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ORIGINAL ARTICLE

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Gender differences in the metabolism and pharmacokinetics of the experimental anticancer agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA)

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Abstract *Purposes:* Marked gender differences in the pharmacokinetics of many drugs have been reported. For the investigational anticancer drug, 5,6-dimethylxanthenone-4-acetic acid (DMXAA), negligible gender differences in the plasma pharmacokinetics have been observed in mice. The gender effects on the plasma pharmacokinetics of DMXAA were further investigated using the rat model. In addition, the in vitro metabolism and plasma protein binding of DMXAA in male and female mice, rats and humans were investigated. *Methods:* DMXAA was administered to male and female rats by intravenous injection. DMXAA and its major metabolites formed in liver microsomes were determined by HPLC. Unbound DMXAA in plasma was separated by ultrafiltration followed by HPLC determination. *Results:* In vivo kinetic studies indicated that female rats had 60%, 55% and 73% higher area under the plasma concentration-time curve (AUC) of DMXAA (2413 ± 188 vs 1505 ± 312 $\mu M \cdot$h, $P < 0.05$), elimination half-life (2.40 ± 0.45 vs 1.55 ± 0.33 h) and maximal plasma concentration (Cmax) (1236 ± 569 vs 716 ± 280 μM), but 61% lower plasma clearance than male rats. In vitro studies indicated that male rats had a 67% higher glucuronidation activity (0.75 ± 0.03 nmol/min per mg) than female rats (0.45 ± 0.01 nmol/min per mg), resulting in a 96% faster intrinsic clearance (CL_{int}) in the males than the females (6.36 ± 0.65 vs 3.24 ± 0.42 ml/min per g, $P < 0.05$). In contrast, female rats had 25% higher 6-methylhydroxylation activity (0.045 ± 0.003 nmol/min per mg) than male rats (0.036 ± 0.002 nmol/min per mg), resulting in a 57% faster intrinsic clearance (CL_{int}) in the females than males (0.36 ± 0.06 vs 0.23 ± 0.05 ml/min per g). Overall, total CL_{int} by both glucuronidation and 6-methylhydroxylation in male rats was 83% higher than in female rats (6.59 ± 2.11 vs 3.60 ± 1.07 nmol/min per g). Men ($n = 4$) had a significantly lower ($P < 0.05$) CL_{int} for glucuronidation than women ($n = 10$), but a higher CL_{int} for 6-methylhydroxylation, resulting in significantly higher total CL_{int} in women than men (5.63 vs 8.33 nmol/min per g). There was no significant difference in either the total plasma protein or albumin concentration between male and female mice, rats or humans. *Conclusion:* There were significant gender-related differences in the metabolism and pharmacokinetics in the rat, in contrast to the mouse. This indicates a limited usefulness of the rat as a model for the study of DMXAA metabolism in relation to gender differences, although the gender differences in the in vitro metabolic capacity for DMXAA may provide an explanation for the gender differences in the pharmacokinetics in rats. Data from human liver microsomes may allow the prediction of gender effects in the in vivo pharmacokinetics of DMXAA.

Keywords DMXAA · Gender difference · Metabolism · Pharmacokinetics

Abbreviations *CYP:* cytochrome P450 · *DMXAA:* 5,6-dimethylxanthenone-4-acetic acid · *DMXAA-G:* DMXAA acyl glucuronide · *6-OH-MXAA:* 6-hydroxymethyl-5-methylxanthenone-4-acetic acid · *UDPGA:* uridine diphosphate glucuronic acid · *UGT:* uridine diphosphate glucuronosyltransferase

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Introduction

Little or no information on gender-related differences in pharmacokinetics is available for a number of drugs.

Therefore, regulatory authorities have recently requested that women be included in all phases of clinical drug development so that potential gender differences in the pharmacokinetics (absorption, distribution, metabolism and elimination; ADME) and pharmacodynamics of drugs can be thoroughly investigated. In females, any processes associated with ADME may differ due to hormonal effects on physiological functions. Marked gender differences in ADME have indeed been reported for many drugs, although these gender differences generally become less marked or disappear in clinical settings [3, 20]. In vitro and animal studies have indicated marked gender differences in the phase I metabolism catalysed by cytochrome P450 (CYP) and the phase II metabolism catalysed by uridine diphosphate glucuronosyltransferase (UGT) of many drugs [3, 9, 20, 34]. For example, the majority of studies indicate that the activity of human liver CYP3A4, an enzyme responsible for the metabolism of over 50% of therapeutic drugs, is higher in women than in men, whereas the activity of many other drug-metabolizing enzymes may be higher in men than in women [9]. Human liver CYP3A4 plays an important role in the metabolism of many important anticancer drugs, including epipodophyllotoxins (etoposide and teniposide), ifosphamide, tamoxifen, paclitaxel, vinca alkaloids (vindesine, vinblastine and vinorelbine) and docetaxel [7, 10, 12, 16]. Given that anticancer drugs are generally administered at doses close to the maximal tolerated doses, gender differences in the pharmacokinetics of newly developed anticancer agents may be of clinical significance.

5,6-Dimethylxanthenone-4-acetic acid (DMXAA, Fig. 1) is an experimental anticancer agent, developed by the Auckland Cancer Society Research Centre (ASCRC). It has recently completed phase I clinical trials in New Zealand and the UK under the direction of the Cancer Research Campaign's Phase I/II Clinical Trials Committee [11]. Unlike conventional chemotherapeutic agents, DMXAA induces rapid vascular collapse and necrosis in transplantable murine tumours, thought to be due to immune modulation and the induction of cytokines, in particular tumour necrosis factor-α, interferons, serotonin and nitric oxide [2, 22, 32, 33]. DMXAA has also shown antiangiogenic effects [4]. DMXAA is highly bound to plasma proteins across species, in a drug concentration-dependent manner [42].

DMXAA is extensively metabolized by acyl glucuronidation, and to a lesser extent by 6-methylhydroxylation [14, 19, 36, 40]. The resultant major metabolites, DMXAA acyl glucuronide (DMXAA-G) and 6-hydroxymethyl-5-methylxanthenone-4-acetic acid (6-OH-MXAA), are excreted in the bile and urine [14, 36]. Enzyme mapping studies have indicated that UGT1A9 and UGT2B7 are responsible for DMXAA acyl glucuronidation [19] and CYP1A2 for 6-methylhydroxylation [40]. In mice, there are insignificant gender differences in the plasma pharmacokinetics, but some quantitative gender differences have been observed in the DMXAA-thalidomide pharmacokinetic interaction, with the area under the plasma concentration-time curve (AUC) of DMXAA increased by 80% and 23% in female and male mice, respectively, when coadministered with thalidomide [41].

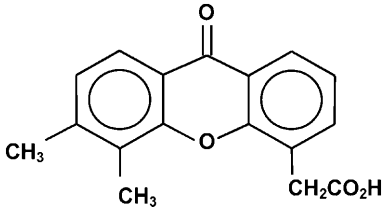

Fig. 1 The chemical structure of DMXAA

The aim of this study was to use the rat model to further investigate the gender effects on the plasma pharmacokinetics of DMXAA in comparison with the pharmacokinetics in the mouse. The rat model was chosen as it allows easier intravenous (i.v.) administration of DMXAA, and repeated blood sampling from a single animal is possible, thus reducing some of the variability observed with the mouse model. In addition, there are a wider variety of more manageable in vitro models (e.g. rat hepatocytes and isolated perfused liver model) available for subsequent studies. The in vitro metabolism in liver microsomes and plasma protein binding of DMXAA in mice, rats and humans (both male and female) were also investigated since it was considered that the resulting data could provide an explanation for the in vivo pharmacokinetic gender differences.

Materials and methods

Chemicals and reagents

DMXAA sodium salt and the internal standard, 2,5-dimethylxanthenone-4-acetic acid (SN24350) (purity >99%, determined by thin-layer chromatography) were synthesized in the ACSRC [23]. DMXAA was protected from light exposure to avoid degradation [24]. Authentic DMXAA-G and 6-OH-MXAA were isolated and purified from the bile and urine of the rats treated with DMXAA by a solid-phase extraction method. Both metabolites had a purity of 99% as determined by high-performance liquid chromatography (HPLC) and their structures identified by liquid chromatography mass spectrometry (LC-MS) and [^{1}H]-nuclear magnetic resonance [14, 40]. Bromcresol green, Brij 35, citric acid, essentially fatty acid-free human serum albumin prepared from fraction V, α-nicotinamide adenine dinucleotide phosphate (reduced form, NADPH) and uridine diphosphate glucuronic acid (UDPGA) were purchased from Roche Diagnostics (Auckland, NZ). D-Saccharic acid 1,4-lactone, bicinchoninic acid reagent, and Brij 58 were obtained from Sigma-Aldrich Chemical Company (Auckland, NZ). The Centrisart micropartition device with a 20,000 molecular weight cut-off was from Sartorious (Goettingen, Germany). All other reagents were of analytical or HPLC grade as appropriate.

Animals

Male and female C57B1/6 mice (25–32 g) and Wistar Kyoto rats (180–230 g) were housed under conditions of constant temperature,

lighting and humidity according to institutional guidelines. Sterile food and water was available ad libitum. All animal procedures were approved by the Animals Ethics Committee of the University of Auckland.

Preparation of plasma and liver microsomes

Fresh heparinized blood was obtained from C57Bl/6 mice and Wistar Kyoto rats (both male and female) and healthy humans. Plasma was separated by centrifugation at 1000 *g* for 15 min. Microsomes were prepared by differential centrifugation from livers from humans ($n = 14$) and Wistar Kyoto rats ($n = 6$, for each gender) as described previously [26]. Human liver samples were donated by 13 individuals who had undergone liver resection for either metastasis of colon cancer or hydatid disease, and by one transplant donor [40]. Histological examination of the resected livers ensured the use of healthy liver tissue. Ethical approval from the Northern New Zealand Research Ethics Committee and written informed consent for liver tissues to be used for research was obtained. Livers and microsomes were stored at -80°C until used. Microsomal protein concentration was determined by the bicinchoninic acid method [29]. Albumin concentrations in the plasma were determined using the bromcresol green dye binding method [8].

In vivo pharmacokinetic studies

Male and female Wistar Kyoto rats ($n = 5$ for each group) were used for in vivo kinetic studies. DMXAA was dissolved in sterile water and administered at 30 mg/kg to rats as an i.v. injection via the tail vein under light anaesthesia with halothane. All experiments were conducted in subdued light. Blood (200 µl) was collected in heparinized tubes by snipping the tip of the tail of the rats at 0.25, 1, 2, 4, 6, 8 and 10 h following drug administration. Plasma was separated immediately by centrifugation for 10 min at 1000 *g* and stored at –20°C until assayed.

Microsomal incubations

The kinetics of the in vitro DMXAA glucuronidation and 6-methylhydroxylation with mouse, rat and human liver microsomes were investigated using optimized incubation conditions [40, 41]. Briefly, typical incubations (200 µl) for DMXAA glucuronidation contained liver microsomal protein (0.1 mg/ml), 10 m*M* UDPGA, 5 m*M* $MgCl_2$, 0.1 mg/ml D-saccharic acid 1,4-lactone, Brij 58 (Brij 58/microsome ratio 0.1-0.4:1 w/w) and DMXAA (5-350 µ*M*) in 0.1 *M* phosphate buffer (pH 6.8). D-Saccharic acid 1,4-lactone was used to inhibit the activity of *β*-glucuronidase in microsomes. Typical incubations (200 µl) for 6-methylhydroxylation contained 1 mg/ml liver microsomes, 5 m*M* $MgCl_2$, 0.5 m*M* NADPH and DMXAA (5–350 µ*M*) in 0.1 *M* phosphate buffer (pH 7.4). All incubations were performed in triplicate, were initiated by the addition of cofactor (UDPGA or NADPH), and were conducted at 37°C in a shaking water bath. Incubations were stopped by cooling on ice and adding two volumes of an ice-cold mixture of acetonitrile/methanol (3:1, v/v) containing 2 µ*M* internal standard (SN24350), and vortexing vigorously. Mixtures were centrifuged (3000 *g* for 10 min) to remove the precipitated microsomal protein. The supernatant was removed and evaporated under nitrogen gas and the residue reconstituted with 200 µl mobile phase, and 25-75 µl injected into the HPLC.

Plasma protein binding assay

The determination of DMXAA unbound fraction (f_u) in plasma from mice, rats and humans has been described previously [42, 43]. Briefly, DMXAA (100 and 500 µ*M*) was incubated with plasma for 30 min at 37°C with shaking. A 400-µl aliquot was then transferred to the Centrisart ultrafiltration device, centrifuged (2000 *g* for 30 min) at 37°C, and the DMXAA concentration in the ultrafiltrate determined by HPLC. A 100-µl sample of ultrafiltrate was mixed with 50 µl of 0.1 *M* phosphate buffer (pH 7.4) containing 10 µ*M* internal standard, and 50 µl injected into the HPLC. A 100-µl aliquot of plasma was also taken to determine the total DMXAA concentration. The aliquot was mixed with 50 µl methanol containing 20 µ*M* internal standard, followed by 0.4 ml ice-cold acetonitrile/methanol (3:1, v/v). After centrifugation (2500 *g* for 15 min) to remove precipitated proteins, the supernatant was removed and evaporated to dryness under nitrogen. The residue was dissolved in 200 µl mobile phase, and 50 µl was injected into the HPLC.

LC-MS and HPLC

The LC-MS identification of the metabolites formed in liver microsomes has been described previously [40]. The LC-MS system was fitted with either an atmospheric pressure chemical ionization or an electrospray interface (Hewlett Packard, Avondale, Pa.). The determination of DMXAA, DMXAA-G and 6-OH-MXAA in plasma and microsomes has been described previously [14, 39]. Briefly, for DMXAA, plasma samples were diluted with 1 m*M* ammonium acetate buffer (pH 5.5) and extracted using an automatic solid-phase extraction system (Gilson, Middleton, Wis.). DMXAA was eluted with 1 ml methanol, and the eluents collected and evaporated under nitrogen. The residues were reconstituted with 200 µl mobile phase and injected into the HPLC system. The latter consisted of model 510A pumps with a WISP 712B sample injector and a Radial Compression Module (Waters Associates, Milford, Mass.) fitted with a C18 5-µm 100×4.6 mm Luna column (Phenomenex Company, Avondale, Calif.) protected by a Luna C18 guard column. The mobile phase (flow rate 2.0 ml/min) was 10 m*M* ammonium acetate buffer/acetonitrile (24:76, v/v) adjusted to pH 5.0 with acetic acid. Fluorescence detection was performed using a Shimadzu RF-530 at excitation and emission wavelengths of 345 nm and 409 nm, respectively (supported by Unicam 4880 data processing software).

The HPLC system for determining DMXAA-G and 6-OH-MXAA in microsomal incubations consisted of a solvent delivery system, a Model SF250 fluorescence detector (excitation and emission wavelengths 345 nm and 409 nm, respectively), a Model 460 autosampler, and a Model D450 data processing system (all from Kontron Instrument Company, Milan, Italy). A Luna C18 guard column and a 5-µm Spherex analytical column (150×4.6 mm) (Phenomenex Company, Avondale, Calif.) were used. The mobile phase was the same as for DMXAA but at a flow rate of 2.8 ml/min. All HPLC methods had acceptable accuracy (85-115% of true values) and precision (intra- and interassay coefficients of variation < 15%).

Data analysis

Data are presented as means ± SD. Several models to describe the kinetics of DMXAA acyl glucuronidation and 6-methylhydroxylation (single binding site and two binding sites, substrate-activator and substrate-inhibitor complex formation models) were fitted and compared using the Prism 3.0 program (Graphpad Software Company, Calif.) [40, 41]. The choice of best model was confirmed by the *F*-test and Akaike's information criterion [38]. The initial statistical analysis to evaluate the differences in the mean kinetic parameters between genders was performed using Student's *t* for within-species comparisons.

Pharmacokinetic parameters were calculated using standard model-independent pharmacokinetic formulas. The elimination half-life ($T_{1/2\beta}$) was estimated by fitting all of the data points on the plasma concentration-time curve to an exponential function, and the $T_{1/2\beta}$ was calculated as 0.693/k, where k is the elimination rate [15]. AUC was calculated using the log trapezoidal rule without extrapolation to infinity. The plasma clearance of DMXAA following i.v. administration was calculated as the total administered dose/AUC. Statistical significance was assessed using Student's

t-test at $P < 0.05$. The initial statistical analysis to evaluate the differences in the mean kinetic parameters among the different species, or within one species, was performed by a two-way analysis of variance (ANOVA) with a Tukey-Kramer test.

Results

Gender differences in the plasma pharmacokinetics of DMXAA in the rat

The plasma concentration-time profiles of DMXAA in male and female rats are shown in Fig. 2. In vivo kinetic studies indicated 60%, 55% and 73% higher plasma DMXAA AUC (2413 ± 188 vs 1505 ± 312 $\mu M \cdot$h, $P < 0.05$), $T_{1/2}$ (2.40 ± 0.45 vs 1.55 ± 0.33 h) and Cmax (1236 ± 569 vs 716 ± 280 μM) in female than in male rats, resulting in a 1.6-fold higher clearance in male rats (0.066 l/kg per h) than female rats (0.041 l/kg per h).

Gender differences in the in vitro metabolism of DMXAA

The kinetic parameters for DMXAA acyl glucuronidation and 6-methylhydroxylation in mouse, rat and human liver microsomes are shown in Table 1. The kinetic parameters for male and female mice were similar. However, male rats had a 67% higher glucuronidation activity (0.75 ± 0.03 nmol/min per mg) than female rats (0.45 ± 0.01 nmol/min per mg), resulting in a 96% faster intrinsic clearance (CL_{int}) in male than female rats (6.36 ± 0.65 vs 3.24 ± 0.42 ml/min per g, $P < 0.05$). In contrast, female rats had 25% higher 6-methylhydroxylation activity (0.045 ± 0.003 nmol/min per mg) than male rats (0.036 ± 0.002 nmol/min per mg), resulting in a 57% faster intrinsic clearance (CL_{int}) in female than male rats (0.36 ± 0.06 vs 0.23 ± 0.05 ml/min per g). Overall, total CL_{int} by both glucuronidation and 6-methylhydroxylation in male rats was 83% higher than in female rats (6.59 ± 2.11 vs 3.60 ± 1.07 nmol/min per g). Men ($n = 4$) had a significantly lower ($P < 0.05$) CL_{int} for glucuronidation than women ($n = 10$) but a higher CL_{int} for 6-methylhydroxylation, resulting in a significantly higher total CL_{int} in women than men (8.33 vs 5.63 nmol/min per g).

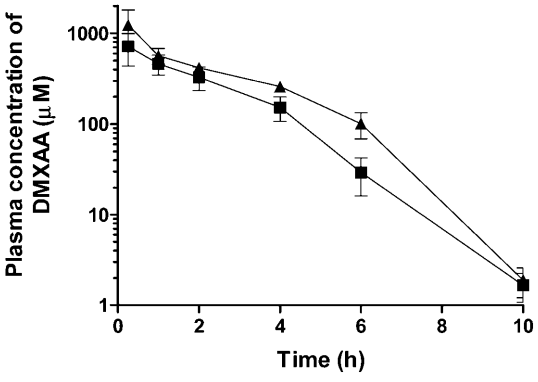

Fig. 2 The plasma concentration-time profile of DMXAA in male rats (*squares*) and female rats (*triangles*)

Gender differences in the in vitro plasma protein binding of DMXAA

There were no significant differences in either the total plasma protein or albumin concentration between male and female mice, rats or humans (Table 2). At 100 or 500 μM DMXAA, the f_u was not significantly different between male and female mice, rats or humans ($P > 0.05$, unpaired *t*-test).

Discussion

This study demonstrated significant gender differences in plasma pharmacokinetics of DMXAA in rats, with a 1.6-fold higher clearance in males than in females, which is in contrast to mice in which no significant gender differences were observed [41]. The reason for the species difference in the gender effects on the pharmacokinetics of DMXAA is unknown. One possible reason is that mice have a much slower DMXAA plasma clearance than rats [14]. These results indicate that the mouse may

Table 1 Comparison of the kinetic parameters for in vitro DMXAA metabolism in male and female mice, rats and humans. The kinetic parameters (means ± SD) for DMXAA metabolism were determined from at least three determinations under optimized incubation conditions. Liver microsomes from rats ($n = 6$) were pooled, but the human data were from individuals. The kinetic parameters for glucuronidation were obtained from detergent-activated liver microsomes

Species	Acyl glucuronidation			6-Methylhydroxylation		
	K_m (μM)	V_{max} (nmol/min/mg)	CL_{int} (ml/min/g)	K_m (μM)	V_{max} (nmol/min/mg)	CL_{int} (ml/min/g)
Mice						
M	144 ± 18	0.05 ± 0.00	0.35 ± 0.04	236 ± 44	0.026 ± 0.002	0.11 ± 0.03
F	120 ± 16	0.05 ± 0.00	0.42 ± 0.06	220 ± 32	0.020 ± 0.001	0.09 ± 0.02
Rats						
M	118 ± 11	0.75 ± 0.03	6.36 ± 0.65	158 ± 21	0.036 ± 0.002	0.23 ± 0.05
F	139 ± 15	0.45 ± 0.01	3.24 ± 0.42	125 ± 13	0.045 ± 0.003	0.36 ± 0.06
Human						
M ($n = 4$)	154 ± 111	0.44 ± 0.17	2.85 ± 3.16	18 ± 4	0.050 ± 0.005	2.78 ± 0.09
F ($n = 10$)	131 ± 68	0.86 ± 0.56	6.56 ± 7.68	22 ± 6	0.039 ± 0.021	1.77 ± 0.34

Table 2 Unbound fraction (%) of DMXAA in plasma from mice, rats and humans. Values are means ± SD. Plasma from mice and rats were pooled, but the human data were from individuals

	Mice		Rats		Humans	
	M ($n=10$)	F ($n=12$)	M ($n=6$)	F ($n=5$)	M ($n=5$)	F ($n=6$)
Total protein (g/l)	53.2 ± 5.0	52.4 ± 6.5	66.3 ± 5.2	65.3 ± 4.8	72.1 ± 4.6	70.1 ± 5.5
Albumin (g/l)	34.5 ± 0.9	36.8 ± 6.2	37.4 ± 0.5	36.5 ± 1.6	44.2 ± 6.9	41.3 ± 6.8
f_u (100 μ*M*)	3.65 ± 0.64	3.13 ± 0.41	2.61 ± 0.22	2.84 ± 0.51	1.68 ± 0.51	2.07 ± 0.42
f_u (500 μ*M*)	4.58 ± 0.66	5.25 ± 0.29	2.64 ± 0.30	3.25 ± 0.41	3.18 ± 0.58	3.51 ± 0.28

be a more appropriate model than the rat for DMXAA metabolism studies in relation to gender differences. Indeed, the mouse was the animal model most extensively used in the preclinical evaluation of DMXAA. The mechanism of action, pharmacokinetic profile, toxicity and drug interactions of DMXAA have mostly been studied in mice [5, 6, 13, 15, 22, 41]. However, the existence of significant differences in the in vitro microsomal metabolism and inhibition [44], pharmacokinetics, tolerability [14] and interactions with other drugs such as thalidomide [41, 45] of DMXAA across species has resulted in difficulty in the choice of appropriate models to predict the pharmacokinetic parameters, tolerability and drug interactions of DMXAA in patients. In particular, the 20-fold differences in the total in vitro CL_{int} of DMXAA between humans or rats and mice indicate that the usefulness of the mouse as a model in DMXAA metabolism studies is also limited.

Our in vitro studies using liver microsomes showed an 83% higher intrinsic clearance of DMXAA in male than in female rats, which was consistent with a faster plasma clearance in vivo in male than in female rats. Thus, the gender differences in the in vitro metabolic capacity for DMXAA may provide an explanation for the gender differences in the pharmacokinetics in rats. This also indicates the importance of the application of liver microsomes as a metabolic model. One factor contributing to gender differences for drugs eliminated mainly by metabolism is differential expression of hepatic drug-metabolizing enzymes [9, 18, 31]. It has been shown that DMXAA is extensively metabolized by glucuronidation and to a lesser extent by 6-methylhydroxylation in rats [14, 36], and UGT2B and the CYP1A subfamily may be involved in the metabolism of DMXAA, as indicated by our inhibition studies using UGT substrates and CYP chemical inhibitors [44]. For example, furafylline, a mechanism-based CYP1A inhibitor [28], significantly inhibits DMXAA 6-methylhydroxylation in rat liver microsomes (k_{inact} 0.21 ± 0.03 min^{-1}, K'_{app} 19.2 ± 7.3 μ*M*), indicating the involvement of the CYP1A subfamily [44]. Therefore, any gender differences in the expression of the enzymes responsible for DMXAA metabolism may contribute to the gender differences in the pharmacokinetics of DMXAA in rats.

Our results also indicate the importance of using human liver microsomes to study the metabolism of DMXAA. This study demonstrated a significantly higher rate of in vitro DMXAA 6-methylhydroxylation (CYP1A2-catalysed) [40] in men than in women, in contrast to a significantly higher rate of in vitro DMXAA glucuronidation (UGT1A9- and UGT2B7-catalysed [19] in women than in men, which appears to be consistent with reports that men have higher CYP1A2 activity [9, 21, 30], but may have lower glucuronidation activity than women. These in vitro data may allow prediction of gender effects on the in vivo pharmacokinetics of DMXAA and the reasonable design of future phase II trials of DMXAA. However, it is unlikely that any conclusions on the effects of gender on in vivo DMXAA clearance can be drawn as only a small number of livers (14) were used in this study.

Our study showed negligible gender differences in the plasma protein binding of DMXAA in all the species examined. This is in agreement with other reports that the influence of gender on plasma protein binding is small [35, 37]. The lack of gender differences in the plasma protein binding of DMXAA in rats excludes the possibility that the slower clearance in male rats was due to an altered free fraction. However, it should be noted that for some drugs, such as diazepam [1, 27], chlordiazepoxide [25] and imipramine [17], marked gender effects on plasma protein binding have been observed. The clinical significance for marked gender effects on plasma protein binding of drugs appears to be minimal, with the possible exception of warfarin for which a small change in free fraction may alter the anticoagulant activity.

In conclusion, these results indicate that there are significant gender-related differences in the metabolism and pharmacokinetics in the rat, in contrast to the mouse. This indicates a limited usefulness of the rat as a model for the study of DMXAA metabolism in relation to gender differences, although the gender differences in the in vitro metabolic capacity for DMXAA may provide an explanation for the gender differences in the pharmacokinetics in rats. Data from human liver microsomes may allow the prediction of gender effects on the pharmacokinetics of DMXAA.

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