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Metabolism of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide in cancer patients undergoing a phase I clinical trial

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Abstract *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA) is an experimental antitumour agent that has just completed phase I clinical trials in New Zealand and the United Kingdom. Urine (0–72 h) was analysed from 20 patients receiving DACA infused over 3 h (dose range 60–1000 mg/m^2, the latter being the highest dose achieved in the trial). Aliquots were analysed for DACA and its metabolites by high-performance liquid chromatography (HPLC). Over 72 h, 44 ± 5% (range 20–60%) of the dose was recovered in the urine, with 0.8 ± 0.3% (range 0–3.1%) occurring as DACA. The major urinary metabolite was DACA-*N*-oxide-9(10*H*)acridone, accounting for 34 ± 3% of the dose. Minor metabolites were identified as *N*-monomethyl-DACA-9(10*H*)acridone (2.0 ± 0.5%), DACA-9(10*H*)acridone (3.3 ± 0.5%), *N*-monomethyl-DACA (0.2 ± 0.1%) and DACA-*N*-oxide (0.5 ± 0.1%). No ring-hydroxylated metabolite was detected. The urinary excretion of metabolites was greatest over 0–6 h in most patients. The composition of urinary metabolites was also independent of the delivered dose. Plasma was sampled at intervals throughout the infusion and at time points up to 48 h post-administration. The major plasma metabolites observed were DACA-9(10*H*)acridone and DACA-*N*-oxide-9(10*H*)acridone. These results indicate that, based on urinary excreted metabolites, the major biotransformation reactions for DACA in humans involve *N*-oxidation of the tertiary amine side chain and acridone formation, both of which appear to be detoxication reactions.

Key words DACA · Metabolism · Urine · Clinical trial · Anticancer agent

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Introduction

N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA; see Fig. 1 for structures) is an experimental antitumour agent developed in the Auckland Cancer Society Research Centre. It is notable for its cytotoxicity towards multidrug-resistant tumour cell lines and its high activity against murine solid tumours such as Lewis lung and Colon 38 carcinomas [1, 2, 10]. Previous studies have shown that DACA is extensively metabolised in vivo in mice, rats and humans, producing complex metabolite profiles [5, 9, 18, 20, 24]. In rodents the major urinary and biliary metabolites have been identified as 7-hydroxy-9(10*H*)acridone derivatives of DACA and the *N*-demethylated metabolite of DACA, *N*-monomethyl-DACA, which are excreted as either aglycones or glucuronides. In addition, the tertiary amine side-chain *N*-oxide derivative of DACA has been detected in both urine and bile. From these observations as well as studies using subcellular and enriched enzyme fractions from rat liver and isolated rat hepatocytes [22, 23, 25] it has been established that in these animals, DACA is mainly metabolised by aldehyde oxidase to the 9(10*H*)acridone, followed by 7-hydroxylation and conjugation with the glucuronide. A similar sequence of events occurs after DACA has been *N*-demethylated, but not after *N*-oxidation of the tertiary amine side chain. In humans utilising [^{11}C]-DACA, seven radioactive metabolites are found in plasma within 1 h of DACA administration, with one being identified as DACA-*N*-oxide [18].

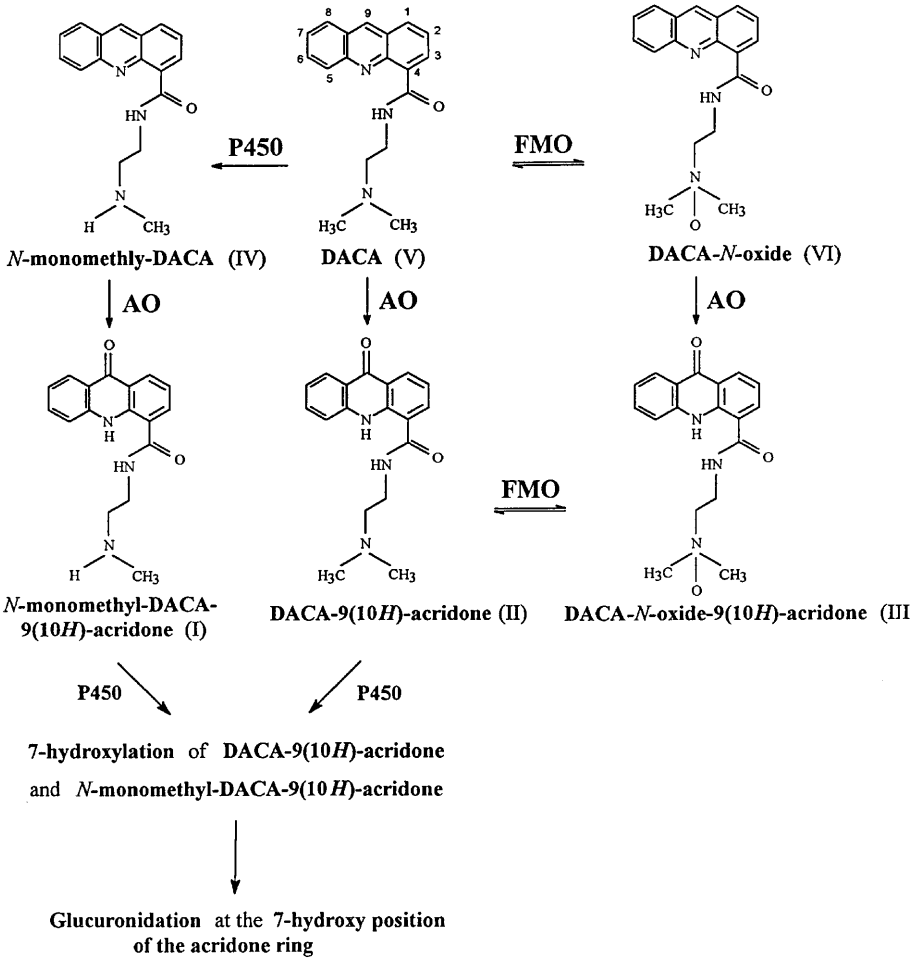

Fig. 1 Putative human and rodent metabolism pathways for DACA (*AO* Aldehyde oxidase, *FMO* flavin monooxygenase, *P450* cytochrome P450 oxidising system)

Phase clinical trials of DACA have been conducted in both New Zealand (NZ) [17] and the United Kingdom (UK) (Twelves et al., submitted for publication) under the auspices of the Cancer Research Campaign, UK. To investigate the metabolism of DACA in humans, we studied urinary and plasma metabolites from patients treated with DACA in the NZ trial. A number of metabolites were identified and the metabolic pathways in rodents and humans were compared.

Materials and methods

Chemicals

DACA-9(10*H*)acridone, *N*-monomethyl-DACA, DACA-*N*-oxide and 7-hydroxy-DACA were synthesised in the Auckland Cancer Society Research Centre [1, 19] and kindly provided by Professor W.A. Denny. The synthesis of DACA-*N*-oxide-9(10*H*)acridone, *N*-monomethyl-DACA-9(10*H*)acridone, 7-hydroxy-*N*-monomethyl-DACA-9(10*H*)acridone and 7-hydroxy-DACA-9(10*H*)acridone has been described elsewhere [24]. Metabolites were characterised by [^{1}H]-nuclear magnetic resonance (NMR) spectra using a Bruker AM-400 spectrometer (400 MHz) and fast atom bombardment/mass spectrometry (FAB/ms) on a Varian UG70-SE mass spectrometer from a glycerol/thioglycerol matrix as previously presented and reported [23, 24]. Absorption spectra of these metabolites were also obtained during high-performance liquid chromatography (HPLC) with the Waters 490E variable-wavelength detector at various wavelengths (250, 254 and 261 nm) and were used to identify the metabolites in the urine samples. β-Glucuronidase type H-2 (1–5% residual sulfatase activity) was obtained from Sigma Chemical Co. (St. Louis, Mo.). All other reagents and solvents were of analytical or HPLC grade.

Patient's data and sample collection

Samples were obtained from patients entered in the phase I clinical trial at the Department of Clinical Oncology, Auckland Hospital [17]. These patients received DACA (as the dihydrochloride trihydrate salt) infused into an arm vein in 0.9% saline with a target duration of 3 h over the dose range of 18–1000 mg/m^2. Urine was collected immediately before the infusion and then over the periods of 0–6, 6–12, 12–18, 18–24, 24–48 and 48–72 h after the commencement of the infusion. At the end of each collection period the volume was measured and an aliquot (50–60 ml) stored at −20 °C. Samples from the first seven patients were not analysed because of the low concentrations of DACA and metabolites detected in the urine. Samples from patients 9–29 (details are summarised in Table 1) were thawed at 25 °C and analysed within 4 days of collection. An initial analysis by HPLC was performed after dilution with 100 m*M* triethylammonium phosphate (pH 3) solution containing 3% acetonitrile. Following HPLC analysis the undiluted urine aliquots were frozen at −20 °C and then transferred to −80 °C for storage prior to re-analysis within 1–2 months. No significant difference was observed between the initial and later determinations.

Table 1 Patients' details (*Hc* Hepatocellular carcinoma, *Ls* liposarcoma, *End* endometrium, *U.a.* unknown adenocarcinoma, *BSA* body surface area)

Patient/cycle	Age (years)/sex	Cancer	Ethnic group	Wt (kg)	BSA (m^2)	Dose (mg/m^2)	Total dose (μmol)
8/1	56/F	Lung	Caucasian	67	1.84	60	262
9/1	34/F	End	Caucasian	106	1.95	60	286
10/1	46/F	Rectum	Caucasian	74	1.85	90	381
11/1	66/M	Colon	Caucasian	76	1.92	90	405
12/1	52/F	Ovary	Caucasian	78	1.83	90	393
13/1	33/F	Breast	Caucasian	77	1.91	350	1595
14/1	61/F	Breast	Caucasian	67	1.65	350	1381
15/1	73/F	Ovary	Caucasian	60	1.60	350	1333
16/1	45/M	U.a.	Polynesian	90	2.00	480	2286
17/1	63/F	Hc	Caucasian	63	1.72	480	1964
18/1	49/F	Ovary	Caucasian	70	1.75	480	2000
20/1	56/M	Colon	Caucasian	89	1.92	480	2190
21/1	48/F	Pancreas	Chinese	49	1.50	575	2048
22/1	59/F	U.a.	Caucasian	57	1.65	575	2262
24/1	64/M	U.a.	Polynesian	105	2.20	575	3012
25/1	51/F	Ovary	Caucasian	63	1.60	750	2857
26/1	47/M	Lung	Caucasian	100	2.10	750	3750
27/1	64/M	Rectum	Caucasian	76	1.80	750	3214
28/1	57/F	Ls	Caucasian	45	1.40	1000	3333
29/1	67/F	Renal	Caucasian	64	1.65	1000	3929

Plasma that was surplus to the pharmacokinetics study requirements [16] was available for five patients. Aliquots (200 μl) were extracted with ice-cold methanol (2 × 5 vol.), and the methanol extracts were taken to dryness using a centrifugal evaporator (Speed-Vac, Savant Instruments Inc., Farmingdale, N.Y., USA). The residue was analysed by HPLC after being dissolved in 0.37 *M* ammonium formate solution (pH 4.5) containing 27% acetonitrile. Faeces collection was performed for one patient (patient 27) out to 7 days. Samples were extracted with ice-cold methanol and then treated in the same way as the urine samples.

HPLC analysis

Urine samples in triplicate were analysed by reversed-phase HPLC using an acetonitrile gradient (5–20% in triethylammonium phosphate, 100 m*M*, pH 3.0) as previously described [24] except that the gradient was linear. A Hewlett-Packard diode-array detector was used to record the spectra of the eluted components. DACA and its metabolites were identified by comparison of their retention times and UV/visible spectra with those of the synthetic standards. Spectral details for these compounds have been reported elsewhere [23, 24]. Addition of synthetic metabolites to human urine indicated that this assay had good accuracy (≤15% deviation from true values) with acceptable precision (CV < 10% for major peaks such as DACA-*N*-oxide-9(10*H*)acridone for triplicate analyses; CV < 20% for minor peaks such as DACA-9(10*H*)acridone). The limit of quantitation of these compounds was taken as 0.07 μ*M*.

Plasma extracts in triplicate were analysed using a Waters C_{18} μBondapak Radial Pak column and a linear gradient of acetonitrile (27–50% over 14 min) and ammonium formate (pH 4.5, 0.37–0.18 *M*) with diode-array detection (Hewlett Packard 1040 A).

Analysis for glucuronides

The possible presence of glucuronide metabolites of DACA in patients' urine (pre-infusion, 0–6 h and 6–12 h) was assessed by hydrolysis in ammonium acetate (50 m*M*, pH 5) with crude β-glucuronidase (*Helix pomatia*, ~5000 U/ml) at 37 °C for 1, 4, 8 and 16 h. Urine from DACA-treated mice was used as a positive control [24]. Samples were subsequently analysed by HPLC.

Results

Analysis of urine samples

Representative pre-infusion and post-infusion (0–6 h) HPLC profiles (from patient 13, cycle 1) are shown in Fig. 2. Most compounds eluting before 15 min were present in the pre-infusion urine. From the retention time and spectral comparison against synthetic standards plus the mass spectral data, the major component was identified as DACA-*N*-oxide-9(10*H*)acridone (III; λ_{max} 410 nm) and the minor components were identified as *N*-monomethyl-DACA-9(10*H*)acridone (I; λ_{max} 352 nm), DACA-9(10*H*)acridone (II; λ_{max} 408 nm), *N*-monomethyl-DACA (IV; λ_{max} 362 nm), DACA (V; λ_{max} 362 nm) and DACA-*N*-oxide (VI; λ_{max} 362 nm). The urinary excretion of these compounds for collection periods up to 72 h is shown for patient 13, cycle 1, in Fig. 3. The maximal excretion of the major metabolite, DACA-*N*-oxide-9(10*H*)acridone (III), occurred at 0–6 h in 12 patients, at 6–12 h in 5 cases, and at 12–24 h in the remaining patients. A summary of the urinary excretion (0–72 h) of these components as a percentage of the delivered dose is shown in Fig. 4 and details are given in Table 2. The extent of urinary excretion of metabolites was independent of the dose given (Fig. 5). Treatment of urine with β-glucuronidase for 1, 4, 8 and 16 h resulted in minimal changes in the HPLC profiles, indicating the absence of glucuronide conjugates in these samples. In contrast, concurrent treatment of urine from mice that had received DACA resulted in a reduction in glucuronide peaks with a corresponding increase in aglycone metabolite peaks as shown in a previous publication [24].

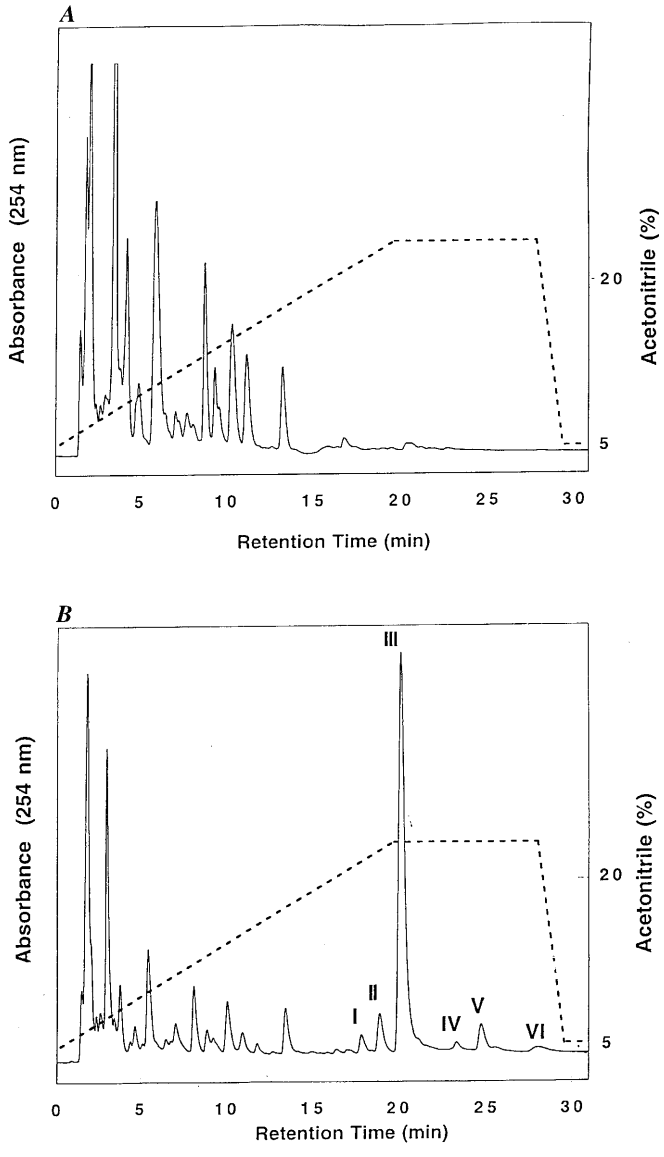

Fig. 2A, B Representative **A** pre-infusion and **B** post-infusion (0–6 h) HPLC profile of DACA and metabolites in human urine (patient 13, cycle 1). Injection volumes were **A** 50 and **B** 30 µl. The *dashed line* represents the acetonitrile gradient. (*Peak I N*-Monomethyl-DACA-9(10*H*)acridone, *peak II* DACA-9(10*H*)acridone, *peak III* DACA-*N*-oxide-9(10*H*)acridone, *peak IV N*-monomethyl-DACA, *peak V* DACA, *peak VI* DACA-*N*-oxide)

Analysis of plasma samples

The analysis of DACA in the plasma pharmacokinetics study of these patients utilised a solid-phase extraction method that was developed specifically for the measurement of DACA [28]. However, the method afforded poor and variable recoveries of DACA metabolites. Nevertheless, the chromatograms resulting from this study indicated the presence of DACA-9(10*H*)acridone (II) and DACA-*N*-oxide-9(10*H*)acridone (III), with maximal concentrations occurring between 3 and 6 h. Methanol extraction of synthetic metabolites (2.5 µ*M*) added to human plasma gave reproducible and relatively constant recoveries of 52% for DACA, 46% for DACA-*N*-oxide-9(10*H*)acridone (III), 52% for DACA-9(10*H*)acridone (II), 47% for *N*-monomethyl-DACA-9(10*H*)acridone (I) and 52% for DACA-*N*-oxide (VI). After correction for these recoveries the accuracy, precision and reproducibility were similar to those of the urinary assay. This method plus an HPLC system with a diode-array detector was used to examine plasma samples taken at 4 or 4.5 h after DACA administration from five patients (patients 13–17). Significant amounts of DACA-9(10*H*)acridone (II) and DACA-*N*-oxide-9(10*H*)acridone were identified in these samples (Table 3). For example, in the three patients who received 350 mg/m^2, after correction for recoveries the concentrations of DACA-*N*-oxide-9(10*H*)acridone (III) and DACA-9(10*H*)acridone (II) were 2.1 ± 0.8 and 2.0 ± 0.4 µ*M*, respectively, as compared with DACA at 2.0 ± 0.2 µ*M*.

Analysis of faecal samples

A preliminary investigation was made of faecal samples collected over 5 days from patient 27. No DACA-*N*-oxide-9(10*H*)acridone (III) was detected, and only small amounts of DACA-9(10*H*)acridone (II) and *N*-monomethyl-DACA-9(10*H*)acridone (I) were observed. The recoveries were not quantitated.

Discussion

These results indicate that DACA is extensively metabolised in humans, with approximately 44% of the dose being excreted in the urine over 72 h. This compares with approximately 25% of the dose ([^{3}H]-DACA: 410 µmol/kg i.p. or 30 µmol/kg i.v.) excreted over 48 h in mouse urine [9, 20]. The difference is not surprising as the molecular-weight threshold for biliary excretion, around 300 for rats and believed to be about 500 in humans [8], favours greater biliary excretion in rodents. The pattern of DACA metabolites seen in humans also appears to be different from that observed in rodents. The major urinary metabolite in humans is DACA-*N*-oxide-9(10*H*)acridone (III), with minor amounts of *N*-monomethyl-DACA, DACA and their 9(10*H*)acridones and DACA-*N*-oxide (VI), being detected. In mice and rats the major urinary and biliary metabolites have been identified as 7-hydroxy-9(10*H*)acridone derivatives of DACA and *N*-monomethyl-DACA, which are excreted as either aglycones or glucuronides [24]. In humans there is no evidence of the aromatic ring-hydroxylated derivatives. Thus, it appears that the metabolism of DACA may be less complex in humans than in rodents, with *N*-oxidation of DACA and acridone formation predominating over *N*-demethylation, ring hydroxylation and glucuronidation (Fig. 1).

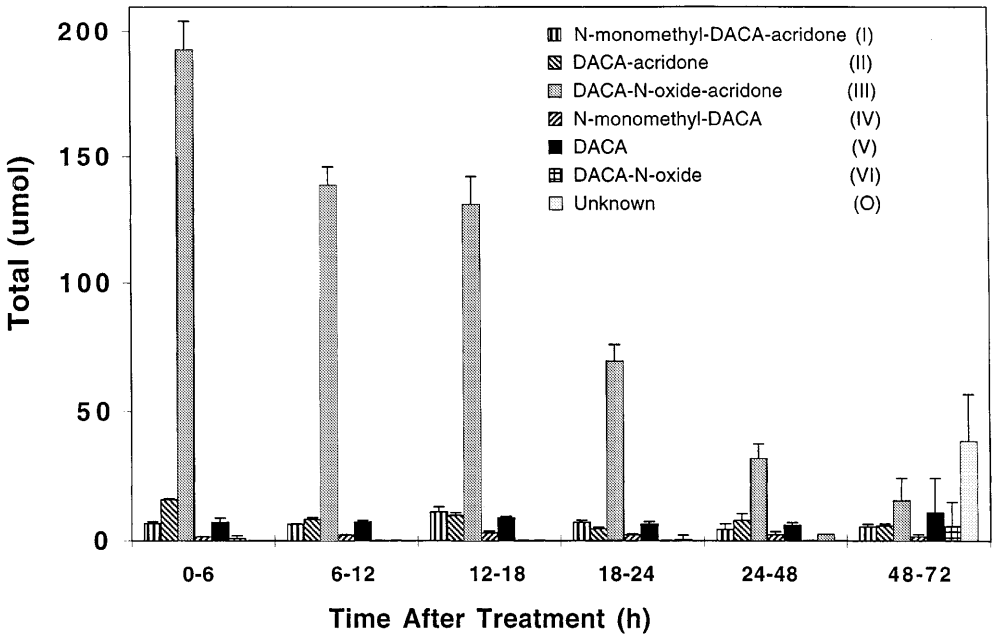

Fig. 3 Quantitation of DACA and metabolites in urine of a typical patient (patient 13, cycle 1–350 mg/m^2). The values (mean ± SD, $n = 3$) are the product of the concentration of the respective components × the volume (ml) of urine collected in the respective intervals

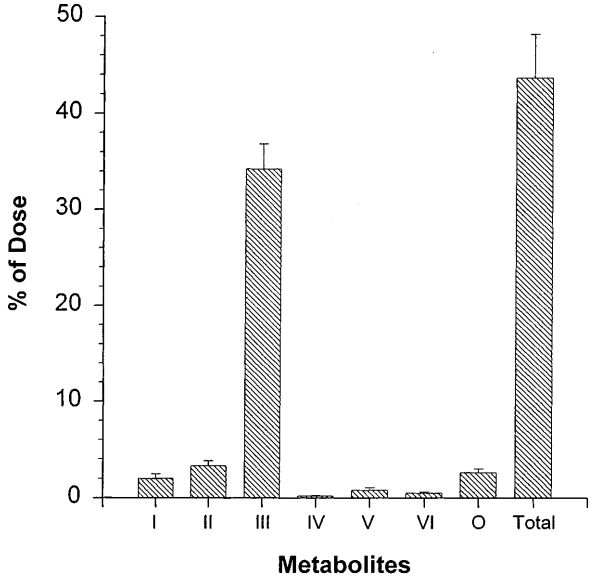

Fig. 4 Mean (±SD) urinary excretion of DACA and metabolites as a percentage of the delivered dose in 20 patients(*Peak I–peak VI* see Fig. 2, *O* = unidentified)

Previously, *N*-oxidation has been regarded as a minor pathway of drug metabolism but one of more importance to humans than to rats [13]. For example, chlorpromazine, whose structure bears some similarities to that of DACA (i.e. three aromatic rings with an aliphatic tertiary amine side chain), undergoes comparable metabolism with a similar reported species difference. *N*-Oxidation of the tertiary amine side chain has been clearly established as a relatively important biotransformation pathway for chlorpromazine in humans, with both chlorpromazine-*N*-oxide and chlorpromazine *N*, S-dioxide being observed in urine [11, 26]. In contrast, these products were not detected in rats [15]. It is not known whether this might be due to the rat's lack of capacity for *N*-oxidation as compared with humans or to its greater capacity to reduce the *N*-oxide back to the parent amine [14]. The latter explanation appears more likely for DACA, as studies with isolated rat hepatocytes indicate significant back-reduction of DACA *N*-oxide to DACA followed by further *N*-demethylation and acridone formation [25]. However, chlorpromazine also undergoes significant aromatic ring hydroxylation and glucuronidation in humans [26, 27]. The possibility exists that the corresponding reactions also occur for DACA in humans but that the products are excreted in bile and then subjected to further extensive metabolism by digestive enzymes and the bacterial flora in the gut before their excretion in faeces. This occurs in the case of chlorpromazine, where various metabolites such as 7-hydroxychlorpromazine *O*-glucuronide have been identified in rat bile but have not been observed in the faeces [15]. It is also well known that *N*-oxides can undergo reduction during transit down the gastrointestinal tract [7]. In the preliminary investigation of faecal samples from patient 27, no DACA-*N*-oxide-9(10*H*)acridone (III) was detected, and only small amounts of DACA-9(10*H*)acridone (II) and *N*-monomethyl-DACA-9(10*H*)acridone (I) were observed. It is possible that unidentified DACA metabolites are present in faeces, and further investigation is necessary.

In plasma the major metabolites of DACA are DACA-*N*-oxide-9(10*H*)acridone (III) and DACA-9(10*H*)acridone (II). These compounds were also identified in the HPLC traces used for the quantitation of DACA after solid-phase extraction in the phase I plasma pharmacokinetics study [16]. Despite the latter's low and variable recoveries, it was clear that peak concentrations of DACA-*N*-oxide-9(10*H*)acridone (III) occurred between 3 and 6 h and declined thereafter. The possibility

Table 2 Percentage of the dose excreted in urine between 0 and 72 h as DACA and metabolites[a] (*n.d.* Not detected)

Dose (mg/m^2)	Patient	*N*-mono methyl-DACA-acridone (I)[b]	DACA-acridone (II)	DACA-*N*-oxide-acridone (III)	*N*-mono methyl-DACA (IV)	DACA (V)	DACA-*N*-oxide (VI)	Unknowns (O)	Totals
60	8/1	3.6	0.4	33.7	n.d.	0.1	0.3	3.1	41.2
60	9/1	4.2	14.8	26.8	n.d.	n.d.	0.6	1.6	48.0
60	Mean	3.9 ± 2.2	7.6 ± 1.2	30.3 ± 2.4	n.d.	0.05 ± 0.04	0.5 ± 0.3	2.3 ± 0.6	44.6 ± 6.8
90	10/1	0.6	0.6	37.2	0.1	0.2	0.3	2.3	41.3
90	11/1	0.7	2.0	25.7	n.d.	0.2	0.4	3.0	32.0
90	12/1	1.2	1.2	31.0	n.d.	n.d	0.4	1.8	35.5
90	Mean	0.8 ± 0.4	1.3 ± 0.9	31.3 ± 3.1	0.05 ± 0.03	0.15 ± 0.09	0.4 ± 0.1	2.4 ± 0.4	36.2 ± 4.9
350	13/1	2.6	3.3	36.3	0.8	2.9	0.4	2.9	49.1
350	14/1	2.4	2.3	48.7	0.6	1.5	0.9	3.3	59.7
350	15/1	0.9	6.8	27.2	0.5	1.8	0.3	0.2	37.7
350	Mean	2.0 ± 0.3	4.1 ± 0.6	37.4 ± 3.1	0.6 ± 0.2	2.1 ± 1.1	0.5 ± 0.3	2.1 ± 0.3	48.8 ± 6.0
480	16/1	2.5	3.2	42.7	0.7	3.1	1.7	0.8	54.5
480	17/1	2.6	3.0	28.7	0.4	0.8	0.5	3.4	39.3
480	18/1	1.0	0.7	37.6	0.2	0.2	0.3	2.1	41.8
480	20/1	2.1	3.4	36.5	n.d.	0.5	0.8	2.8	45.5
480	Mean	2.1 ± 0.2	2.8 ± 0.3	36.5 ± 2.7	0.4 ± 0.2	1.1 ± 0.2	0.8 ± 0.1	2.8 ± 0.3	47.7 ± 4.3
575	21/1	1.9	1.7	30.9	n.d.	n.d.	0.4	6.4	41.3
575	22/1	0.7	2.2	31.9	0.1	0.5	0.7	3.0	39.1
575	24/1	1.1	4.4	38.9	n.d.	0.4	0.6	2.2	46.6
575	Mean	1.3 ± 0.2	2.8 ± 0.5	33.9 ± 1.5	0.03 ± 0.01	0.3 ± 0.02	0.6 ± 0.02	3.9 ± 0.30	42.7 ± 2.5
750	25/1	3.8	4.2	36.5	n.d.	1.1	0.6	4.3	50.5
750	26/1	3.3	6.5	47.3	n.d.	1.3	0.6	2.9	61.9
750	27/1	1.8	0.7	24.9	n.d.	0.1	0.1	1.5	29.0
750	Mean	3.0 ± 0.6	3.8 ± 0.3	36.2 ± 2.7	n.d.	0.8 ± 0.2	0.4 ± 0.1	2.9 ± 0.4	47.1 ± 4.2
1000	28/1	1.5	1.4	49.3	n.d.	0.3	0.7	2.4	55.3
1000	29/1	1.3	3.0	12.9	n.d.	0.4	0.1	2.2	19.9
1000	Mean	1.4 ± 0.1	2.2 ± 0.2	31.1 ± 2.3	n.d.	0.3 ± 0.03	0.4 ± 0.04	2.3 ± 0.3	37.6 ± 2.9
Range	(n = 20)	0.6–4.2	0.4–14.8	12.9–49.3	0.1–0.8	0.1–3.1	0.1–1.7	0.2–6.4	19.9–59.7
Total	Mean	2.0 ± 0.5	3.3 ± 0.5	34.2 ± 2.6	0.2 ± 0.06	0.8 ± 0.3	0.5 ± 0.1	2.6 ± 0.4	43.5 ± 4.5

[a] Mean values are expressed for individual patients (n = 3) and mean values ± SD are provided for each dose level
[b] See Fig. 2

of back-reduction of *N*-oxide metabolites by denatured haemoglobin exists [21]. However, no obvious increase in DACA concentration was observed in haemolysed plasma samples collected from patients in the pharmacokinetics study [16].

In summary, the critical reactions in the metabolism and elimination of DACA in humans, based on urinary excreted and identified metabolites, appear to be *N*-oxidation of the tertiary amine side chain and acridone formation. Both *N*-oxidation and acridone formation typically represent detoxification reactions [3, 6]. Metabolites such as DACA-*N*-oxide (VI) and DACA-9(10*H*)acridone (II) have been shown to have little toxicity to rat hepatocytes, P388 leukaemia cells and Lewis lung tumour cells and are less toxic than DACA to the mouse in vivo [25]. The *N*-oxidation of the aliphatic tertiary amine side chain of DACA is most likely catalysed by flavin-containing mono-oxygenase, as this reaction can be inhibited by methimazole in rat hepatocytes [6, 25]. However, this does not rule out the possible involvement of the cytochrome P450 oxidising system, as methimazole has also been reported to inhibit oxidation by cytochrome P450 [12]. Acridone formation appears to be carried out by aldehyde oxidase [3, 22, 23]. Studies using enriched preparations from post-mortem liver (unpublished observations) indicate that DACA is a substrate for human aldehyde oxidase, although the apparent K_m values are 4- to 8-fold higher than those observed for similar preparations of the rat enzyme [23]. DACA-*N*-oxide has not been studied as a substrate for human aldehyde oxidase, but in rat liver cytosol the apparent K_m for oxidation of DACA-*N*-oxide to its acridone is 12 times greater than that recorded for DACA. Although the kinetics and interrelation of these reactions require further study to determine the order of metabolism and the likely rate-limiting step, the identi-

Table 3 Concentrations of DACA and metabolites detected in plasma at 4 or 4.5 h after DACA administration[a]

Dose (mg/m^2)	Patient	DACA-acridone (II)	DACA-*N*-oxide-acridone (III)	DACA (V)	Unknowns (O)
350	13/1	2.86	2.06	2.13	0.00
350	14/1	1.49	1.62	1.88	0.05
350	15/1	1.72	3.77	1.73	0.02
350	Mean	2.03 ± 0.73	2.48 ± 1.14	1.92 ± 0.20	
480	16/1	1.95	2.03	1.85	0.05
480	17/1	2.47	1.48	1.89	0.12
480	Mean	2.21 ± 0.36	1.76 ± 0.39	1.87 ± 0.03	0.08 ± 0

[a] Data for individual patients are expressed as mean values ($n = 3$)

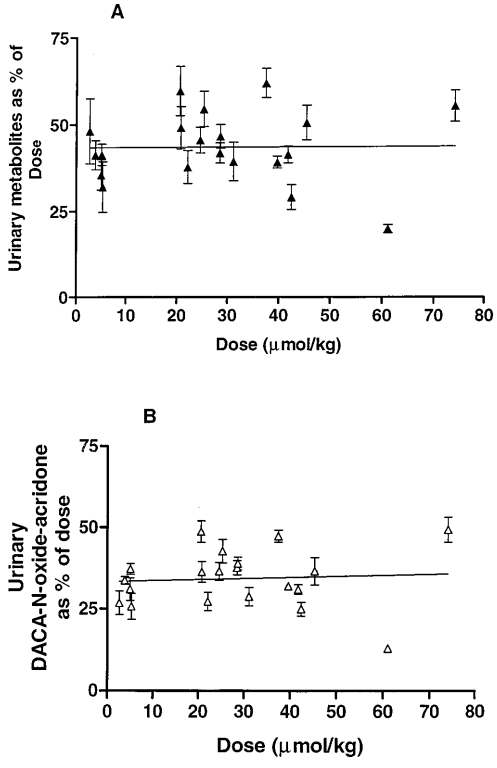

Fig. 5A, B Correlation between the dose delivered (μmol/kg) and urinary excretion. **A** Urinary excretion of metabolites expressed as a percentage of the dose ($n = 20$; $y = 0.0040x + 43.35$, $r^2 = 0.00005$). The slope is not significantly different from 0 ($P = 0.98$). **B** Urinary excretion of DACA-*N*-oxide-9(10*H*)acridone (III) expressed as a percentage of the dose ($n = 20$; $y = 0.0312x + 33.35$, $r^2 = 0.005$). The slope is not significantly different from 0 ($P = 0.78$)

fication of the major metabolic pathways of DACA will prove useful in future clinical studies of DACA, which are likely to involve relatively high doses given by constant infusion [4].

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