Cancer Chemother Pharmacol (2000) 45: 417–422

ORIGINAL ARTICLE

Graeme J. Finlay · Bruce C. Baguley

Effects of protein binding on the in vitro activity of antitumour acridine derivatives and related anticancer drugs

Received: 20 July 1999 / Accepted: 5 November 1999

Abstract *Purpose*: We set out to measure drug binding to serum proteins. These have been shown to reduce the free plasma concentrations of a number of anticancer drugs, particularly of those of complex organic structure, in both experimental studies and clinical trials. *Methods*: We have used cultures of murine Lewis lung carcinoma cells as sensors of available drug to measure the effects of two drug-binding plasma proteins, α-acid glycoprotein (AAG) and human serum albumin (HSA), as well as of bovine serum albumin (BSA) on drug activity. *Results*: The concentrations required for 50% growth inhibition (IC_{50} values) of a number of anticancer drugs were found to be linear functions of the added proteins. Assuming that cells respond to free drug, the data provide estimates of the product $K \cdot n$, where K is the binding constant of the protein and n is the number of drug binding sites per protein molecule. Amsacrine, the amsacrine analogue asulacrine, camptothecin, DACA (*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide), doxorubicin, etoposide, mitoxantrone, paclitaxel and vincristine were tested. The $K \cdot n$ values for AAG were 30, 2400, 8.7, 340, 29, 290×10^3 M^{-1} and 120×10^3 M^{-1}, respectively, and the $K \cdot n$ values for HSA were 16, 580, 530, 10, 6.2, 4.3×10^3 M^{-1} and 0.0×10^3 M^{-1}, respectively. The combined data allowed the estimation of free fractions of drug in plasma, assuming that AAG and HSA contributed most to protein binding. The data were in general comparable with that reported using equilibrium dialysis and ultrafiltration. Data for drug binding to BSA were different from those for HSA, in some cases by a large factor with values for HSA generally higher. The applicability of the method to analogue development was illustrated by examining the binding to AAG of a series of DACA analogues, and binding was found to be primarily related to lipophilicity. *Conclusion*: IC_{50} determinations provide a rapid means of estimating drug binding to plasma proteins and have utility in the assessment of new anticancer drugs.

Key words α-Acid glycoprotein · Albumin · Cytotoxic drugs

G. J. Finlay (✉) · B. C. Baguley
Auckland Cancer Society Research Centre,
University of Auckland School of Medicine,
Private Bag 92019, Auckland, New Zealand
e-mail: g.finlay@auckland.ac.nz
Tel.: +64-9-3737599 ext. 6140; Fax: +64-9-3737502

Introduction

The binding of anticancer drugs to plasma proteins can affect both their pharmacokinetics and pharmacodynamics [7]. Since the extent of drug binding may vary considerably in different species, protein binding measurements are important in predicting doses for clinical studies of new anticancer drugs from preclinical studies in experimental animals [18, 22]. This is particularly important in the testing of relatively complex organic molecules that poison topoisomerase enzymes and tubulin. For instance, in the case of the amsacrine analogue asulacrine and the acridine derivative *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA), free plasma drug fractions in mice were much higher than in humans, and the starting doses in the human trials could have been more precisely predicted by consideration of free rather than total plasma drug concentrations [19, 26]. It is generally assumed that the cellular response is a function of free rather than total drug concentrations, and free drug rather than total drug concentrations are also of importance in pharmacologically-guided dose escalation [9].

Two of the main drug binding proteins in plasma, α-acid glycoprotein (AAG) and human serum albumin (HSA) are synthesized by the liver and are of particular importance to the cytotoxic actions of anticancer drugs. AAG, an acute phase protein also known as orosomucoid [23, 28] is often elevated in the plasma of cancer patients [22, 32]. Because of its acidic nature,

AAG is likely to bind to basically charged drugs such as amsacrine, doxorubicin and mitoxantrone. HSA, which is often present in reduced concentrations in plasma of cancer patients, is known to bind to a variety of anticancer drugs including paclitaxel [24], etoposide [25] and teniposide [31].

Equilibrium dialysis or ultrafiltration, combined with analysis of drug concentrations by high-performance liquid chromatography has generally been used to determine drug-free fractions. In this communication, we have taken advantage of the intrinsic cytotoxicity of anticancer drugs and have measured IC_{50} values (concentrations required for 50% growth inhibition) for a number of anticancer drugs in the presence of a range of concentrations of AAG and albumin. The method is rapid and can be used in studies on a series of drug analogues, obviating the need to develop specific analytical methods for each drug. The results help to validate the assumption that cancer cells respond to free rather than total drug.

Materials and methods

Materials

DACA and its analogues [2, 12, 33, 35], as well as amsacrine and asulacrine [4], were synthesized in the Auckland Cancer Society Research Centre. Camptothecin was from Sigma Chemicals (St. Louis, Mo., USA). Doxorubicin, etoposide, mitoxantrone and vincristine were obtained as the clinical formulations. Paclitaxel was kindly provided by Bristol-Myers-Squibb (Wallingford, Conn., USA). AAG (human; 99% pure from Cohn fraction VI; Sigma Chemicals), HSA (fraction V; Sigma Chemicals) and BSA (bovine serum albumin; low endotoxin; Immunochemical Products, Auckland, New Zealand) were dissolved in culture medium to concentrations of 0.6, 48 mg/ml and 48 mg/ml, respectively. Albumin solutions were dialysed against growth medium before use. BSA also required titration to a pH of 7.4 by addition of NaOH before use. The molecular weights of AAG, HSA and BSA were assumed to be 42,000, 57,000 and 57,000, respectively.

Culture techniques

LLTC murine Lewis lung carcinoma cells were seeded at 500 cells per well in 96-well plates, using modified minimal essential medium supplemented with 10% foetal bovine serum (FBS). IC_{50} values (drug concentrations required for 50% inhibition of growth, as determined by sulforhodamine B staining) were measured for the indicated drugs after growth for 4 days [15] in the presence of different amounts of AAG, HSA or BSA.

Calculations

The IC_{50} value, corrected for drug binding by BSA present in the growth medium, was assumed to be equivalent to free drug concentration. The drug binding constant, K, is given by the following equation:

$$K = \frac{Db}{Df \cdot Pf \cdot n} \tag{1}$$

Db and Df represent the concentrations of bound and free drug, Pf represents the free protein concentration and n represents the number of binding sites per protein molecule. Since the proportion of protein sites bound by drug is small, Pf can be approximated to P, the total protein concentration. The equation can be rearranged in terms of the bound drug concentration, Db, to provide an expression for the total added drug concentration Dt:

$$Dt = Df + Db \tag{2}$$

$$Dt = Df + K \cdot Df \cdot P \cdot n \tag{3}$$

At constant IC_{50} values (i.e. constant Df), the total added drug concentration Dt should be linearly related to the added protein concentration P, with a slope corresponding to the product $K \cdot n$ and an intercept corresponding to Df. To facilitate comparison, data were plotted as normalized values ($Df = 1$) to provide a series of lines with an intercept of 1.0 and a slope of $K \cdot n$. In the calculation of plasma-free drug fractions (Df/Dt) at physiological protein concentrations, the plasma concentrations of AAG and HSA were assumed to be 38 g/l and 0.75 g/l. Rearrangement of the binding equation gave:

$$\frac{Df}{Dt} = \frac{1}{1 + K \cdot n \cdot P + K' \cdot n' \cdot P'} \tag{4}$$

K and K' are the binding constants of the two proteins (AAG and HSA), P and P' are their respective concentrations, and n and n' the drug binding sites per molecule. Because the culture medium contains 10% FBS, the data for drug binding to BSA was used as a means of estimating the free drug fraction in growth medium alone.

Results

Relationships between anticancer drug IC_{50} values and concentrations of α-acid glycoprotein, human serum albumin and bovine serum albumin

The IC_{50} values of the drugs in cultures of LLTC cells are shown in Table 1. The effect of added human AAG, HSA and BSA on drug IC_{50} values was measured. For each of these proteins, addition in the absence of drug had a negligible effect on the IC_{50} value over the range of concentrations studied. In the presence of drug, linear relationships between added protein and IC_{50} values were obtained. Normalized values are shown in Fig. 1. Binding of drugs to BSA allowed correction of the IC_{50} data for free drug. The slopes of the lines allowed estimation of the bound drug, and the ratio of bound to free drug concentrations provided the value ($K \cdot n$), which is the product of the binding constant and the number of bound drug molecules per protein molecule. The calculated values for AAG, HSA and BSA are shown in Table 1. These values were used to calculate the free drug fraction in the presence of physiological concentrations of AAG and HSA, and also the free drug fraction in the presence of BSA at the concentration used in the culture medium (Table 1).

Measurement of α-acid glycoprotein binding of drug analogues

DACA, a dual topoisomerase I/II poison [5, 16], has formed the basis for an extensive analogue development programme [2, 12, 33, 35]. Since the free fraction of DACA in plasma is predominantly determined by binding to AAG, rapid estimation of drug binding is a useful adjunct to drug development. We tested a number

Table 1 Calculated binding data for anticancer drugs. *IC_{50}* Drug concentrations required for 50% growth inhibition, *AAG* α-acid glycoprotein, *HSA* human serum albumin, *BSA* bovine serum albumin, *DACA* *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide

Drug	IC_{50} (n*M*)	$K \cdot n_{AAG}$ ($\times 10^3\ M^{-1}$)	$K \cdot n_{HSA}$ ($\times 10^3\ M^{-1}$)	$K \cdot n_{BSA}$ ($\times 10^3\ M^{-1}$)	% Free drug in plasma[b]	% Free drug in medium
Amsacrine	13	30	16	6.2	8.2	71
Asulacrine	6.1	2400	580	61	0.23	20
Camptothecin	40	8.7	530	0.33	0.28	98
DACA	160	340	10	1.1	7.3	93
Doxorubicin	20	29	6.2	3.8	18	80
Etoposide	220	29	4.2	0.20	23	99
Mitoxantrone	0.51	240	4.3	2.2	11	87
Paclitaxel	36	140	5.1	ND[a]	14	77[a]
Vincristine	3.5	120	0.0	0.80	32	95

[a] *ND* not determined. Value for HSA was used in the estimation of the free fraction in growth medium
[b] Calculated assuming concentrations of AAG and HSA of 38 g/l and 0.75 g/l, respectively

of DACA analogues to determine the relationship between drug structure and AAG binding. The results (Table 2) show that small changes in structure have quite large effects on protein binding. Figure 2 shows the relationship between binding constants for AAG and relative chromatographic mobility measurements as determined from thin-layer chromatography. These are in turn related to logarithmic octanol-water partition coefficients [6].

Discussion

We have described here the use of cultured cells as sensors for available drug, and have used this property to measure the binding of cytotoxic drugs to plasma proteins. The method is rapid, reproducible and can be semi-automated. Drug binding is measured at physiological conditions, and degradation of drug during the course of the experiment, providing it is independent of added protein concentration and does not produce a more cytotoxic drug species, has minimal effect on the result. Of particular interest are the large species differences in the drug binding properties of albumin. In general, HSA bound drugs more strongly than did BSA (Table 1), and very large differences were observed for some drugs such as camptothecin (1600-fold), etoposide (21-fold) and asulacrine (9.5-fold). Species differences in

Fig. 1 Relationship between normalized IC_{50} values (see Table 1 for IC_{50} values in the absence of added proteins) and added protein concentration for AAG (**A**), HSA (**B**) and BSA (**C**). Symbols indicate for amsacrine (●), asulacrine (○), camptothecin (▲), doxorubicin (■), DACA (□), etoposide (▼), mitoxantrone (▽), paclitaxel (◆) and vincristine (△). *IC_{50}* Drug concentrations required for 50% growth inhibition, *AAG* α-acid glycoprotein, *HSA* human serum albumin, *BSA* bovine serum albumin, *DACA* *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide

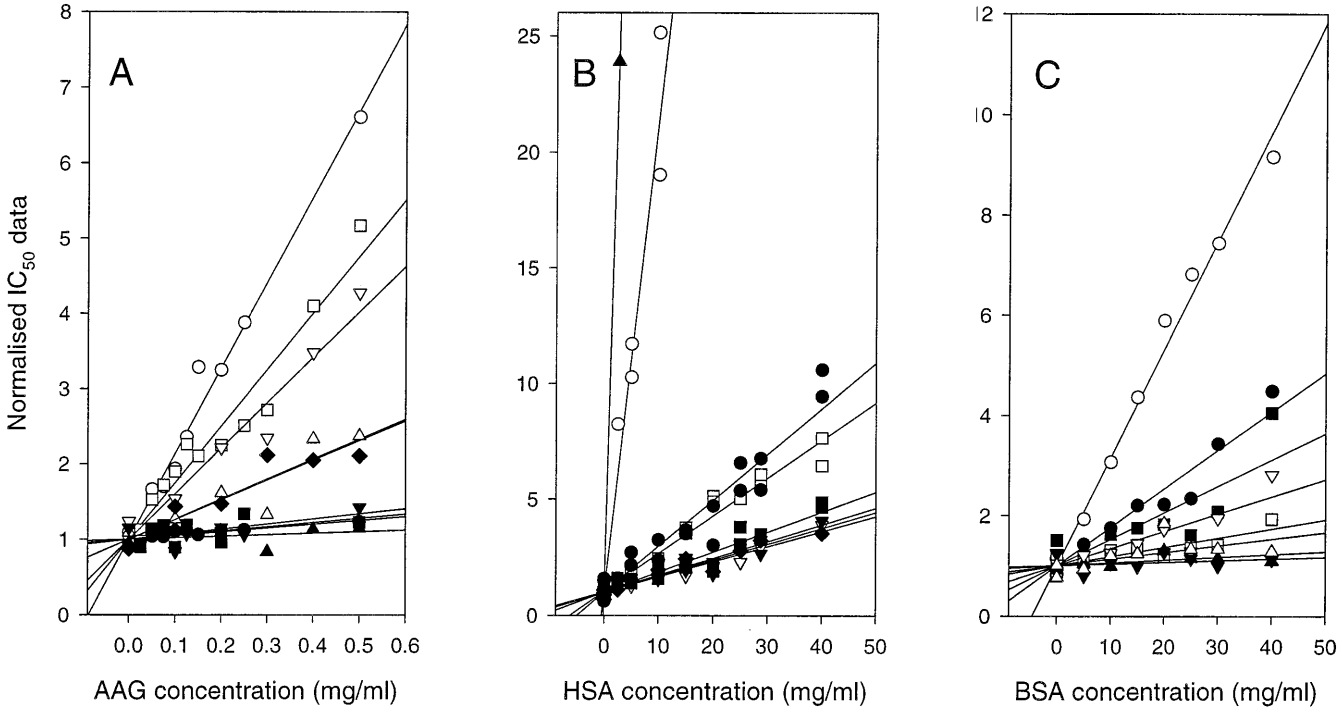

Table 2 Calculated α-acid glycoprotein (*AAG*) binding data (uncorrected for binding to proteins in culture medium) for *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide analogues. IC_{50} Drug concentrations required for 50% growth inhibition

Substituent	IC_{50} (n*M*)	$K \cdot n_{AAG}$ ($\times 10^3$ M^{-1})	Relative mobility[a]	Calculated log P[b]
Parent	160	320	−0.2	3.101
2-Chloro	39	97	+0.03	3.880
5-Chloro	2.8	360	−0.21	3.837
6-Chloro	110	390	−0.06	3.837
7-Chloro	420	62	−0.03	3.837
7-Methyl	680	180	−0.05	3.600
7-Methoxy	850	65	−0.22	3.375
7-Hydroxy	290	18	−0.5	3.124
9-Amino	5.9	18	−1.11	2.795
9-Amino, 5-methylsulphone	28	12	−1.35	1.602
5,9-Diamino	19	67	−1.1	2.235
6,9-Diamino	300	1.7	−1.24	2.235
7,9-Diamino	87	0.0	−1.64	2.235

[a] Relative chromatographic mobility determined by thin-layer chromatography. Values are quoted from previous publications [3, 12]
[b] Calculated using CLOGP version 3.05 (courtesy of Daylight Chemical Information Systems, Mission Viejo, Calif., USA)

the binding of camptothecin to HSA and BSA have previously been reported [27].

Three clinical acridine-derived topoisomerase poisons were investigated. Amsacrine is currently used in the treatment of leukaemia [1], while asulacrine [19] and DACA [26] have completed phase I trial. The free fractions of amsacrine in physiological concentrations of purified AAG and HSA were determined from the data in Table 1 to be 65% and 9%, respectively. These values are comparable with those (69% and 10%, respectively) determined by equilibrium dialysis [29]. The binding constant for DACA for AAG has been previously determined by equilibrium dialysis as 7.8×10^4 M^{-1} with a binding site concentration of 3.8 drug molecules per protein molecule [14]. The corresponding $K \cdot n$ value of 2.96×10^5 M^{-1} is in good agreement with Table 1.

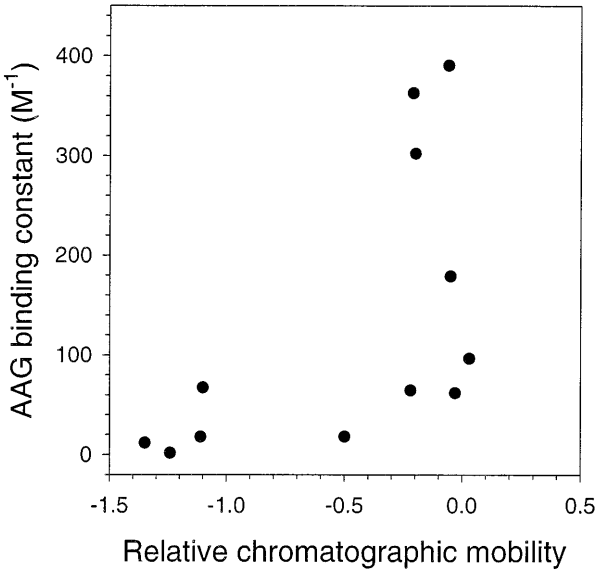

Fig. 2 Relationship between AAG binding constant and lipophilicity for DACA and its derivatives. *AAG* α-Acid glycoprotein, *DACA N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide

The reported free fractions for amsacrine and asulacrine in plasma from patients in clinical trials are 3% and 0.1%, respectively [21, 30], as compared with the calculated values of 8.2% and 0.2% in Table 1. The free fraction of DACA in plasma from normal volunteers was 3.3% [14], again somewhat lower than the calculated value of 7.3% in Table 1. Plasma free drug fractions in patients in a Phase I clinical trial are generally lower than those from normal volunteers because of increased plasma AAG concentrations [22], and other proteins, such as lipoproteins, may also make binding contributions in plasma [8].

Doxorubicin and mitoxantrone are further examples of DNA intercalating anticancer agents. The free drug fractions for doxorubicin in the presence of physiological concentrations of purified AAG and HSA were calculated from Table 1 to be 65% and 20%, respectively. Published values determined by ultrafiltration are 68% and 38%, respectively [8]. The free fraction of doxorubicin in HSA was also determined by equilibrium dialysis to be 62% [13]. The reported free fraction in plasma from healthy volunteers is 28% for doxorubicin [13], as compared with 18% in Table 1. The free drug fractions for mitoxantrone in the presence of physiological concentrations of purified AAG and HSA were calculated from Table 1 to be and 16% and 26%, respectively, compared with published values from ultrafiltration of 49% and 35%, respectively [8]. The reported plasma free fraction for mitoxantrone in a clinical trial was 3% [20], compared with 11% in Table 1.

The binding properties of two topoisomerase-binding proteins, camptothecin and etoposide, were investigated (Table 1). The calculated plasma free drug fraction of 0.28% (Table 1) was comparable with the value of 0.36% obtained by ultrafiltration for a solution of 63 *M* camptothecin in human plasma taken in a Phase I clinical trial [10]. The binding constant for camptothecin binding to HSA, determined by equilibrium dialysis, was reported to be 6×10^7 M^{-1} [10], which is higher than the value in Table 1 and inconsistent with the free drug

fraction. It is also much higher than that (5.5×10^3 M^{-1}) determined by fluorescence spectrometry [27]. The free fraction of etoposide in plasma is highly variable in cancer patients with a range of 3–20% [25]. These values are lower than the value of 23% in Table 1, and the difference might be explained partially on the basis of elevated AAG levels in cancer patients, and partially on the basis of bilirubin binding [17].

The binding of two mitotic poisons, vincristine and paclitaxel, was also investigated. Significant binding of vincristine to HSA was not detected and the calculated free fraction in plasma of 32% (Table 1) is comparable with the published value of 29% [11]. As measured by equilibrium dialysis, the free fraction of vincristine in the presence of 2 g/l AAG was 48% [37], compared with 32% from Table 1. Paclitaxel was found from the values in Table 1 to bind to similar extents to AAG and HSA at physiological concentrations, and to provide a calculated free fraction in plasma of 11%. As determined by equilibrium dialysis, paclitaxel is 90–95% bound in plasma [34], with similar values obtained in healthy volunteers and cancer patients, and with approximately equal contributions from AAG and HSA binding [24].

The practicability of the IC_{50} method in comparing protein binding within a drug series was tested using analogues of the drug DACA. Binding was compared with experimentally determined measurements of lipophilicity in Fig. 2, and a similar relationship was found to exist for calculated lipophilicity values (Table 2). The results show that lipophilic character is necessary but not sufficient for a high degree of binding to AAG. The position of substituents on the acridine ring markedly affects AAG binding, as shown by the 2-chloro and 6-chloro derivatives, which have similar lipophilicity but differ by almost four-fold in AAG binding. The role of pKa in binding to AAG [36] is more difficult to assess because of the limited data available in this series [12]. The addition of a 9-amino group to DACA, which strongly reduces AAG binding, causes a large increase in pKa (from 3.54 to 8.3), raising the question of whether pKa is more important than lipophilicity. However, addition of the 5-methylsulphone group to the 9-amino compound reduces pKa back to 5.15 but does not increase AAG binding. Conversely, addition of a 5-amino group, which has only small effects on lipophilicity and pKa, increases AAG binding (Table 2). It is likely that drug lipophilicity plays the major part in the binding of these drugs to AAG, but that individual substituents on the drug molecule make productive contacts with the drug binding region(s) on this protein.

In conclusion, the overall agreement between protein binding data obtained using this method and results from the literature using other methods justifies consideration of this method for estimating available drug in clinical trials of both existing and new anticancer drugs. While protein-binding species other than AAG and HSA exist in human plasma, the calculation of free drug fractions based on the binding of these two proteins alone provides good estimates. Furthermore, the IC_{50} method here provides strong supporting evidence for the hypothesis that tumour cells respond to free drug rather than total drug. The strong protein binding observed with some of the drugs used in this study emphasizes the need to consider free drug rather than total drug concentrations when extrapolating from in vivo to in vitro conditions.

Acknowledgements This work was supported by the Auckland Division of the Cancer Society of New Zealand and by the Health Research Council of New Zealand. The authors thank Dr. Michael Hay for help with the calculation of log *P* values.

References

1. Arlin ZA (1989) A special role for amsacrine in the treatment of acute leukemia. Cancer Invest 7: 607
2. Atwell GJ, Cain BF, Baguley BC, Finlay GJ, Denny WA (1984) Potential antitumor agents. Part 43. Synthesis and biological activity of dibasic 4-carboxamido-9-aminoacridines, a new class of antitumor agent. J Med Chem 27: 1481
3. Atwell GJ, Rewcastle GW, Baguley BC, Denny WA (1987) Potential antitumor agents. 50. In vivo solid tumor activity of derivatives of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. J Med Chem 30: 664
4. Baguley BC, Denny WA, Atwell GJ, Finlay GJ, Rewcastle GW, Twigden SJ, Wilson WR (1984) Synthesis, antitumor activity, and DNA binding properties of a new derivative of amsacrine, *N*-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)-phenylamino]-4-acridinecarboxamide. Cancer Res 44: 3245
5. Bridewell DJA, Finlay GJ, Baguley BC (1999) Mechanism of cytotoxicity of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide and of its 7-chloro derivative: the roles of topoisomerases I and II. Cancer Chemother Pharmacol 43: 302
6. Cain BF, Atwell GJ, Denny WA (1975) Potential antitumor agents. Part 16. 4′-(Acridin-9-ylamino)methanesulfonanilides. J Med Chem 18: 1110
7. Chabot GG (1994) Factors involved in clinical pharmacology variability in oncology. Anticancer Res 14: 2269
8. Chassany O, Urien S, Claudepierre P, Bastian G, Tillement JP (1996) Comparative serum protein binding of anthracycline derivatives. Cancer Chemother Pharmacol 38: 571
9. Collins JM, Zaharko DS, Dedrick RL, Chabner BA (1986) Potential roles for preclinical pharmacology in phase I clinical trials. Cancer Treat Rep 70: 73
10. Creaven PJ, Allen LM, Muggia FM (1972) Plasma camptothecin (NSC-100880) levels during a 5-day course of treatment: relation to dose and toxicity. Cancer Chemother Rep 56: 573
11. de Lannoy IA, Mandin RS, Silverman M (1994) Renal secretion of vinblastine, vincristine and colchicine in vivo. J Pharm Exp Ther 268: 388
12. Denny WA, Atwell GJ, Rewcastle GW, Baguley BC (1987) Potential antitumor agents. 49. 5-Substituted derivatives of *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide with in vivo solid tumor activity. J Med Chem 30: 658
13. Eksborg S, Ehrsson H, Ekqvist B (1982) Protein binding of anthraquinone glycosides, with special reference to Adriamycin. Cancer Chemother Pharmacol 10: 7
14. Evans SMH, Robertson IGC, Paxton JW (1994) Plasma protein binding of the experimental antitumour agent acridine-4-carboxamide in man, dog, rat and rabbit. J Pharm Pharmacol 46: 63
15. Finlay GJ, Marshall ES, Matthews JHL, Paull KD, Baguley BC (1993) In vitro assessment of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide, a DNA-intercalating antitumour drug with reduced sensitivity to multidrug resistance. Cancer Chemother Pharmacol 31: 401

16. Finlay GJ, Riou J-F, Baguley BC (1996) From amsacrine to DACA (*N*-[2-(dimethylamino)ethyl]acridine 4-carboxamide): selectivity for topoisomerases I and II among acridine derivatives. Eur J Cancer 32 A: 708
17. Fleming RA, Evans WE, Arbuck SG, Stewart CF (1992) Factors affecting in vitro protein binding of etoposide in humans. J Pharm Sci 81: 259
18. Graham MA, Kaye SB (1993) New approaches in preclinical and clinical pharmacokinetics. Cancer Surv 17: 27
19. Hardy JR, Harvey VJ, Paxton JW, Evans PC, Smith S, Grillo-Lopez A, Grove W, Baguley BC (1988) A phase I trial of the amsacrine analog 9-[[2-methoxy-4-[(methylsulfonyl)amino]-phenyl]amino]-*N*,5-dimethyl-4-acridinecarboxamide (CI-921). Cancer Res 48: 6593
20. Hu OY, Chang SP, Law CK, Jian JM, Chen KY (1992) Pharmacokinetic and pharmacodynamic studies with mitoxantrone in the treatment of patients with nasopharyngeal carcinoma. Cancer 69: 847
21. Kestell P, Paxton JW, Evans PC, Young D, Jurlina JL, Robertson IGC, Baguley BC (1990) Disposition of amsacrine and its analogue 9-((2-methoxy-4-[(methylsulfonyl)-amino]phenyl)-amino)-N,5-dimethyl-4-acridinecarboxamide (CI-921) in plasma, liver, and Lewis lung tumors in mice. Cancer Res 50: 503
22. Kestell P, Dunlop I, McCrystal MR, Evans BD, Paxton JW, Gamage RSKA, Baguley BC (1999) Plasma pharmacokinetics of *N*-[2-(dimethylamino)-ethyl]-acridine-4-carboxamide (DACA) in a phase I trial. Cancer Chemother Pharmacol 44: 45
23. Kremer JM, Wilting J, Janssen LH (1988) Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol Rev 40: 1
24. Kumar GN, Walle UK, Bhalla KN, Walle T (1993) Binding of Taxol to human plasma, albumin and alpha-1-acid glycoprotein. Res Commun Chem Pathol Pharmacol 80: 337
25. Liu B, Earl HM, Poole CJ, Dunn J, Kerr DJ (1995) Etoposide protein binding in cancer patients. Cancer Chemother Pharmacol 36: 506
26. McCrystal MR, Evans BD, Harvey VJ, Thompson PI, Porter DJ, Baguley BC (1999) Phase I study of the cytotoxic agent *N*-[2-dimethylamino)ethyl]acridine-4-carboxamide. Cancer Chemother Pharmacol 44: 39
27. Mi Z, Burke TG (1994) Marked interspecies variations concerning the interactions of camptothecin with serum albumins: a frequency-domain fluorescence spectroscopic study. Biochemistry 33: 12540
28. Paxton JW (1983) Alpha$_1$-acid glycoprotein and binding of basic drugs. Methods Find Exp Clin Pharmacol 5: 635
29. Paxton JW, Jurlina JL, Foote SE (1986) The binding of amsacrine to human plasma proteins. J Pharm Pharmacol 38: 432
30. Paxton JW, Hardy JR, Evans PC, Harvey VJ, Baguley BC (1988) The clinical pharmacokinetics of N-5-dimethyl-9-[(2-methoxy-4-methyl-sulfhonylamino)phenylamino]-4-acridine carboxamide (CI-921) in a phase I trial. Cancer Chemother Pharmacol 22: 235
31. Petros WP, Rodman JH, Relling MV, Christensen M, Pui CH, Rivera GK, Evans WE (1992) Variability in teniposide plasma protein binding is correlated with serum albumin concentrations. Pharmacotherapy 12: 273
32. Raveendran R, Heybroek WM, Caulfield M, Abrams SML, Wrigley PFM, Slevin M, Turner P (1992) Protein binding of indomethacin, methotrexate and morphine in patients with cancer. Int J Clin Pharmacol Res 12: 117
33. Rewcastle GW, Atwell GJ, Chambers D, Baguley BC, Denny WA (1986) Potential antitumor agents. 46. Structure-activity relationships for acridine mono-substituted derivatives of the antitumor agent *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide. J Med Chem 29: 472
34. Sonnichsen DS, Relling MV (1994) Clinical pharmacokinetics of paclitaxel. Clin Pharmacdokinet 27: 256
35. Spicer JA, Gamage SA, Atwell GJ, Finlay GJ, Baguley BC, Denny WA (1997) Structure-activity relationships for acridine-substituted analogues of the mixed topoisomerase I/II inhibitor *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. J Med Chem 40: 1919
36. Urien S, Bree F, Testa B, Tillement JP (1991) pH-dependency of basic ligand binding to alpha 1-acid glycoprotein (orosomucoid). Biochem J 280: 277
37. Woodcock BG, Abdelrahman MS, Wosch F, Harder S (1993) Effect of D,L -verapamil, verapamil enantiomers and verapamil metabolites on the binding of vincristine to alpha1-acid glycoprotein. Eur J Cancer 29 A: 559