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Specificity of Substrate and Inhibitor Probes for Human Cytochromes P450 1A1 and 1A2

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ABSTRACT

Kinetic and inhibitor studies using cDNA-expressed enzymes and human liver microsomes have characterized the specificity of a range of cytochrome P450 (CYP) 1A substrate and inhibitor probes towards the two isoforms comprising this subfamily. Expressed CYP1A1 and CYP1A2 both catalyzed the O-deethylation of phenacetin, although the apparent K_m was about 4-fold lower for CYP1A2 (25 *vs.* 108 μM). Phenacetin O-deethylation exhibited biphasic kinetics in human liver microsomes, and the apparent K_m for the high-affinity component (9 ± 6 μM) was consistent with the involvement of CYP1A2 in this reaction. The prototypic CYP1A xenobiotic inhibitor and substrate probes α-naphthoflavone, ellipticine, 7-ethoxycoumarin and 7-ethoxyresorufin all inhibited CYP1A1- and CYP1A2-mediated phenacetin O-deethylation as well as the high-affinity component of human liver phenacetin O-deethylase activity. α-Naphthoflavone and 7-ethoxycoumarin were, however, approximately 10-fold more potent as inhibitors of CYP1A2 than CYP1A1. Other putative human CYP1A xenobiotic substrates and inhibitors, including caffeine, 5- and 8-methoxypsoralen, nifedipine, paraxanthine, propranolol and theophylline similarly inhibited CYP1A1- and 1A2-catalyzed phenacetin O-deethylation and the high-affinity human liver phenacetin O-deethylase. In contrast, the monoclonal antibody MAb 1-7-1, raised against 3-methylcholanthrene-inducible rat cytochromes 450, almost abolished CYP1A1-mediated phenacetin O-deethylation, but had no effect on human liver microsomal- or CYP1A2-catalyzed phenacetin dealkylation. Together with previous data, the results indicate that the majority of human CYP1A xenobiotic inhibitor and substrate probes are nonspecific in their recognition of CYP1A1 and CYP1A2, although selectivity is apparent for some compounds.

The catalytic function of the two known PAH-inducible CYP 1 family enzymes CYP1A1 and CYP1A2 has attracted considerable attention. Apart from a role in the dealkylation of phenacetin (Sesardic *et al.*, 1988) and the methylxanthines caffeine (Butler *et al.*, 1989; Kalow and Campbell 1988; Tassaneeyakul *et al.*, 1992), theophylline (Robson *et al.*, 1987a), theobromine (Miners *et al.*, 1985) and paraxanthine (Kalow and Campbell, 1988), human CYP1A2 has the capacity to activate xenobiotic arylamines, 2-acetylaminofluorene and food-derived heterocyclic amines to mutagenic metabolites (Kadlubar and Hammons, 1987; Guengerich 1990; McManus *et al.*, 1990). Recent cDNA expression studies have demonstrated that human CYP1A1 may similarly activate 2-acetylaminofluorene and food-derived heterocyclic amines (McManus *et al.*, 1990).

Of additional significance is the tissue distribution of the CYP1A enzymes. Although CYP1A2 is normally expressed in liver, only 50% of human livers appear to express CYP1A1 mRNA (McKinnon *et al.*, 1991). Moreover, the level of expression of CYP1A1 in this tissue is substantially lower than for CYP1A2. Importantly, however, PAH treatment induces CYP1A1 in the liver, lung, kidneys and gut of rats (Sesardic *et al.*, 1990a), and there is an apparent association between cigarette smoking and CYP1A1 activity in human lung and placenta (McLemore *et al.*, 1990; Sesardic *et al.*, 1990b). The contribution of CYP1A1 to the metabolism of procarcinogens and other xenobiotics may, therefore, be of importance for individuals exposed to PAH-like inducers.

To investigate possible links between xenobiotic-related toxicity and CYP1A2 activity, a number of compounds have been investigated as *in vitro* or *in vivo* probes for this enzyme in humans. Caffeine, phenacetin and theophylline have been used to assess both *in vivo* and *in vitro* CYP1A2 activity or regulation in humans (Butler *et al.*, 1989; Kalow and Tang, 1991; Guengerich 1990; Robson *et al.*, 1987a), and 7-ethoxycoumarin, 7-ethoxyresorufin, ellipticine and α-naphthoflavone have been utilized as substrate or inhibitor probes *in vitro* (Murray and Reidy, 1990; Rodrigues and Prough, 1991). However, during an investigation of the molecular basis for the use of caffeine as a

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ABBREVIATIONS: CYP, cytochrome P450; HPLC, high-performance liquid chromatography; PAH, polycyclic aromatic hydrocarbon.

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CYP1A2 probe, it became apparent that this compound was also a substrate for human CYP1A1 (Tassaneeyakul *et al.*, 1992). Thus, in the present study, the substrate specificity of phenacetin and the inhibitor specificity of a range of putative CYP1A xenobiotic probes toward the separate 1A subfamily isoforms have been investigated using cDNA expression and human liver microsomal kinetic and inhibitor techniques. In addition, the inhibitor specificity of a monoclonal antibody (designated MAb 1-7-1), which recognizes both rat CYP1A1 and 1A2 (Friedman *et al.*, 1985; Fujino *et al.*, 1984), has been assessed using the cDNA-expressed human isoforms. Whereas MAb 1-7-1 was a specific inhibitor of CYP1A1-mediated phenacetin O-deethylation, the results indicate that available CYP1A xenobiotic probes are nonspecific in their recognition of the two isoforms.

Methods

Chemicals. Acetaminophen, caffeine, ellipticine, 7-ethoxycoumarin, 7-ethoxyresorufin, 5- and 8-methoxypsoralen, paraxanthine and phenacetin were purchased from the Sigma Chemical Co. (St Louis, MO), α-naphthoflavone from the Aldridge Chemical Co (Milwaukee, WI) and N-butyryl-*p*-aminophenol from ICN (Costa Mesa, CA). Nifedipine was obtained from Bayer Pharmaceutical (Sydney, Australia), propranolol HCl from ICI Australia (Melbourne, Australia) and theobromine and theophylline from Hamilton Laboratories (Adelaide, Australia).

cDNAs and human livers. CYP 1A1 and 1A2 cDNAs were isolated as described previously (Quattrochi *et al.*, 1986; Quattrochi and Tukey, 1989), subcloned into the pCMV4 expression vector and transfected into COS-7 cells (Tassaneeyakul *et al.*, 1992). Cells were harvested 48 hr post-transfection, resuspended in 0.1 M phosphate buffer (pH 7.4) containing 20% (v/v) glycerol and stored at −70°C until used. The frozen lysates were generally used within 1 month of transfection; phenacetin O-deethylase and caffeine 3-demethylase activities were, however, shown to be stable over at least 6 months of storage. Nascent enzyme was labeled with [^{35}S]methionine and immunoadsorbed with an anti-rabbit CYP1A2 antibody, which recognizes both human CYP 1A1 and 1A2 (McManus *et al.*, 1990), and analyzed on 10% sodium dodecyl sulfate-polyacrylamide gels. Gels were dried and exposed to autoradiographic film. In this manner, the relative expression of CYP1A1 and CYP1A2 was shown to be 1.3:1. Cells transfected with vector alone served as negative controls for incubations of expressed enzymes.

Human liver samples were obtained from renal transplant donors and permission was obtained from the Flinders Medical Ethical Review Committee to use human liver tissue for xenobiotic metabolism studies. Relevant details of the donors of livers used in the present study (H7, H8, H10, H15) have been published elsewhere (McManus *et al.*, 1990). Human liver microsomes were prepared as described previously (Robson *et al.*, 1987b).

Enzyme Assays

Phenacetin O-deethylase activity. The phenacetin O-deethylase activity of expressed CYP1A1 and 1A2 and human liver microsomes was determined by measurement of product acetaminophen. A HPLC procedure, based on the method of Borm *et al.* (1983), was used for the quantification of acetaminophen. Incubations contained human liver microsomal (0.2 mg) or COS-7 cell (0.5 mg) protein, reduced nicotinamide adenine dinucleotide phosphate-generating system (1 mM nicotinamide adenine dinucleotide phosphate, 10 mM glucose-6-phosphate, 2 IU of glucose 6-phosphate dehydrogenase and 5 mM $MgCl_2$) and phenacetin (2.5–2000 μM) in phosphate buffer (0.1 M, pH 7.4; total volume 1.0 ml). Incubations were started by the addition of generating system and were performed in air at 37°C for 30 min. Reaction rates were shown to be linear under the conditions used. Incubations were terminated by the addition of 1.0 M NaOH (0.12 ml), which increases the pH to 12 to 13, and cooling on ice. The assay internal standard N-butyryl-*p*-aminophenol (0.1 ml of a 2 mg/l solution: caution-protect from direct light) was added to the incubation mixture, which was then extracted with chloroform (10 ml, rotary mixer for 20 min) to remove unreacted phenacetin. The aqueous layer (1 ml) was separated by centrifugation, the pH decreased to 3 to 3.5 by the addition of 1.0 M HCl (0.17 ml), and extracted twice with 10 ml of diethylether. The combined ether extracts were evaporated under a stream of N_2 and reconstituted in 0.2 ml of the HPLC mobile phase; a 0.1-ml aliquot was injected onto the HPLC. The instrument used was fitted with a Waters-Millipore μ-Bondapak C-18 column (30 cm × 3.9 mm i.d. 10-micron particle size; Millipore, Milford, MA), which was eluted with methanol-water (18:82, pH adjusted to 3.5 using orthophosphoric acid) at a flow rate of 2.0 ml/min. Retention times of acetaminophen and internal standard, which were monitored by ultraviolet detection at 254 nm, were 4 and 13 min, respectively. Standard curves were constructed in the acetaminophen concentration range 0.1 to 5 μM. The standard curve samples were treated in the same manner as incubation samples. Within-day coefficients of variation for the assay, determined by measuring acetaminophen formation in 10 separate incubations of the same batch of hepatic microsomes at two added phenacetin concentrations (10 and 2000 μM), were <5%.

Caffeine 3-demethylase activity. Caffeine 3-demethylation by human liver microsomes and expressed CYP1A2 was determined by the method of Tassaneeyakul *et al.* (1992).

Kinetic and inhibitor studies. Phenacetin O-deethylation kinetics were determined over the following concentration ranges: human liver microsomes (2.5–2000 μM) (12 points); expressed CYP1A2 (3.3–80 μM) (6 points); and expressed CYP1A1 (33.3–400 μM) (7 points). Inhibitory effects of xenobiotics on expressed CYP1A2 and the high-affinity component of human liver microsomal phenacetin O-deethylase activity were determined at a substrate (phenacetin) concentration of 10 μM, whereas effects on expressed CYP1A1 were determined at a substrate concentration of 100 μM (see under "Results"). The xenobiotics screened, and their added concentrations in incubations, are given in figures 3 and 4.

Immunoinhibition experiments with MAb 1-7-1 were performed by preincubating antibody (as ascites fluid) with expressed protein or human liver microsomes at 37°C for 15 min before measurement of phenacetin O-deethylase or caffeine 3-demethylase activity. Control incubations at each antibody concentration contained the same amount of preimmune ascites protein. Phenacetin concentrations utilized in the immunoinhibition studies were the same as those used in the xenobiotic inhibition studies (*viz.* 10 μM for expressed CYP1A2 and human liver microsomes and 100 μM for CYP1A1). A substrate concentration of 100 μM was used in the study investigating the effects of MAb 1-7-1 on human liver microsomal and CYP1A2-catalyzed caffeine 3-demethylation. To determine the relative recognition of expressed CYP 1A1 and 1A2 by MAb 1-7-1, equivalent amounts of [^{35}S]methionine-labeled P450 protein were immunoadsorbed with MAb 1-7-1 as described under "cDNAs and human livers." For comparative purposes, expressed CYP1A1 and 1A2 protein were also immunoadsorbed with a polyclonal anti-rabbit CYP1A2 antibody, which recognizes both CYP1A1 and 1A2 (see under "cDNAs and human livers").

Analysis of results. The Michaelis-Menten parameters K_m and V_{max} were obtained by fitting kinetic data to a one- or two-enzyme model using MK Model, an extended least squares modeling program (Holford, 1985).

Results

Both expressed human CYP1A1 and CYP1A2 catalyzed the O-deethylation of phenacetin (fig. 1). The respective apparent K_m and V_{max} values were 108 μM and 75 pmol/min/mg for CYP1A1, and 25 μM and 98 pmol/min/mg for CYP1A2. In contrast to the expressed enzymes, phenacetin O-deethylation kinetics exhibited biphasic kinetics in human liver microsomes

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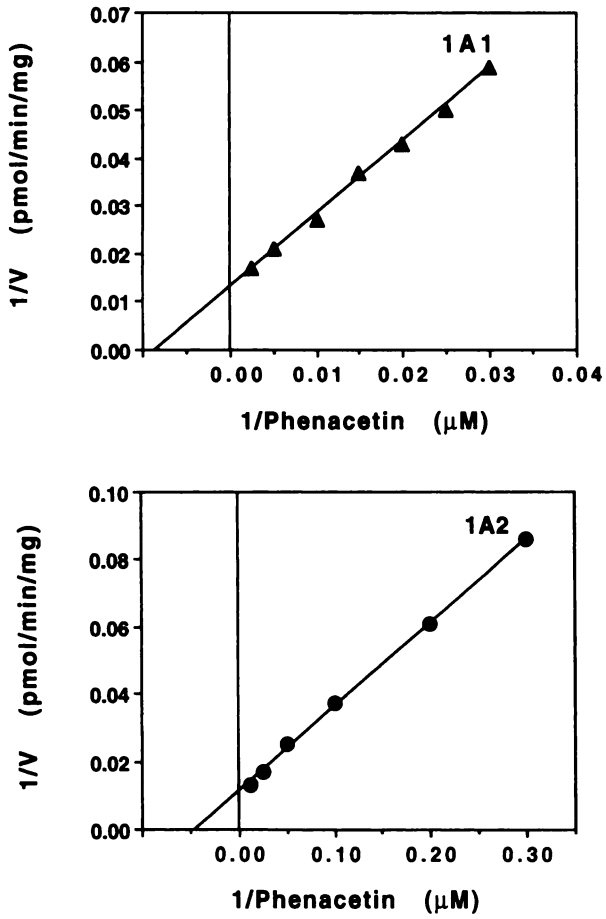

Fig. 1. Kinetic plots for phenacetin O-deethylation by expressed human CYP 1A1 (top panel) and 1A2 (bottom panel).

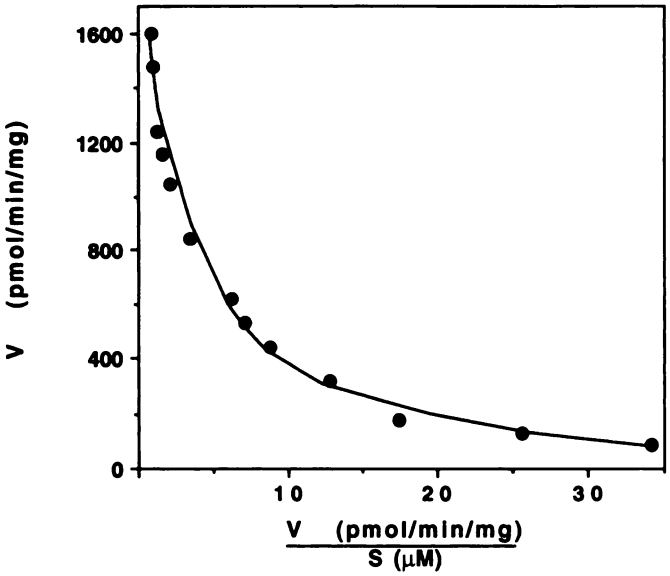

Fig. 2. Representative Eadie-Hofstee plot for phenacetin O-deethylation by human liver microsomes (liver H7). Points are experimentally determined values, and the solid line is the computer-generated curve of best fit for a two-enzyme model.

(fig. 2). Mean (±SD) apparent K_m values for the high- and low-affinity components of liver microsomal (n = 4 livers) phenacetin O-deethylase were 9 ± 6 and 322 ± 188 μM, respectively. The respective mean V_{max} values for the high- and low-affinity liver microsomal enzyme activities were 382 ± 219 and 1128 ± 335 pmol/min/mg.

Xenobiotic inhibition studies with human liver microsomes and CYP1A2 were performed at a phenacetin concentration of 10 μM, which is the approximate K_m value for the high-affinity liver microsomal phenacetin O-deethylase and for expressed CYP1A2. Similarly, inhibition studies with expressed CYP1A1 were performed at a substrate concentration of 100 μM, the approximate apparent K_m for phenacetin O-deethylation by this enzyme. It should be noted that substitution of the K_m and V_{max} values in the Michaelis-Menten equation for a two-enzyme system indicated that on average, 82% (range 74–91%) of the microsomal phenacetin O-deethylase activity of the four human livers investigated here was due to the high-affinity enzyme at a substrate concentration of 10 μM. At an added concentration of 5 μM, the xenobiotics ellipticine, 7-ethoxyresorufin, 5- and 8-methoxypsoralen and α-naphthoflavone essentially abolished expressed CYP1A1- and CYP1A2-mediated phenacetin O-deethylation and the high-affinity component of human liver microsomal phenacetin O-deethylation (fig. 3). 7-Ethoxycoumarin, nifedipine and propranolol were somewhat less potent as inhibitors, with concentrations of around 0.1 mM being necessary to abolish phenacetin O-deethylase activity (figs. 3 and 4). A concentration of 1 mM of each of the methylxanthines caffeine, paraxanthine and theophylline was necessary to diminish the three enzyme activities by approximately 50%. The methylxanthine theobromine had little or no inhibitory effect, even at 1 mM.

To determine the selectivity of inhibition by the prototypic CYP1A probes 7-ethoxycoumarin, 7-ethoxyresorufin, ellipticine and α-naphthoflavone, IC_{50} values were determined for each compound (fig. 4). Selectivity of inhibition was apparent for 7-ethoxycoumarin and α-naphthoflavone, but not for ellipticine and 7-ethoxyresorufin. IC_{50} values for α-naphthoflavone were an order of magnitude lower for expressed CYP1A2 and human liver microsomes (0.01–0.02 μM) compared to expressed CYP1A1 (0.15 μM). Similarly, IC_{50} values for 7-ethoxycoumarin were about 8-fold lower for expressed CYP1A2 and human liver microsomes (3 μM) compared to expressed CYP1A1 (25 μM). The IC_{50} values for 7-ethoxyresorufin and ellipticine inhibition of both isoforms was approximately 0.1 to 0.2 μM.

MAb 1-7-1 inhibited CYP1A1 catalyzed phenacetin dealkylation by approximately 80 to 90% at ascites protein to COS cell protein ratios of 0.2:1 or more (fig. 5). In contrast, MAb 1-7-1 was without effect on CYP1A2-mediated phenacetin O-deethylation and the high-affinity component of human liver microsomal phenacetin O-deethylase activity, even at an ascites protein to COS cell/liver microsomal protein ratio of 5:1 (fig. 5). To exclude the possibility that inhibition of phenacetin O-deethylase activity by MAb 1-7-1 was markedly substrate concentration dependent, the effect of the antibody at a single ascites protein to COS cell protein ratio (*viz.* 2.5:1) was also determined at saturating phenacetin concentrations (*i.e.*, 125 μM for CYP1A2 and 500 μM for CYP1A1). At these saturating concentrations, CYP1A2- and CYP1A1-mediated phenacetin O-deethylase activities were 86 and 11% of control, respectively; these values are similar to those observed (97 and 11% of control, respectively; fig. 5) when the substrate concentration approximated the apparent K_m. As was the case with phenacetin O-deethylation, MAb 1-7-1 at ascites protein to COS cell/liver microsomal protein ratios up to 5:1 caused <10% inhibition of

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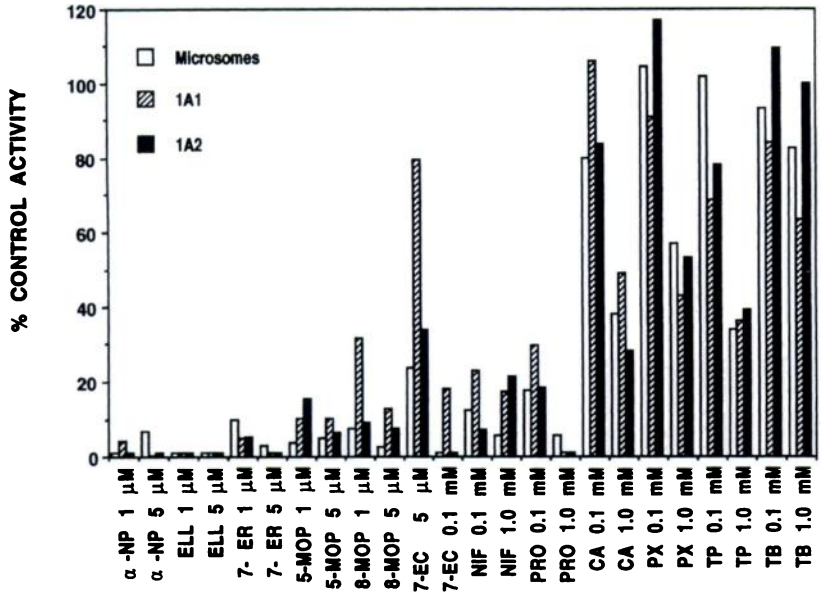

Fig. 3. Inhibitory effects of various xenobiotics on expressed CYP 1A1- and 1A2-mediated phenacetin O-deethylation and on the high-affinity component of human liver microsomal phenacetin O-deethylase activity. See under "Methods" for experimental details: Each bar represents the mean of duplicate measurements for expressed CYP 1A1 and 1A2 and the mean of triplicate estimations for human liver microsomes (livers H7, H10, H15). Concentrations refer to the final concentration of each xenobiotic inhibitor in the incubations. Abbreviations: αNP, α-naphthoflavone; ELL, ellipticine; 7-ER, 7-ethoxyresorufin; 5-MOP, 5-methoxypsoralen; 8-MOP, 8-methoxypsoralen; 7-EC, 7-ethoxycoumarin; NIF, nifedipine; PRO, propranolol; CA, caffeine; PX, paraxanthine; TP, theophylline; TB, theobromine.

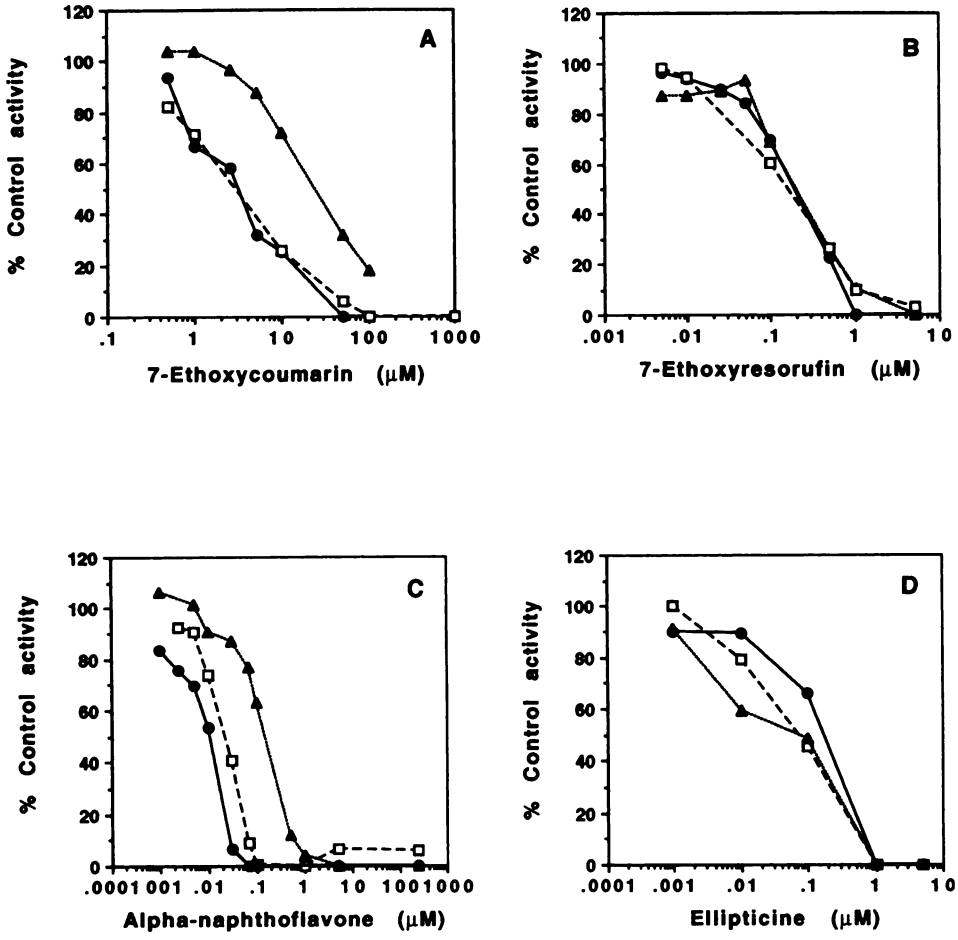

Fig. 4. Inhibitory effects of 7-ethoxycoumarin (A), 7-ethoxyresorufin (B), α-naphthoflavone (C) and ellipticine (D) on phenacetin O-deethylation by expressed CYP1A2 (●—●), expressed CYP1A1 (▲·····▲) and high-affinity human liver microsomal activity (□-□). Each point represents mean of duplicate measurements for expressed CYP 1A1 and 1A2 and mean of triplicate estimations for human liver microsomes (livers H7, H10, H15).

CYP1A2-catalyzed caffeine 3-demethylation and the high-affinity component of human liver microsomal caffeine 3-demethylase activity. (Limitations of assay sensitivity precluded accurate assessment of the effects of MAb 1-7-1 on CYP1A1-mediated caffeine 3-demethylation). The relative recognition of ^{35}S-labeled expressed CYP1A1 and 1A2 protein MAb 1-7-1, as determined by immunoadsorption and autoradiography, was 4:1 (fig. 6).

Further studies of the low-affinity component of human hepatic phenacetin O-deethylase activity were not undertaken because even at relatively high substrate concentrations (≥5 mM), the low- and high-affinity deethylases could not be adequately differentiated.

Discussion

Largely as a consequence of the proposed roles of CYP1A1 and CYP1A2 in chemical carcinogenesis and mutagenesis, there

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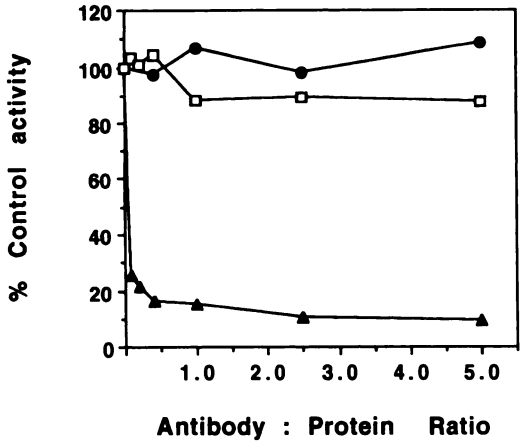

Fig. 5. Effects of MAb 1-7-1 on phenacetin O-deethylation by expressed CYP1A1 (▲—▲), expressed CYP1A2 (●—●) and high-affinity human liver (H10) microsomal activity (□—□). Each point represents mean of duplicate estimations. "Antibody" refers to ascites fluid protein.

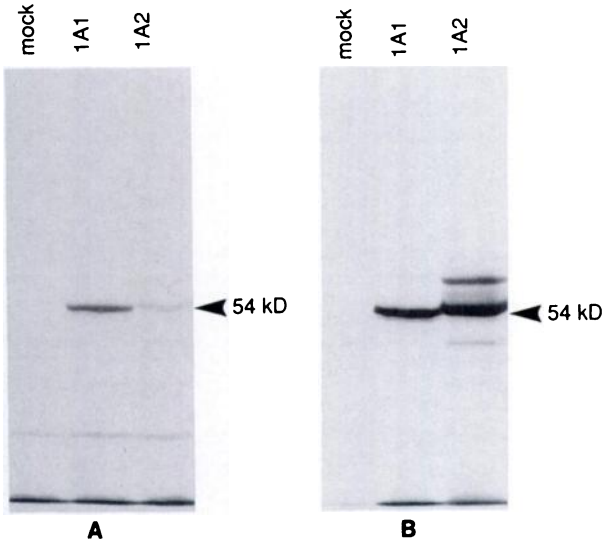

Fig. 6. Immunoadsorption of cDNA-expressed CYP 1A1 and 1A2 by MAb 1-7-1 (A) and a nonspecific anti-rabbit CYP1A antibody (see under "Methods") (B).

has been considerable interest in the development of probes for the investigation of the two enzyme activities in humans, with most attention focused on CYP1A2. Both caffeine and phenacetin have found widespread use as CYP1A2 probes, but in a recent study, we demonstrated that caffeine was a nonspecific 1A substrate (Tassaneeyakul *et al.*, 1992). With the use of expressed human CYP1A1 and CYP1A2, the present study has demonstrated conclusively that both enzymes also effectively catalyze the O-deethylation of phenacetin. Moreover, the prototypic xenobiotic substrate and inhibitor probes 7-ethoxycoumarin, 7-ethoxyresorufin, ellipticine and α-naphthoflavone were shown to inhibit both CYP1A1- and CYP1A2-catalyzed phenacetin O-deethylation, although selectivity was observed with 7-ethoxycoumarin and α-naphthoflavone. Other compounds known to inhibit CYP1A2-mediated hepatic drug metabolism in humans (5- and 8-methoxypsoralen, nifedipine and propranolol) similarly inhibited both CYP1A1- and CYP1A2-mediated phenacetin O-deethylation. In contrast, the anti-rat CYP1A monoclonal antibody MAb 1-7-1 was a specific inhibitor of human CYP1A1-catalyzed phenacetin dealkylation.

The apparent K_m for phenacetin O-deethylation by expressed CYP1A1 (108 μM) was 4.3-fold higher than that of expressed CYP1A2 (25 μM). The value of the apparent K_m for phenacetin O-deethylation by cDNA-expressed CYP1A2 is in the range of values reported here and elsewhere (*viz.* 6.3–36 μM) for the high-affinity component of human liver microsomal phenacetin O-deethylase activity (Boobis *et al.*, 1981; Distlerath *et al.*, 1985; Sesardic *et al.*, 1990d). An apparent K_m of 43 μM has been obtained for phenacetin O-deethylation by purified CYP1A2 (Distlerath *et al.*, 1985). Assuming essentially equivalent expression of catalytically active CYP 1A1 and 1A2 by COS cells (see under "Methods"), phenacetin intrinsic clearance (given by V_{max}/K_m) by CYP1A2 is about 5-fold higher than that by CYP1A1. Although this indicates that phenacetin is a better substrate for CYP1A2, both CYP1A isoforms will nevertheless contribute to phenacetin O-deethylation in tissues in which they are coordinately expressed. The CYP1A nonspecificity of phenacetin demonstrated here is supported by previous indirect studies which have shown phenacetin O-deethylation in rat and human tissues which differentially express the two isoforms (Sesardic *et al.*, 1988, 1990a,b).

The biphasic liver microsomal phenacetin O-deethylation kinetics observed in the present study is consistent with the results of previous rat and human *in vitro* kinetic studies (Boobis *et al.*, 1981; Sesardic *et al.*, 1990c), and the Michaelis-Menten parameters reported here are very close to those derived by Boobis *et al.* (1981). Apparent K_m values for phenacetin O-deethylation by expressed CYP1A2 and the high-affinity human liver enzyme were of a similar order, and inhibition profiles of these two enzyme activities by 7-ethoxycoumarin, 7-ethoxyresorufin, ellipticine and α-naphthoflavone were essentially superimposable. These observations are in agreement with the proposal (Sesardic *et al.*, 1988) that CYP1A2 is the enzyme responsible for the high-affinity component of human liver phenacetin O-deethylation and are also consistent with low expression of CYP1A1 in uninduced human liver.

α-Naphthoflavone and ellipticine are used widely as CYP1A inhibitor probes, whereas 7-ethoxycoumarin and 7-ethoxyresorufin are used commonly as substrate probes *in vitro*. Although it is now known that 7-ethoxycoumarin may also be metabolized by other non-CYP1A subfamily isoforms (Yun *et al.*, 1991), 7-ethoxyresorufin has been suggested to be a specific CYP1A1 substrate (Rodrigues and Prough, 1991). In the present study, α-naphthoflavone, 7-ethoxycoumarin, 7-ethoxyresorufin and ellipticine all inhibited CYP1A1- and CYP1A2-mediated phenacetin O-deethylase activity, although α-naphthoflavone and 7-ethoxycoumarin were more potent in their effects on CYP1A2. Thus, at least with phenacetin as substrate, an α-naphthoflavone concentration of 0.025 μM could differentiate the contributions of the separate human CYP1A isoforms to total metabolic activity. Although it is acknowledged that substrate specificity cannot always be inferred from inhibition data, recently published work supports the conclusion that 7-ethoxycoumarin and 7-ethoxyresorufin are non-specific CYP1A substrates. Independent studies have demonstrated that 7-ethoxyresorufin is metabolized by expressed human CYP1A1 (Eugster *et al.*, 1990), whereas 7-ethoxycoumarin and 7-ethoxyresorufin are deethylated by expressed human CYP1A2 (Fisher *et al.*, 1992). Furthermore, apparent K_m values for 7-ethoxyresorufin were similar for both enzymes, consistent with the lack of inhibitor selectivity observed here for this compound. Furafylline, a proposed specific CYP1A2 inhibitor (Sesardic *et al.*, 1990b,c), is not available generally for investigation.

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A number of drugs, including 5- and 8-methoxypsoralen, nifedipine and propranolol, are known to inhibit the human hepatic CYP1A2-catalyzed demethylation of caffeine *in vivo* and/or *in vitro* (see Tassaneeyakul *et al.*, 1992). As with the prototypic *in vitro* probes, all four compounds inhibited CYP1A1- and CYP1A2-mediated phenacetin O-deethylation. The relatively potent inhibition of CYP1A activities observed with nifedipine is interesting because this compound is metabolized largely by human CYP3A4 (Guengerich *et al.*, 1986; Gonzalez *et al.*, 1988). It is unknown whether the CYP1A inhibitory effects of nifedipine arise from metabolism along a minor pathway or from a noncatalytic interaction. Caffeine, a known CYP1A substrate, and the putative CYP1A2 substrates theophylline and paraxanthine also inhibited both CYP1A isoforms. The lesser inhibition observed with caffeine and theophylline is consistent with the relatively high apparent K_m values observed for these compounds (approximately 0.2–0.6 mM) in human liver microsomes (Robson *et al.*, 1987b; Tassaneeyakul *et al.*, 1992). Although induction of metabolism by cigarette smoking is suggestive of a role of CYP1A2 in the *in vivo* metabolism of the related methylxanthine theobromine (Miners *et al.*, 1985), this compound had little or no effect on phenacetin O-deethylation. This may reflect a lower affinity for CYP1A or the involvement of non-1A subfamily P450s in theobromine metabolism.

MAb 1-7-1 is a monoclonal antibody raised against a 3-methylcholanthrene-inducible rat liver P450. It has been used widely to probe the presence of CYP1A isoforms in both animals and humans (Friedman *et al.*, 1985). Immunoabsorption studies performed here demonstrated that MAb 1-7-1 recognized both expressed human CYP1A1 and 1A2 (in the ratio 4:1), but over the range of antibody concentrations investigated, inhibition was observed only for the CYP1A1-catalyzed O-deethylation of phenacetin. The antibody was without effect on CYP1A2-mediated (cDNA-expressed enzyme or human liver microsomes) phenacetin deethylation or caffeine 3-demethylation. Thus, at least with phenacetin as substrate, MAb 1-7-1 may differentiate CYP 1A1 and 1A2. Although consistent with the results of recent studies of CYP1A expression in human lung, liver and placenta (Pelkonen *et al.*, 1986; Wheeler *et al.*, 1991), the specific inhibition of CYP1A1-mediated phenacetin O-deethylation contrasts the effects of MAb 1-7-1 on human acetanilide hydroxylase activity reported by Liu *et al.* (1991). These authors demonstrated that MAb 1-7-1 inhibited acetanilide hydroxylation by expressed human CYP1A2 and by human liver microsomes. Antibody-protein concentrations required for inhibition were, however, higher than those necessary to inhibit the acetanilide hydroxylase activity of 3-methylcholanthrene-induced rat liver microsomes or expressed murine CYP1A2 (Liu *et al.*, 1991) and the expressed human CYP1A1 phenacetin O-deethylase activity of the present study. (It should be noted that purified MAb 1-7-1 immunoglobulin G was used by Liu *et al.*, whereas ascites protein was used in the present study. Assuming an immunoglobulin G yield from ascites protein of 10%, the normalized IC_{50} for MAb 1-7-1 inhibition of CYP1A1-catalyzed phenacetin O-deethylation determined here was approximately 30-fold lower than that reported by Liu *et al.* for inhibition of CYP1A2-catalyzed acetanilide hydroxylation.) Although MAb 1-7-1 recognizes an epitope common to human CYP 1A1 and 1A2 (fig. 6), it would appear that the antibody is a more potent inhibitor of CYP1A1-mediated reactions.

In summary, it has been demonstrated that both human CYP1A isoforms catalyze the O-deethylation of phenacetin, although the affinity of this compound for CYP1A2 is approximately 4-fold higher than for CYP1A1. Nonspecific inhibition was also apparent for all other putative CYP1A xenobiotic substrates and inhibitors investigated. Taken together with the results of other recent CYP1A substrate specificity studies with caffeine (Tassaneeyakul *et al.*, 1992), 2-acetylaminofluorene and food-derived heterocyclic amines (McManus *et al.*, 1990), it would appear that most, if not all, CYP1A xenobiotic substrates and inhibitors are nonspecific in their recognition of the two isoforms comprising this subfamily. Selectivity does, however, occur with some compounds, and it may be possible to differentiate CYP1A1 and CYP1A2 activities with the use of a selective inhibitor such as α-naphthoflavone. The monoclonal antibody MAb 1-7-1, although not completely specific for CYP1A1, may also potentially provide a means of differentiating the two human CYP1A isoform activities under appropriate experimental conditions. Results presented here emphasize the difficulties of interpreting enzyme activities based on the use of substrate probes in tissues which potentially express both CYP 1A1 and 1A2.

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