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Pharmacokinetics and toxicity of the antitumour agent *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide after i.v. administration in the mouse*

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Summary. The pharmacokinetics, tissue distribution and toxicity of the antitumour agent *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide(AC) were studied after i.v. administration to mice. Over the dose range of 9–121 μmol/kg (3–40 mg/kg), AC displayed linear kinetics with the following model-independent parameters: clearance (*C*), 21.0 ± 1.9 l h^{-1} kg^{-1}; steady-state volume of distribution (V_{ss}), 11.8 ± 1.4 l/kg; and mean residence time (MRT), 0.56 ± 0.02 h. The plasma concentration-time profiles for AC fitted a two-compartment model with the following parameters: C_c, 19.4 ± 2.3 l h^{-1} kg^{-1}; V_c, 7.08 ± 1.06 l/kg; $t_{1/2\alpha}$ 13.1 ± 3.5 min; and $t_{1/2Z}$, 1.60 ± 0.65 h. AC displayed moderately high binding in healthy mouse plasma, giving a free fraction of 15.9%–25.3% over the drug concentration range of 1–561 μM. After the i.v. administration of 30 μmol/kg [^{3}H]-AC, high radioactivity concentrations were observed in all tissues (especially the brain and kidney), showing a high $t_{1/2c}$ value (37–59 h). At 2 min (first blood collection), the AC concentration as measured by high-performance liquid chromatography (HPLC) comprised 61% of the plasma radioactivity concentration (expressed as AC equivalents/l). By 48 h, 73% of the dose had been eliminated, with 26% and 47% of the delivered drug being excreted by the urinary and faecal routes, respectively; <1% of the total dose was excreted as unchanged AC in the urine. At least five distinct radiochemical peaks were distinguishable by HPLC analysis of plasma extracts, with some similar peaks appearing in urine. The 121- μmol/kg dose was well tolerated by mice, with sedation being the only obvious side effect and no significant alterations in blood biochemistry or haematological parameters being recorded. After receiving a dose of 152 μmol/kg, all mice experienced clonic seizures for 2 min (with one death occurring) followed by a period of sedation that lasted for up to 2 h. No leucopenia occurred, but some mild anaemia was noted. There was no significant change in blood biochemistry. A further 20% increase in the i.v. dose (to 182 μmol/kg) resulted in mortality, with death occurring within 2 min of AC administration.

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Introduction

N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AC, NSC 601316; Fig. 1) is an experimental antitumour agent that was developed in the Cancer Research Laboratory, University of Auckland Medical School [6]. It was synthesised in a continuation of a programme that produced two other acridine derivatives: amsacrine, which is now used in the treatment of acute leukemia [1], and its 4-methyl-5-(*N*-methyl-carboxamide) analogue, CI-921, which has completed phase I clinical trials [9] and is now undergoing phase II evaluation against various solid tumours. Amsacrine exhibits only marginal antitumour activity in vivo against the Lewis lung carcinoma in mice, whereas CI-921 displays high activity [2] and AC is curative [5]. It has been suggested that the high activity of AC against solid tumours in mice could partly be explained by better distributive properties, as it is more lipophilic than amsacrine or CI-921 and the ionisation of its acridine nitrogen is almost completely suppressed at physiological pH [6]. A reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the determination of AC in biological fluids [16]. Using this method and [^{3}H]-AC, we investigated the pharmacokinetics and tissue distribution of AC after i.v. administration to the mouse and compared the results with those obtained in previous studies on amsacrine and CI-921 [12].

N-(2-(DIMETHYLAMINO)ETHYL)ACRIDINE-4-CARBOXAMIDE

AC

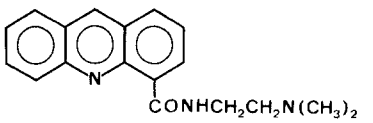

Fig. 1. Structure of *N*-[2-(dimethylamino)-ethyl]acridine-4-carboxamide

Materials and methods

Materials. The dichloride salts of AC, the internal standard *N*-[2-(diethylamino)ethyl]acridine-4-carboxamide, some putative metabolites of AC and the radiochemical starting material (acridine-4-carboxylic acid sodium salt) were synthesised by Prof. W. A. Denny in the Cancer Research Laboratory, University of Auckland Medical School [6]. The latter compound was tritiated by catalytic exchange by Amersham International (Amersham UK) and then conjugated with 2-dimethylaminoethylamine to form the di-HCl salt of AC (in the Cancer Research Laboratory). The chemical and radiochemical purity was confirmed by thin-layer chromatography (TLC) on silica gel plates (Whatman LK6D, 20 × 5 cm) using a solvent system consisting of butanol: acetic acid: water (4 : 1 : 1, by vol. followed by scanning for radioactivity using an automatic TLC linear analyser (Tracemaster 20; Berthold Analytical Instruments Inc., Nashua, N. H., USA) and also by HPLC (conditions described below) using concurrent fluorescence and radiochemical detection (Raytest Ramona LS Radioactivity Monitor, Straubenhardt, FRG). The [^{3}H]-AC was >98% pure, exhibiting a specific activity of 164.6 μCi/μmol, and was used without further purification. The scintillation fluid was ACS II (Amersham UK). Protosol (NEN, Boston, Mass., USA) was used to dissolve tissue before the counting of radioactivity. The solvents were HPLC-grade acetonitrile and methanol (Mallinckrodt Inc., Paris, Ky., USA). The ammonium acetate, heptane sulfonic acid (sodium salt), triethylamine, glacial acetic acid (all from BDH Chemicals, Poole, UK) and phosphoric acid (J. T. Baker, Phillipsburg, NJ, USA) were all Analar grade. All aqueous solutions were prepared using Millipore Milli-Q water.

Animals. Male BDF_1 mice (20–25 g) were maintained under constant temperature and humidity, with sterile bedding and food and water being provided according to institutional guidelines. All animal procedures were approved by the Animal Ethics Committee, University of Auckland.

Drug formulation and administration. A stock AC solution (10 mM) was prepared in sterile saline and diluted to the concentration required to provide an injection volume of 5 μl/g at doses of 9, 30, 91 and 121 μmol/kg (i. e. 3, 10, 30 and 40 mg/kg). The optimal single i. v. dose for the cure of Lewis lung carcinoma in the mouse was 91 μmol/kg [6]. [^{3}H]-AC was included in the 30 μmol/kg dose for distribution and excretion studies. AC (100–125 μl) was injected into the tail vein over 15 s. With the animals under ether anaesthesia, blood was collected into heparinised tubes by ocular extrusion at 2, 15 and 30 min and at 1, 1.5, 2, 3, 4, 5 and 6 h (3 mice per time point), and the mice were swiftly killed by cervical dislocation. The liver, gallbladder (when possible), kidneys, spleen and brain were rapidly removed from mice that had received [^{3}H]-AC), blotted dry and then weighed. After the centrifugation of blood, the plasma was removed and both plasma and packed red blood cells (and tissues) were stored frozen at –80° C until analysis. Nine additional mice that had been given [^{3}H]-AC were held individually in glass or plastic metabolic cages, which enabled the separate collection of urine and faeces. At the end of 8, 24 and 48 h, the mice were killed and blood and tissues were obtained as described above.

HPLC analysis. A specific reversed-phase HPLC method using *N*-[2-(diethylamino)ethyl]acridine-4-carboxamide as an internal standard has been developed for the quantitation of AC in plasma [16]. Briefly, the compounds of interest were extracted from mouse plasma (50–200 μl) after protein precipitation with acetonitrile. This acetonitrile supernatant was further purified on C18 solid-phase extraction Bond Elut columns (Analytichem International, Harbor City, Calif., USA). After elution with acetonitrile-ammonium acetate buffer and subsequent evaporation, the final separation was carried out on a C18 μBondapak stainless steel column (length, 30 cm; inside diameter, 0.05 cm; Waters Associates, Milford, Mass., USA) using a mobile phase of acetonitrile: water (32 : 68, v/v) containing 5 mM heptane sulfonic acid and 10 mM triethyl-ammonium phosphate (final pH, 3.5). Detection was carried out by fluorescence emission at 475 nm, with 358 nm being the excitation wavelength (the extracts containing [^{3}H]-AC were also monitored by the radiochemical detector). The accuracy (i. e. relative recovery of drug) of the method varied from 97% to 105% with acceptable precision, the intra- and inner-run coefficients of variation (CV) being <4.1% and 7.7%, respectively, over the concentration range of 0.1–5 μM. The lowest concentration that could be measured in 200 μl mouse plasma with acceptable accuracy (90%–110%) and precision (CV, <10%) was 0.02 μM. The same method was used for the quantitation of AC in mouse urine except that the urine was diluted 1 : 10 (v/v) with water and 25 μl was used for the analysis.

Plasma (from mice treated with [^{3}H]-AC) that was left over after HPLC was pooled from individual mice at each collection time and aliquots (100–250 μl) were subjected to protein precipitation with acetonitrile or methanol. Supernatant samples (50 μl) were analysed by HPLC using radiochemical detection. Aliquots of urine were also subjected to similar HPLC analysis. In addition, the precipitated plasma proteins were further washed with 6 × 2.5 ml acetonitrile followed by 3 × 2.5 ml methanol. The protein pellet was then digested and assessed for radioactivity.

Radioactivity quantitation. Total radiochemical equivalents of AC in plasma and in various tissues were determined by liquid scintillation counting with quench correction. Plasma (10 μl) and urine (10 μl) were counted in 10 ml ACS II. Weighed tissue samples, red blood cells and gallbladders were digested with protosol (0.5–1.5 ml) overnight and decolorised with 0.5 ml hydrogen peroxide when necessary. ACS II (10 ml) was added and the pH was neutralised with glacial acetic acid (100–200 μl) to reduce chemiluminescence before counting.

Free fraction of AC in plasma. The free AC fraction was determined in mouse plasma by equilibrium dialysis using a Dianorm dialyser (Diachema, Switzerland) and a cellulose acetate membrane with a 12,000-Da molecular-weight cutoff (Visking, Union Carbide Corporation, Chicago, Ill., USA). The protein concentration in each plasma sample was determined before and after each dialysis event using the method of Lowry et al. [13]. AC and [^{3}H]-AC were added to plasma from healthy, untreated mice to give a concentration of 1–561 μM. In addition, plasma samples from mice that had received an i. v. dose of 91 μmol/kg AC were collected at 1 and 3 h and 0.01 μM [^{3}H]-AC was added as a tracer. When appropriate, AC was further added such that all final concentrations were approx. 1 μM. The plasma was adjusted to pH 7.35 with CO_2 and then dialysed for 3 h at 37° C against isotonic phosphate-buffered saline (pH 7.35). After dialysis, the radioactivity in buffer and plasma was determined by liquid scintillation counting. The free fraction fu', calculated as the ratio of the radioactivity in buffer to that in plasma, is independent of any loss of [^{3}H]-AC to the apparatus or membrane but does not take into account the volume changes that occur due to colloidal osmotic fluid shift during dialysis [15]. The free fraction fu, which does take these volume changes into account, was calculated by the equation proposed by Huang [11]:

$$fu = \frac{fu' \times R}{fu'R + 1 - fu'}$$

where R represents the ratio of plasma protein concentration after/before dialysis.

Toxicological monitoring. Serum biochemical (creatinine, bilirubin, albumin, total protein, aspartate transaminase and alkaline phosphatase)

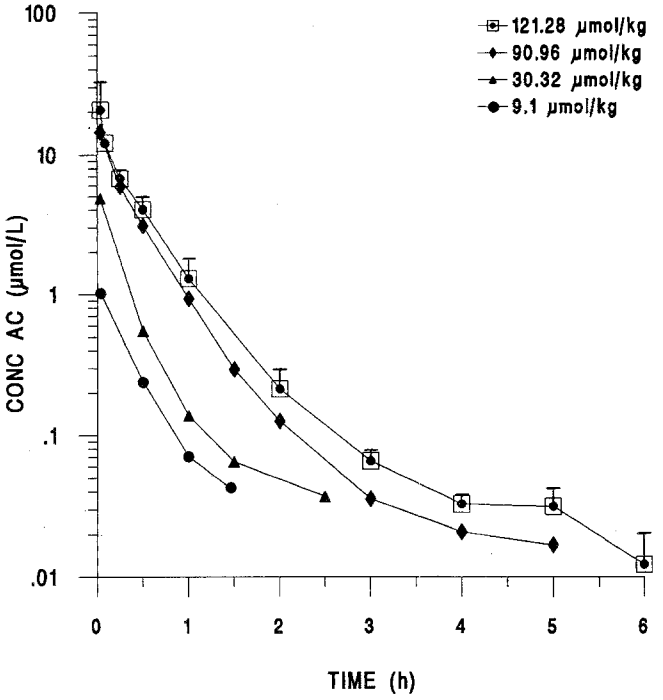

Fig. 2. Mean plasma AC concentration-time profiles in mice after i.v. doses of 9, 30, 91 and 121 μmol/kg. The bars represent +1SD and for clarity are shown only for the highest dose

and haematological (haematocrit, haemoglobin and red blood cell, white blood cell, lymphocyte and platelet counts) tests were carried out in mice after saline injection (n = 12) and at 1, 2, 3, 5 and 7 days after i.v. AC administration (121 and 152 μmol/kg). These tests were carried out by routine methods in the Clinical Chemistry Unit and Haematology Department of Auckland Hospital.

Pharmacokinetic methods. The area under the plasma concentration-time curve (AUC) was computed using the log trapezoidal rule and was extrapolated to infinity by addition of the value C_t/Z, where C_t represents the concentration at the last time point and Z indicates the terminal slope determined by linear regression. The model-independent pharmacokinetic parameters clearance (C), steady-state volume of distribution (V_{ss}) and mean residence time (MRT) were calculated by the following equations: C = Dose/AUC; V_{ss} = (Dose × AUMC)/AUC^2, and MRT = AUMC/AUC, where AUMC represents the total area under the first moment of the plasma concentration-time curve computed in a fashion similar to that used for AUC [7]. The mean concentration-time profiles at the four dose levels were fitted to one- or two-compartment models with linear kinetics using MKMODEL, an extended least-squares modelling system, and the models were compared by the Schwarz criterion [10]. Elsewhere, data were compared using Student's t-test, with differences being considered to be significant if P <0.05.

Results

AC plasma pharmacokinetics

The mean concentration-time profiles of AC in mouse plasma after i.v. administration (Fig. 2) typically exhibited biphasic kinetics. The modelling programme indicated that the two-compartment model provided the best fit. A summary of the pharmacokinetic data is given in Table 1. There were significant linear correlations between both dose and AUC ($y = 0.052x - 0.070$; P <0.0001; $df = 3$; $r = 0.9986$) and dose and C_{max} ($y = 0.170x - 0.327$; P <0.0001; $df = 3$; $r = 0.9987$), indicating that the kinetics in mice are independent of the dose up to 121 μmol/kg. Over this 13-fold dose range, the C, V_{ss} and MRT remained relatively constant, the mean values being 21.0±1.9 l h^{-1} kg^{-1}, 11.8±1.4 l/kg and 0.56±0.02 h, respectively.

Plasma protein binding

AC displayed moderately high binding in healthy mouse plasma, with the free fraction amounting to 15.9%–25.3% over the concentration range of 1–561 μM. The free fraction increased significantly (P <0.001) at AC concentrations of ≥56 μM (Table 2). In plasma collected from mice at 1 or 3 h after i.v. administration of AC, the free fraction (18.4%±0.9% and 20.8%±0.4% of the delivered dose, respectively) was significantly greater (P <0.001) than the control values (14.6%±0.5%).

Radioactivity distribution

The time course of radioactivity in plasma and various tissues after i.v. administration of 30 μmol/kg [^{3}H]-AC is reported in Table 3. Radioactivity concentrations in the plasma remained remarkably constant (7–8 μM) over the 24-h period following AC administration. At 2 min (the first sampling time), the AC concentration (4.9±0.5 μM as determined by HPLC) was 61% of the radiochemical AC

Table 1. Model-independent and -dependent pharmacokinetic parameters for AC in mice

Dose (μmol/kg)	Model-independent parameters					Model-dependent parameters			
	AUC (μmol h l^{-1})	c_{max} (μM)	C (l h^{-1} kg^{-1})	V_{ss} (l/kg)	MRT (h)	C_c (l h^{-1} kg^{-1})	V_c (l/kg)	$t_{1/2\alpha}$ (min)	$t_{1/2Z}$ (h)
9.1	0.41	1.03±0.08	22.2	13.2	0.59	18.3±4.4	7.95±0.72	12.4±4.3	2.54±1.65
30.3	1.32	4.86±0.54	22.9	12.8	0.56	22.8±1.3	5.6 ±0.36	8.4±0.7	1.05±0.40
91	4.82	14.5 ±0.9	18.9	10.5	0.56	18.0±1.2	7.11±0.70	15.4±0.9	1.31±0.43
121	6.06	21.0 ±5.8	20.0	10.7	0.54	18.7±1.4	7.75±0.84	16.0±1.3	1.49±0.27
Mean			21.0±1.9	11.8±1.4	0.56±0.02	19.4±2.3	7.08±1.06	13.1±3.5	1.60±0.65

Data represent mean values (±SD where appropriate). C_c, Total plasma clearance from a 2-compartment model; V_c, volume of distribution of the central compartment

Table 2. AC free fraction in plasma from healthy and AC-treated mice

	AC concentration (μM)	% Free fraction (±SD)	*n*	*P* value
Untreated mice:	1	15.9±0.6	4	
	11	15.8±0.2	4	NS
	56	18.6±0.9	4	$P<0.001$
	112	20.7±0.7	4	$P<0.0001$
	561	25.3±2.6	4	$P<0.0001$
Treated mice[a]:				
Controls[b]	1	14.6±0.5	3	–
1 h	1	20.8±0.4	3	$P<0.001$
3 h	1	18.4±0.9	3	$P<0.001$

[a] Treated mice received an i. v. dose of 91 μmol/kg and were bled at 1 or 3 h. At 1 h, the plasma concentration of AC was estimated to be approx. 1 μM; at 3 h this value was estimated to be approx. 0.04 μM. Therefore, AC was added to give an approximate final concentration of 1 μM

[b] Control mice received a saline injection and were bled at 1 h; AC was then added to give a plasma concentration of 1 μM

NS, Not significant

equivalents (8±0.3 μM) in plasma, indicating rapid metabolism of AC in the mouse. HPLC analysis using radiochemical detection of aliquots from acetonitrile and methanol extracts of mouse plasma collected up to 1.5 h after [^{3}H]-AC administration indicated at least five major radioactive peaks distinct from that of the standard [^{3}H]-AC. After the precipitated plasma proteins had been washed with acetonitrile and methanol; <1% of the total plasma radioactivity remained in the protein pellet, suggesting that AC is not covalently bound to plasma proteins in vivo. The highest concentrations of radioactivity were observed in the kidney (103.3±6.7 μmol/kg) and the brain (89.6±6.6 μmol/kg) at the first timepoint (2 min). The brain concentrations fell rapidly from this peak, reaching concentrations similar to those in plasma within 1 h, whereas the kidney concentrations of radioactivity declined more slowly over 24 h. Peak radioactivity concentrations occurred in the spleen (71.1±9 μmol/kg) and liver (34.5±1.3 μmol/kg) at 30 min. As in the kidney, radioactivity concentrations in the liver declined slowly over the following 24 h to reach concentrations equivalent to those in plasma. From 24 to 48 h, the apparent $t_{1/2Z}$ values for the removal of radioactivity from plasma and tissues were long and variable, ranging from 37 h in plasma to 59 h in red blood cells, with a mean value of 48±8 h being obtained for the tissues studied.

Excretion of radioactivity

After i.v. administration of 30 μmol/kg [^{3}H]-AC, <1% of the total dose was excreted in the urine as unchanged AC. Five major radioactive peaks distinct from that of [^{3}H]-AC were distinguishable by HPLC analysis of the urine specimens. The cumulative excretion of total radioactivity expressed as a percentage of the delivered dose is given in Table 4. The principal excretory pathway was the faecal route (47%±8%), and this value was approximately twice that for urinary excretion. By 24 h, 70% of the dose had been eliminated from the body, with little increase in this value being noted at 48 h.

Toxicological monitoring

Administration of 182 μmol/kg i.v. AC was lethal, with seizures and death occurring within 2 min in both of the mice who received this dose. Of the 18 mice that received an i.v. dose of 152 μmol/kg, 1 died within 2 min after AC administration. The remaining 17 experienced clonic seizures followed by a period of sedation that lasted for up to 2 h. Thereafter, the mice commenced grooming and eating and appeared normal. Sedation (but no obvious seizures) was observed in 18 mice that received 121 μmol/kg AC. No significant weight loss was recorded over 28 days after AC administration at either dose. There was no significant alteration in biochemical parameters over the 7-day monitoring period after dosing as compared with the 12 control mice that received vehicle. Mild anaemia was apparent in the mice after a dose of 152 μmol/kg, with significant reductions in haematocrit ($P = 0.005$, $df = 23$), RBC count ($P = 0.005$, $df = 23$) and haemoglobin concentration ($P < 0.0001$, $df = 25$) being noted, but no leucopenia was observed. There was no significant reduction in the platelet count.

Table 3. Tissue concentrations of radioactivity after an i. v. dose of 30 μmol/kg [^{3}H]-AC

	Concentration (μmol/l or /kg)								Half-life[a] (h)
Time (h)	0.03	0.5	1	1.5	2.5	8	24	48	
Plasma	8.0±0.3	7.9± 0.5	7.9± 0.6	7.7± 0.2	8.2± 1.2	8.0± 0.6	6.8±0.9	4.3±0.4	37± 8
RBC	11.8±2.6	6.5± 0.4	5.8± 0.3	5.0± 0.1	5.5± 0.7	4.8± 0.9[b]	4.3±0.2[b]	3.3±0.1	59± 5
Brain	89.6±6.6	16.2± 0.7	9.4± 0.4	7.4± 0.5	7.3± 1.5	7.2± 0.4	7.1±0.8	5.3±1.0	54±21
Spleen	19.7±4.3	71.1± 9.0	28.2± 5.1	24.0± 2.7	10.2± 2.0	4.6± 2.1	4.2±1.4	3.8±0.7	–
Kidney	103.3±6.7	46.5± 4.9	27.3± 12.2	19.0± 5.5	12.4± 2.3	11.5± 3.6	7.1±2.7	4.8±0.7	42±30
Liver	26.5±4.0	34.5± 1.3	26.4± 2.6	23.3± 3.9	20.4± 6.8	16.3± 3.6	8.1±1.1	5.6±1.0	46±13
Gallbladder	8.9±5[b]	794 ±619[b]	598 ±330	740 ±517	494 ±591[b]	108. ±142[b]	3 ±1[b]	4 ±1	–

Data represent mean values ± SD ($n = 3$) expressed in μmol AC equivalents per litre or kg

[a] The half-life for the elimination of radioactivity was calculated from 24 to 48 h. This parameter was not calculated for the spleen or the gallbladder, as there was little difference between the 24- and the 48-h concentrations

[b] $n = 2$

Table 4. Excretion of radioactivity after i.v. administration of 30 μmol/kg [^{3}H]-AC to mice

	Cumulative % of dose		
Excretion period (h)	Urine	Faeces	Total
0– 8	13±9	24±13	37±13
0–24	21±6	49± 9	70± 7
0–48	26±4	47± 8	73± 6

Data represent mean values ± SD ($n = 3$)

Discussion

These studies indicate that AC is eliminated in the mouse mainly by biotransformation and excretion of the metabolites via the bile into the faeces and, to a lesser extent, by the kidney into the urine. At least five distinct metabolites were observed in plasma, and further work is under way to identify these metabolites. The concentration-time profile for unchanged AC in the mouse was more similar to that of amsacrine than to that of CI-921 [12]. A 30-μmol/kg AC dose resulted in a plasma C_{max} value of 4.9 μM and an AUC of 1.3 μmol h l^{-1} vs the values of 6.5 μM and 1.9 μmol h l^{-1} previously found for a similar molar dose of amsacrine [12]. Using a haematocrit value of 40% and a blood-plasma concentration ratio of 1 (from the addition of [^{3}H]-AC to normal mouse blood), a blood clearance value of 18 l h^{-1} kg^{-1} was calculated. The hepatic blood flow in the mouse is reported to be 5 l h^{-1} kg^{-1} [3], which suggests that AC undergoes a high degree of hepatic extraction and/or that other non-hepatic pathways play a significant role in its removal.

Similarly high blood clearance (21 l h^{-1} kg^{-1}) has been found for amsacrine in mice, but amsacrine undergoes significant covalent binding to plasma and tissue proteins, which is considered to be a major additional pathway that significantly contributes to the high clearance value for this drug in mice [12]. However, there was no evidence of covalent binding of AC to mouse plasma proteins. Similarly, the prolonged elimination half-life of plasma radioactivity could not be explained by covalent binding of AC to plasma proteins and subsequent retention of radioactivity within the circulation. Possible explanations for the persistence of radioactivity in plasma and tissue include the slow release of AC/metabolite from high-affinity binding sites within the tissues, the formation of a metabolite(s) that is slowly eliminated, or perhaps enterohepatic recirculation of metabolites. In contrast to amsacrine and CI-921, whose pharmacokinetics are dose-dependent in mice over the dose range of 14–58 μmol/kg [12], AC exhibited dose-independent (i.e. linear) kinetics at doses of up to 121 μmol/kg, which we considered to be the maximal tolerated dose given via the tail vein to healthy BDF_1 mice.

The free (i.e. dialysable) fraction of AC in mouse plasma was considerably greater than those reported for amsacrine (6.72%±0.72%) and CI-921 (0.63%±0.11%) [12]. The significant increase in the AC free fraction that occurred at 56 μM suggests that a protein with a low plasma concentration (such as α_1-acid glycoprotein) may play a more important role in plasma binding than does albumin (present in mouse plasma at approximately 400 μM) [14]. Studies using human α_1-acid glycoprotein have indicated that this protein is of major importance for the binding of AC in human plasma (unpublished data). In addition, the significant increase in the AC free fraction in mouse plasma at 1 and 3 h after drug administration suggests that metabolites of AC may bind to the same protein, possibly with greater affinity that exhibited by the parent drug. It may be surprising that these alterations in plasma protein binding did not result in non-linear kinetics, but it is possible that they were insufficient to cause a significant change in the total AC concentrations in plasma.

In prior investigations of the antitumour activity of AC, 91 μmol/kg was found to be the optimal single i.v. dose for the cure of s.c. Lewis lung tumours in BDF_1 mice [6]. This was also the maximal tolerated i.v. dose in these tumour-bearing mice (Baguley, personal communication). In our study on healthy mice, 121 μmol/kg was the maximal dose that could be given by tail-vein injection (over 15 s) without producing adverse effects other than sedation. A 25% increase in the dose to 152 μmol/kg resulted in clonic seizures in all mice and in one death. An additional 20% increase to 182 μmol/kg resulted in seizures and death in the two mice that received this dose. This lethal toxicity appeared to be related to a peak concentration phenomenon, as animals died within 2 min of drug injection. This may be associated with the rapid entry and uptake of AC into the brain. Using Oldendorf's technique, we have observed a high brain-uptake index (similar to that of diazepam) and retention of AC in the brain in mice [4].

The ability of AC to cross the blood-brain barrier rapidly suggests a role for the drug in the treatment of brain tumours. With the exception of nitrosoureas, few of the antitumour agents currently in use possess the physicochemical properties required for adequate penetration of the blood-brain barrier [8]. Conversely, this could be a disadvantage if AC is found to be useful for the treatment of non-cerebral tumours. However, the apparent acute neurotoxicity of AC can be circumvented by the use of a different method of administration. For example, mice have tolerated an approximate 4-fold increase in the AC dose (to 455 μmol/kg) following i.p. injection, with sedation being the only obvious side effect [6]. It is not known whether the reduction in acute toxicity after i.p. administration is attributable to reduced bioavailability due to the route of administration used or to altered distribution (e.g. the rate and amount of uptake into the brain). Further studies are under way to investigate these possibilities. However, it is unlikely that this acute toxicity will clinically prove to be a problem, as AC will preferably be given by slow infusion. A major advantage of AC administration may be reflected in the absence of leucopenia in our mice after a single curative dose.

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References

1. Arlin Z (1983) Current status of amsacrine combination chemotherapy programs in acute leukaemia. Cancer Treat Rep 67: 967
2. Baguley BC, Denny WA, Atwell GJ, Finlay GJ, Rewcastle GW, Twigden SJ, Wilson WR (1984) Synthesis, antitumour 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
3. Boxenbaum H (1982) Interspecies scaling, allometry, physiological time and the ground plan of pharmacokinetics. J Pharmacokinet Biopharm 10: 201
4. Cornford EM, Young D, Paxton JW (1991) A comparison of brain and liver penetration of acridine antitumor drugs. Cancer Chemother Pharmacol (in press)
5. Finlay GJ, Baguley BC (1989) Selectivity of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide towards Lewis lung carcinoma and human tumour cell lines in vitro. Eur J Cancer Clin Oncol 25: 271
6. Finlay GJ, Rewcastle GW, Baguley BC, Denny WA (1987) Potential antitumour agents: 50. In vivo solid tumour activity of derivatives of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. J Med Chem 30: 664
7. Gibaldi M, Perrier D (1982) Noncompartment analysis based on statistical moment theory. In: Gibaldi M, Perrier D (eds) Pharmacokinetics. Marcel Dekker, New York
8. Greig MH (1987) Chemotherapy of brain metastases: current status. Cancer Treat Rep 11: 157
9. Hardy JR, Harvey VJ, Paxton JW, Evans PC, Smith S, Grove W, Grillo-Lopez AJ, Baguley BC (1988) A phase I trial of the amsacrine analog 9-[[2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino]-*N*,5-dimethyl-4-acridine-carboxamide (CI-921). Cancer Res 48: 6493
10. Holford NHG (1985) MKMODEL, a modelling tool for microcomputers – a pharmacokinetic evaluation and comparison with standard computer programs (abstract). Clin Exp Pharmacol [Suppl 9]: 95
11. Huang JD (1983) Errors in estimating the unbound fraction of drugs due to volume shifts in equilibrium dialysis. J Pharm Sci 72: 1368
12. 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-acridine-carboxamide (CI-921) in plasma, liver and Lewis lung tumors in mice. Cancer Res 50: 503
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265
14. Paxton JW (1983) $Alpha_1$-acid glycoprotein and binding of basic drugs. Methods Find Exp Clin Pharmacol 25: 353
15. 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
16. Young D, Evans PC, Paxton JW (1990) Quantitation of the antitumour agent *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide in plasma by high performance liquid chromatography. J Chromatogr Biomed Appl 528: 385