence, the possibility of CI-941-induced cardiotoxicity following repeated administration cannot be excluded. However, free-radical-mediated tissue damage is unlikely to occur with CI-941 [14], and myelosuppression is anticipated to be the major dose-limiting toxicity in patients.

At high doses (>30 mg/kg), acute lethalities in the form of assynchronous, clonic, generalised seizures, the cause of which remains unclear, occurred within seconds of the injection. An epileptic-type seizure has been considered as a possible mechanism. In this respect, the anthrapyrazoles are structurally similar to a number of nitroacridone compounds possessing a dialkylaminoalkyl side chain in the N-10 position [22]. Mayer and Bain [23] have demonstrated that compounds of this type accumulate in the nucleoli of cells in the CNS, inducing seizures in mice and rats following i.v. injection. However, CI-941 was undetectable in the brains of mice, presumably due to the failure of the drug to penetrate the blood-brain barrier. This failure to penetrate the CNS is probably due to the charged nature of the side chains on the molecule at physiological pH. Moreover, pre-treatment of the mice with the anti-convulsant drugs phenytoin, phenobarbitone or sodium valproate failed to prevent the seizures when the animals were challenged with a convulsant dose of CI-941 [13]. Because an epileptic-type convulsion is deemed unlikely, the possibility of a seizure mediated peripherally to the CNS may be considered.

An effect of CI-941 on the cardiovascular system is another possible mechanism by which this drug may induce seizures. Although lethally convulsant doses of CI-941 did not stop the heart from beating, the drug may cause vasoconstriction or vasodilatation, which may ultimately induce an anoxic state. However, a toxicity of this nature is felt to be unlikely at clinically relevant doses, as a number of anthrapyrazoles, including CI-941, have demonstrated antitumour activity in mice at doses (5–10 mg/kg) well below those that induce seizures (25–30 mg/kg) [19, 32].

The pharmacokinetics of CI-941 were studied in an attempt to correlate drug toxicity with drug exposure, measured as the area under the CI-941 plasma vs time curve, or AUC. The kinetics were determined at 20 mg/kg (LD_{10}), 15 mg/kg, 10 mg/kg and 1.5 mg/kg to investigate whether the increase in AUC was linear with respect to the dose. The AUC was found to be proportional to the dose, up to and including 15 mg/kg (Table 1). However, a 33% increase in dose to 20 mg/kg resulted in a 2.5-fold increase in the AUC. This non-linearity in the kinetics above 15 mg/kg suggested the saturation of a clearance mechanism which, in turn, led to a high AUC that may be responsible for the steep dose-lethality relationship.

CI-941 was rapidly cleared from the plasma at a rate approaching the cardiac output of the mouse [36], and a three-compartmental, open model could be fitted to the data. Mitozantrone also displays triexponential pharmacokinetics when given as a single i.v. bolus injection to either dogs [21] or patients [34]. After its plasma clearance, the major fate of CI-941 was its uptake by tissues. As evidenced by renal CI-941 levels, there appeared to be a limit to the extent of tissue accumulation, beyond which a further increase in dose did not result in higher tissue concentrations. CI-941 was found to bind to proteins to similar extents in mouse and human plasma. However, in view of the rapidity of plasma drug clearance and the extent of tissue distribution, small variations in plasma protein binding are unlikely to influence unduly the extrapolation of the target AUC value to patients.

Like anthracyclines and anthraquinones, the anthrapyrazoles strongly interact with DNA [8, 12, 18]; thus, saturation of the DNA-binding of CI-941 could explain the tissue saturation phenomena. However, other mechanisms may be operative; in particular, the mechanism for the unusual renal accumulation of CI-941 is the subject of current investigations. Taken together, the pharmacokinetic data suggest that the capacity of tissues to accumulate CI-941 will play a major role in determining the plasma AUC and hence the MTD of the drug in patients.

The present study addressed a number of the objectives regarding the design of pre-clinical investigations of new anti-cancer drugs [7, 11]. Firstly, the LD_{10} of CI-941 was determined in the mouse. From this determination, the phase-I starting dose of 5 mg/m^2 was derived, i.e. the dose equivalent to 1/10 of the LD_{10} in the mouse. Secondly, a pharmacokinetic study was carried out at four dose levels from 1/10 of the LD_{10} to the LD_{10} to investigate linearity and define the target AUC for clinical studies. The results demonstrated that the kinetics were linear with respect to the dose, up to 15 mg/kg, but became non-linear at 20 mg/kg. The AUC at 15 mg/m^2 (110 $\mu M \times$ min) was chosen as the target AUC. An additional toxicity study (carried out parallel to the pharmacokinetic study and in the same group of randomised animals) was included to control for variables, such as sex and strain differences and variations between groups of animals, which can compromise the comparison of toxicity data with pharmacokinetic data [9]. Thirdly, the protein binding of CI-941 was examined in both mouse and human plasma and found to be similar in both species. This is an important variable to eliminate, as species variations in protein binding can complicate the interpretation of pharmacokinetic data [9]; particularly if the intent is to reach a target AUC in patients based on the data accrued from studies in mice, the comparison of AUC values between the species may be invalid if differences in protein binding are not taken into account.

In conclusion, a dose of 5 mg/m^2 CI-941 is recommended as a safe phase-I starting dose for single administration schedules. In view of the non-linear pharmacokinetics at the LD_{10} in mice and the steep dose-lethality relationship, a pharmacokinetically guided dose-escalation strategy based on acheiving an AUC of 110 $\mu M \times$ min is proposed. Should toxicity not be encountered at this AUC in patients, the dose should be escalated using established methods until the MTD is acheived. Myelosuppression is anticipated to be the major dose-limiting toxicity in patients. The application of a pharmacokinetically guided escalation strategy for the phase-I trial of CI-941 should expedite the clinical evaluation of this new anti-cancer drug.

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