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Short communication

Pharmacokinetics of acridine-4-carboxamide in the rat, with extrapolation to humans

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Summary. The pharmacokinetics of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (AC) were investigated in rats after i.v. administration of 18, 55 and 81 μmol/kg [^{3}H]-AC. The plasma concentration-time profiles of AC (as measured by high-performance liquid chromatography) typically exhibited biphasic elimination kinetics over the 8-h post-administration period. Over this dose range, AC's kinetics were first-order. The mean (±SD) model-independent pharmacokinetic parameters were: clearance (Cl), 5.3±1.1 l h^{-1} kg^{-1}; steady-state volume of distribution (Vss), 7.8±3.0 l/kg; mean residence time (MRT), 1.5±0.4 h; and terminal elimination half-life ($t_{1/2Z}$), 2.1±0.7 h (n = 10). The radioactivity levels (expressed as AC equivalents) in plasma were 1.3 times the AC concentrations recorded at 2 min (the first time point) and remained relatively constant for 1–8 h after AC administration. By 6 h, plasma radioactivity concentrations were 20 times greater than AC levels. Taking into account the species differences in the unbound AC fraction in plasma (mouse, 16.3%; rat, 14.8%; human, 3.4%), allometric equations were developed from rat and mouse pharmacokinetic data that predicted a Cl value of 0.075 (range, 0.05–0.10; 95% confidence limits) l h^{-1} kg^{-1} and a V_{ss} value of 0.63 (range, 0.2–1.1) l/kg for total drug concentrations in humans.

Introduction

N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (AC; NSC 601316) is an experimental antitumour agent developed in the Cancer Research Laboratory, University of Auckland Medical School [2, 5]. AC is presently being considered for phase I clinical trials by the Cancer Research Campaign (UK). Its predecessors were amsacrine (now used to treat leukaemia) and its 4-methyl-5-(*N*-methyl-carboxamide) analogue (CI-921), which is undergoing phase II evaluation against solid tumours [1, 6]. We have previously demonstrated the use of allometric equations that allowed a reasonable prediction of the pharmacokinetics of amsacrine and CI-921 in patients from animal data [10]. In particular it was observed that the mouse and rat data were close to the best-fit prediction line, suggesting the possibility of making reasonable pharmacokinetic predictions for patients entering a phase I trial from just the mouse and the rat. The pharmacokinetics of AC in mice have previously been reported [11]. The aim of this study was to investigate the pharmacokinetics of AC in the rat and, with the mice data, to predict the pharmacokinetic parameters for AC in humans.

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Materials and methods

The drug formulation, pharmacokinetic analysis, high-performance liquid chromatography (HPLC) of AC and radioactivity ([^{3}H]-AC equivalents) measurements in plasma have been described elsewhere [3, 11, 12]. Male Wistar rats (350–400 g) were given [^{3}H]-AC by tail-vein injection at three dose levels (18, 55 and 81 μmol/kg), with 3–4 rats being studied at each dose. The maximal dose for rats was calculated from the maximum tolerated dose in mice using body weight raised to the power 0.75 ($W^{0.75}$). Blood (0.5 ml) was collected at 2 and 30 min and at 1, 2, 4, 6 and 8 h from the carotid artery, which had been cannulated with PE50 polyethylene tubing under anaesthesia (Nembutal, 60 mg/kg i.p.) the previous day. This cannula was tunneled to the back of the neck, which permitted the animal to be awake and taking food and fluids ad libitum during the 8-h period of blood sampling. The cannula was flushed with saline equivalent to the volume of blood removed. The total volume of blood collected (apart from the last sample, when the rat was exsanguinated) did not exceed 3 ml, which is approximately 10% of the total blood volume. All animal procedures were approved by the Animal Ethics Committee, University of Auckland.

Results

AC pharmacokinetics at different dose levels

The concentration-time profiles of AC in rat plasma after i.v. administration typically exhibited biphasic kinetics (Fig. 1). A summary of the model-independent pharmaco-

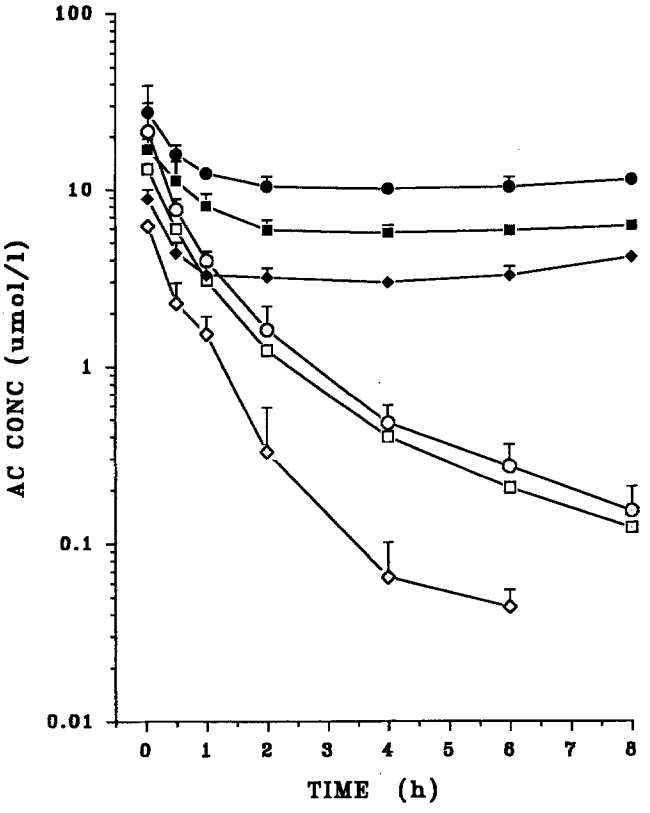

Fig. 1. Mean plasma AC concentration-time profiles obtained after i. v. bolus injection of 18 (◇), 55 (□) and 81 μmol/kg (○) in plasma of normal rats. For the sake of clarity, only +SD values have been included for the low and high doses. The *solid symbols* represent the respective mean radioactive concentrations (+1 SD) expressed as AC equivalents

kinetic data is presented in Table 1. There were significant linear correlations between dose and AUC ($y = 0.052x - 0.070$; $P < 0.0001$; $df = 3$, $r = 0.9986$) and between dose and C_{max} ($y = 0.170x - 0.327$; $P < 0.0001$; $df = 3$; $r = 0.9987$), indicating that AC exhibits first-order kinetics in the rat at doses of up to 81 μmol/kg. Over this dose range, the clearance (Cl), steady-state volume of distribution (V_{ss}) and mean residence time (MRT) remained relatively constant, with mean (±SD) values being 5.3±1.1 l h^{-1} kg^{-1}, 7.8±3.0 l/kg and 1.5±0.4 h, respectively.

Radioactivity profile

The time courses of radioactivity in plasma are also shown in Fig. 1. At 2 min (the first sample) the AC concentration determined by HPLC was 70%–78% of the radiochemical AC equivalents, indicating rapid metabolism of AC in the rat. After 1 h, the radioactivity concentrations in the plasma remained remarkably constant and by 6 h were greater than 20 times the AC concentrations.

Extrapolation of AC pharmacokinetics to humans

Model-independent pharmacokinetic parameters were used for extrapolation of the data to humans. Previously we have shown that better allometric correlations are obtained for Cl and V_{ss} with amsacrine and CI-921 if species differences in plasma protein binding are taken into account [10]. The mean percentage (±SD) of the unbound fraction for AC in rat plasma is 16.3%±0.9%, that in mouse plasma is 14.8%±0.8%, and that in human plasma is 3.4%±0.2% [4]. These values were used to calculate the clearance (Cl_u) and steady-state volume of distribution (V_{ss_u}) of the unbound plasma concentration for mice and rats. The relationship between body weight and both Cl_u, and V_{ss_u} from mouse and rat data resulted in the following allometric equations: $Cl_u = 19W^{0.49}$ and $V_{ss_u} = 40W^{0.82}$. Substituting 70 kg for W in these equations gave a Cl_u value of 2.2 (range, 1.5–2.9; 95% confidence limits) l h^{-1} kg^{-1} and a V_{ss_u} value of 18.6 (range, 5.2–32) l/kg for a 70-kg patient. The V_{ss} and Cl of the total drug concentration in the plasma can then be calculated from the latter values by multiplying by 0.034 (the unbound fraction in human plasma) to give 0.075 (range, 0.05–0.1) l h^{-1} kg^{-1} (Cl) and 0.63 (range, 0.2–1.1) l/kg (V_{ss}).

Discussion

The plasma pharmacokinetics of AC were first-order up to the maximum tolerated dose in rats, which is in agreement with the results of our mouse studies [11]. The maximum tolerated i. v. dose in rats (81 μmol/kg) was calculated from that in mice (121 μmol/kg) by extrapolation using $W^{0.75}$. This rat dose was appropriate, resulting in a maximal concentration (C_{max}) of 21.3±2.6 as compared with 21.0±5.8 μmol/l in mice [11]. As with mice, minor

Table 1. Model-independent pharmacokinetic parameters for AC in rats

i. v. Dose (μmol/kg)	AUC (μmol h l^{-1})	C_{max} (μmol/l)	Cl (l h^{-1} kg^{-1})	V_{ss} (l/kg)	$t_{1/2Z}$[a] (h)	MRT (h)
18 ($n = 3$)	3.84±1.29	6.20±0.42	5.18±1.41	5.04±0.80	2.13±0.88	1.04±0.25
55 ($n = 3$)	11.1 ±2.2	13.1 ±1.9	5.20±1.01	9.42±2.73	2.44±0.65	1.77±0.21
81 ($n = 4$)	15.0 ±2.5	21.3 ±2.6	5.55±0.84	9.00±2.79	1.93±0.39	1.55±0.42
Overall mean ($n = 10$)			5.33±1.10	7.82±2.98	2.10±0.66	1.46±0.43

[a] Calculated from the terminal slope determined by linear regression
Data represent mean values ±SD where appropriate

seizures (2/4 rats) and sedation were observed at this dose in rats. In two rats that received an increased i.v. dose (100 μmol/kg), seizures followed by death resulted within minutes of drug administration. This was similar to our experience with mice. Although AC displays concentration-dependent protein binding in both mouse and rat plasma [11], concentrations of ≥ 20 μmol/l are necessary for a significant change (reduction) in the AC free fraction. In this study the C_{max} value was 21.3 ± 2.6 μmol/l, falling rapidly to <10 μmol/l within 30 min. Thus, concentration-dependent alterations in plasma protein binding would not be expected to have any significant effect on the pharmacokinetics of AC at these dose levels.

In a previous retrospective study of two structural analogues of AC, we have demonstrated using allometric equations based on data from four animal species (mice, rats, rabbits and dogs) that reasonable predictions of human pharmacokinetics were possible despite the observation that both agents were eliminated primarily by biotransformation [10]. These predictions for patients would have been useful in the estimation of a more appropriate starting dose and, in particular, might have resulted in potential savings in escalation steps during phase I clinical testing. In that study it was noted that the mouse and rat data were close to the best-fit line of prediction. That observation in conjunction with the guidelines (preclinical toxicology to be based on mice and rats) established by the European Organization for Research and Treatment of Cancer (EORTC) and the Cancer Research Campaign (CRC) [7] suggested the merit of extrapolating our mouse and rat data to predict the pharmacokinetic parameters in humans, especially as a phase I clinical trial for AC is planned for 1993.

The use of allometric equations to extrapolate pharmacokinetic parameters from rodent data gave predicted values of 0.63 l/kg and 0.075 l h^{-1} kg^{-1} for V_{ss} and Cl, respectively, suggesting an elimination half-life ($t_{1/2}$) of approximately 6 h in humans. However, one factor that could interfere with these predicted values is the presence of α_1-acid glycoprotein (AAG), the major binding protein for AC in plasma. Levels of this acute-phase reactant protein may be significantly elevated in cancer patients [9]. We have shown that in situations of elevated AAG levels (4.5 g/l), the unbound AC free fraction in plasma may be reduced 3-fold [4]. Thus, the predicted pharmacokinetic parameters may represent a 3-fold over-estimation of the true values in patients. Although there was a significant correlation between AAG concentrations and the AC plasma unbound fraction [4], it may be difficult to predict the free fraction in cancer patients as, although they typically have elevated AAG levels, they usually have decreased concentrations of albumin, which is also involved in the plasma protein binding of AC. In addition, metabolites of AC may alter AC's plasma protein binding [4] and counteract any increase in plasma binding due to increased levels of AAG. In both mouse and rat plasma, high concentrations of radioactivity with prolonged half-lives were observed. The persistence of radioactivity in plasma was not due to covalent binding of AC (or its metabolites) to plasma proteins, which has been observed for amsacrine in mouse plasma [8]. Alternative explanations include enterohepatic recirculation or, more likely, the appearance of a metabolite(s) with a slow elimination rate.

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