Cancer Chemother Pharmacol (1989) 23: 291–295

Cancer Chemotherapy and Pharmacology

The effect of cimetidine, phenobarbitone and buthionine sulphoximine on the disposition of *N*-5-dimethyl-9-[(2-methoxy-4-methyl-sulphonylamino) phenylamino]-4-acridinecarboxamide (CI-921) in the rabbit

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Summary. *N*-5-dimethyl-9-[(2-methoxy-4-methylsulphonylamino)phenylamino]-4-acridinecarboxamide (CI-921) is an amsacrine analogue currently undergoing phase II clinical trials as an antitumor drug. Significant alterations in the plasma clearance (CL) of amsacrine have been demonstrated in rabbits after pretreatment with cimetidine (CT), phenobarbitone (PB) and buthionine sulphoximine (BSO). In the present study, the influence of these agents on the disposition of CI-921 was investigated in rabbits. After a short infusion of CI-921 (12.7 μmol/kg), blood (8 × 3 ml) was collected up to 12 h and the total plasma concentration of CI-921 determined by HPLC. Model-independent pharmacokinetic parameters were compared by Student's paired *t*-test. CT pretreatment significantly ($P = 0.011$) increased the AUC (mean, 21%; range, 3%–43%) and significantly ($P = 0.019$) decreased the CL (mean, 17%; range, 4%–30%). The induction effect of PB pretreatment was not confirmed with CI-921. No significant reduction in AUC or increase in CL was apparent. BSO pretreatment caused a small but significant ($P = 0.049$) increase in AUC (mean, 20.5%; range, 4%–59%) but had no effect on CL. Although more modest changes in kinetic parameters were observed with CI-921 than with amsacrine, these results suggested the involvement of the hepatic mixed-function oxidase system but not PB-inducible cytochrome P-450 isozymes in the elimination of CI-921 in the rabbit. As with amsacrine, a reduction in hepatic glutathione (GSH) concentrations in the body also appeared to have a modest effect on the disposition of CI-921.

Introduction

CI-921 (Fig. 1) is a second generation antitumour agent currently undergoing phase II clinical trials. Amsacrine, its predecessor, was clinically effective against leukaemia but had little activity against most solid tumours [1, 3–5, 10]. CI-921 had significantly greater activity than amsacrine in various in vivo and in vitro solid tumour test systems [2]. It is structurally similar to amsacrine, with an additional carboxamide and methyl group at the 4 and 5 positions, respectively, of the acridine nucleus. Data from patients have indicated that amsacrine is eliminated mainly by hepatic metabolism [7, 9]. From studies in rats, Shoemaker et al. [17–19] have suggested that amsacrine was oxidised to a reactive intermediate that further reacted with glutathione at the 4 or 5 position of the anilino ring to form a hepatic glutathione (GSH) adduct, which was then eliminated in the bile. We have also shown in rabbits that pretreatment with buthionine sulphoximine (BSO), a specific depletor of GSH [6, 12], significantly reduced the plasma clearance (CL) of amsacrine [14]. Significant changes in CL were also observed after pretreatment with phenobarbitone (PB) and cimetidine (CT), an inducer and inhibitor, respectively, of the cytochrome P-450-dependent mixed-function oxidase system [14]. In the present study, we investigated the influence of these agents on the disposition of CI-921 in the rabbit.

Materials and methods

A total of 13 New Zealand white rabbits (12 males and 1 female weighing 2.5–5.8 kg) were infused with CI-921 (7.5 mg/kg = 12.7 μmol/kg in 20 ml 5% dextrose solution) by a previously published procedure [15]. Groups of six rabbits were pretreated with saline, BSO (444 mg/kg i.p. 10 h before and 111 mg/kg i.v. 0.5 h before CI-921) or CT (150 mg/kg i.p. 1 h before CI-921) in a balanced crossover study. For the PB study, four rabbits from the BSO group, two from the CT group, plus one additional rabbit (I46) were pretreated with PB (20 mg/kg i.p.) for 8 days before the CI-921 infusion. Rabbit I46 received 8 days of pretreatment with an equivalent volume of i.p. saline as the control, but for the remaining rabbits the previous saline controls were used. A minimal period of 1 month was allowed between CI-921 infusions, as this had previously been shown to be adequate for recovery between successive doses [15].

On completion of the CI-921 infusion, 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 postinfusion. Plasma was separated immediately by centrifugation and stored at −20° C in capped glass vials until analysis. CI-921 concentrations were determined in duplicate 0.5-ml aliquots by our HPLC method [8]. This assay is accurate, with recoveries ranging from 98.3% to 106.6% over the range of 0.05–40 μmol/l in plasma, and precise, with coefficients of variation (CV) of 2.6%, 4.3% and 3.8% ($n = 9$, intra-assay) and 7.3%, 4.0% and 4.0% ($n = 66$, inter-assay) at plas-

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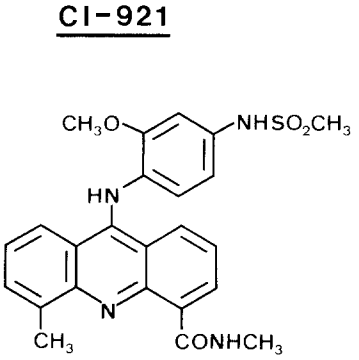

Fig. 1. Structure of CI-921

ma concentrations of 0.1, 1.0 and 15 μmol/l, respectively. Where possible, the control and treated samples were assayed together to eliminate any inter-assay variations. Model – independent pharmacokinetic parameters (total plasma clearance, CL; apparent volume of distribution at steady state, V_{ss}; mean residence time, MRT; elimination half-life, $t_{½\beta}$) were calculated from the plasma concentration-time curve for each rabbit as previously described [14, 15].

Whole blood GSH concentrations were measured by a spectrophotometric method based on that described by Tietze [20]. Venous blood samples were collected before pretreatment with BSO or saline, and then again 6 h after the CI-921 infusion. Based on duplicate measurements in duplicate samples, the CV of each GSH measurement varied from 1.5% to 13.0%, with a mean value of 5%.

Results were compared by the two-tailed, paired Student's *t*-test. Differences were considered significant when the probability (*P*) value was <0.05.

Results

CT pretreatment

The mean plasma CI-921 concentration-time profiles after saline and CT pretreatment in six rabbits are shown in Fig. 2, and the kinetic parameters for individual rabbits, in Table 1. CT pretreatment caused a significant increase ($P = 0.011$, 5 *df*) in the area under the extrapolated concentration-time curve (AUC), with individual increases ranging from 3% to 43% (mean, 21%) and a significant decrease ($P = 0.019$, 5 *df*) in CL ranging from 4% to 30% (mean, 17%) in all six rabbits. This was accompanied by a significantly prolonged $t_{½\beta}$ ($P = 0.036$) and MRT ($P = 0.038$). There was no significant difference in maximal concentration (C_{max}) or V_{ss} after CT treatment.

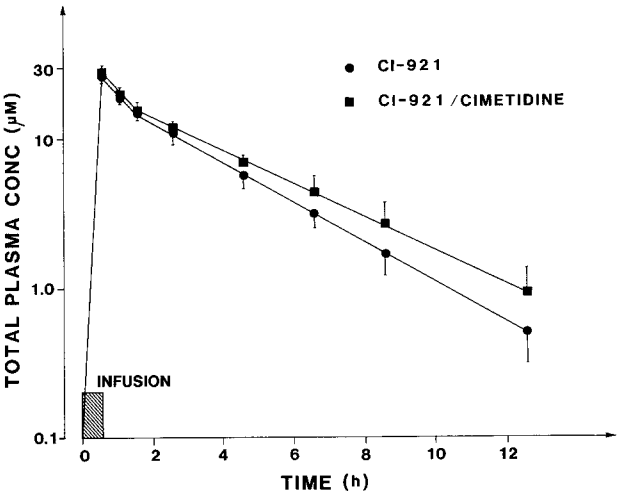

Fig. 2. The effect of CT on the postinfusion plasma CI-921 concentration-time profiles. Each point is the mean (±SD) for six rabbits who received saline (●) or CT pretreatment (■)

PB pretreatment

Seven rabbits received PB pretreatment. The mean CI-921 concentration-time profiles for control and PB-treated animals are illustrated in Fig. 3 and individual kinetics are reported in Table 2. PB pretreatment tended to increase the AUC and decrease the CL, although this did not reach statistical significance at the 5% level. There was a small but significant increase in the MRT from a mean value of 2.3 to 2.9 h, but no significant alterations in C_{max} or V_{ss} occurred.

BSO pretreatment

The mean CI-921 plasma concentration-time profiles for six rabbits following saline and BSO pretreatment are shown in Fig. 4, and the individual kinetic parameters are reported in Table 3. BSO caused an increase in the AUC ranging from 7% to 59% of the control values in five of the six animals. In one rabbit a 4% decrease in AUC was observed. Overall, the mean increase in the AUC for the six

Table 1. Effect of CT pretreatment on the kinetics of CI-921 in six rabbits

Rabbits	(Sex)	C_{max} (μmol/l)		$t_{1/2\beta}$ (h)		MRT (h)		CL (ml/h per kg)		V_{ss} (ml/kg)		AUC (μmol h/l)	
		C	CT	C	CT	C	CT	C	CT	C	CT	C	CT
B52	(M)	23.3	24.9	2.6	2.8	3.2	3.9	202	141	656	555	62.8	89.8
B55	(M)	27.8	23.4	2.5	2.9	3.4	4.2	147	130	502	547	86.7	97.6
K41	(M)	27.3	30.1	1.8	1.8	2.4	2.7	201	150	492	411	63.1	84.5
K46	(F)	26.0	32.8	2.1	2.5	3.1	3.2	157	151	485	489	81.1	83.9
K62	(M)	20.8	26.2	2.2	3.2	3.4	4.9	177	141	596	693	71.8	90.3
H43	(M)	27.6	29.2	2.3	2.4	3.1	3.3	162	148	501	490	78.4	85.7
Mean		25.5	27.8	2.2	2.6	3.1	3.7	174	144	539	531	74.0	88.6
SD		2.8	3.5	0.3	0.5	0.4	0.8	23	8	71	95	9.8	5.1
Paired Student's *t*-test		NS		$P = 0.036$		$P = 0.038$		$P = 0.019$		NS		$P = 0.011$	

C, saline control; CT, cimetidine; NS, not significantly different at 5% level

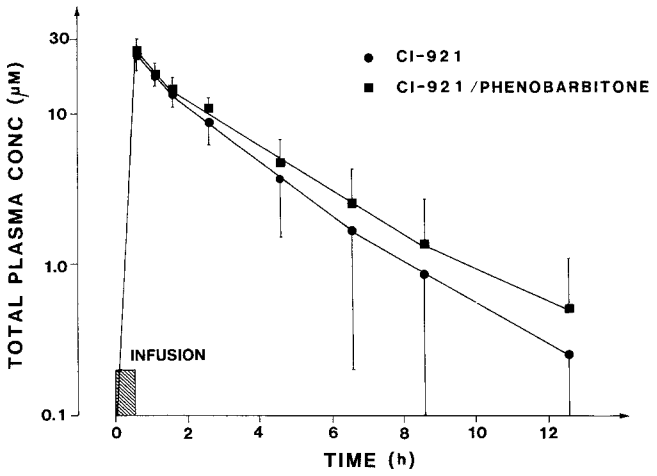

Fig. 3. The effect of PB on the postinfusion plasma CI-921 concentration-time profiles. Each point is the mean (±SD) for seven rabbits who received saline (●) or PB pretreatment (■)

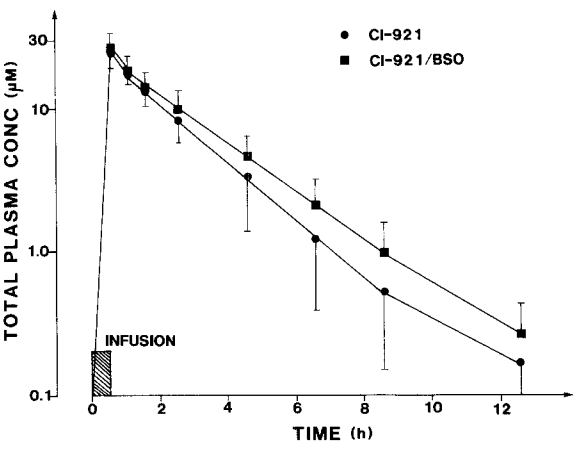

Fig. 4. The effect of BSO on the postinfusion plasma CI-921 concentration-time profiles. Each point is the mean (±SD) for six rabbits who received saline (●) or BSO pretreatment (■)

rabbits was 20.5% (significant at the 5% level). A small but significant increase in the mean MRT from 2.1 to 2.5 h was also observed. A decrease in total plasma CL, ranging from 6% to 37%, was recorded in five of the six rabbits but was not significant for the group. Similarly, there was no significant difference in C_{max}, $t_{½\beta}$ or V_{ss} after BSO pretreatment.

Pretreatment blood GSH concentrations were extremely variable between rabbits, ranging from 329 to 1005 μg/ml, with a mean of 665 μg/ml. There was no significant difference in blood GSH concentrations before saline pretreatment (mean, 565 μg/ml) and 6 h after CI-921 (mean, 535 μg/ml). There was a highly significant difference ($P = 0.0026$, 5 *df*) between GSH concentrations

Table 2. Effect of PB treatment on the kinetics of CI-921 in seven rabbits

Rabbit	(Sex)	C_{max} (μmol/l)		$t_{1/2\beta}$ (h)		MRT (h)		CL (ml/h per kg)		V_{ss} (ml/kg)		AUC (μmol h/l)	
		C	PB	C	PB	C	PB	C	PB	C	PB	C	PB
G20	(M)	27.6	16.7	2.2	1.9	1.8	2.6	256	254	459	655	49.5	50.0
G29	(M)	14.4	23.0	1.8	2.0	2.5	2.6	233	213	592	545	54.5	59.7
I12	(M)	23.4	23.9	2.4	2.0	1.6	2.6	315	225	517	593	40.3	56.5
I46	(M)	22.1	24.3	2.2	2.5	2.1	3.0	247	180	525	541	51.4	70.5
R1	(M)	28.6	24.8	2.1	4.5	1.6	2.0	305	310	494	627	41.7	41.0
B55	(M)	27.8	31.4	2.5	3.7	3.4	4.8	147	109	502	522	86.7	116.3
K46	(F)	26.0	33.9	2.1	2.1	3.1	2.8	157	157	485	433	81.1	81.0
Mean		24.3	25.4	2.2	2.7	2.3	2.9	237	207	511	559	57.9	67.9
SD		5.0	5.7	0.2	1.0	0.7	0.9	65	66	42	74	18.5	25.0
Paired Student's *t*-test		NS		NS		$P = 0.031$		NS		NS		NS	

C, saline control; PB, phenobarbitone; NS, not significantly different at 5% level

Table 3. Effect of BSO pretreatment on the kinetics of CI-921 in six rabbits

Rabbit	(Sex)	C_{max} (μmol/l)		$t_{1/2\beta}$ (h)		MRT (h)		CL (ml/h per kg)		V_{ss} (ml/kg)		AUC (μmol h/l)	
		C	BSO	C	BSO	C	BSO	C	BSO	C	BSO	C	BSO
G20	(M)	27.6	25.9	2.2	2.1	1.8	2.0	256	240	459	472	49.5	52.9
S81	(M)	25.4	21.4	2.1	2.2	2.7	3.0	170	157	457	464	74.7	81.0
G29	(M)	14.4	19.2	1.8	1.8	2.5	2.6	233	244	592	635	54.5	52.0
S83	(M)	32.4	39.1	2.4	2.2	2.4	2.6	184	138	443	365	69.1	92.1
I12	(M)	23.4	22.9	2.4	1.6	1.6	2.7	315	198	517	536	40.3	64.0
R1	(M)	28.6	28.6	2.1	2.2	1.6	2.2	305	228	494	512	41.7	55.7
Mean		25.3	26.2	2.2	2.0	2.1	2.5	244	201	494	497	55.0	66.3
SD		6.1	7.2	0.2	0.2	0.5	0.4	60	45	55	89	14.2	16.7
Paired Student's *t*-test		NS		NS		$P = 0.045$		NS		NS		$P = 0.049$	

C, saline control; NS, not significantly different at 5% level

before BSO pretreatment (mean, 766 μg/ml) and at 6 h after CI-921 (mean, 370 μg/ml), with reductions ranging from 34% to 66% and a mean of 50%. However, the percent increases in AUC did not correlate with percent reductions in whole blood GSH levels or with GSH concentrations at 6 h after BSO treatment.

Discussion

The metabolic pathways of CI-921 have not previously been reported. However, since its structure is similar to that of amsacrine, it was thought that similar pathways might prevail, i.e. hepatic oxidation by the cytochrome P-450-dependent mixed-function oxidase system to the reactive quinone diimine intermediate, followed by conjugation with glutathione and excretion in the bile [17–19].

CT pretreatment reduced the total plasma CL of CI-921 by 18% compared with 33% for amsacrine, suggesting a lesser dependence on the cytochrome P-450-dependent mixed-function oxidase system for the elimination of CI-921. Alternatively, this reduced effect might be due to the lower dose of CT used in this study. The i.v. administration of CT (50 mg/kg) was associated with the death of two rabbits, the first animal in this study and one in the previous amsacrine study [14]. Consequently, the previous CT pretreatment schedule (150 mg/kg i.p. 10 h before and 50 mg/kg i.v. 0.5 h before CI-921) was replaced by a single i.p. dose (150 mg/kg) 1 h before the start of the infusion.

The tendency of PB to increase the AUC and prolong the MRT was surprising and is difficult to explain. It certainly suggests that the PB-inducible cytochrome P-450 isozymes are not involved to any great extent in the elimination of CI-921, in contrast to amsacrine. PB also has a number of non-specific effects such as increasing liver mass, liver blood flow, bile flow and biliary excretion [11], but all might be expected to increase hepatic CL rather than decrease it as in the present study. One mechanism by which PB might increase the AUC and reduce the CL of CI-921 could be by promoting an increase in the plasma protein binding of the latter; increases in the plasma protein binding of some drugs have been reported after PB treatment [16]. Although we observed no change in the plasma binding of amsacrine in rabbits receiving PB pretreatment, binding changes may well occur with CI-921, which is more highly bound than amsacrine in rabbit plasma [13]. As both amsacrine and CI-921 are low hepatic extraction drugs [13], the total body CL would be influenced by the degree of plasma protein binding; hence it is possible that for CI-921 any inducing effect of PB may be opposed by an increase in plasma protein binding. Further studies on the plasma protein binding of CI-921 and the effect of other inducing agents such as β-naphthaflavone are under way.

In our previous study [14], BSO caused significant increases in AUC, MRT and $t_{½\beta}$ and a significant reduction in amsacrine CL. With CI-921, the same BSO regimen caused more modest increases in AUC and MRT that were barely significant. Although there was a tendency towards reduced CL of CI-921, this was not significant at the 5% level.

In conclusion, the present results suggest a lesser involvement of the hepatic cytochrome P-450-dependent mixed-function oxidase system in the elimination of CI-921 than in that of amsacrine, and that glutathione status may play a less important part in the elimination of CI-921 in the rabbit. Alternative metabolic pathways may exist for CI-921.

Acknowledgements. This study was supported by a grant from the Medical Research Council of New Zealand. Thanks are extended to Zea Eagle for typing this manuscript.

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Received December 24, 1987/Accepted September 25, 1988