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# Dose-dependent pharmacokinetics of *N*-5-dimethyl-9-[(2-methoxy-4-methylsulphonylamino)phenylamino] -4-acridinecarboxamide (CI-921) in rabbits*

**J. W. Paxton, P. C. Evans, and R. M. Singh**

Department of Pharmacology and Clinical Pharmacology, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand

**Summary.** *N*-5-dimethyl-9-[(2-methoxy-4-methylsulphonylamino)phenylamino]-4-acridinecarboxamide (CI-921), which is an analogue of amsacrine, has entered phase 1 clinical trials as an antitumour drug. The plasma pharmacokinetics of CI-921 has been studied in six rabbits after short i. v. infusions of 6.35, 12.7 and 25.4 μmol/kg. Total plasma concentrations of CI-921 were determined by a high-performance liquid chromatography method for up to 12 h post infusion. Comparison of pharmacokinetic parameters for each rabbit by within-subject analysis of variance indicated that with a four-fold increase in the dose from 6.35 to 25.4 μmol/kg there was a 44% increase in the area under the concentration-time curve normalised to dose ($P<0.001$) and a 43% increase in the elimination half-life ($P<0.005$), and a 30% decrease in the total plasma clearance ($P<0.001$). Dose had no effect on the end of infusion concentration normalised to dose, or on the steady-state volume of distribution. These results indicate that CI-921 experiences dose-dependent elimination kinetics in the rabbit.

## Introduction

*N*-5-dimethyl-9-[(2-methoxy-4-methylsulphonylamino)-phenylamino]-4-acridinecarboxamide (CI-921) (Fig. 1) is a second-generation anticancer agent which is currently in phase 1 clinical trials. Amsacrine, its predecessor, was clinically effective against leukaemia [2, 4] and certain lymphomas, [10] but had little activity against most solid tumours [3, 4]. Further studies have been undertaken to identify analogues of amsacrine which might offer a broader clinical antitumour spectrum. From this programme has emerged CI-921, which has significantly greater activity than amsacrine against solid tumour test systems [1]. This compound is more lipophilic, is a weaker base and experiences more plasma protein binding than amsacrine [7]. Previously we have shown that amsacrine undergoes dose-dependent kinetics in the rabbit at doses greater than 12.7 μmol/kg [6]. The aim of this study was to investigate whether CI-921 experiences dose-dependent kinetics over a similar dose range.

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*Offprint requests to:* J. W. Paxton

## Materials and methods

CI-921 in the form of the isethionate salt (from Dr. B. Baguley, Cancer Research Labs, University of Auckland Medical School) was dissolved in lactic acid solution to give a concentration of 12.7 mmol/l. The appropriate amount of CI-921 was then diluted with 5% dextrose solution to 20 ml for infusion.

Six New Zealand white rabbits (weight range 2.7–5.4 kg) were maintained on commercial rabbit pellets and water ad libitum. Each received CI-921 doses of 12.7, 6.35 and 25.4 μmol/kg, in that order, infused over 35 min into a marginal ear vein at 0.58 ml/min. Two additional rabbits also received identical 12.7 μmol/kg doses administered 1 month apart. On completion of the infusion, the catheter line was flushed with 4 ml 5% dextrose solution containing 5 units/ml sodium heparin. Venous blood (3 ml) was collected from the opposite ear into heparinised tubes at 0, 0.5, 1, 2, 4, 6, 8 and 12 h post infusion. Plasma was separated immediately by centrifugation and stored at −20° C in capped glass vials until analysis. A minimum recovery period of 1 month was allowed between consecutive doses. No significant loss of body weight occurred over the experimental period.

Plasma CI-921 concentrations were determined in duplicate 0.5 ml aliquots by our HPLC method [5]. This assay has good accuracy with recoveries ranging from 98.3% to 106.6% over the range 0.05–40 μmol/l in plasma and excellent precision with intra-assay coefficients of variation ($n=9$) of 2.6%, 4.3% and 3.8% and interassay coefficients of variation of 7.3%, 4.6% and 3.8% ($n=56$) at plasma concentrations of 0.1, 1.0 and 15 μmol/l respectively.

Model-independent pharmacokinetic parameters were calculated from the plasma concentration-time curve for each dose for all rabbits. The area under the plasma concentration-time curve (AUC$^{\infty}$) and the area under the first moment of the concentration-time curve (AUMC$^{\infty}$) were determined using the trapezoidal rule while successive

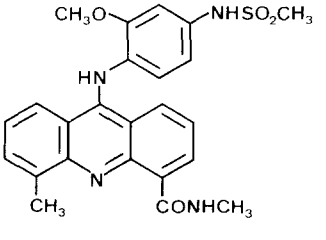


**Fig. 1.** Structure of CI-921

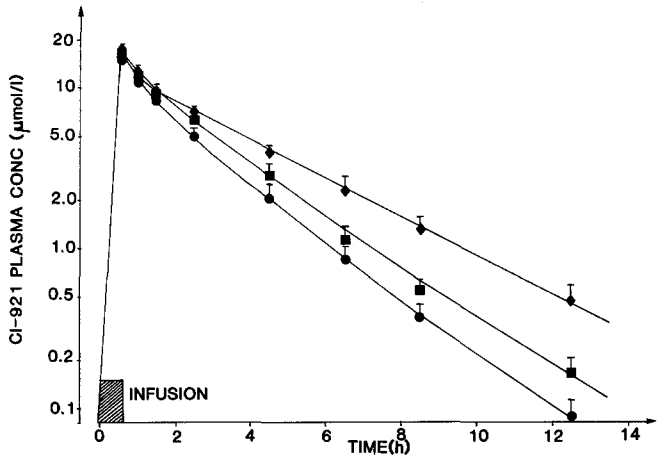


**Fig. 2.** Mean CI-921 plasma concentration-time profiles normalised to the 6.35 μmol/kg dose (●). The 12.7 and 25.4 μmol/kg doses are represented by ■ and ◆ respectively. The *bars* represent the SEM

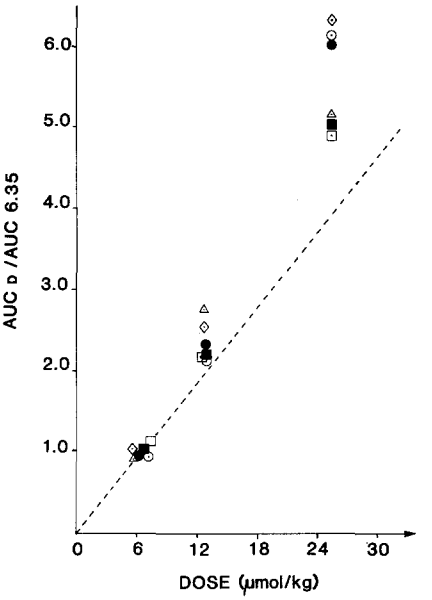


**Fig. 3.** Relationship between dose and the ratio of the $AUC^{\infty}$ for each dose ($AUC_D$) to the AUC for the lowest dose (6.35 μmol/kg) in each rabbit. Each symbol represents an individual rabbit. The *dashed line* represents the predicted values, if the kinetics were linear

concentration values were increasing and the log trapezoidal rule when successive concentration values were decreasing after the maximum. Both were extrapolated to infinity by the usual methods [8]. The terminal half-life ($t^{1}/_{2}$) was calculated by 0.693/k. Other pharmacokinetic parameters calculated were the total plasma clearance ($Cl = DOSE/AUC^{\infty}$) and the steady-state volume of distribution ($Vss = Cl\ (AUMC^{\infty}/AUC^{\infty})-T/2$) where T was the infusion time for this short-term constant rate i. v. infusion.

The pharmacokinetic parameters were compared for each rabbit for each dose using within-subject one-way analysis of variance. Further a posteriori comparisons between the different dose groups were undertaken using the Newman-Keuls test. Differences were regarded as significant at a $P$ value less than 0.05.

**Table 1.** Pharmacokinetic parameters after three different dose infusions of CI-921 in rabbits

| Rabbit | Dose (μmol/kg) | Cmax (μmol/l) | $Cmax_n$ (μmol/l) | $t^{1}/_{2}$ h | Cl (ml/h/kg) | Vss (ml/kg) | $AUC_0^{\infty}$ (μmol · h/l) | $AUC_n$ (μmol · h/l) |
|---|---|---|---|---|---|---|---|---|
| A34 | 6.35 | 13.5 | 13.5 | 1.47 | 222 | 337 | 28.5 | 28.5 |
| | 12.7 | 31.0 | 15.5 | 1.51 | 193 | 322 | 65.9 | 33.0 |
| | 25.4 | 59.7 | 14.9 | 1.95 | 148 | 404 | 172.0 | 43.0 |
| R1 | 6.35 | 18.7 | 18.7 | 1.61 | 251 | 309 | 25.3 | 25.3 |
| | 12.7 | 31.5 | 15.8 | 1.64 | 226 | 351 | 56.3 | 28.2 |
| | 25.4 | 66.9 | 16.7 | 1.68 | 201 | 338 | 126.3 | 31.8 |
| X22 | 6.35 | 21.0 | 21.0 | 1.72 | 141 | 284 | 44.9 | 44.9 |
| | 12.7 | 48.1 | 24.1 | 1.93 | 102 | 228 | 124.4 | 62.2 |
| | 25.4 | 54.2 | 13.6 | 3.27 | 110 | 518 | 231.4 | 57.8 |
| K43 | 6.35 | 16.9 | 16.9 | 1.82 | 180 | 356 | 35.2 | 35.2 |
| | 12.7 | 36.9 | 18.4 | 1.89 | 142 | 272 | 89.6 | 44.8 |
| | 25.4 | 83.7 | 20.9 | 2.44 | 114 | 373 | 221.8 | 55.5 |
| K44 | 6.35 | 15.8 | 15.8 | 2.08 | 154 | 360 | 41.2 | 41.2 |
| | 12.7 | 29.5 | 14.8 | 2.08 | 145 | 370 | 87.6 | 43.8 |
| | 25.4 | 75.7 | 18.9 | 2.65 | 101 | 379 | 251.9 | 63.0 |
| K46 | 6.35 | 19.5 | 19.5 | 1.55 | 166 | 281 | 38.2 | 38.2 |
| | 12.7 | 30.5 | 15.3 | 2.08 | 145 | 370 | 80.5 | 40.8 |
| | 25.4 | 69.9 | 17.5 | 2.65 | 113 | 424 | 225.4 | 56.4 |
| Mean | 6.35 | 17.6 | 17.6 | 1.71 | 186 | 321 | 35.6 | 35.6 |
| SD | | 2.7 | 2.7 | 0.22 | 42 | 35 | 12.1 | 12.1 |
| Mean | 12.7 | 34.6 | 17.3 | 1.82 | 161 | 318 | 84.0 | 42.1 |
| SD | | 7.1 | 3.6 | 0.21 | 43 | 56 | 35.6 | 11.8 |
| Mean | 25.4 | 68.3 | 17.1 | 2.44 | 131 | 406 | 204.8 | 51.3 |
| SD | | 10.6 | 2.6 | 0.56 | 38 | 62 | 85 | 11.6 |

## Results

The observed concentration-time profiles from the two rabbits who received identical 12.7 μmol/kg doses 1 month apart were superimposable with no obvious trends in any of the kinetic parameters, indicating that a 1-month recovery period between doses was acceptable.

The CI-921 plasma concentration-time profiles were normalised by dividing the plasma concentration by the appropriate multiple of the lowest dose (6.35 μmol/kg). The mean normalised profiles are shown in Fig. 2. The 12.7 μmol/kg and 25.4 μmol/kg dose profiles were not superimposable on the lowest dose, giving higher than expected profiles, suggesting non-linear kinetics. This is also evident from Fig. 3, which depicts the relationship between dose and the ratio of the AUC∞ for each dose to the AUC∞ for the 6.35 μmol/kg dose. If the AUC∞ increased proportionally with dose according to linear kinetics, the points would have been scattered normally about the dashed line at the higher doses.

The pharmacokinetic parameters for each rabbit are presented in Table 1. Within-subject one-way analysis of variance indicated that with increasing dose, there were significant increases in the AUC∞ normalised to dose ($AUC_n$; $P<0.001$) and the t½ ($P<0.005$), and a significant decrease in Cl ($P<0.001$). Dose had no effect on Vss or on the end of infusion concentration normalised to dose ($Cmax_n$). Further comparisons using the Newman-Keuls test indicated that significant differences ($P<0.005$) existed for Cl and $AUC_n$ between the 6.35 and 12.7 μmol/kg doses and between the 12.7 and 25.4 μmol/kg doses. A significant difference was also observed for the t½ between the 12.7 and the 25.4 μmol/kg dose and between the 6.35 and the 25.4 μmol/kg dose, but not between the 6.35 and the 12.7 μmol/kg dose.

## Discussion

We have previously shown that amsacrine, an analogue of CI-921, exhibited dose-dependent kinetics at doses greater than 12.7 μmol/kg when infused into rabbits using this same regimen [6]. There is also some evidence to suggest that amsacrine undergoes dose-dependent kinetics in patients [9]. This present study indicated that CI-921 also experiences dose-dependent kinetics in rabbits and this occurs at lower doses than with amsacrine. The dose-dependent kinetics are due to a reduction in total plasma clearance of CI-921 with increasing dose but the mechanism is unknown at present. As CI-921 is very highly protein bound [7], further studies are underway to investigate whether changes in binding are involved in the dose-dependent kinetics. This would appear unlikely, as we have shown by equilibrium dialysis using tritiated CI-921 that the binding of CI-921 is independent of concentration over the range 1–101 μmol/l in blood bank plasma (unpublished observations).

Our results suggest that dose-dependent kinetics might be expected during the phase 1 clinical trials of CI-921 in cancer patients.

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