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ORIGINAL ARTICLE

Philip Kestell · James W. Paxton
Gordon W. Rewcastle · Ingrid Dunlop
Bruce C. Baguley

Plasma disposition, metabolism and excretion of the experimental antitumour agent 5,6-dimethylxanthenone-4-acetic acid in the mouse, rat and rabbit

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Abstract 5,6-Dimethylxanthenone-4-acetic acid (DMXAA), an experimental antitumour agent currently undergoing phase I clinical trial, has a maximum tolerated dose (MTD) in male BDF_1 mice of 99 μmol/kg. We have found the male Sprague-Dawley rat and the New Zealand White rabbit to have greater tolerance to DMXAA, with MTDs being 990 and 330 μmol/kg, respectively. To investigate the causes of this difference, we measured plasma and urine DMXAA concentrations by high-performance liquid chromatography (HPLC) after single i.v. bolus injections of 99 and 990 μmol/kg in the rat and following a bolus dose of 99 μmol/kg and a 10-min infusion of 330 μmol/kg in the rabbit. Following administration of DMXAA at the MTD in the mouse, rat and rabbit the maximal concentrations were 600, 2,200 and 1,708 μ*M*, respectively, whereas areas under the concentration-time curves were 2,400, 19,000 and 2,400 μ*M*h, respectively, for unchanged DMXAA. Data obtained for mice and rabbits were satisfactorily fitted to a two-compartment model with Michaelis-Menten kinetics. DMXAA was highly bound to plasma proteins, with the highest degree of binding being found in the rabbit. A small proportion of the total dose (7.8%, 0.6% and 12.4%, respectively) was excreted unchanged in urine over 24 h. This proportion increased (to 11.6%, 3.5% and 72.4%, respectively) following alkaline hydrolysis, suggesting the presence of glucuronide metabolites. Examination of rat and mouse urine by HPLC revealed the presence of two metabolites, which were characterized by mass spectrometry and nuclear magnetic resonance to be the acyl glucuronide of DMXAA and 6-(hydroxymethyl)-5-methylxanthenone-4-acetic acid. Thus, both mice and rats metabolise DMXAA by similar pathways. The results demonstrate considerable interspecies variations in tolerance to DMXAA that cannot be explained by differences in pharmacokinetics.

Key words Antitumour agents · Pharmacokinetics · Glucuronidation · Interspecies variation

P. Kestell (✉) · G.W. Rewcastle · I. Dunlop · B.C. Baguley
Cancer Research Laboratory, University of Auckland
School of Medicine, Private Bag 92019,
Auckland, New Zealand
Tel.: (64-9) 3737 599 ext. 6140, Fax: (64-9) 3737 502

J.W. Paxton
Department of Pharmacology and Clinical Pharmacology,
University of Auckland School of Medicine,
Private Bag 92019, Auckland, New Zealand

Introduction

5,6-Dimethylxanthenone-4-acetic acid (DMXAA, Fig. 1) is an experimental antitumour agent representative of a new class of anticancer drugs that act as biological response modifiers. DMXAA is particularly active against subcutaneous murine colon 38 tumours, causing haemorrhagic necrosis [5, 17]. This characteristic feature, as revealed by histological examination of tumours, has been associated with the ability of DMXAA to reduce tumour blood flow and cause subsequent vascular collapse [22]. In addition, DMXAA induces the synthesis of mRNA for the cytokine tumour necrosis factor-α (TNFα) [6, 14, 15] as well as TNFα protein itself [15], nitric oxide [19, 20] and serotonin [3]. The relationships between these and the mechanism of antitumour activity are complex and not yet fully understood. However, the ability of DMXAA to stimulate human cells to produce TNF mRNA in vitro has provided the justification for evaluation in humans and phase I clinical trials are currently under way in the United Kingdom and New Zealand. Prior to its clinical evaluation, acute toxicology studies of DMXAA in rodents revealed that the rat could tolerate 10-fold greater doses than the mouse, regardless of whether the drug was given i.v. or i.p. In mice, DMXAA exhibited non-linear plasma pharmacokinetics over a 10-fold dose range of up to 99 μmol/kg (30 mg/kg), the maximum tolerated dose (MTD) [11]. Data obtained from the isolated perfused rat-liver model indicated that glucuronidation of the

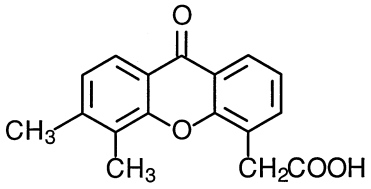

Fig. 1 Chemical structure of 5,6-dimethylxanthenone-4-acetic acid (DMXAA)

acetic acid side chain was the major elimination pathway for DMXAA [21].

The aim of this study was to establish whether the greater tolerance of DMXAA in the rat might arise from species differences in the pharmacokinetics and/or metabolic profile of this drug. The rabbit was included in the study as there is evidence that it provides a more appropriate model for the study of the metabolism and excretion of weak organic acids (e.g. clofibric acid) due to its higher molecular-weight requirement for biliary excretion, which is similar to that of humans [7].

Materials and methods

Chemicals

The sodium salts of DMXAA and 5-propoxyxanthenone-4-acetic acid were synthesised in our laboratory using published methods and were >99% pure when analysed by thin-layer chromatography (TLC) [1, 17]. Authentic DMXAA acyl glucuronide (DMXAA-G) was isolated from the bile of rats treated with DMXAA and was characterised by mass spectrometry [21]. All other chemicals or solvents were of analytical grade.

Animals, dosing and sample collection

Male B6D2F$_1$ mice (20–30 g), Sprague-Dawley rats (200–300 g) and New Zealand White rabbits (2.8–3.4 kg) were housed under constant temperature and humidity using sterile bedding, water and food according to institutional guidelines. The Animal Ethics Committee of the University of Auckland approved all animal procedures. DMXAA was formulated in sterile water and protected from light to prevent photolytic decomposition [16]. DMXAA was given to rats and mice as a tail-vein bolus injection and to rabbits by infusion into a marginal ear vein (0.6 ml/min) using a Harvard Infusion Pump (Model 975A). At the end of the infusion (approximately 10 min), the catheter line was flushed with 4 ml 5% dextrose containing sodium heparin at 5 units/ml. All animals were individually housed in metabolism cages enabling the separate collection of urine and faeces. Blood samples (200–300 µl) were obtained from rats by sampling at the end of the tail and were collected into heparinised tubes at approximate times over a 24-h period after drug administration. Rabbit venous blood (1 ml) was collected from the opposite ear into heparinised tubes at 0. 0.5, 1, 2, 4, 6, 8 and 12 h after infusion. After centrifugation, plasma was removed and stored at −20 °C until analysis. Urine was collected at regular intervals in vessels containing 5 ml 10 m*M* ammonium acetate buffer at pH 5 and was immediately stored at −80 °C.

High-pressure liquid chromatography

High-performance liquid chromatography (HPLC) analyses were performed using a Waters WISP 712B sample injector, a 6000A high-pressure pump, a Model 440 UV absorbance detector (Waters Associates, Milford, Mass., USA), a Model RF-530 Shimadzu fluorescence detector (Shimadzu, Kyoto, Japan) and a Bondclone C18 stainless steel column (300 × 3.9 mm; Phenomenex, Torrence, Calif., USA). Data from each detector were acquired and processed by a Unicam 4880 Chromatography data station (Unicam Ltd, Cambridge, UK).

Quantitation for DMXAA in plasma and urine

Quantitation of DMXAA in plasma and urine was achieved with our previously published HPLC method [11] that used 5-propoxyxanthenone-4-acetic acid as an internal standard. DMXAA was extracted from acidified samples using toluene. A Speed-Vac solvent concentrator (Savant Instruments, Farmingdale, N.Y., USA) was used to remove the toluene, and the resulting residues were analysed by HPLC using fluorescence detection. Analyses were carried out in duplicate. The accuracy of the method varied from 99% to 102% with acceptable precision [intra- and inter-assay coefficients of variation (CV) were 3% and 5%, respectively] over a concentration range of 0.5–20 µ*M*. The lower limit of quantitation for the assay was 0.1 µ*M*.

Alkaline hydrolysis of urine

Aliquots of urine (50–200 µl) collected from each species after i.v. administration of DMXAA (99 µmol/kg) were treated with 500 µl of 0.1*M* sodium hydroxide and then incubated in darkness at 37 °C for 1 h. The amount of DMXAA in each incubation was then determined as described above [11].

Plasma protein binding

DMXAA (100–200 µ*M*) was added to either mouse, rat or rabbit plasma and the unbound fraction was determined by ultrafiltration as previously described [11].

Extraction and isolation of urinary metabolites

Initial HPLC metabolite profiling of urine was performed on samples collected from animals during the 24-h period before and after treatment with DMXAA. Aliquots of urine (20 µl) were acidified with 2 ml of 0.5% trichloroacetic acid and shaken with 5 ml ethyl acetate. The organic layer was transferred to a glass tube and the solvent was evaporated using a Speed-Vac solvent concentrator. The residues were then dissolved in 200 µl mobile phase [14 m*M* diammonium hydrogen phosphate buffer (pH 5.0): isopropyl alcohol, 80: 20(v/v)]. Aliquots (25–50 µl) were injected onto the HPLC column and compounds were detected by UV absorbance at 254 nm with the mobile phase at a flow rate of 1 ml/min. Metabolites were isolated from the remaining urine by semi-preparative chromatography according to the conditions and procedures described by Webster et al. [21].

Mass spectrometry

Mass spectrometry was performed using a VG-7070-EQ mass spectrometer (VG Analytical, Manchester, UK) operating either in the electron impact mode (EI) at 70 eV or in the fast atom bombardment mode (FAB) at 6 kV. FAB spectra were obtained from samples in a glycerol matrix using xenon atoms generated from an atom gun set at 6 kV and 1.2 mA. Cesium iodide was used for calibration. Peak matching was achieved using glycerol and its sodium clusters as internal standards.

Nuclear magnetic resonance spectrometry

[^{1}H-] and [^{13}C]-nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AM-400 spectrometer operating at 400 MHz. Tetramethylsilane was used as an internal standard in dimethyl sulfoxide (DMSO) for the [^{1}H]-NMR spectra. The chemical shift values are reported in parts per million and the coupling constants (J values) are expressed in Hertz.

Pharmacokinetic parameters

The concentration-time profiles were fitted to different pharmacokinetic models using MKMODEL (Elsevier-Biosoft, Cambridge, UK). The fit was evaluated by log likelihood, with the Schwarz criterion providing a means of comparing the different models used to fit the data sets. The two-compartmental model with capacity-limited elimination was defined by the following pharmacokinetic parameters: V_{max}, the maximal elimination rate; K_m, the central compartment concentration associated with elimination at half V_{max}; CL_{dist}, the distribution clearance defining the relationship between the drug concentration in the compartments and the rate of transfer from one compartment to the other; V_{ss}, the volume of distribution at steady-state equilibrium (and equal to the volume of the central and peripheral compartments); and V_1, the volume of the central compartment. The area under the concentration-time curve (AUC) was computed using the log trapezoid 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).

Results

Dose tolerance of DMXAA in rat and rabbit

Rats treated with i.v. DMXAA at 99 and 990 μmol/kg did not exhibit any adverse effect. Following a dose of 1,150 μmol/kg there were observable signs of toxicity, with animals becoming sedated for several minutes. In contrast, 990 μmol/kg was lethal in one rabbit. A reduction in dose to 330 μmol/kg was tolerated, but during the infusion the rabbits ($n = 3$) experienced tachypnea followed by a period of sedation lasting for 1–2 h. These animals also did not void any urine throughout the 24-h period after DMXAA administration. The AUC found in rabbits after a dose of 330 μmol/kg was 2,400 μ*M*h, the same as that observed after the MTD (99 μmol/kg) in mice [11]. In contrast, the AUC observed after a dose of 990 μmol/kg in rats was 19,000 μ*M*h, a value 8-fold greater than the AUC seen in mice and rabbits at their MTD.

Plasma pharmacokinetics

Plasma concentration-time profiles obtained for DMXAA in the rat and rabbit are shown in Figs. 2 and 3, respectively. Data previously published for mice [11], augmented by further data obtained at the lower doses, are shown in Fig. 4. These data sets were analysed using MKMODEL and were best fitted by a two-compartment open model with capacity-limited elimination. The corresponding pharmacokinetic parameters are presented in Table 1. The capacity-limited elimination of DMXAA was further supported by a disproportionate increase in AUC with increasing dose. A 10-fold increase in dose resulted in 14- and 16-fold increases in AUC in the rat and mouse, respectively, whereas a 3.7-fold increase in AUC was observed in the rabbit after a 3.3-fold dose increase. The opposite effect was observed for C_{max}, with smaller than expected increases being seen in this parameter. For instance, a 10-fold dose increase resulted in a 3.4- and 4.4-fold increase in C_{max} in the rat and mouse, respectively, whereas a 3.3-fold dose increase produced a 2.5-fold increase in C_{max} in the rabbit. Following administration of DMXAA at 99 μmol/kg, a similar range of plasma C_{max} values was observed for all species (703 ± 27, 645 ± 48 and 600 ± 148 μ*M* for the rabbit, rat and mouse, respectively). This was reflected in similar values for V_{ss} in all three species, but there were marked differences in Michaelis-Menten

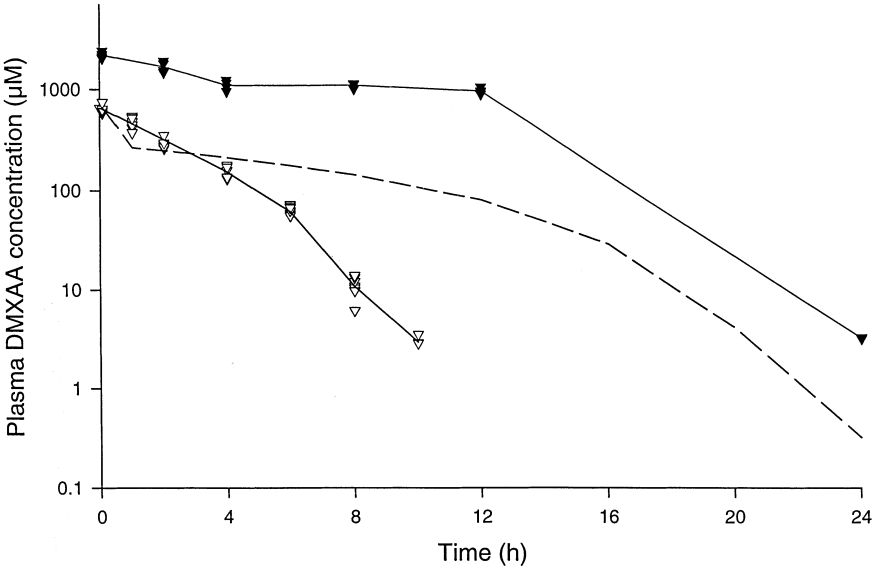

Fig. 2 Plasma DMXAA concentrations detected in rats [(i.v. 99 ($n = 4$, ▽) and 990 μmol/kg ($n = 2$, ▼)] and in mice for comparison (i.v. 99 μmol/kg, *dashed line*). Data obtained at the low dose were fitted to a Michaelis-Menten two-compartment pharmacokinetic model

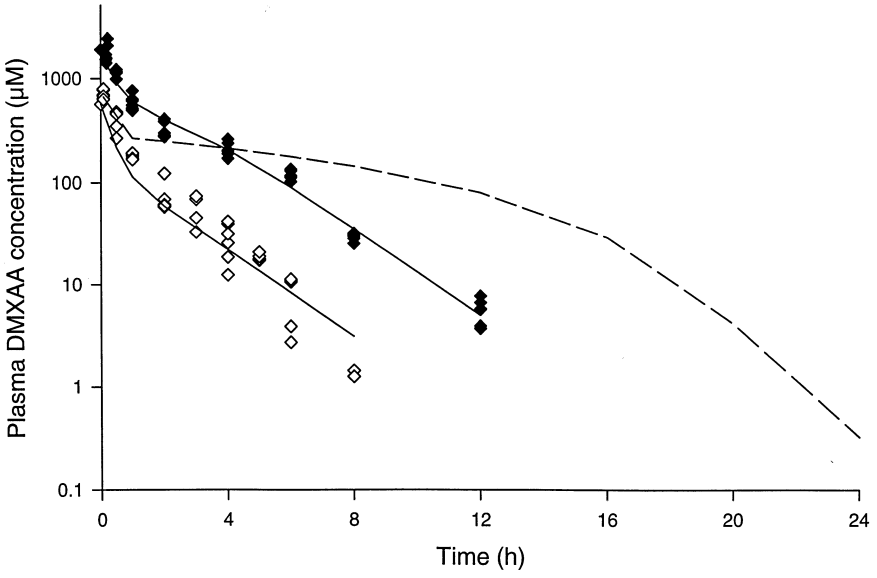

Fig. 3 Plasma DMXAA concentrations detected in rabbits [(i.v. 99 ($n = 3$, ◇) and 330 μmol/kg ($n = 3$, ◆)] and in the mice for comparison (i.v. 99 μmol/kg, *dashed line*). Data were fitted to a Michaelis-Menten two-compartment pharmacokinetic model

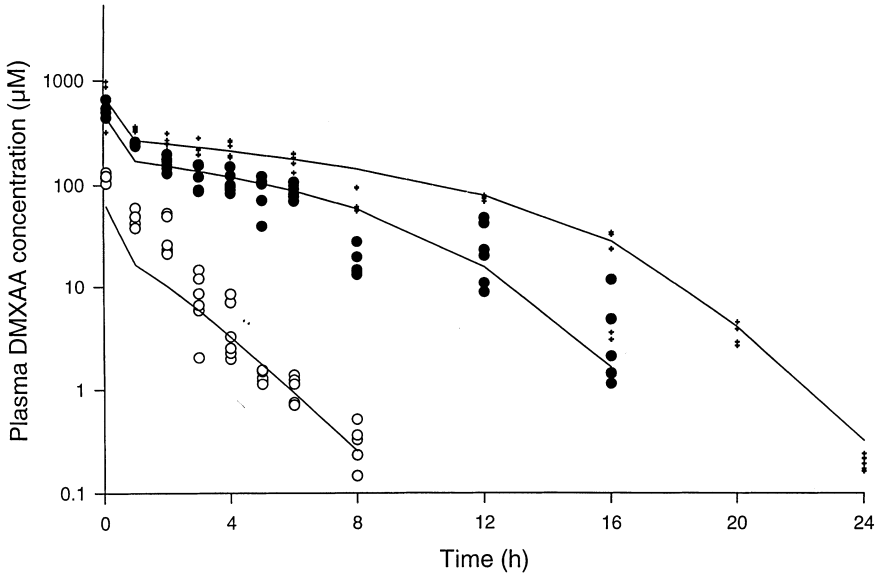

Fig. 4 Plasma DMXAA concentrations detected in mice [i.v. 9.9 (○), 66 (●) and 99 (+) μmol/kg]. Data were fitted to a Michaelis-Menten two-compartment pharmacokinetic model

pharmacokinetic parameters, with the rabbit exhibiting an approximately 10-fold greater capacity to eliminate DMXAA as compared with the mouse and rat.

Plasma protein binding

DMXAA was highly bound in plasma from all three species (Fig. 5). The results extend the findings of previously published work [11]. The free fractions recorded at an added DMXAA concentration of 0.5 m*M* were 4.0%, 1.2% and 1.8% for the mouse, rat and rabbit, respectively. Above 0.5 m*M*, marked increases in free fractions were observed, with the mouse having the highest value (26% at 2 m*M*). Scatchard analysis indicated that the protein binding was particularly high in the rabbit (Fig. 5).

Excretion of and metabolite profile of DMXAA in urine

After i.v. administration of 99 μmol/kg DMXAA a small percentage of the total dose [mean values ± SE 7.8 ± 1.9%, 0.6 ±0.1% and 12.4 ± 0.1% ($n = 3$) for the mouse, rat and rabbit, respectively] was excreted in urine as unchanged DMXAA. After alkaline hydrolysis of the urine this increased to 11.6 ± 1.1% in the mouse, 3.5 ± 0.1% in the rat and 72.4 ± 0.2% in the rabbit.

Ethyl acetate extracts of acidified urine obtained from mice, rats and rabbits during the 24-h period before and after DMXAA administration were analysed by HPLC. Representative chromatograms (Fig. 6) indicated two major metabolites, M1 and M2. Chromatographic profiles of mouse and rat urine extracts

Table 1 DMXAA pharmacokinetic parameters (means ± SE) derived from a Michaelis-Menten two-compartment model

Species	Dose (μmol/kg)	AUC (μ*M*h)	C_{max} (μ*M*)	Pharmacokinetic model values[a]				
				Vmax (μmol h^{-1} kg^{-1})	K_m (μ*M*)	V_{ss} (l/kg)	V_1 (l/kg)	CL_{dist} (l h^{-1} kg^{-1})
Mouse	9.9 (*n* = 24)	148[b]	137 ± 5					
	66 (*n* = 27)	1,400[b]	540 ± 39	6.67 ± 0.05	23.6 ± 0.82	0.34 ± 0.002	0.06 ± 0.01	0.74 ± 0.08
	99 (*n* = 33)	2,400[b]	600 ± 45					
Rat	99 (*n* = 4)	1,339 ± 91	645 ± 24	8.65 ± 1.27	4.63 ± 1.00	0.45 ± 0.12	0.15 ± 0.004	0.04 ± 0.002
	990 (*n* = 2)	19,000 ± 12	2,200 ± 45					
Rabbit	99 (*n* = 3)	641 ± 12	703 ± 15					
	330 (*n* = 3)	2,400 ± 5	1,708 ± 127	82.9 ± 4.0	238 ± 23	0.45 ± 0.01	0.17 ± 0.01	0.27 ± 0.02

[a] Model parameter values were calculated from data obtained at all dose levels for mice and rabbits but only from results recorded at the 99-μmol/kg dose for rats since the 990-μmol/kg dose could not be fitted to the two-compartment model
[b] Standard errors could not be calculated because each sample was obtained from a different animal

were similar at both 99 and 990 μmol/kg. Metabolite M2 was identified as being the acyl glucuronide, DMXAA-G, since both M2 (mouse) and M2 (rat) co-eluted with authentic DMXAA-G (retention time 14 min) and disappeared after urine samples had been subjected to alkaline hydrolysis, as did authentic DMXAA-G when treated similarly. In contrast, the M1 metabolite was stable under the conditions used for alkaline hydrolysis. Although unchanged DMXAA was not observed in the profiles shown in Fig. 6, it was rendered measurable by increases in the sensitivity of the detector. Chromatographic profiles of rabbit urine extracts were more complex, with up to six metabolites being detected. The rabbit M3 metabolite (retention time 13.8 min) co-eluted with authentic DMXAA-G and disappeared after alkaline hydrolysis, as did peaks M2, M4, M5 and M6. This was associated with a substantial increase in the DMXAA peak. The rabbit M1 peak co-eluted with mouse and rat M1 and was not affected by alkaline hydrolysis.

Characterisation of the M1 metabolite in rat and mouse urine

The M1 metabolite was isolated from mouse and rat urine extracts by semi-preparative chromatography and was analysed by high-resolution electron-impact mass spectrometry. Both spectra had mass ions of m/z = 298.0837, which, with corrected peak matching, is consistent with the formula $C_{17}H_{14}O_5$ (theoretical $C_{17}H_{14}O_5$ requires 298.0841), indicating the addition of one oxygen atom to DMXAA. Sufficient material was isolated from rat urine to enable analysis by [^{1}H] and [^{13}C]-NMR spectrometry.

The [^{1}H]-NMR spectrum [$(CD_3)_2SO$; δ 8.09 (dd, J = 8.0, 1.6 Hz, 1 H, H-1), 8.02 (d, J = 8.2 Hz, 1 H, H-8), 7.79 (dd, J = 7.3, 1.6 Hz, 1 H, H-3), 7.54 (d, J = 8.2 Hz, 1 H, H-7), 7.41 (t, J = 7.6 Hz, 1 H, H-2), 4.67 (s, 2 H, CH_2O), 3.97 (s, 2H, CH_2CO_2), 3.90 (br, exchangeable with D_2O, 1 H, OH) and 2.39 (s, 3 H, CH_3)] showed that the five aromatic protons of

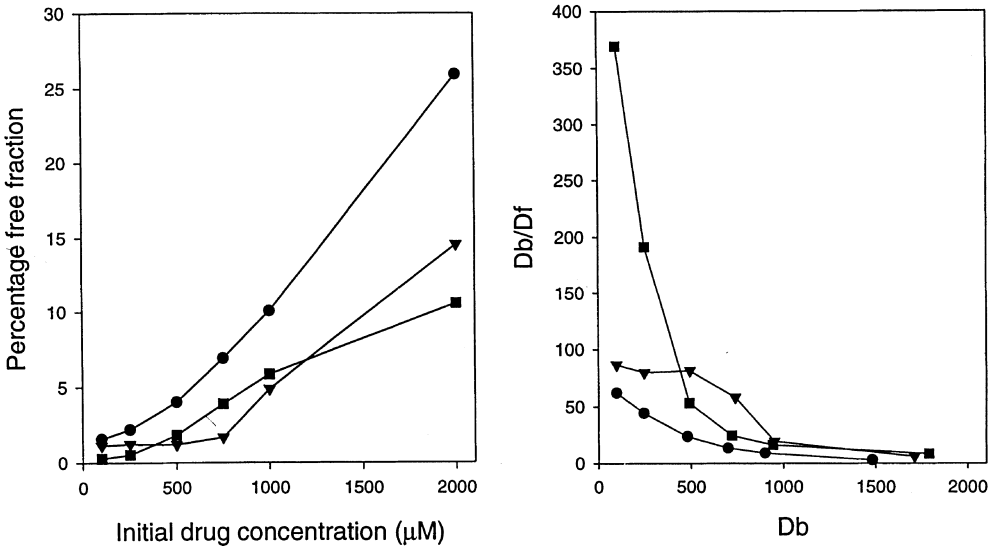

Fig. 5 Percentage of free DMXAA fraction detected in plasma of the mouse (●), rat (▼) and rabbit (■) as a function of added drug. The *right panel* shows same data as a Scatchard plot

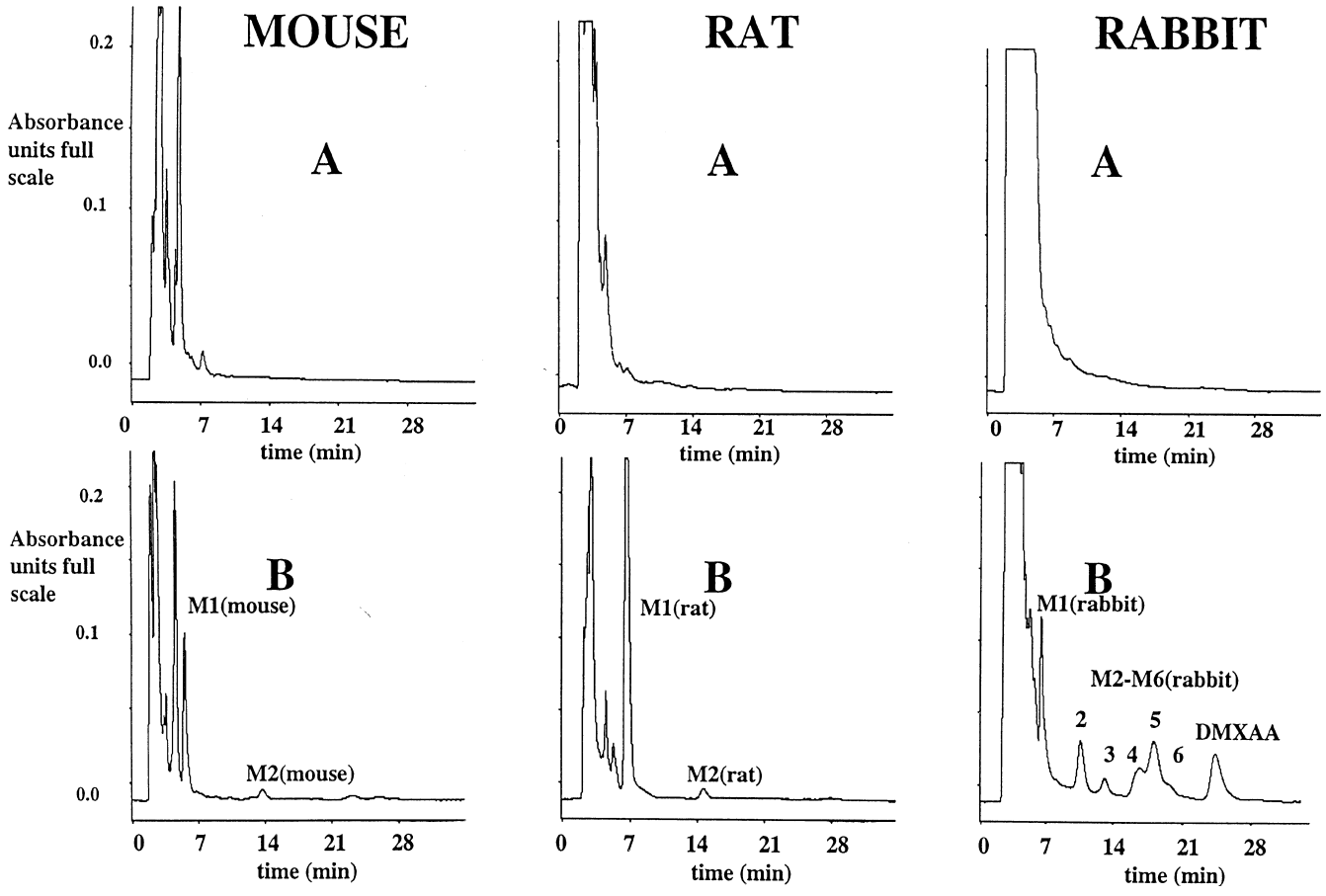

Fig. 6A,B HPLC metabolite profiles obtained for ethyl acetate extracts of acidified urine collected from mice, rats and rabbits during the 24-h period **A** before and **B** after i.v. administration of DMXAA (99 μmol/kg)

DMXAA remained present but that one of the two methyl signals had disappeared and had been replaced with a singlet at 4.67 ppm, which integrated for two protons. A broad (exchangeable) signal was also seen at 3.90 ppm, indicating the possible presence of a hydroxy group. The [^{13}C]-NMR spectrum [δ 176.2 (C = O), 171.9 (CO_2), 153.8 (C), 153.0 (C), 148.2 (C), 136.5 (CH), 125.5 (C), 124.5 (CH), 123.7 (C), 123.6 (CH), 122.6 (CH), 122.3 (CH), 120.5 (C), 119.2 (C), 60.8 (CH_2O), 35.6 (CH_2) and 9.9 (CH_3)] confirmed the presence of five protonated aromatic carbons and one methyl group, whereas a methylene signal at 60.8 ppm implied the addition of a hetero atom at that position. Both spectra confirmed that the acetic acid side chain remained present and unchanged. Taken in combination, these results indicate the replacement of one of the methyl groups of DMXAA by a hydroxymethyl group. Therefore, the structure of the hydroxylated species is either structure A [6-(hydroxymethyl)-5-methyl-xanthenone-4-acetic acid] or structure B [5-(hydroxymethyl)-6-methylxanthenone-4-acetic acid as shown in Fig. 7.

For determination of the relative stereochemistry of the methyl and hydroxymethyl groups, COSYLR homonuclear shift-correlated two-dimensional NMR experiments were conducted with delay periods designed to emphasise long-range or small couplings [4]. The results were consistent with M1 being 6-(hydroxymethyl)-5-methylxanthenone-4-acetic acid (structure A). Strong correlations were observed between the methylene protons of the hydroxymethyl group and H-7 and between the methyl group and H-8, whereas a slightly weaker correlation was observed between H-3 and the methylene group of the acetic acid side chain. Weak correlations were also seen between the hydroxymethyl

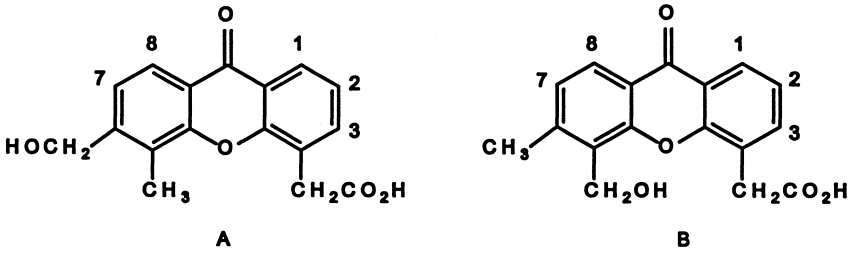

Fig. 7A,B Postulated structures of the major urinary metabolite M1 of DMXAA in the rat

group and H-8, between the methyl group and H-7 and between H-1 and the methylene group of the acetic acid side chain.

Finally, a very weak correlation was also observed between H-2 and the methylene group of the acetic acid side chain.

Discussion

These studies indicate that the considerable species variation in the toxicity of DMXAA is not a consequence of altered pharmacokinetics and must therefore reflect other factors in the response. This species variation is of particular relevance to current clinical trials of DMXAA because the optimal dose cannot adequately be predicted from animal data. Indeed, the rat has the lowest K_m for elimination of all three species, with its capacity for DMXAA elimination being similar to that of the mouse, and might thus be expected to be the animal most susceptible to DMXAA toxicity. However, the rat can tolerate an 8-fold higher level of exposure to DMXAA as compared with the mouse and rabbit. It is also apparent that the C_{max} of DMXAA is not associated with toxicity, as the rat (which was not susceptible) experienced a 3.7-fold higher C_{max} after the MTD as compared with the mouse. Free-drug C_{max} values recorded following administration of the MTD, as based on protein binding studies, provided values of 33, 330 and 140 μM, respectively, for the mouse, rat and rabbit. It is also apparent from the data that the free drug AUC recorded for the rat is much higher than that noted for the mouse, with the rabbit having an intermediate value. Thus, differences in plasma protein binding of DMXAA between the species do not explain the differences in toxicity, and a pharmacodynamic explanation must be sought for these interspecies differences in susceptibility to DMXAA toxicity.

In addition, these studies show that metabolism is the major pathway of elimination in all species, as only a small percentage of the total dose appeared as unchanged DMXAA in the urine. HPLC metabolite profiles together with alkaline hydrolysis revealed that the excretion of biotransformed products was different in the rabbit as compared with the mouse and rat. After alkaline hydrolysis of rabbit urine, 72% of the total dose was unchanged DMXAA, suggesting that glucuronidation of DMXAA followed by urinary excretion is the major elimination route. In the mouse and rat, biliary excretion may be the primary route of excretion, as only small amounts of DMXAA were detected in their urine after similar treatment. This is supported by isolated perfused rat-liver studies [21] and is consistent with the molecular-weight threshold for biliary excretion of anions in rats and rabbits of about 325 and 425, respectively [8, 10, 12] (the molecular weight of DMXAA-G is 458). Thus, it is likely that glucuronidation is a major biotransformation pathway for DMXAA in all species but that the metabolites are excreted by different routes.

Another feature characteristic of the metabolism in all three species was that they excreted significant amounts of 6-(hydroxymethyl)-5-methylxanthenone-4-acetic acid in their urine. This metabolite presumably arises from hydroxylation of the 6-methyl group by a cytochrome P450 isozyme. Conclusive identification of the chemical structure of this compound was not achieved in this study. However, results from the COSYLR homonuclear shift-correlated two-dimensional NMR experiments [4] with M1 (rat) provided strong evidence for structure A (Fig. 7), where the hydroxymethyl and methyl groups are at the 6- and 5-positions, respectively. It is known that larger benzylic couplings occur to *ortho* and *para* protons (i.e. across 4 or 6 bonds) than to *meta* protons (5 bonds) [9]. In contrast, structure B would require stronger coupling between the hydroxymethyl group and H-8 and between the methyl group and H-7 and, therefore, weaker coupling between the hydroxymethyl group and H-7 as well as between the methyl group and H-8 (the reverse of what was actually seen).

These studies also indicate significant species differences in pharmacokinetics, with all three animals exhibiting non-linear pharmacokinetics. A two-compartment model with Michaelis-Menten kinetics was satisfactory fitted to the data sets for the individual species (except for the rat at 990 μmol/kg). Saturation of the glucuronidation pathway may be partly responsible for DMXAA's dose-dependent kinetics. The model-dependent K_m and V_{max} values indicate that the rabbit has a 10-fold greater capacity to eliminate DMXAA from plasma than do the other species. However, these V_{max} and K_m values must be interpreted with caution as the effect of non-linear plasma binding (i.e. an increase in free fraction with increasing concentration) is a tendency for the log concentration-time curve to become convex. The smaller-than-expected C_{max} achieved with increasing dose can be explained by the reduction in plasma protein binding at high concentration (i.e. the increasing free fraction). This is the probable reason for the inability of the model to fit the 990-μmol/kg rat data, since the free fraction recorded at the C_{max} (2200 μ*M*) was approximately 20% as compared with 1% at less than 500 μ*M*. The development of a two-compartment model with Michaelis-Menten kinetics incorporating non-linear plasma/tissue binding would seem more appropriate.

Many factors may contribute to DMXAA's dose-dependent pharmacokinetics. The glucuronide metabolite DMXAA-G, following biliary excretion, may be hydrolysed in the gut and reabsorbed with the formation of a futile cycle. DMXAA induces in vivo the synthesis of tumour necrosis factor (TNF) [15], nitric oxide [2] and serotonin [3], consistent with changes in blood flow. These agents not only cause marked haemodynamic changes but also suppress hepatocyte cytochrome P450- and UDP glucuronosyl transferase-dependent enzyme activities [13, 18] thus potentially altering the clearance of DMXAA and its metabolites. Further studies are

necessary to clarify the mechanisms responsible for the non-linear pharmacokinetics of DMXAA in these animal models and to identify the reason for the greater tolerance to DMXAA shown by the rat.

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