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The effect of buthionine sulphoximine, cimetidine and phenobarbitone on the disposition of amsacrine in the rabbit*

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Summary. Evidence suggests that the main elimination pathway for amsacrine is hepatic oxidation to the quinone diimine derivative followed by conjugation with glutathione (GSH) and excretion in the bile. If this is so, amsacrine elimination should be susceptible to induction by phenobarbitone (PB) and inhibition by cimetidine (CT) and perhaps by buthionine sulphoximine (BSO), a specific depleter of tissue GSH. This study was carried out in groups of six rabbits. Each rabbit acted as its own control and received pretreatment with saline or PB, CT, or BSO, followed by an amsacrine infusion. Blood (8 × 3 mL) was collected up to 12 h and total plasma amsacrine concentrations determined by HPLC. PB pretreatment resulted in a significant increase in amsacrine's Cl (mean 46%, range 25%–70%) and also in the Vd (mean 58%, range 25%–117%), but had no effect on $t_{½\alpha}$, $t_{½\beta}$ or MRT_{ni}. In addition, there was no change in the plasma protein binding of amsacrine after PB pretreatment. CT pretreatment had the opposite effect, resulting in a significant decrease in amsacrine's Cl (mean 33%, range 21%–38%) and a decrease in Vd, although this latter decrease was not significant at the 5% level. As with PB, the time parameters were not significantly changed. BSO pretreatment resulted in a significantly reduced Cl (mean 22%, range 15%–30%), no effect on Vd or on $t_{½\alpha}$, but significantly prolonged $t_{½\beta}$ and MRT_{ni}. BSO pretreatment was also associated with a significant reduction in red blood cell GSH concentration. These results are consistent with the involvement of the hepatic mixed function oxidase system and GSH status in the elimination of amsacrine in the rabbit.

Introduction

Amsacrine, 4'-(9-acridinylamino)methanesulphon-*m*-anisidide, is a novel anticancer agent which has proved to be an effective clinical drug for the treatment of various disseminated tumours, especially acute leukaemia and certain lymphomas [14, 20, 21, 38]. Data recorded in patients and rats suggest that the main pathway of elimination is by metabolism in the liver followed by excretion in the bile. In patients we have observed up to 10% of the total dose excreted as unchanged drug in the urine during the 24 h after drug administration [16]. Hall et al. [11] reported a prolongation of amsacrine's elimination half-life and reduction in clearance in patients with hepatic dysfunction. Most evidence for the hepatic metabolism of amsacrine has been obtained by in vitro and in vivo studies in rats by Shoemaker et al. [32–34]. Using rat liver microsomes, these workers showed that amsacrine is oxidised to N^1-methanesulphonyl-$N^{4'}$- (9-acridinyl)- 3'-methoxy-2'-5'-cyclohexadiene-1',4'-diimine (*m*-AQDI), which further reacts with glutathione (GSH) at the 5'-position of the anilino ring to form an amsacrine GSH conjugation product, which has previously been identified as the principal biliary metabolite in rat in vivo studies (Fig. 1) [34]. We have used rabbits for several in vivo studies of the pharmacokinetics of amsacrine and analogues [26, 27], and we wished to investigate whether a similar metabolic route might be a major elimination pathway for amsacrine in our rabbit model. For this purpose we studied the effects on amsacrine pharmacokinetics of phenobarbitone (PB) and cimetidine (CT), an inducer and inhibitor, respectively, of the hepatic mixed function oxidase system [18, 22, 29] and buthionine sulphoximine (BSO), a specific inhibitor of α-glutamylcysteine synthetase which results in depletion of reduced glutathione (GSH) in the liver [5, 10, 19, 24].

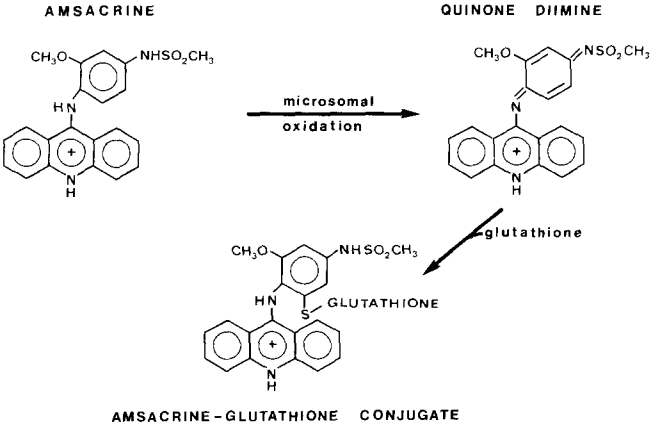

Fig. 1. The proposed pathway for amsacrine metabolism

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Table 1. Effect of PB pretreatment on the kinetics of amsacrine in six rabbits

Rabbit	$t_{1/2\alpha}$ (h)		$t_{1/2\beta}$ (h)		MRT_{ni} (h)		Cl (l h^{-1} kg^{-1})		Vd (l kg^{-1})		AUC_0^∞ (μmol l^{-1} h)	
	C	PB	C	PB	C	PB	C	PB	C	PB	C	PB
A32	1.14	0.69	4.20	3.49	4.87	3.02	0.37	0.56	2.25	2.82	34.1	22.7
A36	1.04	0.88	2.35	2.35	3.23	3.08	0.62	0.97	2.12	3.28	20.3	13.1
A38	0.57	0.42	2.01	2.37	2.28	2.77	0.56	0.70	1.62	3.51	22.3	18.4
A47	0.31	0.47	2.54	2.60	3.71	3.04	0.40	0.68	1.47	2.55	31.6	18.7
A64	0.57	0.61	2.40	2.63	3.23	3.19	0.42	0.54	1.45	2.06	30.4	23.4
H43	0.14	0.35	2.24	3.12	3.23	4.17	0.39	0.38	1.27	1.72	32.2	33.1
Mean	0.63	0.57	2.62	2.76	3.43	3.21	0.46	0.64	1.70	2.66	28.5	21.6
SD	0.39	0.20	0.79	0.45	0.85	0.49	0.10	0.20	0.40	0.69	5.7	6.8
Paired *t*-test	NS		NS		NS		$P=0.009$		$P=0.004$		$P=0.02$	

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

Materials and methods

Groups of six NZ white rabbits received either saline or a treatment with PB (20 mg kg^{-1} i.p. for 8 days), or CT (150 mg kg^{-1} i.p. 10 h before and 50 mg kg^{-1} i.v. 0.5 h before amsacrine) or BSO (444 mg kg^{-1} i.p. 10 h before and 111 mg kg^{-1} 0.5 h before amsacrine) followed by an amsacrine infusion (5 mg kg^{-1} = 12.7 μmol kg^{-1} in 20 ml 5% dextrose solution). Details of the amsacrine infusion have previously been published [26]. The PB study was a balanced crossover design, but both the CT and BSO studies were carried out after prior establishment of control values with saline. A minimum period of 1 month was allowed between amsacrine infusions. This has previously been shown to be an adequate recovery period between successive doses of amsacrine [26].

After the amsacrine 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, and the plasma separated and stored at −20 °C until analysed. Total plasma amsacrine concentrations were determined in duplicate 0.5-ml aliquots by our previously reported HPLC method [15]. This assay has good accuracy, with recoveries ranging from 104%–115% over the range 0.5–10 μmol l^{-1} and excellent precision with mean values for intra- and interassay coefficients of variation less than 5%.

As PB treatment has been reported to affect the binding of some drugs [31], the plasma protein binding of amsacrine was determined in PB-treated and control rabbits. This was carried out by equilibrium dialysis of 800 μl rabbit plasma adjusted to pH 7.4 with CO_2, and dialysed for 4 h at 37 °C against isotonic phosphate buffer containing ^{14}C-amsacrine (S. A. 19.6 mCi $mmol^{-1}$, from SRI International, Menlo Park, Calif). Further details on the determination of the plasma protein binding of amsacrine have been published [28].

GSH concentrations were also measured in red blood cells (RBC) of rabbits before and after BSO treatment by a colorimetric method [3].

The pharmacokinetic parameters were calculated from the total plasma concentration-time profiles using MKMODEL version 1.5 [13] on an Apple IIe computer. The area under the plasma concentration-time curve (AUC_0^∞) was computed using the trapezoidal rule when successive concentration values were increasing, and the log trapezoidal rule when successive concentration values were decreasing after the maximum. The area was extrapolated to infinity by addition of the value of C_t/β, where β was the terminal slope estimated by unweighted least-squares regression of the terminal linear portion of the log concentration-time curve, and C_t was the estimated concentration at the last time point calculated from the terminal relationship. The elimination half-life ($t_{½\beta}$) was calculated by 0.693/β. The mean residence time (MRT_{ni}), which is a measure of the average, or median, time [4] the parent drug molecule remains unchanged in the body, was calculated from the following equation; $MRT_{ni} = AUMC_0^\infty / AUC_0^\infty$, where $AUMC_0^\infty$ represented the total area under the first moment of the plasma concentration-time curve, and was computed in a similar fashion to the AUC_0^∞. This MRT_{ni} value for noninstantaneous input represented the sum of the median residence times for drug infusion and elimination [8]. Other model-independent parameters were calculated by the following equations:

Plasma clearance (Cl) = Dose/AUC_0^∞ and apparent volume of distribution (Vd) = Dose/β AUC_0^∞

Data recorded in the control and treated rabbits were compared by the paired *t*-test. Differences were considered significant when the probability *(P)* value was less than 0.05 by a two-tailed *t*-test.

Results

Phenobarbitone pretreatment

The mean total amsacrine concentration-time profiles after saline or PB treatment are illustrated in Fig. 2, and the individual pharmacokinetic parameters for each rabbit are given in Table 1. Pretreatment with PB resulted in a decreased AUC_0^∞ ranging from 18%–41% (mean 30%) in five of the six rabbits, but had no significant effect on $t_{½\alpha}$, $t_{½\beta}$ or MRT_{ni}. Individual increases in plasma Cl of amsacrine ranged from 25% to 70% (mean 46%) in these five rabbits, which was highly significant ($P<0.01$, 5 *d.f.*) for the whole group. A significant increase ($P<0.005$, 5 *d.f.*) in the Vd of amsacrine ranging from 25% to 117% (mean 58%) also occurred in all six rabbits after PB pretreatment. This was not associated with any plasma protein-binding changes, as the mean percent unbound amsacrine fraction (3.67% ± 0.33%) in control rabbits was not significantly different from that in PB-treated animals (mean, 3.90% ± 0.57%) according to the paired *t*-test.

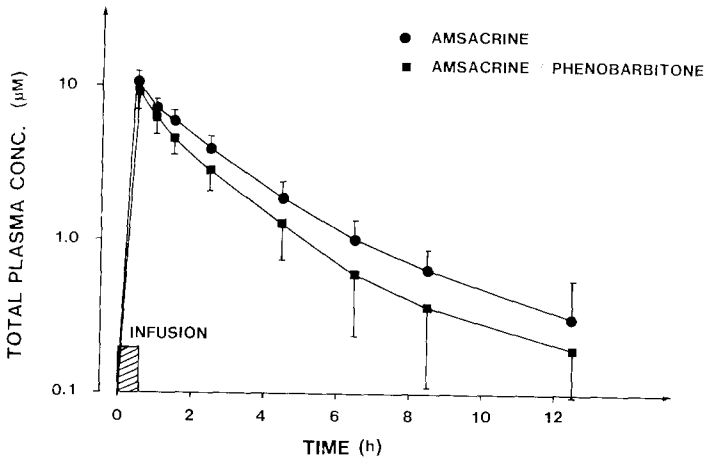

Fig. 2. The effect of PB on the postinfusion plasma amsacrine concentration-time profiles. Each *point* is the mean (±SD) for six rabbits that received saline (●) or PB (■) pretreatment

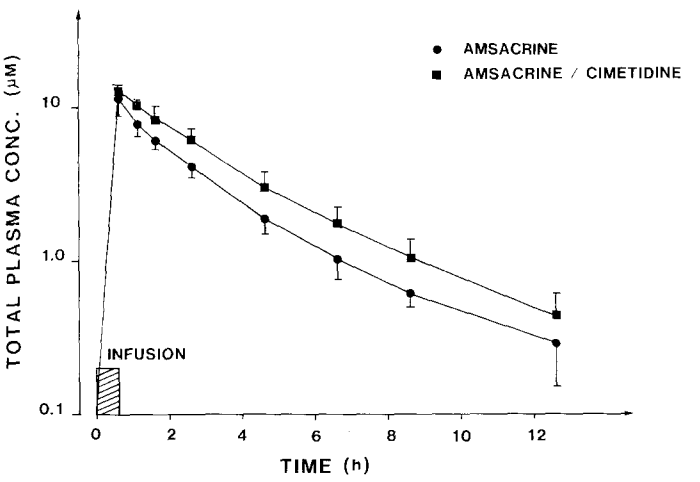

Fig. 4. The effect of CT on the postinfusion plasma amsacrine concentration-time profiles. Each *point* is the mean (±SD) for five rabbits who received saline (●) or CT pretreatment (■)

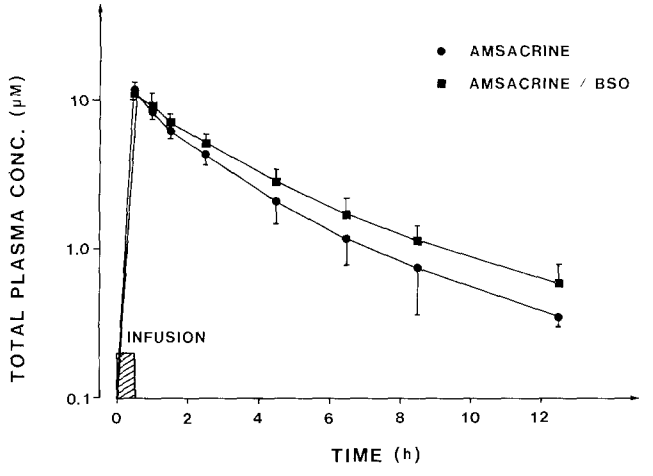

Fig. 3. The effect of BSO on the postinfusion plasma amsacrine concentration-time profiles. Each *point* is the mean (±SD) for six rabbits that received saline (●) or BSO (■) pretreatment

BSO pretreatment

The mean amsacrine plasma concentration-time profiles for control and BSO-treated rabbits are shown in Fig. 3, and individual kinetic parameters in Table 2. After BSO pretreatment all rabbits showed an increased AUC_{∞} ranging from 17% to 45% (mean 30%). This was accompanied by a significantly prolonged $t_{½\beta}$ ($P<0.05$, 5 *d.f.*) and MRT_{ni} ($P<0.005$, 5 *d.f.*), and a significantly reduced Cl, with individual reductions ranging from 15% to 30% (mean 22%). Pretreatment with BSO had no significant effect on $t_{½\alpha}$ or on the Vd of amsacrine. The GSH concentrations in rabbit red blood cells (RBC) after BSO treatment (mean 2.77 ± 1.66 μmol l^{-1}) were significantly lower ($P<0.025$, 5 *d.f.*) than before treatment (mean 4.18 ± 1.62 μmol l^{-1}), but the magnitude of the reduction differed widely between rabbits, ranging from 5% to 47%. The relationship between the percentage reduction in amsacrine Cl and the percentage reduction in RBC GSH concentration had a low linear correlation coefficient (0.4939) and was not significant.

Cimetidine pretreatment

Three rabbits from each group also received CT pretreatment, but one died before receiving the amsacrine infusion. The mean amsacrine concentration-time profiles after CT treatment in the remaining five rabbits are shown in Fig. 4 and the pharmacokinetic parameters in Table 3. The values previously obtained after the saline pretreatment were used as controls. In all five rabbits an increase in the

Table 2. Effect of BSO pretreatment on the kinetics of amsacrine in six rabbits

Rabbit	$t_{1/2\alpha}$ (h)		$t_{1/2\beta}$ (h)		MRT_{ni} (h)		Cl (l h^{-1} kg^{-1})		Vd (l kg^{-1})		AUC_{∞} (μmol l^{-1} h)	
	C	BSO	C	BSO	C	BSO	C	BSO	C	BSO	C	BSO
A21	0.10	0.49	2.88	5.85	4.73	6.21	0.31	0.25	1.42	2.16	40.6	49.7
A28	0.50	1.56	2.00	4.29	2.18	4.54	0.56	0.39	1.62	2.44	22.3	32.2
K47	0.52	0.62	2.49	2.85	3.55	3.84	0.42	0.36	1.51	1.47	30.3	35.6
B52	0.44	1.02	3.43	4.43	3.61	4.84	0.40	0.32	1.97	2.05	32.0	39.7
B55	0.50	1.10	3.65	3.75	4.12	5.04	0.39	0.30	2.06	1.63	32.5	42.0
R2	0.97	0.49	3.05	3.26	3.77	4.72	0.38	0.28	1.67	1.34	33.5	44.7
Mean	0.52	0.88	2.92	4.07	3.68	4.87	0.41	0.32	1.71	1.85	31.9	40.6
SD	0.28	0.42	0.60	1.06	0.81	0.78	0.08	0.05	0.25	0.43	5.9	6.3
Paired *t*-test	NS		$P=0.033$		$P=0.005$		$P=0.001$		NS		$P<=0.001$	

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

Table 3. Effect of CT pretreatment on the kinetics of amsacrine in five rabbits

Rabbit	$t_{1/2\alpha}$ (h)		$t_{1/2\beta}$ (h)		MRT_{ni} (h)		Cl (l h^{-1} kg^{-1})		Vd (l kg^{-1})		AUC_0^∞ (μmol l^{-1} h)	
	C	CT	C	CT	C	CT	C	CT	C	CT	C	CT
B52	0.44	1.07	3.43	2.88	3.61	3.64	0.40	0.29	1.97	1.20	32.0	43.8
B55	0.50	0.78	3.65	2.91	4.12	4.08	0.39	0.24	2.06	1.03	32.5	52.1
A36	1.04	0.59	2.35	2.61	3.23	3.32	0.62	0.40	2.12	1.49	20.3	32.6
A64	0.57	0.91	2.40	2.84	3.23	3.78	0.42	0.33	1.27	1.37	30.4	38.3
A43	0.14	1.14	2.24	3.63	3.23	4.59	0.39	0.26	1.45	1.36	32.2	48.6
Mean	0.54	0.90	2.81	2.97	3.48	3.89	0.44	0.30	1.77	1.29	29.48	43.1
SD	0.32	0.22	0.67	0.39	0.39	0.48	0.10	0.064	0.39	0.18	5.20	7.83
Paired *t*-test	NS		NS		NS		$P=0.002$		NS		$P=0.002$	

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

AUC_0^∞ was observed, ranging from 26% to 61% (mean 47%), after CT pretreatment. There was no significant change in any of the time parameters (i.e. $t_{½\alpha}$, $t_{½\beta}$ and MRT_{ni}), but the Cl was significantly reduced, ranging from 21% to 38% (mean 33%) in individual rabbits. The mean Vd also decreased, but this was not significant at the 5% level.

Discussion

The effects of the compounds studied on the total-body clearance of amsacrine in the rabbit will depend on which is the rate-limiting step in the sequential pathway proposed by Shoemaker et al. [34]. As the liver has a high content of GSH (5–10 mmol l^{-1}) and GSH-S-transferase [17], the oxidation step appears more likely to be rate-limiting. We have previously shown that amsacrine undergoes low hepatic extraction in the rabbit [27], and thus it might be expected that PB would increase amsacrine's hepatic clearance due to induction of a biotransformation pathway rather than any effect on blood flow to the liver. However, reservations on the exact mechanism involved in this interaction must be retained, as the effects of PB treatment are many. The inducing effect is not confined to hepatic cytochrome P-450 but has been reported for a number of enzymes, including α-glutamyl and glucuronyl transferases and cytoplasmic glutathione *S*-transferase [9, 37]. In addition, PB has been reported to increase liver mass, liver blood flow, bile flow and biliary excretion [22] and also the protein binding of some drugs [31]. This last mechanism can be disregarded, as no significant change of amsacrine binding was observed between controls and PB-treated animals in our study. Overall consideration of the effects of PB and CT on amsacrine elimination suggests that the hepatic mixed function oxidase system is involved and may be the rate-limiting step in amsacrine's clearance. One could also speculate that the opposite effects of PB and CT on amsacrine's volume of distribution might be due to changes in blood flow caused by these agents, as cimetidine and other H_2-receptor antagonists have been reported to cause reductions in blood flow [6, 7].

If oxidation of amsacrine to *m*-AQDI is the rate-limiting step in the clearance of amsacrine, it might be expected that depletion of GSH and reduction in the rate of conjugation of *m*-AQDI with GSH would have no effect on amsacrine's clearance. However, we observed a significant reduction in clearance after BSO treatment. The most likely explanation is an indirect effect, as there is evidence that GSH depletion results in increased hepatic lipid peroxidation, a major consequence of which is inhibition of cytochrome P-450-catalysed reactions [23]. Other explanations, such as feedback inhibition of the oxidation process by *m*-AQDI or back-reduction of *m*-AQDI to amsacrine with NADPH, are possible, but accumulation of *m*-AQDI within the cell appears unlikely as *m*-AQDI is very reactive and would conjugate rapidly with nucleophilic groups on proteins to give covalently bound adducts [34]. No matter which mechanism is involved, our results do suggest that glutathione status plays an important part in the overall elimination of amsacrine in the rabbit, and if a similar mechanism occurs in man this raises some interesting possibilities. For instance, in our study of amsacrine kinetics in leukaemia patients [16], one could speculate that the reduction in amsacrine clearance and prolongation of elimination half-life observed after the third of three daily infusions might have been due to gradual GSH depletion over this period. Decreases of liver GSH content have also been reported to occur with ageing in mice [12, 36] and may provide an explanation for the lower clearances of amsacrine observed in our older patients. In addition, in cancer patients the situation might be further aggravated by the markedly lowered plasma GSH levels which have been reported to occur in a variety of malignant disorders, including leukaemia [2]. Obviously further studies on the GSH status of cancer patients before and after amsacrine treatment are warranted. This raises the intriguing possibility of using a compound such as *N*-acetylcysteine as an antidote to amsacrine toxicity, as is done with paracetamol poisoning [25, 30]. Paracetamol toxification has been shown to result in depletion of liver GSH and hepatoxicity [35]. Hepatoxicity, fatal in some cases, has also been associated with amsacrine [1, 20, 39], and it may be that patients susceptible to amsacrine's hepatotoxic effects have excessively low GSH levels and might benefit from concomitant treatment with an agent which would raise their GSH levels.

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