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Comparison of the pharmacokinetics and protein binding of the anticancer drug, amsacrine and a new analogue, N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide in rabbits*

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Summary. Amsacrine (NSC 249992) is a new anticancer drug which, although effective for the treatment of various disseminated tumors, has shown disappointing activity against most solid tumors. A new analogue, N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridine-carboxamide (CI-921, NSC 343499) has been identified, which might offer a broader clinical antitumor spectrum. This analogue is more lipophilic (0.5 log p units) and is also a considerable weaker base (pKa 6.40) than amsacrine (pKa 7.43). This study compared the pharmacokinetics of total and unbound amsacrine and CI-921 in plasma after equimolar dose infusions (12.7 μmol/kg) in a balanced crossover design in six rabbits. Drug concentrations were determined by high-pressure liquid chromatography and the unbound fraction by equilibrium dialysis. Threefold higher total plasma concentrations were achieved with CI-921 than with amsacrine. However, the unbound fraction was significantly less for CI-921 (0.33% ± 0.04) than for amsacrine (2.78% ± 0.53). There was no significant difference between distribution and elimination half-life and mean residence time, but the apparent volume of distribution (means, 121 vs 45 l/kg) and clearance (means, 46.6 vs 16.3 l h^{-1} kg^{-1}) of unbound CI-921 were threefold greater than the corresponding parameters for unbound amsacrine. We suggest that despite higher binding in plasma, the greater distribution or tissue uptake of CI-921 may be partly responsible for its greater anticancer activity in vivo.

Introduction

Amsacrine, 4'-(9-acridinylamino)methanesulfon-*m*-aniside (NSC 249992) (Fig. 1) was first synthesized and shown to be active in a variety of experimental tumors by Cain and Atwell [4]. This agent has proved to be an effective clinical drug for the treatment of various disseminated tumors, especially acute leukemia [2, 13] and certain lymphomas [18] However, a number of clinical trials have shown it to be ineffective or only marginally effective against a wide range of solid tumors in man [3, 6]. Further studies have been undertaken to identify analogues of amsacrine that might offer a broader clinical antitumour spectrum. From this program a new analogue, N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridine-carboxamide (CI-921; NSC 343499) (Fig. 1) emerged, which has significantly greater activity than amsacrine in solid tumor test systems [1]. The most promising feature was vastly superior in vivo activity towards Lewis lung carcinoma growing either in the lung or subcutaneously in the mouse, with similar efficacy to cyclophosphamide. This compound will enter phase 1 clinical trials in 1985. Some comparative physicochemical and biological properties of amsacrine and CI-921 are given in Table 1 [1]. We wished to compare the pharmacokinetics of these two agents to check the basic tenet that the superior activity of CI-921 was in part due to more favorable pharmacokinetics. In addition, as we have previously studied the phar-

Table 1. Some physicochemical and biological properties of amsacrine and CI-921

Property	Amsacrine	CI-921
pKa	7.43	6.40
Lipophilicity (log p)[a]	0.60	1.10
DNA binding (Ka)[b]	$1.3 \times 10^5 M^{-1}$	$2.1 \times 10^6 M^{-1}$
In vitro P388; ID_{50}[c]	14 n*M*	12 n*M*
In vitro Lewis lung carcinoma; ID_{50}	27 n*M*	13 n*M*
In vivo P388; ILS%[d]	72 (IV)[e]	136 (IV)[e]
In vivo P388; ILS%	62 (PO)[f]	164 (PO)[g]
In vivo Lewis lung carcinoma; ILS%	58 (IV)[e]	127 (IV)[e]
In vivo Lewis lung carcinoma; ILS%	38 (PO)[h]	132 (PO)[g]

[a] Logarithm of the partition coefficients between *n*-octyl alcohol and water

[b] Association constant for binding to double-stranded calf thymus DNA

[c] ID_{50}, drug concentration required to decrease the cell density at the end of cell culture by 50% relative to that of untreated control cultures

[d] ILS, increase in life-span of treated tumor-bearing animals relative to that of untreated control mice

[e] Optimal dose by IV route (30 mg/kg) in mice inoculated with P388 leukemia or Lewis lung carcinoma

[f, g, h] Optimal doses given by the oral route were [f] 65 mg/kg, [g] 50 mg/kg and [h] 75 mg/kg

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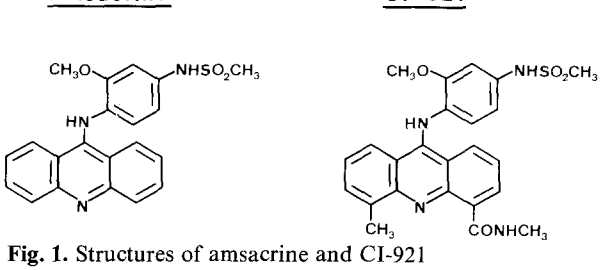

Fig. 1. Structures of amsacrine and CI-921

macokinetics of amsacrine in rabbits [14] and human patients [12], a knowledge of the pharmacokinetics of CI-921 in the rabbit may be of use in the more rational choice of dosage regimen in phase 1 clinical trials of CI-921, whose pharmacokinetics have not been reported in any species to date.

Materials and methods

A balanced crossover design was used in six New Zealand white rabbits (weight range 3.4–5.0 kg) maintained on commercial rabbit pellets and water ad libitum. One month was allowed between the crossover, which has previously been shown to be an adequate recovery period for successive doses of amsacrine [14]. The rabbits' body weight did not change appreciably over the experimental period.

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 lactic acid solution to give equimolar concentrations (12.7 m*M*). The appropriate amount of drug was then diluted to 20 ml with 5% dextrose solution and infused (12.7 μmol/kg, equivalent to 5 mg/kg amsacrine) into a marginal ear vein at 0.58 ml/min via a small vein infusion set (25 g × 3/4″, thin wall needle, McGraw Laboratories Inc., Puerto Rico) using a Harvard Infusion pump (model 975A). At the end of the infusion (approximately 35 min) the catheter line was flushed with 4 ml 5% dextrose containing 5 units/ml sodium heparin. Venous blood (3 ml) was collected from a marginal ear vein on the opposite ear into heparinized tubes at 0, 0.5, 1, 2, 4, 6, 8, and 12 h after infusion. Plasma was separated by centrifugation at 1720 g for 15 min at 10 °C and stored at −20 °C in capped glass vials until analyzed.

Total plasma amsacrine and CI-921 concentrations were determined in duplicate 0.5 ml aliquots by our previously reported high-pressure liquid chromatographic (HPLC) methods [10, 11]. These assays have good accuracy over the range 0.5–10 μ*M*, with recoveries ranging from 99% to 115%, and excellent precision with mean values for intra- and interassay coefficients of variation less than 5%.

The plasma protein binding of amsacrine and CI-921 was determined by equilibrium dialysis using a Dianorm Dialyser (Diachemia, Switzerland) and cellulose membranes (Visking, Union Carbide Corp., New York, molecular weight cutoff 12000–14000). The ^{14}C-amsacrine (S.A., 19.6 mCi/mmol) was obtained from SRI International, Menlo Park, Calif, and ^{3}H-CI-921 (S.A., 59.3 mCi/mmol) from Dr Baguley (Cancer Research Labs, Auckland, NZ). The radiochemical purity of each label was verified as >99% in our HPLC systems. With CI-921 no inhomogeneity was detected, but with amsacrine a small secondary peak eluting after amsacrine was observed. This peak did not correspond to any of the oxidation products of amsacrine [10], and was not associated with any radioactivity. In addition no inhomogeneity of either label was detected by thin-layer chromatography using various different solvent systems. Subsequent autoradiography following TLC indicated >99% radiochemical purity for CI-921 and amsacrine. The plasma remaining after concentration determinations was pooled for each rabbit, adjusted to pH 7.4 with CO_2, and dialyzed for 4 h at 37 °C against isotonic phosphate buffer pH 7.4 containing the appropriate radiolabel. As no loss of radiolabel to the membrane or apparatus was apparent, the unbound fraction (fu) was determined by the ratio of D_d': $(D_d - D_d')$, where D_d and D_d' are the dpm/ml in buffer before and after dialysis, respectively. This equation takes into account the volume changes which occur due to colloidal osmotic fluid shift during dialysis [17].

As no significant change in fu occurs with either agent over the concentration range observed in our rabbits, the value for each rabbit was used to calculate the free concentrations.

Pharmacokinetic analyses were carried out on an Apple IIe computer using MKMODEL version 1.3, an extended least-squares modeling program developed by Dr Holford, Department of Pharmacology and Clinical Pharmacology, University of Auckland School of Medicine, NZ. The distribution and elimination half-life ($t_{½\alpha}$ and $t_{½\beta}$) were calculated by the ratio 0.693 : slope. Further model-independent parameters were calculated by the method of statistical moments from the following equations [7]:

$$\text{Plasma clearance (Cl)} = \text{Dose}/AUC_0^\infty, \text{ E. (1)}$$

The area under the plasma concentration-time curve (AUC_0^∞) was computed using the trapezoidal rule while successive concentration values were increasing, and the log trapezoidal rule while successive concentration values were decreasing after the maximum. The area was extrapolated to infinity by addition of the value of C_t/β, where C_t was the estimated concentration at the last time point calculated from the terminal linear relationship and β was the terminal slope. The mean residence time (MRT_{inf}), which is a measure of the average time the parent drug molecule remains unchanged in the body, was calculated from the following equation:

$$MRT_{inf} = AUMC_0^\infty/AUC_0^\infty, \text{ E. (2)}$$

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_{inf} value for a noninstantaneous input represented the sum of the mean residence times for drug infusion and elimination. The apparent volume of distribution of drug at steady-state (Vss) was calculated from the equation:

$$Vss = Cl\,(MRT_{inf} - T/2), \text{ E. (3)}$$

where T was infusion time for this short-term constant rate IV infusion. The kinetic parameters calculated from both total and free concentrations were compared by the paired *t*-test. Differences were considered significant when the probability (*P*) value was less than 0.05.

Results

Two of the six rabbits received two infusions of CI-921 one month apart. The AUCs for total drug after the first

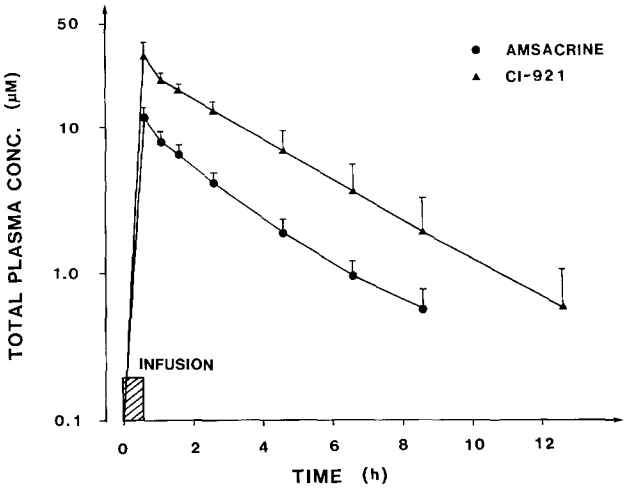

Fig. 2. Mean total plasma concentrations (+SD) of amsacrine (●) and CI-921 (▲) after equimolar dose infusions (12.7 μmol/kg) in six rabbits

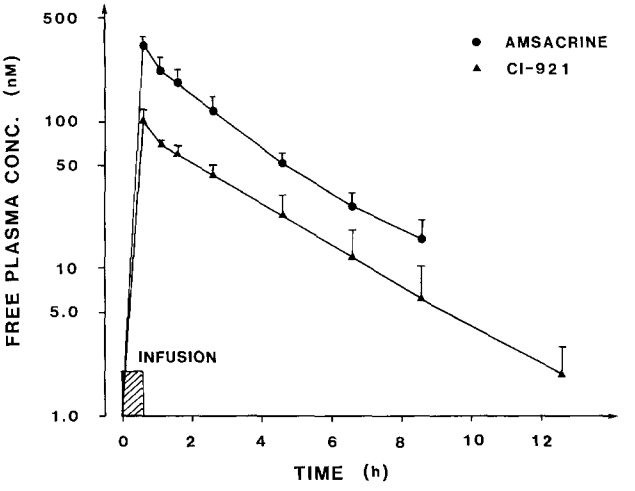

Fig. 3. Mean unbound (free) plasma concentrations (+SD) of amsacrine (●) and CI-921 (▲) after equimolar dose infusions in six rabbits

and second infusions were within 10% of each other (61 vs 65 and 100 vs 91 μmol h^{-1} l^{-1} for the first and second infusions, respectively, in each rabbit). There was no trend in the direction of change of any of the pharmacokinetic parameters with the second infusion, suggesting that as for amsacrine, 1 month is sufficient for recovery after CI-921, with no subsequent effects on drug kinetics.

Individual postinfusion elimination curves for both total and free amsacrine and CI-921 were best fitted by a biexponential expression, except after two CI-921 infusions, when a monoexponential expression was more appropriate. Mean total plasma concentrations of amsacrine and CI-921 observed in the six rabbits are shown in Fig. 2. The mean pharmacokinetic parameters computed from the total concentration-time profiles are given in Table 2. Equimolar doses of amsacrine and CI-921 administered at the same rate resulted in total CI-921 concentrations in plasma that were three times those of amsacrine. However, total plasma concentrations depend on two independent variables, the unbound plasma concentration (which is the pharmacologically active fraction) and the plasma protein binding. Our studies indicated that CI-921 is significantly more highly bound than amsacrine in plasma, with an unbound fraction approximately one-tenth of that found for amsacrine (Table 2). The profiles for the mean unbound plasma concentrations of amsacrine and CI-921 in rabbits are shown in Fig. 3. Comparison of pharmacokinetic parameters calculated from the unbound concentrations indicated no significant difference between distribution and elimination half-life ($t_{½\alpha}$ and $t_{½\beta}$) and MRT_{inf} for the two compounds, but a threefold increase in the clearance and volume of distribution of unbound CI-921 (Table 2).

Table 2. Mean (± S.D.) pharmacokinetic parameters calculated from total and unbound concentrations after equimolar doses of amsacrine and CI-921 in 6 rabbits

Parameter		Amsacrine	CI-921
% fu		2.78 ±0.53	0.33 ±0.04*
$t_{1/2\alpha}$ (h)	Total	0.52 ±0.51	0.62 ±0.35
	Unbound	0.49 ±0.51	0.61 ±0.34
$t_{1/2\beta}$ (h)	Total	2.25 ±0.39	2.03 ±0.67
	Unbound	2.25 ±0.39	2.14 ±0.55
MRT_{inf} (h)	Total	3.02 ±0.53	3.29 ±0.82
	Unbound	3.09 ±0.44	3.29 ±0.82
Cl ($l\ h^{-1}\ kg^{-1}$)	Total	0.45 ±0.08	0.15 ±0.04*
	Unbound	16.3 ±3.3	46.6 ±13.7*
Vss (l/kg)	Total	1.18 ±0.16	0.40 ±0.08*
	Unbound	45 ± 11	121 ± 21*

* Significant difference ($P < 0.005$; paired t-test, 5 df)

Discussion

In vitro CI-921 is similar to amsacrine in its potency against a number of mouse leukemia cell lines, but more potent against various solid tumor cell lines. In vivo these differences in activity are accentuated, with CI-921 providing considerably higher life extensions against both inoculated leukemia and solid tumor cell lines. An obvious explanation for the in vivo superiority of CI-921 appears to be more favorable pharmacokinetics. Addition of a lipophilic methyl group and a hydrophilic *N*-methylcarboxamide group to amsacrine to give CI-921 resulted in a threefold increase in the lipid solubility of the molecule. The latter substitution also reduced the pKa by 1 unit. Since these analogues are weak bases, the lower pKa of CI-921 will ensure that at physiological pH, almost twice as much circulating CI-921 as amsacrine will be the un-ionized species, which is the most appropriate form for diffusion across biological membranes. Thus, both substitutions will tend towards more rapid diffusion and more widespread distribution of CI-921 out of the systemic circulation into the body. This is in agreement with the threefold greater apparent volume of distribution shown by unbound CI-921, which is regarded as the pharmacologically active fraction in plasma. The possiblity that the larger apparent volume of distribution of CI-921 might be due to greater tissue uptake in a specific organ cannot be dismissed. One could speculate that the increased reversible binding to DNA exhibited by CI-921 might also contribute to the larger apparent volume of distribution by increasing the tissue uptake of CI-921 and retention within cells. In addition, as CI-921 undergoes greater binding to plasma proteins, it would not be unreasonable to assume similarly greater binding to tissue proteins also contributing to its larger volume of distribution. With regard to the determi-

nation of the plasma protein binding of these compounds, it is obvious that as both compounds are very highly bound, the presence of a small amount of radiochemical impurity, even as much as 0.1%, could have a significant effect on the value determined for the free fraction, especially that of CI-921. However, no radiochemical impurity was detected in either radioactive drug sample, and each was taken as 100% pure.

It might be expected that the greater apparent volume of distribution of unbound CI-921 than of amsacrine would lead to a longer elimination half-life of the former. However, no significant difference was observed in elimination half-life or mean residence time between the two analogues. The apparent reason for this is that CI-921 experiences a greater intrinsic clearance rate than amsacrine. As elimination half-life is directly proportional to volume of distribution and inversely proportional to clearance, a similar increase in each parameter will lead to no overall change in elimination half-life. This greater intrinsic clearance of CI-921 may be due to greater diffusion of CI-921 into the hepatocyte or a greater affinity for the metabolism enzyme.

The greater relevance of the pharmacokinetic parameters calculated from the unbound concentrations must be emphasized. It is the unbound concentration that distributes throughout the body water and it is this concentration that is eliminated from the vascular system. However, the clearance of total drug from blood is useful to give an estimate of the extraction ratio across an eliminating organ. The clearance of a drug from blood can be estimated from the total plasma clearance value and a knowledge of the blood-to-plasma concentration ratio [15]. Experimentally we determined the blood-to-plasma concentration ratio as 0.85 ± 0.05 (SD) for amsacrine and 0.55 ± 0.03 for CI-921 over the plasma concentration range observed in our rabbits. From these ratios and our total plasma clearance values, total blood clearance of drug was estimated to be 0.52 and 0.28 l h^{-1} kg^{-1} for amsacrine and CI-921, respectively. Evidence has suggested that amsacrine is mainly eliminated by hepatic metabolism and biliary excretion in mice, rats and humans [5, 12, 16]. Assuming that the blood clearance for both drugs is predominantly hepatic in the rabbit, with a rabbit liver blood flow of 2.6–3.5 l h^{-1} kg^{-1} [8, 9] our results indicate that both drugs undergo low hepatic extraction, with estimated extraction ratios of <0.2 for amsacrine and <0.1 for CI-921. According to theoretical considerations, the total drug clearance of these analogues should thus depend on the unbound fraction, decreasing with increasing plasma protein binding, i.e., decreasing as the unbound fraction decreases. Thus, increased plasma binding may well explain the observed smaller clearance of total CI-921 than of amsacrine, despite greater intrinsic hepatic clearance for the analogue.

In summary, our study suggests that CI-921, despite significantly higher binding in the plasma, undergoes greater distribution or tissue uptake within the body, which may partly explain its superior in vivo activity against tumors compared with amsacrine. Despite its larger apparent volume of distribution of unbound drug, no significant change was observed in elimination half-life, due to the greater intrinsic clearance experienced by CI-921.

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References

1. 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: 3245–3251
2. Bodey GP, Jacquillat C (1983) In: "Amascrine: Current perspectives and clinical results with a new anti-cancer agent". Communications Media for Education, New Jersey
3. Bukowski RM, Legha SS, Saiki J, Eyre HJ, O'Bryan RO (1982) Phase II trial of m-AMSA in hepatocellular carcinoma: A Southwest Oncology Group Study. Cancer Treat Rep 66: 1651–1652
4. Cain BF, Atwell GJ (1974) The experimental antitumor properties of three congeners of the acridylmethanesulphonanilide (AMSA) series. Eur J Cancer 10: 539–549
5. Cysyk RLD, Shoemaker D, Adamson RH (1977) The pharmacologic disposition of 4'-(9-acridinylamino)methanesulfon-*m*-anisidide in mice and rats. Drug Metab Dispos 6: 579–590
6. Drelichman A, Decker DA, Al-Sarraf M, Dhabuwala CO (1982) m-AMSA in disseminated prostatic carcinoma: A phase II study. Cancer Treat Rep 66: 1993–1994
7. Gibaldi M, Perrier D (1982) Noncompartment analysis based on statistical moment theory In: Pharmacokinetics. Dekker, New York
8. Guentert TW, Jin-Ding H, Oie S (1982) Disposition of quinidine in the rabbit. J Pharm Sci 71: 812–815
9. Hiura M, Nakagawa S, Kawashima M, Kaneniwa N (1984) Effect of lipid solubility on hepatic first-pass metabolism of barbiturates in rabbits. Int J Pharm 20: 73–85
10. Jurlina JL, Paxton JW (1983) A high-performance liquid chromatographic method for the determination of 4'(acridinylamino)methanesulfon-*m*-anisidide (AMSA) in plasma. J Chromatogr 276: 367–374
11. Jurlina JL, Paxton JW (1985) A high-performance liquid chromatographic assay for plasma concentrations of *N*-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide (CI-921, NSC 343499). J Chromatogr 342: 431–435
12. Jurlina JL, Varcoe AR, Paxton JW (1985) Pharmacokinetics of amsacrine in patients receiving combined chemotherapy for treatment of acute myelogenous leukemia. Cancer Chemother Pharmacol 14: 21–25
13. Legha SS, Keating MJ, McCredie KB, Bodey GP, Freireich EJ (1982) Evaluation of AMSA in previously treated patients with acute leukemia. Results of therapy in 109 adults. Blood 60: 484–490
14. Paxton JW, Jurlina JL (1985) Elimination kinetics of amsacrine in the rabbit. Evidence of nonlinearity. Pharmacology 31: 50–56
15. Rowland M, Tozer TN (1980) Clearance and renal exretion. In: Clinical pharmacokinetics: concepts and applications. Lea and Febiger, Philadelphia
16. Shoemaker DD, Cysyk RL, Gormley PE, De Souza JSV, Malspeis L (1984) Metabolism of 4'-(9-acridinylamino)methansulfon-*m*-anisidide by rat liver microsomes. Cancer Res 44: 1939–1946
17. Tozer TN, Gambertoglio JG, Furst DE, Avery DS, Holford NHG (1983) Volume shifts and protein binding estimates using equilibrium dialysis: Application to prednisolone binding in humans. J Pharm Sci 72: 1442–1448
18. Warrel RP, Strauss DJ, Young CW (1980) Phase II trial of 4'-(9-acridinylamino)methansulfon-*m*-anisidide in the treatment of advanced non-Hodgkin's lymphoma. Cancer Treat Rep 64: 1157–1158

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