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ORIGINAL INVESTIGATION

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Phenacetin *O*-deethylation by human liver microsomes in vitro: inhibition by chemical probes, SSRI antidepressants, nefazodone and venlafaxine

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Abstract Biotransformation of phenacetin via *O*-deethylation to acetaminophen, an index reaction reflecting activity of Cytochrome P450-1A2, was studied in microsomal preparations from a series of human livers. Acetaminophen formation was consistent with a double Michaelis-Menten system, with low-K_m (mean K_{m1} = 68 μM) and high-K_m (mean K_{m2} = 7691 μM) components. The low-K_m enzyme accounted for an average of 96% of estimated intrinsic clearance, and was predicted to contribute more than 50% of net reaction velocity at phenacetin concentrations less than 2000 μM. Among index inhibitor probes, α-naphthoflavone was a highly potent inhibitor of the low-K_m enzyme (K_{i1} = 0.013 μM); furafylline also was a moderately active inhibitor (K_{i1} = 4.4 μM), but its inhibiting potency was increased by preincubation with microsomes. Ketoconazole was a relatively weak inhibitor (K_{i1} = 32 μM); quinidine and cimetidine showed minimal inhibiting activity. Among six selective serotonin reuptake inhibitor (SSRI) antidepressants, fluvoxamine was a potent inhibitor of 1A2 (mean K_{i1} = 0.24 μM). The other SSRIs were more than tenfold less potent. Mean K_{i1} values were: fluoxetine, 4.4 μM; norfluoxetine, 15.9 μM; sertraline, 8.8 μM; desmethylsertraline, 9.5 μM; paroxetine, 5.5 μM. The antidepressant nefazodone and four of its metabolites (*meta*-chloro-phenylpiperazine, two hydroxylated derivatives, and a triazoledione) were very weak inhibitors of P450-1A2. Venlafaxine and its *O*- and *N*-desmethyl metabolites showed minimal inhibitory activity.

Key words Phenacetin · In vitro metabolism · Cytochrome P450-1A2 · α-Naphthoflavone · Furafylline · Ketoconazole · Quinidine · Cimetidine · Fluoxetine · Norfluoxetine · Sertraline · Desmethylsertraline · Paroxetine · Fluvoxamine · Nefazodone · Venlafaxine

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Introduction

The Cytochrome P450-1A (CYP1A) subfamily has two isoforms, 1A1 and 1A2. Although many substrate and inhibitor probes are nonspecific in their recognition of the two isoforms (Tassaneeyakul et al. 1993), the relative tissue distributions of 1A1 and 1A2 differ significantly, with 1A2 being found in the liver and 1A1 expressed primarily at extrahepatic sites after induction (Wrighton et al. 1993; Gonzalez and Gelboin 1994).

P450-1A2 is a well-conserved, constitutively expressed protein. Levels of expression in human liver are highly variable and have been reported to follow a trimodal distribution (Sesardic et al. 1988; Schweikl et al. 1993; Kadlubar 1994). P450-1A2 is responsible for metabolic activation of arylamine carcinogens and heterocyclic arylamine mutagens (Gonzalez and Gelboin 1994), and contributes importantly to the metabolism of caffeine, theophylline, imipramine, tacrine, and clozapine (Lemoine et al. 1993; Ohmori et al. 1993; Bertilsson et al. 1994; Sarkar and Jackson 1994; Tassaneeyakul et al. 1994a; Pirmohamed et al. 1995; Spaldin et al. 1995; Tjia et al. 1996).

Phenacetin *O*-deethylation (Fig. 1) is a highly specific index reaction for 1A2 activity in in vitro systems derived from hepatic tissue (Distlerath et al. 1985; Tassaneeyakul et al. 1993). Previous work has documented its biphasic nature, with low-K_m and high-K_m components (often termed high- and low-affinity) (Boobis et al. 1981). Several potent in vitro inhibitors of the low-K_m component have been described, including α-naphthoflavone, furafylline and fluvoxamine (Murray and Reidy 1990; Brøsen et al. 1993b; Chang et al. 1994;

Halpert et al. 1994; Halpert 1995; Newton et al. 1995; Bourrié et al. 1996). The latter, a selective serotonin reuptake inhibitor (SSRI) antidepressant, is of clinical significance considering the widespread use of antidepressant medications and the potential for toxicity of 1A2 substrates in vivo (Brøsen 1995; Brøsen et al. 1993b).

Nefazodone is a recently approved antidepressant which acts by blocking 5-HT_2 receptors and inhibiting serotonin reuptake (Eison et al. 1990). Its chemical structure is similar to trazodone and etoperidone, and one metabolite of nefazodone, *meta*-chloro-phenylpiperazine (mCPP), is also a metabolite of trazodone, etoperidone, and mepiprazole (Mayol et al. 1994). We examined the ability of nefazodone and four major metabolites to inhibit phenacetin *O*-deethylation in human liver microsomal preparations. In addition to mCPP, the metabolites include two hydroxy derivatives with hydroxylations on aliphatic and ring positions, and a triazoledione. The recently introduced antidepressant venlafaxine acts by inhibition reuptake of norepinephrine and serotonin (Holliday and Benfield 1995). Venlafaxine and its *O*- and *N*-desmethyl metabolites were studied in the same system. Results were compared to the inhibitory capabilities of α-naphthoflavone, furafylline, ketoconazole, quinidine, and cimetidine. Also evaluated was the inhibitory activity of fluvoxamine compared to that of other SSRIs including fluoxetine, sertraline, paroxetine and the major metabolites of fluoxetine and sertraline (norfluoxetine and desmethylsertraline).

Materials and methods

In vitro incubation procedures

Liver samples from five human donors with no known liver disease were provided by the International Institute for the Advancement of Medicine, Exton, Pa. Microsomes were prepared by ultracentrifugation; microsomal pellets were suspended in 0.1 M potassium phosphate buffer containing 20% glycerol and stored at −80°C until use. Chemical reagents and drug entities were purchased from commercial sources or kindly provided by their pharmaceutical manufacturers.

Incubation mixtures contained 50 mM phosphate buffer, 5 mM Mg^{2+}, 0.5 mM $NADP^+$, and an isocitrate/isocitric dehydrogenase regenerating system (von Moltke et al. 1993, 1994a, b, 1995a, 1996a, b; Schmider et al. 1995, 1996a). Varying quantities of phenacetin in methanol solution, to yield final incubate concentrations ranging from 0 to 10 mM, were added to a series of incubation tubes. The solvent was evaporated to dryness at 40°C under conditions of mild vacuum. Solubility of phenacetin in microsome-free incubation mixtures at 37°C was verified by the linear relation of added phenacetin concentration and HPLC peak height, using the analytic procedure described below. In addition, data points from actual microsomal incubations were used in subsequent kinetic analyses only if the phenacetin/internal standard peak height ratio remained linearly related to added phenacetin concentration.

Incubations were also performed with co-addition of two concentrations each of a series of potential metabolic inhibitors, each at three different substrate concentrations. These inhibitors included compounds identified as "index" probes, the SSRI antidepressants and their metabolites, and the serotonin antagonist antidepressant nefazodone and its metabolites. The antidepressant venlafaxine, and its *O*- and *N*-desmethyl metabolites, were tested using fixed concentrations of phenacetin, since their inhibiting potency was very weak.

Reactions were initiated by addition of microsomal protein (approximately 0.25 mg/ml). After 20 min at 37°C, reactions were stopped by cooling on ice and addition of 100 µl acetonitrile. 2-Acetamidophenol was added as internal standard, the incubation mixture was centrifuged, and the supernatant transferred to an autosampling vial for HPLC analysis. The mobile phase consisted of 150 ml of acetonitrile and 850 ml of 50 mM phosphate buffer; the flow rate was 1.5 ml/min. The analytical column was stainless steel, 30 cm × 3.9 mm, containing reverse-phase C-18 micro Bondapak (Waters Associates, Milford, Mass.) Column effluent was monitored by ultraviolet absorbance at 254 nm (Fig. 2). Concentrations of acetaminophen in reaction mixtures were determined based on calibration curves constructed from a series of standards containing varying known amounts of acetaminophen together with internal standard. The rate of formation of acetaminophen was linear with respect to time and protein concentration. Reaction velocities were calculated in units of nmol product formed per minute per mg microsomal protein.

The complex concentration-dependence of α-naphthoflavone inhibition of phenacetin *O*-deethylation was evaluated using a fixed concentration of phenacetin, and concentrations of α-naphthoflavone ranging from 0 to 50 µM. This relationship was contrasted to the effects of α-naphthoflavone on alprazolam 4-hydroxylation, a reaction used as an index for activity of P450-3A isoforms (von Moltke et al. 1994b, 1995a, b; Schmider et al. 1996a).

All of the above studies were done without preincubation of inhibitors with microsomal protein. The effect of preincubation on the inhibitory activity of furafylline and α-naphthoflavone was studied by preincubation of varying concentrations of furafylline (up to 10 µM) or α-naphthoflavone (up to 1.0 µM) for 20 min with microsomal protein and cofactors. Reactions were initiated by addition of phenacetin to a final concentration of 100 µM. Reaction mixtures were then analyzed after 20 min of further incubation as described above. Preincubated samples were compared to an identical series without preincubation, in which reactions were initiated by addition of microsomal protein.

Data analysis

Reciprocal plots (Eadie-Hofstee) of reaction velocities in the absence of inhibitor were biphasic, consistent with a two-enzyme system. Accordingly, the following equation was fitted to the data

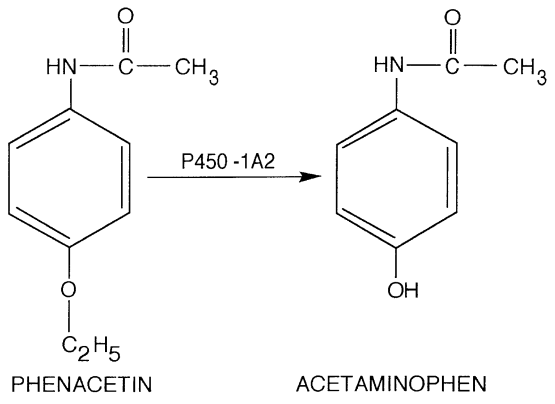

Fig. 1 Structural formula of phenacetin and its principal metabolite, acetaminophen, formed by *O*-deethylation mediated by cytochrome P450-1A2

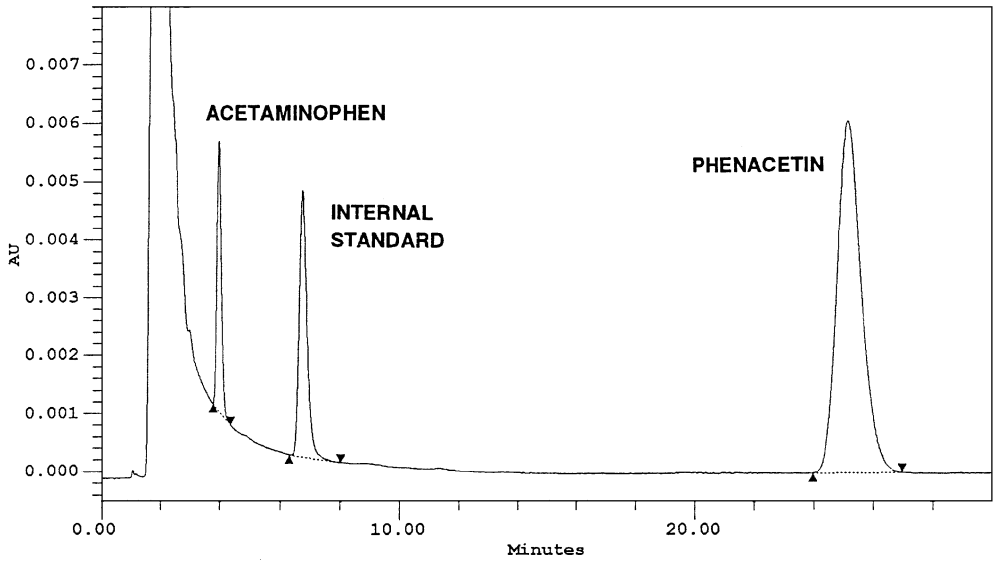

Fig. 2 HPLC tracing of an incubation mixture showing peaks corresponding to phenacetin (50 μM), acetaminophen, and the internal standard, 2-acetamidophenol

points using derivative free nonlinear least squares regression:

$$V = \frac{V_{max1} \cdot S}{S + K_{m1}} + \frac{V_{max2} \cdot S}{S + K_{m2}} \qquad \text{(Equation 1)}$$

where *V* is the reaction velocity corresponding to *S*, the concentration of substrate (phenacetin). Iterated variables were V_{max1} and V_{max2}, the maximum reaction velocities in the two Michaelis-Menten systems, and K_{m1} and K_{m2}, the substrate concentrations corresponding to 50% of the respective V_{max} values. The contribution of the two pathways to net estimated intrinsic clearance (Houston 1994) was determined from the relative magnitude of V_{max1}/K_{m1} and V_{max2}/K_{m2}.

The quantitative inhibiting potency of each inhibitor in each set of human microsomes was determined by nonlinear least squares regression using the following equation:

$$V = \frac{V_{max1} \cdot S}{S + K_{m1} \cdot \left(1 + \frac{I}{K_{i1}}\right)} + \frac{V_{max2} \cdot S}{S + K_{m2} \cdot \left(1 + \frac{I}{K_{i2}}\right)} \qquad \text{(Equation 2)}$$

I is the concentration of inhibitor, and *V* and *S* have the same meaning as in Equation 1. The Michaelis-Menten constants were previously determined from Equation 1 using data without inhibitor. Iterated variables were K_{i1} and K_{i2}, the inhibition constants based on the assumption of competitive inhibition.

Results

For all human liver samples Eadie-Hofstee plots were biphasic, and untransformed data were consistent with Equation 2 (Fig. 3). The mean K_m value for the low-K_m enzyme (K_{m1}) was 68 μM, while that for the high-K_m enzyme (K_{m2}) was 7691 μM (Table 1). The low-K_m enzyme accounted for an average of 96% of estimated intrinsic clearance, and would be predicted to contribute more than 50% of the net velocity of acetaminophen formation at phenacetin concentrations less than 2000 μM (Fig. 4).

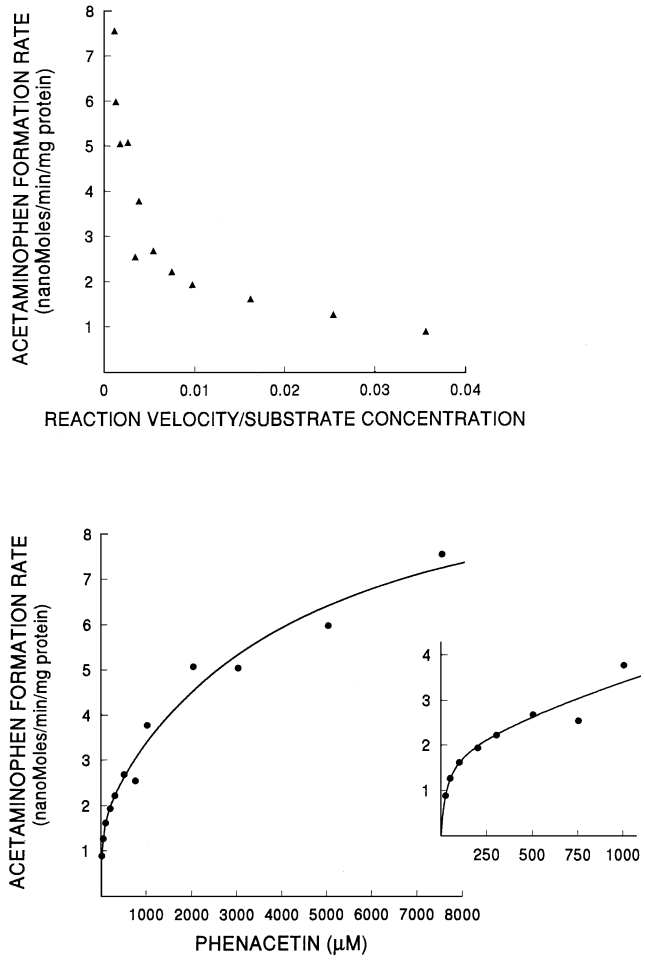

Fig. 3 *Above*: Eadie-Hofstee plot of reaction velocity (y-axis) vs. reaction velocity/substrate concentration ratio (x-axis) for a human liver sample. *Below*: reaction velocity (y-axis) versus substrate concentration (x-axis) for the same human liver sample. *Solid line* was determined by nonlinear least squares regression analysis based on Equation 2. *Insert, lower right*: substrate concentration range of 0–1000 μM shown on an expanded scale

Table 1 Characteristics of acetaminophen formation from phenacetin by human liver microsomes in vitro (n = 5)

	Mean	± SE	Range
Low-K_m enzyme			
V_{max1}[a]	1.62	± 0.32	0.76–2.60
K_{m1}[b]	68.4	± 22.3	35–152
V_{max1}/K_{m1} ratio, × 1000	34.5	± 9.7	5.0–60.6
Percent of total estimated intrinsic clearance	96%	± 1.2%	92–99%
High-K_m enzyme			
V_{max2}[a]	6.35	± 1.60	2.38–9.92
K_{m2}[b]	7691	± 1993	2254–13738
V_{max2}/K_{m2} ratio, × 1000	0.89	± 0.15	0.45–1.33
Percent of total estimated intrinsic clearance	3.8%	± 1.2%	1.4–8.3%

[a] Units of nanoMoles/min per mg protein
[b] Units of μM
[c] Total estimated intrinsic clearance calculated as $V_{max1}/K_{m1} + V_{max2}/K_{m2}$

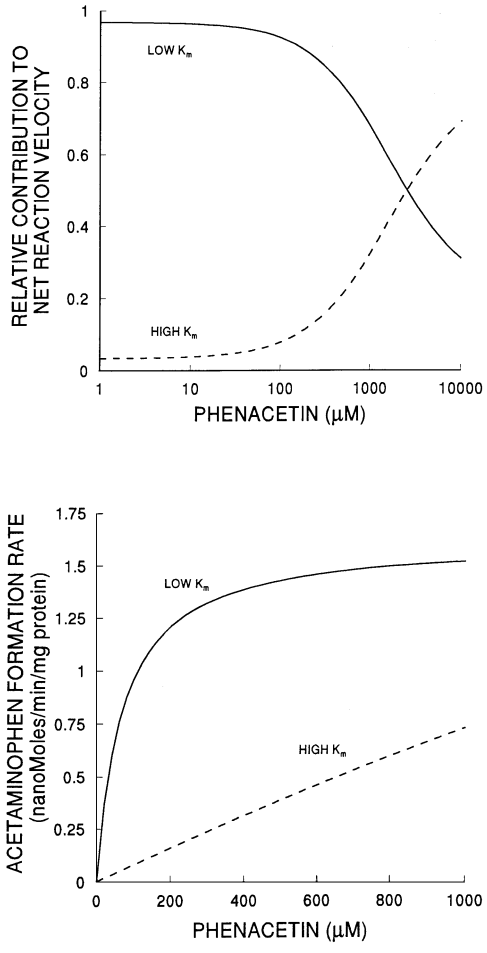

Fig. 4 Predicted contribution of high- and low-affinity enzymes to net velocity of acetaminophen formation, in relation to concentration of the substrate, phenacetin. Predictions are passed on mean values of kinetic parameters shown in Table 1. *Above*: relative contributions of the two sites. Note that the high-affinity site predominates at phenacetin concentrations under 2000 μM. *Below*: absolute contributions of the two sites at substrate concentrations less than 1000 μM

Those compounds that inhibited acetaminophen formation in general were more potent inhibitors of the low-K_m component ($K_{i1} < K_{i2}$). Since the low-K_m enzyme is of greatest quantitative importance, comparisons of inhibiting potency focused on K_{i1} (Table 2). α-Naphthoflavone was the most potent of all inhibitors tested. At low concentrations (< 1 μM), inhibition was consistent with a competitive mechanism (Figs. 5 and 6); however quantitative inhibition of acetaminophen formation was diminished at higher

Table 2 Competitive inhibition of low-K_m (apparent high-affinity) enzyme (K_{i1}) for phenacetin *O*-deethylation in human liver microsomes by a series of compounds

Compound	Mean (± SE) competitive inhibition constant (μM)
Index inhibitors	
a-Naphthoflavone	0.013 (± 0.001)
Furafylline	4.7 (± 1.6)
Ketoconazole	32 (± 17)
Quinidine	> 80
Cimetidine	> 60
SSRI antidepressants	
Fluoxetine	4.4 (± 0.7)
Norfluoxetine	15.9 (± 3.9)
Sertraline	8.8 (± 2.2)
Desmethylsertraline	9.5 (± 1.1)
Paroxetine	5.5 (± 1.6)
Fluvoxamine	0.24 (± 0.18)
Nefazodone and metabolites	
Nefazodone	65 (± 17)
OH-nefazodone(aliphatic)	66 (± 45)
p-OH-nefazodone (aromatic)	> 70
meta-Chlorophenylpiperazine	70 (± 26)
Triazoledione	> 70

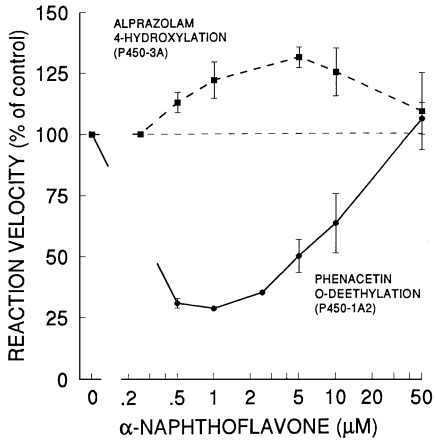

Fig. 5 Effect of varying concentrations of α-naphthoflavone (x-axis) on rate of formation of acetaminophen from phenacetin (200 μM), mediated by P450-1A2, and on formation of 4-hydroxy-alprazolam from alprazolam (100 μM), mediated by P450-3A isoforms. Each point (mean ± SE, n = 3) represents the ratio, expressed in percent, of reaction velocity at the indicated concentration of α-naphthoflavone divided by the reaction velocity with no inhibitor

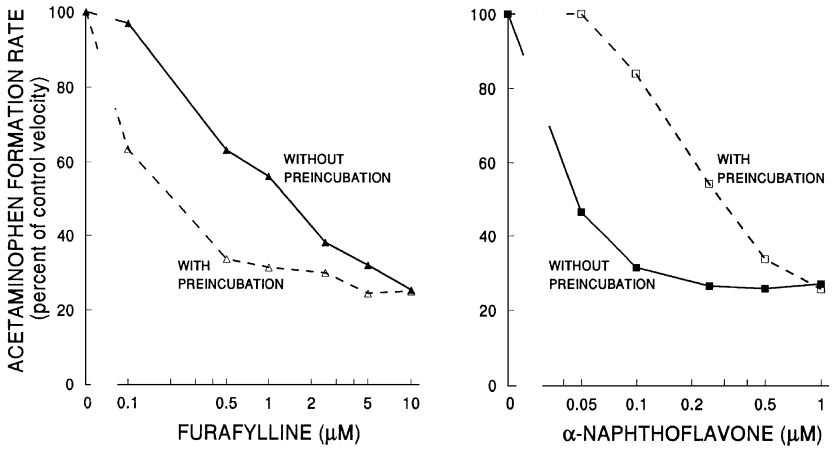

Fig. 6 Effect of a 20-min preincubation period on inhibition of acetaminophen formation from phenacetin (100 μM) in a representative liver sample by furafylline (*left*) or α-naphthoflavone (*right*). Each point represents the ratio, expressed in percent, of reaction velocity at the indicated concentration of inhibitor divided by the reaction velocity with no inhibitor present. The inhibiting potency of furafylline was enhanced by preincubation, while that of α-naphthoflavone was diminished by preincubation

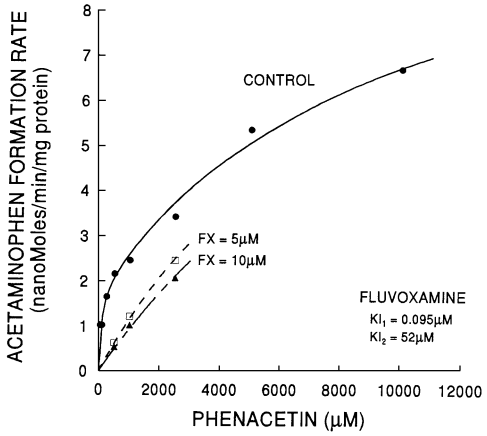

Fig. 7 Rate of formation of acetaminophen in relation to concentration of the substrate, phenacetin, in a representative human liver. Reaction velocities are shown in the control condition, with no inhibitor present, and with coaddition of 5 μM or 10 μM fluvoxamine (*FX*). *Solid line* (control, without inhibitor) represents fitted function based on Equation 1. *Dashed lines*, and K_i values for fluvoxamine, were based on Equation 2

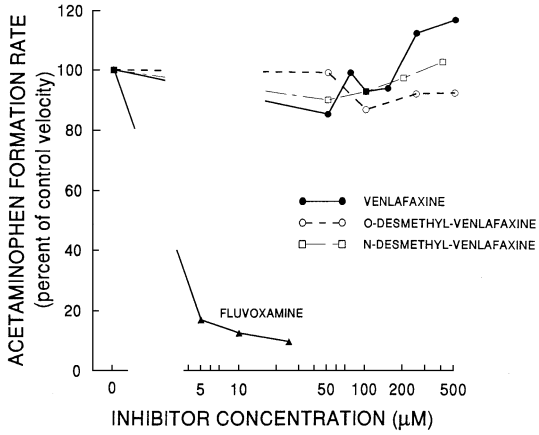

Fig. 8 Effect of varying concentrations of venlafaxine or its two metabolites (*O*- or *N*-desmethyl-venlafaxine) on formation of acetaminophen from phenacetin (100 μM) in a representative liver sample. Each point is the ratio, expressed in percent, of the reaction velocity at the indicated inhibitor concentration divided by the velocity with no inhibitor present. Shown for comparison purposes is the effect of fluvoxamine as an inhibitor

concentrations, and reaction velocities were indistinguishable from control at 50 μM (Fig. 5). At concentrations of 0.5–10 μM, α-naphthoflavone enhanced activity of P450-3A, based on formation of 4-OH alprazolam from alprazolam (Fig. 5).

Although considerably less potent than α-naphthoflavone, furafylline was a moderately potent inhibitor of acetaminophen formation (Table 2). Its inhibiting potency was increased by preincubation, while that of α-naphthoflavone was diminished by preincubation (Fig. 6). Ketoconazole also was an inhibitor of acetaminophen formation, although relatively weak. Quinidine and cimetidine showed minimal inhibiting activity.

All SSRIs were inhibitors of acetaminophen formation (Fig. 7). The potency of fluvoxamine (mean K_{i1} = 0.24 μM) was more than 10-fold greater than any of the other SSRIs (K_{i1} range: 4.4–15.9 μM). Nefazodone and its principal metabolites all were weaker inhibitors of acetaminophen formation than any of the SSRIs. Venlafaxine and its *O*- and *N*-desmethyl metabolites showed no detectable inhibition even at concentrations as high as 400–500 μM (Fig. 8).

Discussion

The kinetics of phenacetin *O*-deethylation to form acetaminophen, a reaction mediated by Cytochrome P450-1A2, was studied in microsomal preparations from a series of human liver samples. This biotransformation is one of several index reactions commonly used as probes to study characteristics of Cytochrome P450-1A2 activity in humans. Other index reactions include: caffeine *N*-3 demethylation, ethoxyresorufin

O-deethylation, tacrine hydroxylation, and theophylline oxidation. As described previously (Boobis et al. 1981; Distlerath et al. 1985; Sesardic et al. 1990; Tassaneeyakul et al. 1993; Schmider et al. 1996a), the profile of phenacetin *O*-deethylation was consistent with a two-enzyme Michaelis-Menten system, with low-K_m (apparent high-affinity) and high-K_m (apparent low-affinity) sites. Although the high-affinity enzyme had somewhat lower capacity ($V_{max1} < V_{max2}$), its estimated intrinsic clearance accounted for more than 90% of total intrinsic clearance, based on V_{max}/K_m ratios for the two enzymes. The high-K_m site was predicted to account for only a small fraction of net reaction velocity except at very high substrate concentrations. Thus, metabolic activity of P450-1A2 in vivo, including properties such as susceptibility to induction and inhibition, are likely to reflect mainly the low-K_m enzyme component. Our K_m values for the low-K_m site in human liver microsomes are in a range similar to that reported in previous studies of human microsomes (Distlerath et al. 1985; Sesardic et al. 1990; Brøsen et al. 1993b; Bourrié et al. 1996) as well as transfected cell lines expressing human P450-1A2 (Jensen et al. 1995). However other reports have suggested somewhat lower K_m values for this site (Boobis et al. 1981; Tassaneeyakul et al. 1993).

α-Naphthoflavone is confirmed as being a highly potent inhibitor of phenacetin *O*-deethylation (Boobis et al. 1981; Tassaneeyakul et al. 1992, 1993; Chang et al. 1994; Bourrié et al. 1996) as well as other reactions mediated by cytochrome P450-1A2 (Boobis et al. 1981; Tassaneeyakul et al. 1992; Chang et al. 1994; Ono et al. 1995; Siess et al. 1995; Tjia et al. 1996). The potency and relative specificity of α-naphthoflavone as an inhibitor of human P450-1A2 may allow this compound to be used as an inexpensive and easily obtained inhibitory chemical probe to identify the potential role of P450-1A2 in specific metabolic reactions in vitro. However, several properties of this compound must be considered. The present study and other reports (Boobis et al. 1981; Tassaneeyakul et al. 1992; Tjia et al. 1996) suggest that inhibition of P450-1A2 by α-naphthoflavone increased with concentration only in a low concentration range; at higher concentrations the inhibitory effect diminishes. The mechanism of this complex relationship is not established. α-Naphthoflavone itself is both a substrate and an activator of P450-3A isoforms (Schwab et al. 1988; Schou et al. 1994). We demonstrated significant enhancement of P450-3A activity, based on velocity of alprazolam 4-hydroxylation, at concentrations of α-naphthoflavone in the range of 1–10 μM. Other studies have shown even greater enhancement of P450-3A activity by α-naphthoflavone (Schwab et al. 1988; Fleming et al. 1992; Andersson et al. 1993, 1994a,b; Patten et al. 1993; Shou et al. 1994). Metabolic degradation by P450-3A may explain why the inhibitory activity of α-naphthoflavone was diminished by preincubation.

Inhibition of P450-1A2 by furafylline in vitro has also been verified (Sesardic et al. 1990). Interaction of furafylline with P450-1A2 substrates has also been demonstrated in clinical studies (Tarrus et al. 1987). Even without preincubation, furafylline was a moderately potent inhibitor in vitro ($K_{i\,1}$ = 4.7 μM), but its inhibiting potency was increased considerably by preincubation with microsomes and cofactors. This is consistent with the identification of furafylline as a mechanism-based inhibitor (Kunze and Trager 1993; Newton et al. 1995; Clarke et al. 1994; Tassaneeyakul et al. 1994b; Bourrié et al. 1996). Thus furafylline is another candidate for a chemical inhibitor probe to identify the role of P450-1A2 in specific metabolic reactions. However, the potential value of furafylline needs to be weighed against the preincubation requirement, and the high cost of this chemical as available from commercial sources.

Ketoconazole was a weak inhibitor of phenacetin *O*-deethylation in vitro. Ketoconazole is well established as a high-affinity inhibitory probe against P450-3A isoforms in vitro, making it a relatively specific inhibitor at low concentrations (Murray and Reidy 1990; Halpert et al. 1994; Baldwin et al. 1995; Halpert 1995; Newton et al. 1995; von Moltke et al. 1995b). The mechanism of ketoconazole inhibition of 3A isoforms appears to be competitive, although this is not unequivocally established (Wrighton and Ring 1994; Bourrié et al. 1996). In any case, inhibitory K_i values for ketoconazole against 3A-mediated reactions generally fall in the nanomolar range (Schmider et al. 1995; von Moltke et al. 1994b, 1995a, 1996a,b; Bourrié et al. 1996). However, the present study and other reports (von Moltke et al. 1994a, 1995b; Newton et al. 1995; Bourrié et al. 1996) emphasize that ketoconazole inhibition of cytochrome activity becomes less selective at higher concentrations. The K_i for ketoconazole against desipramine hydroxylation, a reaction mediated by P450-2D6, averaged 10.3 μM (von Moltke et al. 1994a), and the mean K_{i1} against phenacetin *O*-deethylation was 32 μM. Quinidine, a relatively selective inhibitor probe for Cytochrome P450-2D6 (Newton et al. 1995; Bourrié et al. 1996), was a very weak inhibitor of P450-1A2 in the present study. Cimetidine also was a weak inhibitor of 1A2 activity in vitro, although cimetidine may significantly inhibit clearance of 1A2 substrates, such as caffeine and theophylline, in clinical studies (Gerber et al. 1985; Tröger and Meyer 1995). In vitro inhibiting activity of cimetidine probably would have been greater if the study design had included preincubation with microsomes (Chang et al. 1992; Halpert 1995).

The introduction of the SSRI antidepressants into clinical practice a decade ago was followed by recognition that this class of compounds had the additional property of being inhibitors of human cytochromes P450 (Ciraulo and Shader 1990; von Moltke et al. 1994c). Numerous pharmacokinetic drug interactions with SSRI antidepressants have been reported in

controlled studies (Lemberger et al. 1988; Lasher et al. 1991; Bergstrom et al. 1992; Greenblatt et al. 1992; Brøsen et al. 1993a; Spina et al. 1993; Daniel et al. 1994; Fleishaker and Hulst 1994; Perucca et al. 1994; Preskorn et al. 1994; El-Yazigi et al. 1995). However, the available SSRIs are not equally active inhibitors of any specific cytochrome, nor are the various human cytochromes equally susceptible to inhibition by any specific SSRI. In vitro studies have provided considerable information on this topic (Brøsen and Skjelbo 1991; Crewe et al. 1992; Skjelbo and Brøsen 1992; Brøsen et al. 1993b; Otton et al. 1993, 1996; von Moltke et al. 1994a,b,1995a, 1996a,b; Jensen et al. 1995; Rasmussen et al. 1995; Ring et al. 1995; Schmider et al. 1995). Extrapolation of in vitro data to clinical circumstances of drug coadministration must be done with caution. In vivo inhibition of cytochrome activity depends not only on inhibitory K_i values, but also on the dose and plasma concentration of the inhibitor and its metabolites, partitioning of the inhibitors from plasma to the site of metabolic inhibition, and the pharmacokinetic characteristics of the drug whose metabolism is inhibited (von Moltke et al. 1995b). Nonetheless, in vitro results have largely been consistent with findings from clinical studies. The present in vitro study of human liver microsomes indicates that the four SSRIs available for clinical use in the United States (and the two quantitatively important endogenous metabolites) are inhibitors of human Cytochrome P450-1A2 activity. However, as reported previously (Brøsen et al. 1993b; Rasmussen et al. 1995), the inhibiting potency of fluvoxamine against human P450-1A2 activity exceeds that of the other five SSRIs by an average of at least tenfold. This suggests that fluvoxamine might potentially serve as an inhibitory probe for P450-1A2 activity. Although full reports of interaction studies of fluvoxamine with P450-1A2 substrates are not yet published, anecdotal reports and other data suggest that fluvoxamine may produce quantitatively large in vivo clearance inhibition of drugs such as theophylline, caffeine, and clozapine (Brøsen 1995). The relative inhibiting potency of the SSRIs against P450-1A2 contrasts sharply with effects on other cytochromes. Fluoxetine, norfluoxetine, and paroxetine are highly potent inhibitors of P450-2D6, whereas sertraline, desmethylsertraline, and fluvoxamine are much weaker (Crewe et al. 1992; Otton et al. 1993; von Moltke et al. 1994a, 1995a). For P450-3A isoforms, fluoxetine itself is consistently a weak inhibitor, whereas norfluoxentine has greater inhibiting potency (von Moltke et al. 1994b, 1995a, 1996a,b; Rasmussen et al. 1995; Ring et al. 1995; Schmider et al. 1995).

The serotonin antagonist antidepressant nefazodone is biotransformed in humans to a sequence of metabolites (Schmider et al. 1996b), via reactions mediated principally by P450-3A isoforms (von Moltke et al. 1996c). The triazoledione and the aliphatic hydroxy metabolite are of greatest quantitative importance in human plasma; smaller amounts of mCPP can be detected as well (Greene et al. 1995; van Laar et al. 1995). Nefazodone itself is a relatively potent inhibitor of human P450-3A isoforms in vitro (von Moltke et al. 1996b, c) and significantly impairs in vivo clearance of P450-3A substrates such as triazolam and alprazolam (Barbhaiya et al. 1995; Greene et al. 1995). Potent inhibition of P450-3A isoforms is shared by the two hydroxylated metabolites of nefazodone, but not by mCPP or the triazoledione (von Moltke et al. 1996c). However, nefazodone and all of its identified metabolites are very weak inhibitors of human cytochrome P450-2D6 (Schmider et al. 1996b). The present study indicates that nefazodone and its metabolites also are weak inhibitors of P450-1A2 activity, suggesting that clinically important interactions of nefazodone with P450-1A2 substrates are unlikely.

The mechanism of action of the antidepressant venlafaxine involves a combination of norepinephrine and serotonin reuptake inhibition (Holliday and Benfield 1995). The principal human metabolite of venlafaxine is *O*-desmethylvenlafaxine, formed via P450-2D6 dependent biotransformation (Fogelman et al. 1995; Otten et al. 1996). A second product, *N*-desmethyl-venlafaxine, is dependent at least in part on P450-3A isoforms (Fogelman et al. 1995; Otten et al. 1996). Venlafaxine and its *O*- and *N*-desmethyl metabolites are extremely weak inhibitors of P450-1A2 activity in vitro. Venlafaxine and metabolites also are weak inhibitors of P450-3A isoforms (von Moltke et al., unpublished data) as well as of P450-2D6 (Otten et al. 1996). Thus currently available data suggest that pharmacokinetic drug interactions of venlafaxine with substrates of these cytochromes are unlikely.

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