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Plasma pharmacokinetics of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide in a phase I trial

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Abstract DACA {*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide} is an acridine derivative with high activity against solid tumours in mice and a dual mode of cytotoxic action involving topoisomerases I and II. The plasma pharmacokinetics of DACA were studied in 28 patients with solid tumours in a phase I trial. A single dose was given every 3 weeks, being escalated from a starting dose of 18 mg/m^2 (as the dihydrochloride trihydrate salt) to a maximal dose, limited by severe pain in the infusion arm, of 1000 mg/m^2. Drug was given by constant intravenous infusion with a target delivery period of 3 h. Blood samples were taken from the contralateral arm before, during and for up to 72 h after the infusion. DACA was separated from plasma by solid-phase extraction and was analysed by reversed-phase high-performance liquid chromatography (C18 column) using fluorescence detection. A two-compartment pharmacokinetic model provided the best fit for the concentration-time profiles obtained for most patients showing clearance of 1.00 ± 0.36 l h^{-1} kg^{-1}, a volume of distribution of the central compartment of 0.72 ± 0.55 l/kg, an initial half-life of 0.28 ± 0.19 h and a terminal half-life of 2.04 ± 0.94 h. All pharmacokinetic parameters were independent of dose, indicating first-order kinetics. As DACA binds strongly to α_1-acid glycoprotein, plasma concentrations of this protein were determined and used to estimate free-drug fractions in plasma. Estimated values for the free fraction varied from 0.9% to 3.3% and were lower than those determined by equilibrium dialysis for mice and rats (15% and 16%, respectively). At the maximum tolerated dose (MTD) of 750 mg/m^2, the area under the drug concentration-time curve (AUC) was 46.2 ± 4.4 μ*M* h, exceeding that obtained in mice treated at the MTD (23.4 μ*M* h). On the other hand, the corresponding free-drug AUC was 0.92 ± 0.03 μ*M* h, much lower than the corresponding value (3.5 μ*M* h) determined for mice. These results suggest that free-drug rather than total drug concentrations are more appropriate for interspecies dose comparisons when significant differences exist in the free plasma fraction.

Key words Topoisomerase · Amsacrine · Clinical trial α_1-Acid glycoprotein · DACA

Introduction

N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA; see Fig. 1 for the structure) is a lipophilic DNA-intercalating acridine derivative developed in the Auckland Cancer Society Research Centre, The University of Auckland, New Zealand [1]. In mice the high activity of DACA against murine solid tumours such as Lewis lung and Colon 38 carcinomas distinguishes it from many other topoisomerase-directed drugs [2]. DACA is notable for its ability to overcome resistance in vitro against lines displaying P-glycoprotein or multiresistance protein (MRP) resistance [5, 14, 23] as well as against "atypically" multidrug-resistant lines that express low levels of the enzyme DNA topoisomerase II [9]. These properties appear to be a consequence of the dual specificity for topoisomerases I and II [10]. On the basis of these and other properties, DACA was selected to undergo phase I clinical trial under the auspices of the Cancer Research Campaign, United Kingdom (CRC).

This study was conducted under the auspices of the Cancer Research Campaign's Phase I/II Committee

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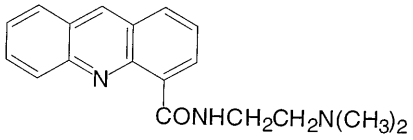

Fig. 1 Structure of DACA as the free base

High-performance liquid chromatography (HPLC) analysis methods have previously been developed for DACA [25], and the plasma pharmacokinetics of DACA have been studied in mice and rats using both radioactively labeled and unlabeled drug [7, 20, 21]. DACA plasma concentration-time profiles typically exhibit biphasic first-order elimination kinetics in both rodent species up to the maximum tolerated dose (MTD). In mice the MTD recorded after a single i.p. dose was approximately 4-fold that noted after i.v. administration. This was not due a reduction in bioavailability after i.p. administration, but the shape of the plasma concentration-time curve was considerably different as reflected by a 3-fold lower maximal concentration (Cmax) in plasma and a longer terminal half-life as compared with the i.v. route. The reduced Cmax and longer time taken to achieve the Cmax are believed to be a major factor in the lower degree of toxicity observed after i.p. administration [7] and suggest that slow infusion (e.g., 3-h infusion) might be more appropriate in patients.

In this plasma pharmacokinetics study of DACA in a phase I trial, doses ranged from 18 to 1000 mg/m^2 infused over 3 h. Plasma concentrations of α_1-acid glycoprotein (AAG) were also measured and used to estimate free (unbound) fractions and, from this, the free-DACA concentrations in plasma. Estimation of the free-drug AUC values of DACA may be important not only in comparison of the plasma pharmacokinetics of DACA among patients but also in the relation of human and rodent studies.

Patients and methods

Patients

This phase I trial of DACA was approved by the Phase I/II Committee of the CRC, the Standing Committee on Clinical Trials in New Zealand and the North Health Ethics Committee, Auckland, New Zealand. A total of 32 patients with solid tumours either refractory to chemotherapy or for whom no useful therapy was available were enrolled in the study. The eligibility criteria and clinical aspects of the trial have been described elsewhere [18]. DACA was formulated in 1- and 2-ml vials as aqueous solution containing 50 mg of the dihydrochloride trihydrate salt (MW 420) per milliliter. Drug was diluted in 0.9% sodium chloride (500 ml) and given by constant infusion in an arm vein with a target period of 3 h using an infusion pump (IMED Corporation, San Diego, Calif., USA). Repeated courses of DACA in the same patient were given where practicable at intervals of 3 weeks. The starting dose of DACA was 18 mg/m^2, corresponding to one-tenth of the i.v. LD_{10} (the dose lethal to 10% of the animals) in mice (calculated in milligrams per square meter of body surface area). A total of 68 courses were completed, with at least 3 patients being entered at each dose, up to a maximal dose of 1000 mg/m^2. One patient (patient 19) refused treatment after enrollment, and in three cases (patients 23, 31 and 32) the infusion was abandoned because of pain. Pharmacokinetics studies were completed in the remaining 28 patients, and in 5 of these a second set of samples was collected at a subsequent treatment.

Blood sampling

Blood samples (5 ml) from a catheter in the contralateral arm were collected in heparinised tubes. Samples were taken before infusion and at 0.5, 1.0, 1.5, 2, 2.5, 3, 3.17, 3.33, 3.75, 4, 4.5, 5, 6, 8, 12, 18, 24, 48 and 72 h after the commencement of infusion. Plasma was separated immediately by centrifugation of samples at 6000 *g* for 5 min, and plasma samples were stored in duplicate snap-top plastic microcentrifuge vials (Sorenson BioScience, Utah, USA) and frozen at −20 °C until analysis.

DACA determination

To accommodate the large number of samples, a solid-phase method for extraction of DACA from plasma was developed using an automatic sample processor (ASPEC XL, Gilson, France). Plasma (0.2 ml) together with *N*-[2-(diethylamino)ethyl]acridine-4-carboxamide as the internal standard (2 μ*M* 50 μl in acetonitrile) were added to tubes containing acetonitrile (0.9 ml) with immediate vortexing. Tubes were centrifuged (600 *g*) for 10 min at 10 °C and the supernatant (1 ml) was transferred to tubes containing 0.05 *M* ammonium acetate buffer (9 ml; pH 5.0). These solutions were loaded onto C18 cartridges (LC-18 SPE tubes, Sepelco, Bellefort, Pa., USA) that had previously been conditioned by washing with pure acetonitrile followed by water. Cartridges were washed successively with 0.05 *M* ammonium acetate buffer (pH 5.0) containing 10%, 20% and 30% acetonitrile. DACA was eluted with 80% acetonitrile: 20% 0.05 *M* ammonium acetate buffer, and the resultant samples were dried using a centrifugal evaporator (Jouan model 1010, Saint Nazaire, France).

Total concentrations of DACA in plasma were determined in triplicate assays (0.2-ml aliquots) by a previously published HPLC method employing fluorescence detection [25]. Reduction of HPLC sample-analysis times was achieved using a mobile phase consisting of 31% aqueous acetonitrile containing 10 m*M* triethylammonium phosphate (final pH 3.0). Dried samples from the solid-phase extraction procedure were dissolved in mobile phase, injected in a volume of 200 μl and eluted through a C18 stainless-steel column (300 × 3.9 mm; Bondclone, Phenomenex, Torrance, Calif., USA). The eluant was analysed by fluorescence detection at 475 nm (excitation wavelength 358 nm). Each analysis typically contained 57 samples from the patient; 16 calibration samples (in duplicate) providing DACA concentrations of 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5 μ*M*; and 9 quality-control samples (in triplicate). Standard curves were obtained for each set of assays (Schwarz criterion typically >20). The within-assay accuracy and precision were acceptable as reflected by relative recoveries of 86–104% and coefficients of variation (CV) of ≤10% ($n = 8$) over the concentration range of 0.05–5 μ*M*. The limit of quantitation was 0.05 μ*M* and concentrations of >5 μ*M* were diluted with control human plasma to levels within the assay range. Quality-control plasma samples were prepared with added DACA concentrations of 0.05, 0.5 and 5 μ*M*; stored at −20 °C; and re-assayed with patients' samples over a period of 16 months. These were shown to be stable only over a period of 10 months, during which 18 assays were performed and gave recoveries that ranged from 97% to 105% with CV of ≤11%. Determinations thereafter gave values that exceeded acceptable limits.

During the preparation of plasma quality-control samples it was discovered that multiple cycles of freezing and thawing could significantly reduce the recoveries of DACA, particularly at concentrations below 0.5 μ*M*. The source of human plasma and the type of plastic or glass tubes in which plasma solutions were prepared and stored also affected recoveries. To minimise this effect,

quality controls were prepared in fresh plasma from healthy donors and samples from patients were not frozen and thawed more than twice before analysis.

Pharmacokinetic analysis

The AUC was computed using the log trapezoid rule and was extrapolated to infinity by addition of the value C_t/Z, C_t representing the concentration at the last measurable (i.e., concentration $\geq 0.05\ \mu M$) time point and Z, the terminal slope determined by linear regression, typically made up of 6–8 time points and measured in triplicate. The model-independent pharmacokinetic parameters plasma clearance (Cl), steady-state volume of distribution (Vss) and mean retention time (MRT) were calculated by the equations $Cl = Dose/AUC$, $Vss = (Dose \times \text{AUMC})/(AUC)^2$ and $MRT = AUMC/AUC - T/2$, respectively, 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 the AUC and T represents the infusion time [12]. Concentration-time profiles were fitted to one- or two-compartment models with linear kinetics using MKMODEL, an extended least-squares modeling system, and models were compared by the Schwarz criterion [15].

Estimation of free-DACA concentrations in plasma

In vitro equilibrium dialysis studies of DACA in healthy human plasma enriched with AAG previously indicated a significant linear relationship between the ratio B/F (where B and F are the concentrations of bound and free DACA, respectively) and the concentration (A) of the major drug-binding protein, AAG [8]. The following relationship was obtained:

$$B/F = (14.2 \times A) + 24.6\ (r = 0.93; P < 0.0001).$$

AAG concentrations were determined using a nephelometric method, and the above equation was used to determine the unbound fraction on plasma and, thus, the free-DACA concentrations.

Results

Pharmacokinetic analysis was carried out on 33 sets of plasma samples obtained during the phase I clinical trial. Plasma concentration-time profiles were generally biphasic (Fig. 2), with concentrations generally falling below detection within 24 h of the commencement of infusion. Model-independent pharmacokinetic parameters (mean and standard deviation) were as follows: Cl 1.02 ± 0.38 l h^{-1} kg^{-1}, Vss 1.75 ± 0.89 l/kg, MRT 1.77 ± 0.72 h and terminal half-life ($t_{1/2}$) 2.05 ± 0.98 h. The model-independent pharmacokinetics are shown in Table 1. One- and two-compartment pharmacokinetic models were also applied to the analysis of the concentration-time profiles. The two-compartment model provided the better fit in most patients, providing the following parameters: Cl 1.00 ± 0.36 l h^{-1} kg^{-1}, volume of distribution of the central compartment (V_1) 0.72 ± 0.55 l/kg, initial half-life ($t_{1/2}1$) 0.28 ± 0.19 h and terminal half-life ($t_{1/2}2$) 2.04 ± 0.94 h.

In five patients a second set of samples was collected in a repeat dose, allowing for comparison of kinetics in two different cycles. No significant difference was found

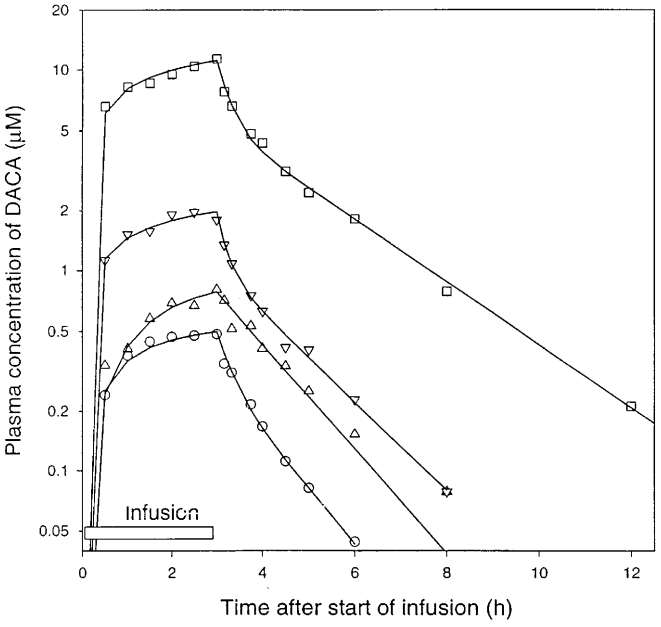

Fig. 2 DACA concentration-time profiles obtained for patient 1, 18 mg/m^2 (○); patient 5, 36 mg/m^2 (▽); patient 10, 90 mg/m^2 (△); and patient 26, 750 mg/m^2 (□)

in the pharmacokinetic parameters recorded for each pair of doses as determined by Student's two-tailed, paired t-test at the $P < 0.05$ level. The relationship of the AUC to the dose was calculated on a double logarithmic scale and is shown in Fig. 3. A high degree of linear correlation was obtained (slope 0.96; $r = 0.97$; $P < 0.0001$). Cmax was highly correlated with the dose

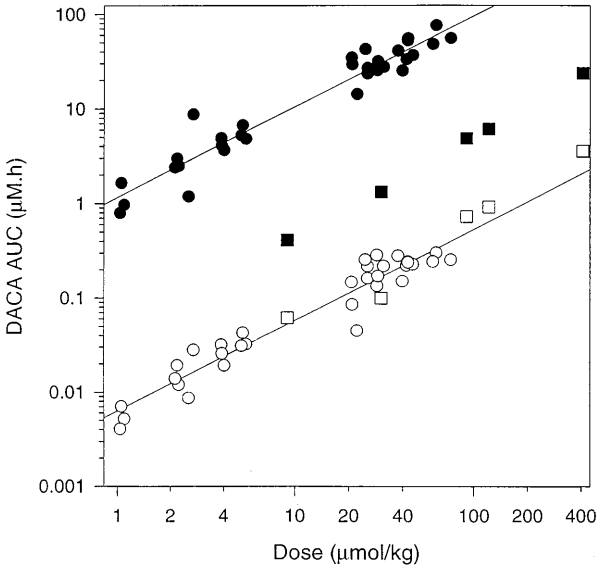

Fig. 3 Relationship of the total DACA AUC in plasma (●) and the free-DACA AUC in plasma (○) to the delivered dose of DACA. The regression lines have slopes of 0.96 (total) and 0.97 (free) and correlation coefficients of 0.97. For purposes of comparison, AUC data determined for plasma from mice are included for total DACA (■) and free DACA (□)

Table 1 Model-independent pharmacokinetic parameters determined for delivered courses of DACA (*AUCF* Estimated free-drug AUC)

Patient/ course	Age (year)/ sex	Dose (mg/m^2)	Dose (μmol/kg)	Cmax (μ*M*)	AUC (μ*M* h)	AAG (g/l)	AUCF (μ*M* h)	Cl ($l\ h^{-1}\ kg^{-1}$)	Vss (l/kg)	$t_{1/2}$ (h)	MRT (h)
1/1	51/F	18	1.05	0.48	1.64	2.96	0.02	0.64	0.70	0.94	1.09
2/1	60/M	18	1.09	0.30	0.97	2.22	0.02	1.13	1.35	0.89	1.15
3/1	63/M	18	1.04	0.21	0.79	1.79	0.02	1.31	1.81	1.33	1.38
4/1	42/F	36	2.53	0.40	1.18	1.40	0.03	2.15	2.08	0.86	0.97
5/1	56/F	36	2.19	0.81	2.96	1.09	0.07	0.74	1.45	1.56	2.02
5/2		36	2.23	0.52	2.48	1.20	0.06	0.90	2.27	2.05	2.59
6/1	49/F	36	2.13	0.77	2.39	2.03	0.04	0.89	1.31	1.67	1.52
7/1	58/F	60	4.03	1.16	3.64	2.41	0.06	1.11	0.48	0.84	0.72
8/1	56/F	60	3.89	1.48	4.88	1.42	0.11	0.80	1.06	2.02	1.33
8/2		60	3.91	1.17	4.10	1.35	0.09	0.95	1.13	1.53	1.25
9/1	34/F	60	2.70	2.11	8.75	3.46	0.12	0.31	0.91	2.72	2.96
10/1	46/F	90	5.13	1.96	6.70	1.38	0.15	0.77	0.97	1.34	1.26
11/1	66/M	90	5.35	1.25	4.81	0.88	0.13	1.11	2.50	2.60	2.50
12/1	52/F	90	5.05	1.69	5.25	1.99	0.10	0.96	1.43	1.81	1.55
13/1	33/F	350	20.85	9.27	29.40	5.87	0.27	0.71	0.89	2.34	1.26
14/1	61/F	350	20.67	8.64	34.60	2.29	0.60	0.60	1.35	2.76	2.26
15/1	73/F	350	22.22	3.66	14.25	3.89	0.18	1.56	2.12	1.23	1.36
16/1	45/M	480	25.40	6.23	23.52	0.86	0.62	1.08	1.95	2.24	1.80
16/2		480	25.40	8.40	26.83	0.86	0.71	0.95	1.11	1.72	1.24
17/1	63/F	480	31.18	6.59	27.56	0.26	0.94	1.13	3.48	3.08	3.07
18/1	49/F	480	28.57	8.59	26.45	0.26	0.90	1.08	1.04	1.05	0.96
20/1	56/M	480	24.67	11.29	42.47	1.25	0.98	0.58	1.07	2.34	1.84
21/1	48/F	575	41.79	11.13	33.30	1.69	0.67	1.25	1.58	1.85	1.26
22/1	59/F	575	39.68	6.50	25.21	1.20	0.59	1.57	2.47	2.04	1.57
24/1	59/M	575	28.68	6.22	25.42	1.41	0.56	1.13	4.05	5.56	3.59
24/2		575	28.99	7.90	31.65	1.41	0.69	0.92	1.97	2.11	2.23
25/1	51/M	750	45.35	9.40	36.75	1.06	0.90	1.23	2.09	2.23	1.70
26/1	47/M	750	37.50	11.60	40.93	1.05	1.01	0.92	1.61	1.97	1.75
27/1	64/M	750	42.46	14.00	52.34	2.21	0.92	0.81	1.15	1.55	1.41
27/2		750	42.57	15.40	54.97	2.71	0.86	0.77	1.11	1.60	1.50
28/1	57/F	1000	74.07	13.21	55.43	1.82	1.08	1.34	2.23	2.27	1.67
29/1	67/F	1000	61.38	18.49	75.61	2.47	1.25	0.81	2.09	3.40	2.58
30/1	40/M	1000	58.83	10.91	47.83	1.31	1.08	1.23	3.76	2.81	2.93

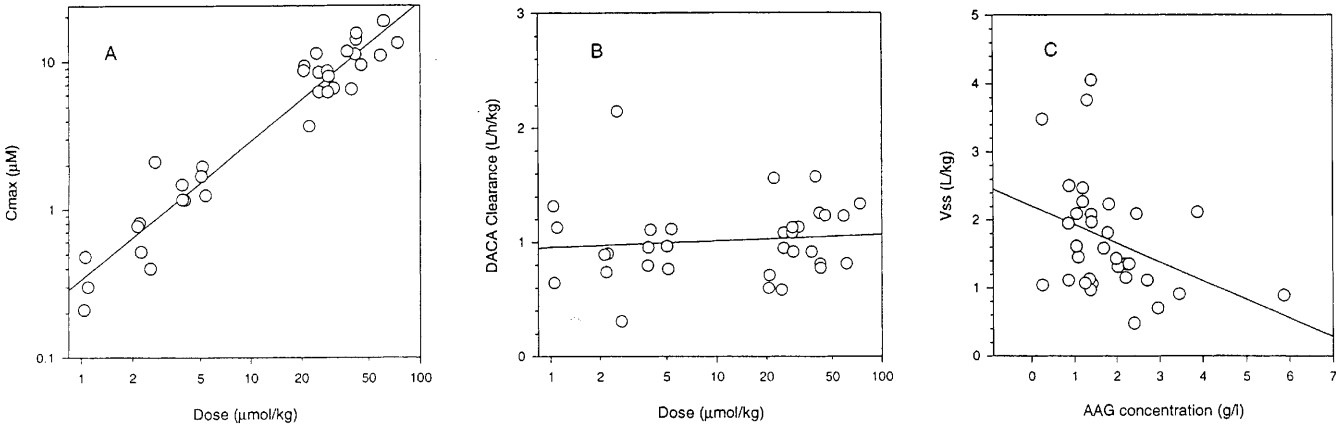

Fig. 4 **A** Relationship of the maximal total DACA concentration determined in plasma to the delivered dose. **B** Relationship of the DACA plasma clearance to the delivered dose. **C** Relationship of the Vss determined for DACA to the concentration of AAG detected in individual plasma samples

(slope 0.93; $r = 0.97$; $P < 0.0001$), whereas Cl was unrelated to the dose (Fig. 4). Vss, MRT and $t_{1/2}$ were also not significantly correlated with the dose, indicating first-order kinetics.

Since DACA is highly bound to AAG, the concentration of the latter was measured in each patient and used to calculate the free-DACA AUC values. These values showed a similar high degree of linear correlation with the dose (slope = 0.99; $r = 0.97$; $P < 0.0001$; Fig. 3). Plasma concentrations of AAG were not significantly correlated with Cl, MRT or $t_{1/2}$ but showed a weak correlation with Vss ($r = 0.36$; $P < 0.05$; Fig. 4).

Discussion

The investigative antitumour drug DACA was given to 31 patients over the dose range of 18–1000 mg/m^2 and the pharmacokinetics were measured in 28 cases. The metabolism of DACA in 20 patients from this trial has been described elsewhere [24]. The pharmacokinetic results demonstrate a biphasic disposition with a terminal half-life of 2 h. Both AUC and Cmax are linearly related to the dose over this range. In five patients in whom the pharmacokinetics were determined in the first infusion and then in a subsequent infusion, the results were the same within experimental error, arguing that drug treatment does not influence subsequent elimination of the drug.

Although the dose-escalation schedule used in this clinical trial of DACA was based on a modified Fibonacci scale, the principle of pharmacologically guided dose escalation (PGDE) as advanced by Collins et al. [4] to reduce the number of required dose escalations was considered in this study [18]. The wisdom of using free-drug rather than total drug AUC values is accepted for PGDE in cases where there are large interspecies differences in protein binding [6, 11], but the application is not common in practice [13]. The free fraction found for DACA (at a concentration of 1 μM) in human plasma (3.8%) is significantly lower than that reported for mouse plasma (15%) or rat plasma (16%) as determined by equilibrium dialysis [8]. This discrepancy may result from differences in the plasma content of AAG, an acute-phase protein that is elevated in cancer patients [22].

Two other acridine-based antitumour drugs, amsacrine and asulacrine (CI-921), also bind tightly to AAG, and their pharmacokinetics have previously been investigated in both rodents and humans. The total drug AUC values determined for mice at the MTD are 6.3 and 31 μM h, respectively [17]. In contrast, the AUC values achieved in therapeutic trials are 52 μM h for amsacrine [16] and 225 μM h (75 μM h $\times$ 3) for asulacrine [19]. In each case the mouse AUC data underestimate the human AUC data by nearly 10-fold. This difference has been hypothesised to result from the large interspecies differences in the measured free-drug fraction as a consequence of drug binding to AAG [17]. For DACA, a double logarithmic plot of AUC measurements obtained in mice and humans versus the dose indicates that the free-drug AUC values recorded for mice fall on the regression line for the free-drug AUC in humans, whereas the those total drug AUC values do not (Fig. 3). The AUC determined for DACA in mice treated at the MTD by a single i.p. injection (410 μmol/kg) is 23.4 μM h [7], which is one-third of the maximal AUC observed in this trial. On the other hand, the free-drug AUC determined for DACA in mice treated i.p. at this dose is 3.5 μM h, corresponding to approx. 3 times the maximal free-drug AUC obtained in patients treated at the maximal dose reached.

If free-drug AUC values are accurate in interspecies predictions, the results suggest that the effective/MTD in humans would be approximately 3000 mg/m^2. The total dose achieved in the phase I trial in the United Kingdom in which DACA was given as by three infusions on consecutive days was 2400 mg/m^2 [Twelves et al., submitted for publication], whereas that achieved in a subsequent 5-day continuous-infusion phase I trial was 3010 mg/m^2 [3].

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