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The effect of food on the bioavailability and kinetics of the anticancer drug amsacrine and a new analogue, *N*-5-dimethyl-9-[(2-methoxy-4-methylsulphonylamino)phenylamino]-4 acridinecarboxamide in rabbits

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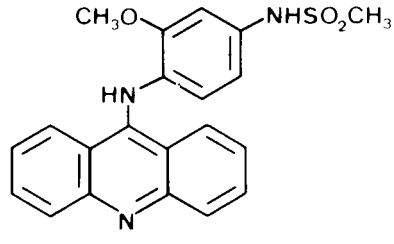
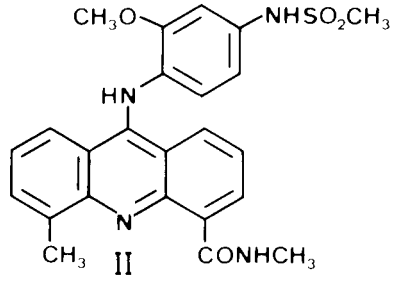
Both amsacrine and its analogue, *N*-5-dimethyl-9-[(2-methoxy-4-methylsulphonylamino)phenylamino]-4-acridinecarboxamide (CI-921) are absorbed from the gastrointestinal tract in rabbits. The mean bioavailability for amsacrine was 50% ± 17 (s.d.) in non-fasting animals, and was significantly increased in fasting animals (mean, 90% ± 10). The bioavailability for CI-921 (mean, 26% ± 11) in the non-fasting animal was significantly less than that found for amsacrine, but this difference disappeared in the fasting animal when the bioavailability of CI-921 was significantly increased to 69% ± 23. Oral administration of both agents resulted in significantly prolonged elimination half-lives and mean residence times compared to the i.v. infusion, but no significant difference was observed in these parameters between the fasting and non-fasting state. This study suggests that oral dosing may be a possible alternative route for the administration of these anticancer agents.

Amsacrine (I) is an effective drug for the treatment of leukaemia (Jacquillat 1983; Legha et al 1982) and certain lymphomas (Warrel et al 1980), but has little effect against solid tumours in man (Bukowski et al 1982; Drelichman et al 1982). A new analogue, *N*-5-dimethyl-9-[2-methoxy-4-methylsulphonylamino)phenylamino]-4-acridinecarboxamide (CI-921) (II) has been synthesized which has significantly greater activity in solid tumour test systems (Baguley et al 1984). Physicochemical studies of it indicated an approximate 3-fold increase in lipophilicity and a decreased acridine base strength (pK_a 6·40) compared with amsacrine (pK_a 7·43) (Baguley et al 1984). The in-vivo experimental antitumour results in mice indicated enhanced activity of CI-921 compared with amascrine after oral dosing (Baguley et al 1984). We wished to ascertain whether this was due to an increase in the absolute bioavailability of CI-921 compared with amsacrine. Amsacrine has been used orally to treat patients, with a reported bioavailability of 32% (De Jager et al 1980). Apart from this abstract, there are no published data on the oral bioavailability of amsacrine or CI-921 either in animals or in man.



I



II

Methods and materials

A balanced crossover study was undertaken in 12 NZ white rabbits (6 for each compound) maintained on commercial rabbit pellets and water. One month was allowed between the crossover, which has previously been shown to be an adequate recovery period (Paxton & Jurlina 1985). After an additional month, the oral administration was repeated in the same rabbits, who were fasted for the 24 h before drug administration and up to 8 h post administration.

Amsacrine as the free base in *N,N*-dimethylacetamide (from Parke-Davis/Warner-Lambert, NZ Ltd.) or CI-921 as the isethionate salt (from Dr B. Baguley, Cancer Research Labs, University of Auckland School of Medicine) were diluted with 5% dextrose solution, and 12·7 μmol kg^{-1} administered to each rabbit, either by infusion into a marginal ear vein (over 35 min), or by administration into the stomach via a stainless steel applicator. Venous blood (3 ml) was collected from the opposite ear into heparinized tubes at 0, 0·5, 1, 2, 4, 6, 8 and 12 h post infusion and 0·25, 0·5, 1·0, 1·5, 2, 4, 6, 8, and 12 h post oral dose. Total plasma amsacrine and CI-921 concentrations were determined in duplicate 0·5 ml aliquots by our reported HPLC methods (Jurlina & Paxton 1983, 1985). These assays have good accuracy over the range

0·5–10 μmol litre^{-1} with recoveries ranging from 99–115%, and excellent precision with mean values for intra- and inter-assay coefficients of variation less than 5%.

Peak concentration (C_{max}) and time to peak concentration (t_{max}) were read from individual concentration-time plots. The area under the concentration-time curve (AUC $^{\infty}$) was computed using the trapezoidal rule and extrapolated to infinity by addition of the value of C_t/β; where β was the slope of the terminal linear portion of the log concentration-time curve estimated by unweighted least squares regression, and C_t was the estimated concentration at the last time point calculated from the terminal relationship. The elimination half-life ($t_{\frac{1}{2}\beta}$) was calculated by 0·693/β. The mean residence time (MRT_{ni}) was calculated from the following equation; MRT_{ni} = AUMC$^{\infty}$/AUC$^{\infty}$, where AUMC$^{\infty}$ represented the total area under the first moment of the plasma concentration-time curve, and was computed in a similar fashion to the AUC$^{\infty}$. This MRT_{ni} value for a non-instantaneous input represented the sum of the median residence times for drug infusion (or absorption) and elimination (Gibaldi & Perrier 1982). The bioavailability was calculated from ratio of the AUC$^{\infty}$ after oral administration compared with i.v. administration, and expressed as a percentage. Comparisons between data groups were made using the Mann-Whitney U-test for unpaired data and the Wilcoxon signed-ranks test for paired values, with a *P*-value less than 0·05 being regarded as significant on a two-tailed test.

Results

Amsacrine. The mean concentration-time profiles after i.v. infusion, and oral administration in the non-fasting and fasting state are illustrated in Fig. 1. After oral administration to non-fasting animals, the concentration-time profiles were variable with a C_{max} ranging from 0·7–2·6 μmol litre^{-1} and t_{max} from 0·25–8 h (Table 1). The mean absolute bioavailability in non-fasting rabbits for amsacrine was 50% with a range from 30–80%. On fasting, all five rabbits showed an

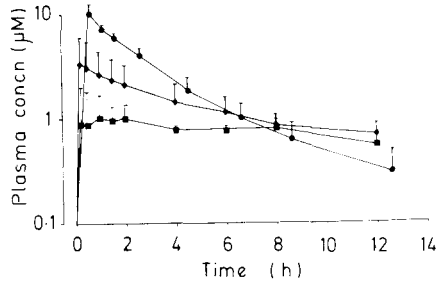


FIG. 1. Mean total plasma concentrations (+ s.d.) of amsacrine (12·7 μmol kg^{-1}) after a 35 min i.v. infusion to non-fasting rabbits (●), and after oral administration to the same non-fasting (■) and fasting rabbits (◆). In the latter group n = 5, but otherwise n = 6.

Table 1. Mean ± s.d. (range) of parameters after i.v. and p.o. administration of amsacrine to 6 rabbits.

Parameter	i.v. non-fasting	p.o. non-fasting	p.o.[b] fasting
C_{max} (μmol litre^{-1})	10·3 ± 2·1 (7·0–13·1)	1·4 ± 0·7 (0·7–2·6)	3·5 ± 2·5 (0·7–6·9)
t_{max}	0·58 (EI)[a]	2·75 ± 2·92 (0·25–8·0)	1·25 ± 1·63 (0·25–4·0)
$t_{\frac{1}{2}\beta}$ (h)	2·91 ± 0·92 (2·01–4·20)	7·04 ± 2·57 (3·94–10·47)	9·44 ± 4·18 (6·44–15·92)
MRT_{ni} (h)	3·60 ± 0·75 (2·67–4·87)	11·44 ± 3·70 (8·27–16·74)	12·08 ± 7·91 (7·17–23·90)
AUC$^{\infty}$ (μmol h litre^{-1})	27·7 ± 6·0 (20·3–34·1)	13·7 ± 6·3 (9·5–26·3)	25·6 ± 6·5 (17·8–33·9)
Bioavailability (%)	100	50 ± 17 (30–80)	90 ± 10 (77–100)

[a] EI = end of infusion; [b] n = 5 in this group.

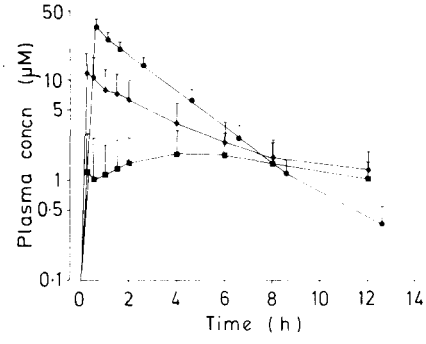


FIG. 2. Mean total plasma concentrations (+ s.d.) of CI-921 (12·7 μmol kg^{-1}) after a 35 min i.v. infusion to non-fasting rabbits (●), and after oral administration to the same rabbits non-fasting (■) and fasting (◆) (n = 6).

increase in AUC$^{\infty}$ and increased bioavailability with a mean value of 90%, which was significant ($P < 0·05$, 4 d.f., Wilcoxon matched-pairs test). An increase in the mean C_{max} and a decrease in the mean t_{max} were also observed in fasting animals, but neither was significant at the 5% level. Oral administration significantly ($P < 0·05$, d.f., Wilcoxon matched-pairs test) prolonged the $t_{\frac{1}{2}\beta}$ and the MRT_{ni} of amsacrine compared with i.v. infusion, but there was no significant difference in either of these parameters in fasting or non-fasting animals.

CI-921. The mean concentration-time profiles after i.v. infusion of CI-921, and oral administration to non-fasting and fasting rabbits are illustrated in Fig. 2. As with amsacrine, concentration-time profiles of CI-921 were variable after oral dosing. In non-fasting animals the C_{max} ranged from 0·5–4·9 μmol litre^{-1} and t_{max} from 0·25–6 h, with a low absolute bioavailability (mean, 26%; range 8–40%) (Table 2). On fasting, the AUC$^{\infty}$ increased in all 6 rabbits giving a mean absolute bioavailability of 69% (range 38–100%), which was significant ($P < 0·05$, 5 d.f., Wilcoxon matched-pairs test). Fasting also significantly increased ($P < 0·05$,

Table 2. Mean ± s.d. (range) of parameters after i.v. and p.o. administration of CI-921 to 6 rabbits.

Parameter	i.v. non-fasting	p.o. non-fasting	p.o. fasting
C_{max} (μmol litre^{-1})	34·4 ± 7·2 (29·5–48·1)	2·8 ± 1·7 (0·5–4·9)	12·0 ± 6·7 (3·7–20·5)
t_{max} (h)	0·58 (EI)[a]	2·79 ± 2·87 (0·25–6)	0·25(FS)[a]
$t_{\frac{1}{2}\beta}$ (h)	2·00 ± 0·60 (1·43–3·17)	6·71 ± 5·4 (2·96–17·7)	7·53 ± 3·8 (4·08–12·99)
MRT_{ni} (h)	2·69 ± 0·36 (2·27–3·14)	11·55 ± 7·50 (4·95–26·28)	9·27 ± 4·00 (4·05–14·02)
AUC^{∞} (μmol h litre^{-1})	83·9 ± 23·6 (56·6–124·3)	20·4 ± 6·6 (9·8–30·9)	56·7 ± 20·8 (24·8–66·6)
Bioavailability (%)	100	26 ± 11 (8–40)	69 ± 23 (38–100)

[a] EI = end of infusion, FS = first sample.

5 d.f., Wilcoxon matched pairs test) the C_{max}, which occurred in the first sample (at 15 min) in all six rabbits. As with amsacrine, oral administration of CI-921 significantly prolonged the $t_{\frac{1}{2}\beta}$ and MRT_{ni} compared with the i.v. infusion, but no significant difference in these parameters was observed between the fasting and non-fasting state.

Comparison of the absolute bioavailabilities of amsacrine and CI-921 by the Mann-Whitney U-test indicated that bioavailability of CI-921 was significantly lower than amasacrine in non-fasting rabbits ($P < 0.05$, 5 d.f.), but this difference disappeared when the rabbits were fasted.

Toxicity. Three rabbits died during this study. All were from the group receiving amsacrine. One death occurred one month after oral amsacrine dosing to the non-fasting animal and did not appear to be drug-related. This death reduced the group to five rabbits for the fasting study. A further two rabbits died at 4 and 5 days after oral amsacrine dosing to the fasting group. A post-mortem revealed a large stomach ulcer in one, but cause of death in either was not obvious.

Discussion

These results suggest that the apparently enhanced activity of CI-921 compared with amsacrine by the oral route in mice (Baguley et al 1984) was not due to greater bioavailability. Although CI-921 is three-fold more lipophilic than amsacrine and a much weaker base, and might be expected to be absorbed at a greater rate and extent than amsacrine, this was not found to be the case, particularly in non-fasting animals. There are several possible explanations for the low and variable bioavailability observed for both agents in non-fasting animals. Significant first-pass hepatic metabolism might occur. However, this appears unlikely as both agents undergo low hepatic extraction (Paxton & Jurlina 1986). The lack of significant first-pass extraction is supported by the high bioavailabilities observed for both amsacrine and CI-921 in fasting animals. Similarly, these high bioavailabilities suggest that breakdown of amsacrine or CI-921 due to the acid environment of the stomach or intestine is unlikely. Stability studies have also shown that amsacrine is most stable in its protonated form, i.e. in an acid environment (Paxton et al 1986) and no detectable breakdown of amsacrine was observed on incubation at 37 °C in buffer pH 7·4 for 8 h. The most likely explanation for the low and variable bioavailability of these agents in non-fasting animals is binding to food particles within the gastrointestinal tract. In a crude experiment, amsacrine or CI-921 (at concentrations equivalent to those administered to rabbits), was incubated for 0·5 h with agitation in dilute HCl solution (pH 3·0) in the presence of a rabbit food pellet (66 mg ml^{-1}). In the presence of the food pellet there was an 80 and 99% reduction in the concentrations of amsacrine and CI-921, respectively. Thus, the greater absorption of CI-921 on to food particles, may explain the significantly lower bioavailability observed for CI-921 in non-fasting animals, which disappeared with fasting. On fasting the bioavailability of both compounds increased, but overall the mean bioavailability of CI-921 remained less than amsacrine, although this was not significant. Perhaps binding to endogenous gastrointestinal substances by CI-921 may play some part in this. Previously we have shown that CI-921 binds to plasma proteins to a greater extent than amsacrine (Paxton et al 1986).

Our results indicate that both drugs are absorbed from the gastrointestinal tract to an acceptable degree, and that oral dosing may be a possible alternative route of administration. Some advantages may be attached to this route due to the prolongation of the elimination half-life and mean residence time of the drug, thus maintaining lower concentrations for longer times. There is considerable evidence of increased antitumour efficacy and decreased toxicity, associated with a more prolonged exposure of the cancer cell to lower concentrations of the antitumour drug, compared with the short-lived high concentrations achieved after conventional i.v. bolus administrations (Carlson & Sikic 1983). However, we found the oral administration of amsacrine to be associated with greater toxicity than the i.v. infusion so further toxicity studies after the oral administration of these agents would be needed.

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The pharmacokinetics of pyrimethamine in the rat: effect of mefloquine

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The pharmacokinetics and tissue distribution of pyrimethamine have been determined in the rat. Following administration of pyrimethamine alone, drug concentrations declined biexponentially. By contrast, in the presence of mefloquine, the decline in pyrimethamine concentration more closely fitted a monoexponential pattern and the AUC_{0-6h} for pyrimethamine was significantly reduced. Significantly more pyrimethamine was recovered from the livers but less from the lungs of the mefloquine-dosed rats compared with control. This study outlines a potentially clinically relevant drug interaction.

Pyrimethamine, in combination with a sulphonamide or sulphone has been widely used in the suppression and treatment of chloroquine-resistant strains of *Plasmodium falciparum* malaria (Leimer 1981). Pyrimethamine/sulphadoxine (Fansidar) has recently been used together with the promising 4-quinoline methanol, mefloquine, in the preparation Fansimef (250 mg mefloquine, 25 mg pyrimethamine, 500 mg sulphadoxine), which is aimed at delaying the emergence of plasmodial resistance to mefloquine (Kofi-Ekue et al 1985). However a number of unexplained treatment failures (WHO 1983) and a lack of published pharmacokinetic data have been associated with this triple combination. Therefore in the present report, we wished to determine the effect of mefloquine on the disposition of pyrimethamine in the whole rat.

Methods

Male Wistar rats (200–250 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) administered intraperitoneally. The left jugular vein, and right carotid artery were exposed and cannulated with polythene tubing (Portex, Hythe, Kent). The trachea was also exposed and cannulated to assist breathing, and heparin sodium was administered (400 units kg^{-1} i.v.). To a first group of animals (n = 5), pyrimethamine (2 mg kg^{-1}) was administered intraperitoneally (i.p.) dissolved in Hartmann's solution (Travenol Laboratories, Thetford, Norfolk) while a second animal group (n = 5) received pyrimethamine (2 mg kg^{-1} i.p.) concurrently with mefloquine (20 mg kg^{-1}, in Hartmann's solution, i.p.). Blood samples (150 µl) were removed from the carotid artery pre dose, then at 15, 30, 60, 120, 180, 240, 300 and 360 min. After centrifugation (1100*g*, 2 min) the plasma was removed and stored at −20 °C before assay for pyrimethamine and its 3-*N*-oxide metabolite by HPLC (Coleman et al 1984). This method was found to be free from chromatographic interference by mefloquine. An equal volume of heparinized saline was then administered via the jugular vein to replace blood volume removed by sampling. At the conclusion of each experiment, the animals were killed and the liver, kidneys, spleen and lungs removed and weighed. The soft organs were each homogenized in three times their weight of 1/15 molar phosphate buffer using a Teflon-in-glass homogenizer. The resulting 25% homogenates were then stored at −20 °C. Before assay for pyrimethamine and pyrimethamine 3-*N*-oxide by HPLC, standard curves for each compound were prepared in blank homogenate for each tissue.

Pharmacokinetic parameters for pyrimethamine, were calculated as previously described (Coleman et al 1985). However, the volume of distribution in the case of a biexponential decline was calculated with the formula $Vd = Dose/(AUC \times \beta)$. In the present study,

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