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Non-specific binding of the experimental anti-cancer drug 5,6-dimethylxanthenone-4-acetic acid (DMXAA) in liver microsomes from various species

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Abstract

Total (added) drug concentrations other than unbound concentrations have been used to estimate the in-vitro enzyme kinetic parameters for 5,6-dimethylxanthenone-4-acetic acid (DMXAA), an experimental anti-cancer drug. This study aimed to investigate the non-specific binding of DMXAA to liver microsomes from various species and to microsomes from human lymphoblastoid cells expressing drug-metabolising enzymes, and to examine the effect of the binding on the estimation of enzyme kinetic parameters for DMXAA in-vitro. The separation of unbound DMXAA was conducted by ultrafiltration and DMXAA concentrations were determined by validated HPLC. The results indicated that DMXAA was bound to liver microsomes and lymphoblastoid cell microsomes to a small extent (free fraction in microsomes, $f_{u(mic)}$, mostly > 0.85). Correction for the unbound DMXAA concentration resulted in slightly lower apparent Michaelis–Menten constant (K_m) values, but with the maximal velocity of reaction (V_{max}) unchanged, leading to slightly higher unbound V_{max}/K_m values. These results indicate that the non-specific binding of DMXAA to microsomes is insignificant and has little impact on the enzyme kinetic estimation in-vitro.

Introduction

The anti-cancer agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) (Figure 1) was developed by the Auckland Cancer Society Research Center (ACSRC) and its Phase-I trial has been recently completed in New Zealand and the UK (Jameson et al 2000). As a biological response modifier, the mode of action of DMXAA is different from most conventional cytotoxic anti-cancer agents. It induces rapid vascular collapse and necrosis in transplantable murine tumours (Zwi et al 1989, 1994). DMXAA has potent immunomodulating activity, and induces the production of various cytokines – in particular, tumour necrosis factor-α, interferons, serotonin and nitric oxide (Thomsen et al 1991; Philpott et al 1995; Baguley et al 1997). In-vitro and in-vivo studies also indicate that DMXAA has an anti-angiogenetic effect (Cao et al 2001). All these effects of DMXAA are considered to contribute to its potent anti-cancer activity. The metabolism of DMXAA has been extensively studied using in-vivo and in-vitro models, including isolated perfused rat liver and hepatic microsomes, and these studies have indicated that uridine diphosphate glucuronosyltransferase (UGT1A2 and UGT2B7)-catalysed glucuronidation on its acetic acid side chain and, to a lesser extent, cytochrome P450 (CYP1A2)-catalysed hydroxylation of the 6-methyl group are its major metabolic pathways (Miners et al 1997; Zhou et al 2000).

During the in-vitro studies on DMXAA's metabolism using microsomes from various sources, it has been assumed that the total (added) concentration of DMXAA is the unbound concentration, without taking into account its non-specific binding to microsomal proteins, resulting in apparent values for the Michaelis–Menten constant (K_m) rather than actual K_m values (Miners et al 1997; Zhou et al 2000). An in-vitro–in-vivo clearance scaling has been conducted, based on these apparent kinetic parameters, but significant under-prediction of in-vivo clearance has been encountered (Zhou et al 2002). Many factors may contribute to the underestimation of DMXAA's clearance. These include: extrahepatic metabolism; loss of UGT enzyme activity during

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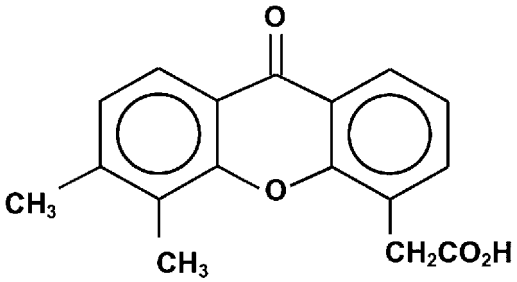

5,6-Dimethylxanthenone-4-acetic acid

Figure 1 The chemical structure of DMXAA.

microsomal preparation; rate-limiting uptake of DMXAA into hepatocytes, leading to overestimation of in-vivo clearance; possible active transport of DMXAA and its metabolites (not taken into account by the in-vitro liver models); and non-specific binding of DMXAA to liver microsomes. It is well known that the non-specific microsomal binding may be an important factor influencing the accuracy of the estimation of enzyme kinetic parameters in-vitro and thus be important in predicting in-vivo drug clearance based on in-vitro data (Tucker 1992; Obach 1997; McLure et al 2000). Generally, non-specific micrsomal binding of a drug results in a higher apparent K_m determined on the basis of the total (added) concentration other than the unbound concentration, but the maximal velocity of reaction, V_{max}, often remains unchanged (Obach 1997; McLure et al 2000). However, in some cases, the non-specific microsomal binding of substrates may have complex effects on the enzyme kinetic parameters (K_m and V_{max}) and inhibition constants, depending on the extent of binding, enzyme type, substrate and microsomal protein concentrations, and the presence of inhibitors. Thus, attempts have been made to investigate the non-specific binding of DMXAA in hepatic microsomes from mouse, rat, rabbit and man, and in microsomes from human lymphoblastoid cells expressing drug-metabolising enzymes, by ultrafiltration followed by validated HPLC methods with fluorescence detection. The impact of the non-specific binding of DMXAA on its in-vitro kinetic estimation was also evaluated.

Materials and Methods

Chemicals and reagents

DMXAA and the internal standard, 2,5-dimethylxanthenone-4-acetic acid (SN24350), were synthesised in the ACSRC (Rewcastle et al 1991). DMXAA was protected from light exposure to avoid degradation (Rewcastle et al 1990). Bicinchoninic acid reagent, Brij 58 and D-saccharic acid 1,4-lactone were purchased from Sigma-Aldrich Chemical Co. (Auckland, NZ). The Centrisart micropartition device with 20 000 molecular weight cut-off was from Sartorious AG (Goettingen, Germany). Human lymphoblast cell microsomes expressing UGT2B7 or CYP1A2 were obtained from Gentest Corp. (Woburn, MA). All other reagents were of analytical or HPLC grade as appropriate.

Hepatic microsomes

Male Wistar Kyoto rats (185–245 g, n = 6), male C57B1 mice (25–32 g, n = 15) and male white New Zealand rabbits (3–3.4 kg, n = 3) were housed under constant temperature (23±1°C), relative humidity (55±5%) and lighting (12-h light–dark cycle) according to institutional guidelines. Sterile food and water were freely available. All animal procedures were approved by the Animal Ethics Committee of the University of Auckland. Hepatic microsomes from various species were prepared by differential centrifugation, as described by Robson et al (1987). Human liver samples (HL6, HL7, HL8, HL12, HL13 and HL14) were donated by individuals who either underwent liver resection for metastasis of colon cancer or hydatid disease, and their details have been published elsewhere (Zhou et al 2000). Histological examination of the resected livers ensured the use of healthy liver tissue. Ethical approval was obtained from the Northern New Zealand Research Ethics Committee, and all donors gave written informed consent for liver tissues to be used for research. Livers and microsomes were stored at −80°C until used. Microsomal protein concentration was determined by the bicinchoninic acid binding method using bovine serum albumin as the standard (Smith et al 1985).

Ultrafiltration

Separation of free DMXAA was done by ultrafiltration using the disposable Centrisart micropartition device. Previous studies had indicated that the adsorption of DMXAA (0.5–500 l M) to the Centrisart filtration device was < 1% (Zhou et al 2001). Filtration studies with liver microsomes from mouse, rat, rabbit and man containing 500 l M DMXAA indicated that centrifugation at 2000 *g* for 30 min at 37°C (Beckman J-6M centrifuge) was appropriate for the determination of the unbound fraction. Microsomal solutions containing 10–1000 l M DMXAA were incubated for 30 min at 37°C. The DMXAA concentrations used for this study were associated with the plasma DMXAA concentrations of cancer patients, where 5–2500 l M has been encountered (Jameson et al 2000). Pooled mouse, rat, rabbit and human liver microsomes (from HL6, HL7, HL8, HL12, HL13 and HL14) were used for the study. Microsomes from human lymphoblastoid cells expressing UGT2B7 or CYP1A2 were also used. A 100-l L sample was taken to determine the total DMXAA concentration by HPLC. A 400-l L sample was transferred to the ultrafiltration device, centrifuged at 2000 *g* for 30 min at 37°C. Samples were capped to minimise changes in pH during ultrafiltration. The DMXAA concentration in the ultrafiltrate was determined by HPLC. The unbound fraction ($f_{u(mic)}$) of DMXAA was calculated by the ratio of the

DMXAA concentration in the ultrafiltrate to that in the microsomal incubation before ultrafiltration.

HPLC

Instrumentation

The concentration of DMXAA was determined by HPLC as described by Zhou et al (2001). Briefly, microsomes or filtrate (100 l L) was mixed with 0.4 mL ice-cold acetonitrile–methanol (3:1, v/v) with 2 l M internal standard. After centrifugation at 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 l L mobile phase, and 50 l L was injected into the HPLC system which consisted of a solvent delivery system, a Model SF250 fluorescence detector (excitation and emission wavelength, 345 nm and 409 nm, respectively), a Model 460 autosampler and a Model D450 data processing system (all from Kontron Instrument Co., Milan, Italy). A Luna C18 guard column and a 5-l m Spherex C18 analytical column (150×4.6 mm; both from Phenomenex, NZ Ltd., Auckland) were used with a mobile phase of acetonitrile–10 mM ammonium acetate buffer (24:76, v/v, pH 5.0) at a flow rate of 2.5 mL min^{-1}.

Calibration curves

Calibration curves (0.5–40 l M) were constructed from the peak area ratio of DMXAA:internal standard vs known DMXAA concentrations in microsomes or 0.1 M phosphate buffer (pH 7.4). Linear least-squares regression analysis was used to determine the slope, intercept and coefficient of determination. Samples with DMXAA concentrations >40 l M were diluted with 0.1 M phosphate buffer (pH 7.4) to ensure that the concentrations were within the assay range.

Sensitivity and selectivity

The limit of quantitation was determined as the minimum concentration that can be accurately and precisely quantified (in practice it is the lowest data point of the calibration curve), and the limit of detection was the amount that could be detected with a signal-to-noise ratio of 3 (Shah et al 2000). The selectivity of the method was examined by determining whether interfering chromatographic peaks were present in blank microsomes.

Accuracy and precision

Quality-control samples of DMXAA were prepared from weighings independent of those used for the preparation of calibration curves. Final concentrations of low, medium and high quality-control samples were 1, 5 and 40 l M. These samples were prepared on the day of analysis in the same way as calibration standards. The performance of the HPLC method was assessed by analysis of at least 12 quality-control samples (4 each of low, medium and high concentrations) on a single assay day to determine intra-day accuracy and precision, and at least 9 quality-control samples (3 each of low, medium and high concentrations) on each of 4 consecutive assay days to determine inter-day accuracy and precision.

In vitro enzyme kinetic parameters of DMXAA

The enzyme kinetic parameters (apparent K_m, V_{max} and V_{max}/K_m) of DMXAA in liver microsomes were obtained previously (Miners et al 1997; Zhou et al 2000) or taken from our unpublished data. A one-enzyme Michaelis–Menten model was used in the determination of enzyme kinetic parameters. Unbound K_m and V_{max} values were calculated from the estimated apparent values of these parameters and free-drug concentrations using estimated free fractions.

Data analysis

Data are presented as mean±s.d. Several models to describe the kinetics of DMXAA's microsomal binding (single- and two-binding site, and a modified two-binding site model (Semmes & Shen 1990)) were fitted and compared using the Prism 3.0 program (Graphpad Software Co., CA) as follows:

$$C_b = \frac{B_{max} \times C_u}{K_d + C_u} \quad (1)$$

$$C_b = \frac{B_{max1} \times C_u}{K_{d1} + C_u} + \frac{B_{max2} \times C_u}{K_{d2} + C_u} \quad (2)$$

$$C_b = \frac{B_{max1} \times C_u}{K_{d1} + C_u} + \frac{B_{max2} \times C_u}{K_{d2}} \quad (3)$$

where C_b is the concentration of bound DMXAA, obtained from subtracting unbound (C_u) from total (C_t) DMXAA concentration; K_d is the apparent dissociation constant for the binding site; B_{max} is the maximal concentration of binding sites on microsomal protein; and subscripts 1 and 2 represent the first and the second type of site. The choice of model was confirmed by comparing and reviewing the relative residuals and the standard error of the parameter estimates from the non-linear regression analysis. The initial statistical analysis to evaluate the differences in the binding kinetic parameters among the different species was performed by a two-way analysis of variance with a Tukey–Kramer test. A P value < 0.05 was considered statistically significant.

Results

Validation of the HPLC methods

Under the chromatographic conditions used for the analysis of DMXAA, the retention times for DMXAA and internal standard were 10.2 and 12.3 min, respectively. The total chromatography run time was 13 min. Matrix-specific interfering peaks that required modification of the mobile-phase composition were not observed in any case, particularly when sample work-up included an extraction step.

Table 1 Accuracy and precision of the HPLC method for the analysis of unbound DMXAA concentrations in human liver microsomes.

Theoretical concn (μM)	Measured concn (mean±s.d.)	% Recovery of theoretical	CV (%)	No. of samples
0.1 M Phosphate buffer				
Intra-assay				
1	0.972±0.047	97.2	4.84	3
5	5.103±0.081	102.1	1.58	3
40	38.54±0.831	96.4	2.16	3
Inter-assay				
1	0.951±0.069	95.1	7.26	4
5	4.931±0.239	98.6	4.85	4
40	40.11±1.114	100.3	2.78	4
Human liver microsomes				
Intra-assay				
1	0.943±0.066	94.3	7.00	3
5	5.001±0.212	100.0	4.24	3
40	39.91±1.511	99.8	3.79	3
Inter-assay				
1	0.924±0.371	92.4	4.02	4
5	4.879±0.320	97.6	6.56	4
40	39.01±1.058	97.5	2.71	4

CV, Coefficient of variation.

Table 2 The free fraction ($f_{u(mic)}$) of DMXAA (200 l M) in liver microsomes from mouse, rat, rabbit and man and lymphoblastoid cell-expressed microsomes.

Microsome source	Concn (mg mL^{-1})	$f_{u(mic)}$	No. of determinations
Mouse liver	0.2	0.952±0.023	3
	1.0	0.885±0.034	6
Rat liver	0.2	0.961±0.032	3
	1.0	0.894±0.028	6
Rabbit liver	0.2	0.961±0.042	3
	1.0	0.910±0.033	6
Human liver	0.2	0.948±0.022	3
	1.0	0.892±0.031	6
Lymphoblastoid cell microsomes expressing UGT2B7	0.1	0.991±0.045	3
	0.2	0.950±0.030	3
Lymphoblastoid cell microsomes expressing CYP1A2	0.5	0.945±0.043	3
	1.0	0.884±0.027	3

Values are mean±s.d.

Extraction efficiency for those microsomal samples expressed as overall mean (±s.d.) percentage for DMXAA (n = 12) and internal standard were 87.3±9.1 and 88.9±7.3, respectively. No concentration dependence was observed. DMXAA is stable under the extraction conditions described. Calibration curves were linear over the concentration range used with mean correlation coefficients being $\geqslant 0.997$ in all types of microsomes and 0.1 M phosphate buffer. The mean y-intercept for DMXAA was < 0.003. The difference between the calculated and the actual concentration and the relative standard deviation was less than 10% at any quality-control concentration. The typical results of the precision and accuracy for non-specific binding assay in human liver microsomes were shown in Table 1. The limit of detection of the assay was 0.20 l M for a 75-l L sample of DMXAA.

Non-specific binding of DMXAA in microsomes

DMXAA was bound to liver microsomes and lymphoblastoid cell microsomes to a small extent ($f_{u(mic)}$, mostly > 0.85). There were no significant differences ($P > 0.05$) in the binding of DMXAA to liver microsomal protein across species. At 0.2 mg mL^{-1} of microsomal protein (a concentration typically used in incubations for DMXAA acyl glucuronidation studies), the $f_{u(mic)}$ was 0.952±0.023,

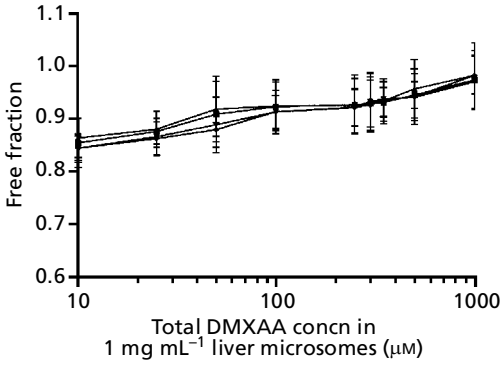

Figure 2 Non-specific binding of DMXAA to liver microsomes from mouse, rat, rabbit and man. Dependence of $f_{u(mic)}$ on DMXAA concentration at 1 mg mL^{-1} microsomal protein. Each point represents the mean±s.d. of three determinations.

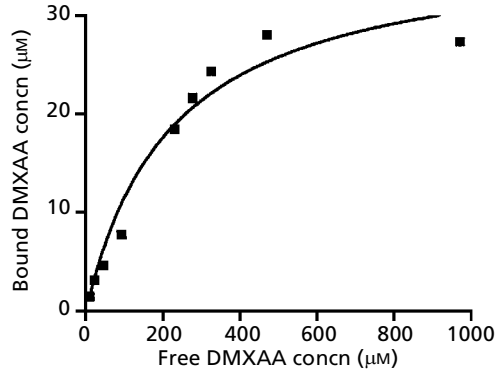

Figure 3 Plot of the bound concentration of DMXAA (C_b) as a function of unbound concentration (C_u) in pooled human liver microsomes (1 mg mL^{-1}). The curve represents the best fit for a one binding-site model. Each point is the mean±s.d. of three determinations.

0.961±0.032, 0.961±0.042 and 0.948±0.022 for mouse, rat, rabbit and man, respectively, whereas the $f_{u(mic)}$ decreased to 0.885±0.034, 0.894±0.028, 0.910±0.033 and 0.892±0.031, respectively, when the microsomal protein concentration was increased to 1 mg mL^{-1} (a concentration typically used in incubations for DMXAA methylhydroxylation studies (Table 2)). The dependence of DMXAA $f_{u(mic)}$ on drug concentration is shown is Figure 2. The $f_{u(mic)}$ progressively decreased as the protein concentration increased and the drug concentration decreased. Figure 3 shows a standard binding plot for DMXAA in human liver microsomes. The one-binding-site model was the best fit for the binding of DMXAA to liver microsomes in all species. The binding kinetic parameters (B_{max} and K_d) determined by this model are shown in Table 3. The binding capacity increased as the microsomal protein concentration increased, whereas the K_d remained approximately unchanged. At the same microsomal protein concentration, similar binding was observed for lymphoblastoid cell microsomes and human liver microsomes.

Table 3 Estimated binding parameters for DMXAA in liver microsomes from mouse, rat, rabbit and man.

Parameters	Mouse	Rat	Rabbit	Man
0.2 mg mL^{-1} microsomal protein				
B_{max} (1 μM)	10.2±2.1	16.7±3.6	9.6±1.8	12.7±1.9
K_d (1 μM)	120.1±61.5	252.3±65.3	100.1±45.1	241.2±64.9
1.0 mg mL^{-1} microsomal protein				
B_{max} (1 μM)	26.7±4.1	40.9±3.6	29.1±4.8	37.4±4.0
K_d (1 μM)	129.1±63.5	261.6±55.3	120.7±65.9	227.6±62.8

Values are mean±s.d.

Impact of non-specifi c microsomal binding of DMXAA on estimation of enzyme kinetic parameters in liver microsomes

The impact of microsomal binding on the kinetics of DMXAA glucuronidation and 6-methylhydroxylation in liver microsomal preparations from mouse, rat, rabbit and man is shown in Table 4. The unbound K_m values for glucuronidation and 6-methylhydroxylation were lower than the apparent K_m in all species, with the V_{max} approximately unchanged, resulting in a slight increase in the unbound V_{max}/K_m values.

Discussion

The validation of the HPLC methods used to determined total and unbound concentration of DMXAA in microsomal incubations indicated acceptable accuracy (85–115% of true values) and precision (intra- and inter-assay coefficients of variation, < 15%). Similar HPLC methods have been used to determine the reversible binding of DMXAA in plasma proteins from various sources (Zhou et al 2001).

Our study has indicated that although DMXAA is bound to liver microsomes from mouse, rat, rabbit and man and to microsomes from human B-lymphoblastoid cells in a microsomal protein and substrate concentration-dependent manner, the binding extent is small with $f_{u(mic)}$ usually > 0.85. This is consistent with the prediction that DMXAA as a weak acid should have insignificant non-specific microsomal binding, since the microsomal membrane has a net negative charge and acidic drugs such as caffeine, tolbutamide and naproxen do not bind significantly to it (McLure et al 2000). Like warfarin (a weak acid with a high pK_a), which has been reported to have a small binding ($f_{u(mic)}$, 0.85 at 1.0 mg mL^{-1} human liver microsomes and 10 1 μM warfarin) with drug concentration-dependence (Obach 1997), DMXAA's binding to liver microsomes was drug concentration dependent over the range of DMXAA concentrations generally used in-vitro. However, concentration-independent human liver microsomal binding of propranolol, imipramine (Obach 1997) and phenytoin (Carlile et al 1999) has also been observed.

Table 4 The effect of non-specific binding of DMXAA to liver microsomes on the estimation of the enzyme kinetic parameters in-vitro.

Metabolic pathway/parameters	Mouse	Rat	Rabbit	Man
Glucuronidation[a]				
Apparent K_m (l M)[b]	144±18	118±11	149±35	132±28
V_{max} (nmol min^{-1} mg^{-1})[b]	0.05±0.00	0.75±0.03	0.94±0.09	0.76±0.11
V_{max}/K_m (mL min^{-1} g^{-1})[b]	0.35±0.04	6.36±0.65	6.31±1.60	5.76±1.24
Unbound K_m[c]	124±15	104±9	126±29	119±21
Unbound V_{max}[c]	0.05±0.00	0.74±0.04	0.91±0.08	0.76±0.10
Unbound V_{max}/K_m	0.40±0.05	7.12±1.00	7.22±2.23	6.39±1.97
Hydroxylation				
Apparent K_m (l M)[b]	236±44	158±21	42±16	21±5
V_{max} (nmol min^{-1} mg^{-1})[b]	0.026±0.002	0.036±0.002	0.097±0.001	0.045±0.002
V_{max}/K_m (mL min^{-1} g^{-1})[b]	0.11±0.02	0.23±0.04	2.31±0.88	2.14±0.61
Unbound K_m[c]	221±36	152±20	40±15	19±5
Unbound V_{max}[c]	0.026±0.002	0.035±0.002	0.098±0.001	0.045±0.001
Unbound V_{max}/K_m	0.12±0.02	0.23±0.04	2.45±0.87	2.36±0.62

[a]The kinetic parameters (mean±s.d.) were obtained from detergent-activated liver microsomes. [b]Apparent K_m, V_{max} and V_{max}/K_m values for glucuronidation and 6-methylhydroxylation were obtained from published data (Zhou et al 2002). [c]Unbound K_m and V_{max} values were calculated from the estimated apparent values of kinetic parameters and free drug concentrations using the determined free fractions.

The DMXAA free fraction progressively decreased with increasing microsomal protein concentration in liver microsomes from all species tested (Table 2). It is thus likely that microsomal binding may contribute in part to the non-linear increase in DMXAA glucuronidation or 6-methylhydroxylation rates with increasing microsomal protein concentrations at protein concentrations of more than 4 mg mL^{-1} (data not shown). DMXAA was also bound to lymphoblastoid cell microsomes expressing UGT2B7 or CYP1A2 to a small extent, leading to minor impact on the kinetic parameter estimation. However, for substrates extensively bound to microsomes, the K_m values determined for the various UGT or CYP isoforms may be biased by the microsomal protein concentration used, resulting in misprediction of the relative contributions of the various isoforms to net intrinsic clearance. The insignificant binding of DMXAA to liver microsomes is remarkably different from its extensive binding to plasma protein binding (Zhou et al 2001).

As indicated by the B_{max} values (Table 3), mouse, rat, rabbit and human liver microsomes contain at least 26.7. 40.9, 29.1 and 37.4 l M of binding sites for DMXAA/mg of microsomal protein, respectively. These concentrations are in great excess of the average molar concentration of total CYP (300–400 pmol (mg protein)$^{-1}$) (Shimada et al 1994) in liver microsomes from these species, suggesting that the observed binding is mainly non-specific and not reflective of specific interactions with the enzyme active site. Although the Scatchard plot (data not shown) for human liver microsomes suggested that two or more binding sites might be involved in the binding of DMXAA to liver microsomes, the fitting of data to various models indicated that a one-binding-site model was the best fit. This may be due to the binding sites for DMXAA having similar binding affinity. Extension of the DMXAA concentration range may reveal the multiplicity of the binding sites.

The binding of DMXAA to liver microsomes results in a slight overestimation of K_m without affecting the metabolic rate at saturating substrate concentrations. In any case, the estimation of K_m values based on unbound drug concentration relies on the assumption that the intrinsic affinity of enzyme for unbound substrate is independent of microsomal protein concentration and that free drug concentration, rather than total concentration, is a better estimate of enzyme-available concentration in-vitro. Although the validity of this assumption remains unclear, correction for microsomal binding using the free fraction in incubation matrices clearly improves the prediction of in-vivo clearance from in-vitro estimates of intrinsic clearance for drugs that are extensively bound to microsomal matrices (Obach 1997, 1999; Carlile et al 1999; Venkatakrishnan et al 2000). Thus, unbound K_m values based on unbound drug concentrations rather than apparent K_m values based on total drug concentrations are expected to be better estimates of the true K_m based on the existing data. Although microsomal binding should in principle affect only the apparent K_m and not alter the rates at saturating substrate concentrations, this may be true only if the kinetics is consistent with monotonic Michaelis–Menten or Hill functions. In addition to its effects on apparent K_m, microsomal binding may also increase the estimated substrate inhibition constant, K_i, for UGT1A9/UGT2B7-mediated DMXAA glucuronidation and CYP1A2-mediated 6-methylhydroxylation. Microsomal binding of inhibitors can theoretically further bias the estimation of in-vitro K_i values in chemical inhibition studies, potentially resulting in underestimation of inhibitor potency. Correction for the unbound fraction in the in-vitro incubation matrix has

improved the prediction of pharmacokinetic clearance estimates in several studies (Obach 1997, 1999; Carlile et al 1999).

Conclusion

Our results indicate that the binding of DMXAA to liver and lymphoblastoid cell microsomes is insignificant, with small impact on the enzyme kinetic estimation in-vitro. However, for those drugs with significant non-specific microsomal binding, the binding should be measured and incorporated in enzyme kinetic analyses so that unbiased kinetic parameters can be determined, based on unbound other than added (total) drug concentrations.

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