J. Pharm. Pharmacol. 1986, 38: 432–438
Received November 11, 1985

© 1986 J. Pharm. Pharmacol.

The binding of amsacrine to human plasma proteins

J. W. PAXTON*, J. L. JURLINA† AND S. E. FOOTE

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

Determination of amsacrine plasma protein binding by both equilibrium dialysis and ultracentrifugation gave similar results and indicated that amsacrine is highly bound (approximately 97%) in human plasma. This binding is independent of amsacrine concentration over the range 1–100 μmol litre^{-1}, but is very sensitive to plasma pH and, to a lesser extent, to temperature. Approximately 20% of the drug appeared to be covalently bound to plasma proteins. Amsacrine was bound by all plasma proteins investigated including albumin, α_1-acid glycoprotein and various γ-globulins. The binding to albumin appeared to occur by two processes, a saturable process at a single site with a K_D of 13·9 μmol litre^{-1} and a non-saturable process. Despite differences in individual protein concentrations, no significant difference was observed in the unbound amsacrine fraction in plasma from patients receiving this drug for treatment of acute myelogenous leukaemia and plasma from healthy individuals.

Amsacrine, 4′-(9-acridinylamino)methane-sulphon-*m*-anisidide, is a new anticancer drug which has significant activity against acute leukaemia in adults (Bodey & Jacquillat 1983) but very little is known about its binding to blood proteins apart from a brief report that approximately 95% is bound to human serum (Luc et al 1981). There is also evidence that amsacrine may form covalent adducts with plasma proteins as the result of nucleophilic attack at the C-9 position of amsacrine by protein thiol groups (Wilson et al 1977). The aim of this study was to investigate the binding of amsacrine to human plasma proteins.

MATERIALS AND METHODS

Materials

Pure amsacrine as the isethionate salt was supplied by Dr B. Baguley, Cancer Research Laboratory, University of Auckland School of Medicine. Amsacrine labelled at the 9-position of the acridine nucleus with ^{14}C (sp. act. 19·6 mCi mmol^{-1}) was a kind gift from Dr M. A. Leaffer, SRI International, Menlo Park, California. High performance liquid chromatography (HPLC) of this radiolabel indicated a secondary peak eluting after amsacrine which did not correspond to any of the oxidation products of amsacrine (Jurlina & Paxton 1983), and did not appear to be associated with any radioactivity. No inhomogeneity could be detected by thin layer chromatography using two different solvent systems ($CHCl_3$–MeOH, 8:2 and n-BuOH–AcOH:H_2O, 4:1:1). Subsequent autoradiography following TLC indicated that 99% of the radioactivity was located in one spot.

Two types of dialysis membranes were used: seamless cellulose tubing (Union Carbide Corp., New York) and Spectrapor 2 sheets with a molecular weight cut-off 10 000 (Spectrum Medical Industries, Los Angeles, USA). Before use, each membrane was soaked in distilled water at 80 °C for 30 min with frequent agitation. After rinsing twice with distilled water, the membrane was soaked in buffer for a minimum of 30 min before use.

Isotonic phosphate buffer (Ehrnebo & Odar-Cederlof 1977) adjusted to pH 7·4 was used for the dialysis experiments and all dilutions. All reagents were of analytical grade.

Two preparations of human serum albumin (HSA), one essentially fatty acid free (EFAF) and the other essentially globulin free (EGF), human α_1-acid glycoprotein (AAG) and various human globulin fractions were purchased from the Sigma Chemical Co., St Louis, MO, USA.

Most binding experiments were performed on pooled blood bank plasma which contained up to 10% of citrate, phosphate and dextrose solution as anticoagulant. Blood samples from healthy volunteers, and patients receiving amsacrine for treatment of acute myelogenous leukaemia (AML), were collected by venepuncture or by catheter into heparinized glass tubes (Venoject, Terumo Corp., Japan). Plasma was separated by centrifugation for 10 min at 2000 rev min^{-1} and 10 °C and stored at −20 °C.

Plasma concentrations of albumin and AAG

* Correspondence.
† Present address: Cancer Research Laboratory, Auckland Medical School, Auckland, New Zealand.

before and after dialysis were measured by radial immunodiffusion using Kallestad Quantiplates and Behringwerke M-Partigen plates, respectively.

Protein binding methods

Equilibrium dialysis. A Dianorm dialysis apparatus (Diachema, Zürich, Switzerland) was used, which allowed the simultaneous dialysis of 20 samples. Each dialysis cell consisted of two 1 ml Teflon chambers separated by a dialysis membrane. Plasma or protein solution was pipetted into one chamber and isotonic phosphate buffer into the other. [^{14}C]Amsacrine (1 μmol litre^{-1}) was added to either the plasma or the buffer. Dialysis was performed by rotating the dialysis cells at 20 rev min^{-1} in a water bath at 37 °C for 2–4 h. Unless otherwise stated, all plasma was titrated to pH 7·4 with CO_2 before dialysis.

After dialysis, duplicate 250 μl aliquots were removed from both chambers of the cell, added to counting vials along with 10 ml scintillation fluid, and counted until accumulation of 10^4 counts. Similar aliquots from the original buffer or plasma solution containing the labelled drug before dialysis were also counted. Quench corrections were undertaken by the external standard channels ratio method. Unbound fraction (fu′) was calculated as D_B/D_P, where D_B and D_P are the disintegrations (d) min^{-1} ml^{-1} in buffer and plasma after dialysis, respectively. Adsorption to the membrane or apparatus does not affect this calculation as sampling from both sides of the membrane was used. However, this equation gave an over-estimation of the unbound fraction due to the dilution of the bound drug concentration with the shift of fluid from the buffer to the plasma side of the membrane under osmotic pressure (Tozer et al 1983). The extent of dilution of the plasma was determined by measurement of albumin (or AAG) concentrations before and after dialysis, and fu′ corrected for volume changes by the following equation (Huang 1983):

$$fu = fu' R/(fu' R + 1 - fu')$$

where R is the ratio of plasma volume before and after dialysis calculated from protein concentrations. There was no apparent loss of albumin due to adsorption to the membrane or to the Teflon cells.

Ultracentrifugation. Ultracentrifugation experiments were undertaken in a Superseed 75 ultracentrifuge (MSE) at 40 000 rev min^{-1} (100 000*g*) for 16 h using polycarbonate tubes in a titanium 10 × 10 ml angle rotor (Kurz et al 1977). After centrifugation of plasma, five distinct layers were observed; a top layer of floating lipoproteins, followed by a protein-free water phase and then three distinct protein fractions. The lowest number of d min^{-1} were found in the protein-free water phase. The unbound fraction was calculated by the ratio of d min^{-1} ml^{-1} in the protein-free water phase to those in the original plasma incubated in a similar centrifuge tube over the same period of time, but without centrifugation. An empirical correction was applied to take account of any sedimentation of free drug. This was done by subjecting two protein-free isotonic phosphate buffer solutions containing 1 μmol litre^{-1} [^{14}C]amsacrine, to the same ultracentrifugation procedure, and comparing the d min^{-1} ml^{-1} at various depths in the centrifuge tubes with an equivalent non-centrifuged tube. These control experiments indicated that the concentration of free amsacrine declined to 86·2% (s.d. = 0·8, n = 4) of its initial value on centrifugation. The unbound fraction was corrected for this decrease and was determined for each run. This ultracentrifugation method was compared with equilibrium dialysis, and was also used to examine the effect of plasma pH on amsacrine binding.

HPLC method

Absolute concentrations of extractable amsacrine in plasma and buffer after equilibrium dialysis were measured by an HPLC method after extraction with ether (Jurlina & Paxton 1983). With 0·5 ml samples, amsacrine concentrations as low as 0·1 μmol litre^{-1} can be determined with a CV <3%. This method is specific for amsacrine with no recorded interference by any metabolites or breakdown products.

RESULTS

Methodological aspects of equilibrium dialysis

Dialysis equilibrium appeared to be established relatively swiftly with plasma containing [^{14}C]amsacrine. No significant difference in fu was apparent after 1, 2, 4 and 8 h dialysis. This was supported by the observation of equivalent d min^{-1} ml^{-1} on each side of the membrane after dialysis of buffer containing radiolabel against drug-free buffer. Addition of [^{14}C]amsacrine to buffer and dialysis against drug-free plasma gave a small significant difference ($P <$ 0·002, 8 d.f.) between the % fu determined after 1 h (4·48 ± 0·20% s.d.) compared with 2 h (3·50 ± 0·23%), but no difference between determinations at 2, 4 or 8 h. From 2 h onwards, addition of the label to either plasma or buffer side gave values for fu which

were not significantly different at the 5% level. As better precision was achieved after the 4 h dialysis, most dialyses were carried out over this period. No significant difference was observed between % fu determined by equilibrium dialysis using Spectrapor 2 membranes (3·32 ± 0·14% s.d., n = 4) compared with Union Carbide cellulose tubing (3·52 ± 0·29%, n = 4). The latter membrane was used for subsequent studies as it was less expensive and more readily available.

When whole plasma was used for dialysis, definite volume changes occurred with transfer of fluid across the membrane from the buffer to the plasma. Before dialysis the plasma albumin concentration was 35·4 ± 1·5 g litre^{-1} (n = 6), falling to 27·9 ± 0·5 (n = 8) after 4 h dialysis. This represented a mean plasma volume increase of 26%. This reduction in albumin concentration was not due to adsorption to the membrane or apparatus, as no significant reduction in albumin concentration was observed in a control set of cells containing plasma on both sides of the membrane. For this reason, calculation of a correction factor for the percent unbound amsacrine was necessary from protein determinations before and after dialysis, to account for this dilution of the plasma.

No significant loss of the radiolabel to the membrane or the cell was apparent after 4 h dialysis of plasma against buffer. Regular recoveries of [^{14}C]amsacrine added to the system ranged from 97·5–104·6% with a mean value of 99·3%.

Further evidence supporting this was provided by the lack of any significant difference in the % fu in patients' plasma (n = 11) calculated by our method (3·08 ± 0·62%) and by the method proposed by Tozer et al (1983) (3·04 ± 0·62%) which assumes no loss of radioactive drug to the membrane or apparatus. In this latter method, the unbound fraction was calculated by the equation, fu = $D_B/(D'_P - D_B)$, where D_B and D'_P represent d min^{-1} ml^{-1} in the buffer after and in plasma before dialysis, respectively.

Using plasma containing 20 μmol litre^{-1} amsacrine and buffer containing 1 μmol litre^{-1} [^{14}C]amsacrine, the % fu fraction was determined after 4 h equilibrium dialysis by radioactivity counting, and also by measurement of the absolute concentration of amsacrine in the plasma and buffer by HPLC. Mean values of 4·10 ± 0·25 (n = 9) and 3·73 ± 0·26% (n = 9) were recorded by each method, which, although significantly different by *t*-test, were within 10% of each other, indicating that the radiolabel was relatively stable over the dialysis period, and that the labelled and unlabelled drug exhibited similar binding properties.

Temperature

The effect of temperature on amsacrine binding in plasma was examined at pH 7·43 after dialysis at 20, 25, 30 and 37 °C. Mean % fu (±s.d.) recorded from eight cells for each temperature was 3·54 ± 0·28, 3·39 ± 0·17, 4·55 ± 0·16 and 4·59 ± 0·22%, respectively, indicating a small reduction in binding with increasing temperature.

Ultracentrifugation

The % fu in plasma obtained by ultracentrifugation (4·72 ± 0·37%, n = 8) and equilibrium dialysis (4·74 ± 0·32%, n = 5) did not differ significantly. The ultracentrifugation experiment did indicate that a small amount of the [^{14}C]amsacrine (6·8 ± 0·48%, n = 8) was bound to the floating, very low-density lipoprotein layer. This method was used to examine the effect of plasma pH on amsacrine binding. The % fu was smallest (0·74%) at the highest pH 9·47, and increased to 3·07% at pH 8·30, 6·69% at pH 7·39 and 21·72% at pH 6·40.

Amsacrine concentration in plasma

The effect of amsacrine concentration on its binding by whole plasma, as determined by equilibrium dialysis, is shown in Fig. 1. Over the range 1–100 μmol litre^{-1}, there was a very small reduction in binding from 97%–96% but no obvious saturation of binding sites. Higher concentrations of amsacrine

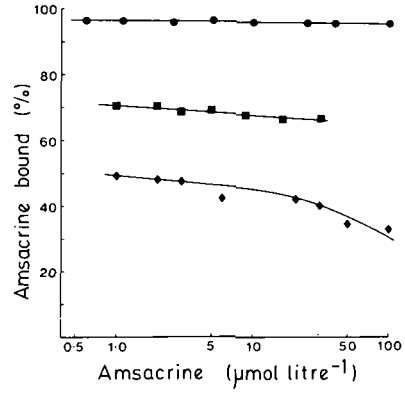

FIG. 1. The effect of amsacrine concentrations on its binding by whole plasma (●), and plasma diluted with buffer to 10% (■) and 2% (◆) solution. Each point is the mean of duplicate determinations.

were not possible due to solubility problems. Dilution of plasma with buffer to 10 and 2% of original concentration resulted in less binding with some increased displacement (Fig. 1). Serial dilutions of plasma indicated a sigmoid relationship between the percent bound and log of protein concentration (Fig. 2).

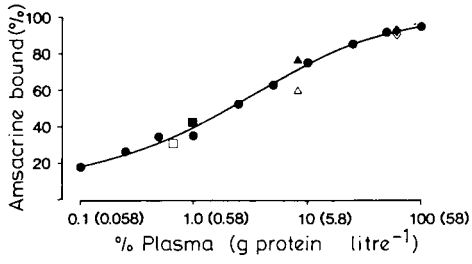

FIG. 2. The effect of protein concentration on the binding of amsacrine. The symbols represent whole plasma and dilutions (●), albumin (EFAF) 36 g litre^{-1} (◆), albumin (EGF) 36 g litre^{-1} (◇), globulin fraction IV-1 (5 g litre^{-1}) (▲), globulin fraction IV-4 (5 g litre^{-1}) (△), AAG at 0·6 g litre^{-1} (■) and 0·39 litre^{-1} (□). Each point is the mean of duplicate determinations.

Individual proteins

Amsacrine was bound by all the human plasma proteins investigated (Table 1). The magnitude of the binding appeared to be mainly dependent on the protein concentration, which was evident from plotting the percent bound against the log concentration of each protein (Fig. 2). However, some variation in binding between proteins does exist, as illustrated by the differences in binding between the same concentrations of different globulin fractions.

Table 1. Percent amsacrine bound by blood bank plasma and some human plasma proteins.

	% Bound	n	CV
Blood bank plasma*	95·3	5	0·3
Albumin (EFAF) [36 g litre^{-1}]	91·8	4	0·5
Albumin (EGF) [36 g litre^{-1}]	90·3	4	0·4
AAG [0·39 g litre^{-1}]	30·6	5	4·6
Globulins [5 g litre^{-1}] fraction IV-1	77·1	5	9·7
Globulins [5 g litre^{-1}] fraction IV-4	59·1	5	3·6

n = No. of determinations.
* Concentrations of the following proteins in blood bank plasma were 58 g litre^{-1} (total protein), 36 g litre^{-1} (albumin) and 0·39 g litre^{-1} (AAG).

A further investigation by equilibrium dialysis of the binding of amsacrine to human serum albumin (10·9 μmol litre^{-1}, 0·72 g litre^{-1}) was carried out over the range 1–87 μmol litre^{-1} (end of dialysis amsacrine concentration). The percent bound decreased from 48·4 to 26·5% with increasing amsacrine concentration. Fig. 3 illustrates the relationship between bound and free amsacrine and indicates that, under these experimental conditions, both saturable and non-saturable binding occur between amsacrine and albumin. Analysis of this curve on an Apple IIe computer by an extended least squares modelling program (MK MODEL, Version 1·4) (Holford 1985) indicated that the most appropriate model for these data was a single saturable site with a K_D (equilibrium dissociation constant), 13·9 μmol litre^{-1} and a B_{max} (the concentration of the binding site), 9·18 μmol litre^{-1}. As the concentration of albumin was 10·9 μmol litre^{-1}, this suggested one single saturable site for the albumin molecule. The non-saturable binding parameters could not be fully resolved as high enough concentrations could not be examined due to limited drug solubility. Binding parameters were not calculated for diluted plasma as sufficient displacement of amsacrine was not obtained (Fig. 1).

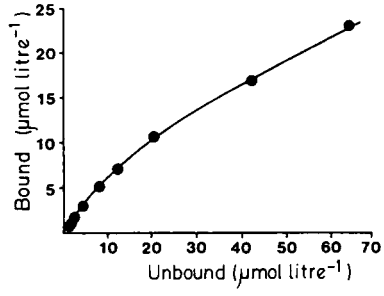

FIG. 3. Relationship between bound and free (unbound) amsacrine over the total concentration range 1–87 μmol litre^{-1} in the presence of HSA (10·9 μmol litre^{-1}). Each point is the mean of 4 determinations.

Drug interaction studies with albumin

These studies were undertaken by equilibrium dialysis. The control solution contained albumin (10·9 μmol litre^{-1}) and amsacrine (10 μmol litre^{-1}) and [^{14}C]amsacrine (1 μmol litre^{-1}). The results are shown in Table 2. Increasing concentrations of warfarin caused small, but significant, increases in the binding of amsacrine, whereas naproxen and

Table 2. The effect of other drugs on the binding of amsacrine by human albumin (10·9 μmol litre^{-1}).

	Mean % fu	s.d.	†Significance
* Control (concn μmol litre^{-1})	66·7 (5)	1·6	
Control + warfarin (5)	64·6 (5)	2·9	NS
Control + warfarin (10)	62·0 (5)	3·6	$P < 0·05$
Control + warfarin (50)	60·6 (5)	3·6	$P < 0·005$
* Control	69·6 (5)	3·0	
Control + naproxen (10)	68·5 (5)	2·7	NS
Control + naproxen (20)	72·5 (5)	1·3	$P < 0·05$
Control + naproxen (50)	74·3 (5)	2·4	$P < 0·025$
* Control	66·9 (4)	3·1	
Control + oleic acid (5)	70·7 (4)	2·0	$P < 0·05$
Control + oleic acid (10)	71·8 (4)	3·2	$P < 0·025$
Control + oleic acid (50)	75·5 (4)	3·2	$P < 0·005$

Number of determinations in parentheses.
* Contained 10 μmol litre^{-1} amsacrine plus 1 μmol litre^{-1} [^{14}C]amsacrine.
† Significantly different from control values by two sample *t*-test.

oleic acid caused small, but significant, reductions in the binding of amsacrine. Phenylbutazone (12 μmol litre^{-1}), which is thought to be specific for the warfarin binding site on albumin, did not cause any significant changes in amsacrine binding.

Covalent binding

Blood bank plasma incubated with 1 μmol litre^{-1} [^{14}C]amsacrine overnight at 37 °C was dialysed against 3 × 1 litre changes of isotonic phosphate buffer over 48 h. Undialysable radioactivity amounted to 20·4 ± 3·1% (s.d.), suggesting that this fraction was covalently bound to plasma proteins. Further evidence for some covalent binding was provided by a study of the effect of time and temperature on the amount of [^{14}C]amsacrine extracted from plasma by ethyl acetate. There was a steady decrease in extractable amsacrine with percent unextractable at 37 °C rising from approximately 1% just after addition of radioactivity, to 4·1% at 1 h, 6·8% at 2 h, 11·5% at 4 h, 13·2% at 8 h and finally 20·6% after 24 h. At ambient room temperature the unextractable radioactivity was reduced by approximately 50% at all time intervals.

Binding in healthy subjects and patients

Despite significantly different albumin and AAG concentrations in normal subjects and patients with acute myelogenous leukaemia (AML), there was no significant difference in amsacrine % fu after addition of [^{14}C]amsacrine to plasma and equilibrium dialysis (Table 3). Inter-individual variability of fu was examined in 6 separate plasma samples obtained after the first and third amsacrine infusions in 6 AML patients during days 4 and 6 of this particular treatment regimen (Jurlina et al 1985). Individual coefficients of variation ranged from 7·9–17·9% with a mean of 12·1%.

DISCUSSION

Two problems were encountered in this study. Firstly, the solubility of amsacrine, does not permit

Table 3. Amsacrine binding and plasma protein concentrations in healthy subjects and patients with acute myelogenous leukaemia.

	AML patients (n = 11)	Healthy subjects (n = 12)	Significance
Mean plasma AAG (g litre^{-1})	1·262	0·674	$P < 0·001$
s.d.	(0·468)	(0·141)	
Mean plasma albumin (g litre^{-1})	36·2	44·17	$P < 0·05$
s.d.	(3·62)	(3·06)	
Mean % fu	3·08	2·98	NS
s.d.	(0·62)	(0·83)	
Range	1·89–3·92	1·67–4·11	

the use of concentrations high enough to saturate the second binding site on albumin or to obtain suitable displacement curves in plasma. The other problem concerned some loss of radioactive label to the membrane at protein concentrations <5·8 g litre^{-1} (i.e. 10% plasma solution) during equilibrium dialysis. For this reason we calculated the unbound fraction after sampling from both sides of the membrane at the end of dialysis and then corrected for volume changes using protein determinations before and after dialysis, rather than use the method suggested by Tozer et al (1983), which assumes no loss of label to apparatus or membrane. In dialysis experiments with low protein concentration and where free amsacrine concentrations will be changing, spurious results may occur. This may well explain the flattening out of the binding curve with dilution of plasma below 1% (Fig. 2).

For most experiments blood bank plasma was used which may contain up to 10% of citrate, phosphate, dextrose solution as anticoagulant. As illustrated by Fig. 2, this small dilution will have little effect on the overall binding of amsacrine, or on the conclusions from the experiments using pooled bank plasma. However this dilution (or also the different anticoagulant) may explain the slightly lower amsacrine binding sometimes observed in blood bank plasma, (e.g. Table 1, mean value 95·3% compared to the range (95·89–98·23%) found in heparinized plasma from 12 healthy subjects).

The dramatic effect of plasma pH might have been expected as amsacrine is a base with a pK_a 7·43, and thus in the transition from pH 6·4–8·4, amsacrine will progress from being mainly protonated to the neutral species at pH 8·4 and above. In addition, at the more basic pH the hydrolysis of amsacrine becomes a distinct possibility, with the higher binding perhaps representing a breakdown product of amsacrine with a higher binding affinity for plasma proteins. Stability studies of amsacrine in buffer over a range of pH values using ethyl acetate extraction followed by HPLC and monitoring of UV absorbance at 435 nm, indicated that a 15% reduction in the amount of amsacrine present occurred after incubation at 37 °C at pH 7·4 for 24 h. Greater reductions were observed at higher pH values, until at pH 10 complete hydrolysis of amsacrine had occurred. No detectable breakdown of amsacrine was, however, observed on incubation at 37 °C pH 7·4 for up to 8 h, indicating that this was not a problem with our 4 h dialysis.

For most drugs, specific binding proteins and binding sites have been identified. However, amsacrine was bound by all plasma proteins examined, with the percent bound being dependent mainly on the concentration of protein. The binding to albumin appeared to occur by two processes, a saturable process at a single site with a moderate affinity constant (K_a = 6·4 × 10^4 litre mol^{-1}) and a nonsaturable process. The possibility that the saturable binding site might correspond to either of the two specific sites which have previously been described for albumin (Sudlow et al 1976; Fehske et al 1981) was tested with warfarin which is specific for site I, and naproxen which is specific for site II, of the benzodiazepine binding site. Both drugs have greater affinity constants for albumin than amsacrine (Sjoholm et al 1979; Kober & Sjoholm 1980) and both caused small changes in amsacrine binding, but in opposite directions. Although these binding interactions would not be clinically significant, speculation on possible mechanisms involved is of interest. Amsacrine binding at site I seems unlikely, as phenylbutazone had no effect on binding, and it could be held that the enhanced amsacrine binding observed in the presence of warfarin is due to a conformational change in albumin induced by warfarin. Displacement of amsacrine by naproxen from site II remains a possibility, with the small reduction observed being due to the displaced amsacrine being mopped up by the non-saturable binding site. This may also provide the explanation for the decreased binding caused by oleic acid, which has been suggested to bind primarily to the benzodiazepine site (Brown et al 1982). An additional complication in the interpretation of amsacrine binding data is the irreversible binding to protein which occurs with this drug.

With regard to the clinical significance, this study has shown that amsacrine is highly bound in plasma, with only 3% of the total plasma concentration consisting of the pharmacologically active fraction. This fraction is independent of amsacrine concentrations achieved clinically, and also of other drugs. Physiological changes in plasma proteins due to disease would also appear not to affect the unbound amsacrine fraction in plasma. The covalent binding of amsacrine to plasma proteins may be of clinical interest, and may be responsible for the allergic skin rashes and other allergy associated adverse side effects which sometimes occur with amsacrine in patients (Bodey & Jacquillat 1983).

Acknowledgements

This study was supported by the Auckland Medical Research Foundation and the New Zealand Medical

Research Council. Thanks are extended to Dr N. H. G. Holford for assistance with the analysis of the amsacrine-albumin binding plot.

REFERENCES

Bodey, G. P., Jacquillat, C. (eds) (1983) in: Amsacrine; Current perspectives and clinical results with a new anti-cancer agent. Communications Media for Education Inc. New Jersey, USA. pp 1–107

Brown, N. A., Wilson, A. G. E., Bridges, J. W. (1982) Biochem. Pharmacol. 31: 4019–4029

Ehrnebo, M., Odar-Cederlof, I. (1977) Eur. J. Clin. Pharmacol. 11: 37–42

Fehske, K. J., Muller, W. E., Schafer, U., Wollert, U. (1981) in: Reitbrook, N., Woodcock, B. G. (eds) Progress in Drug Protein Binding. Friedr Vieweg Sohn Braunschweig/Wiesbaden pp 5–17

Holford, N. H. G. ASCEP Abst. Clin. Expt. Pharmacol. Physiol. in press.

Huang, J. (1983) J. Pharm. Sci. 72: 1368–1369

Jurlina, J. L., Paxton, J. W. (1983) J. Chromatogr. 276: 367–374

Jurlina, J. L., Varcoe, A. R., Paxton, J. W. (1985) Cancer Chemother. Pharmacol. 14: 21–25

Kober, A., Sjoholm, I. (1980) Mol. Pharmacol. 18: 421–426

Kurz, M., Trunk, H., Witz, B. (1977) Drug Res. 27: 1373–1380

Luc, P. V. T., Rosenoff, S., Wooley, P. V. (1981) AACR Abstracts p 176

Sjoholm, I., Ekman, B., Kober, A., Ljungstedt-Pahlman, I., Seiving, B., Sjodin, J. (1979) Mol. Pharmacol. 16: 767–777

Sudlow, G., Birkett, D. J., Wade, D. N. (1976) Mol. Pharmacol. 12: 1052–1061

Tozer, T. N., Gambertoglio, J. G., Furst, D. E., Avery, D. S., Holford, N. H. G. (1983) J. Pharm. Sci. 72: 1442–1448

Wilson, W. R., Cain, B. F., Baguley, B. C. (1977) Chem-Biol. Interactions 18: 163–178