CYP2B6 MEDIATES THE IN VITRO HYDROXYLATION OF BUPROPION: POTENTIAL DRUG INTERACTIONS WITH OTHER ANTIDEPRESSANTS

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ABSTRACT:
The in vitro biotransformation of bupropion to hydroxybupropion was studied in human liver microsomes and microsomes containing heterologously expressed human cytochromes P450 (CYP). The mean (±S.E.) Kₘ in four human liver microsomes was 89 (±14) μM. In microsomes containing cDNA-expressed CYPs, hydroxybupropion formation was mediated only by CYP2B6 at 50 μM bupropion (Kₘ, 85 μM). A CYP2B6 inhibitory antibody produced more than 95% inhibition of bupropion hydroxylation in four human livers. Bupropion hydroxylation activity at 250 μM was highly correlated with S-mephentoin N-demethylation activity (yielding nivadol), another CYP2B6-mediated reaction, in a panel of 32 human livers (r = 0.94). The CYP2B6 content of 12 human livers highly correlated with bupropion hydroxylation activity (r = 0.96). Thus bupropion hydroxylation is mediated almost exclusively by CYP2B6 and can serve as an index reaction reflecting activity of this isozyme. IC₅₀ values for inhibition of a CYP2D6 index reaction (dextromethorphan O-demethylation) by bupropion and hydroxybupropion were 58 and 74 μM, respectively. This suggests a low inhibitory potency versus CYP2D6, the clinical importance of which is not established. Since bupropion is frequently coadministered with other antidepressants, IC₅₀ values (μM) for inhibition of bupropion hydroxylation were determined as follows: paroxetine (1.6), fluvoxamine (6.1), sertraline (3.2), desmethylsertraline (19.9), fluoxetine (59.5), norfluoxetine (4.2), and nefazodone (25.4). Bupropion hydroxylation was only weakly inhibited by venlafaxine, O-desmethylvenlafaxine, citalopram, and desmethylcitalopram. The inhibition of bupropion hydroxylation in vitro by a number of newer antidepressants suggests the potential for clinical drug interactions.

Bupropion hydrochloride is an antidepressant and a non-nicotine aid to smoking cessation that acts by weakly inhibiting the reuptake of dopamine and norepinephrine (Cooper et al., 1994). It is prescribed instead of other antidepressants to patients who have failed to respond to or have not tolerated other agents (Walker et al., 1993). There is evidence that bupropion and selective serotonin reuptake inhibitor (SSRI) combination therapy is more effective for treatment of refractory depression than the use of either agent alone (Bodkin et al., 1997; Nelson, 1998). In addition, bupropion may be used to treat attention-deficit/hyperactivity disorder when other agents are not effective (Cantwell, 1998).

Recently the FDA approved the use of sustained release bupropion (Zyban; Glaxo Wellcome, Research Triangle Park, NC) as an anti-smoking agent. In a clinical trial, bupropion was more effective than placebo in smoking cessation (Hurt et al., 1997). In another trial, there was a significant improvement in cessation of smoking with bupropion alone or bupropion combined with the nicotine patch compared with the nicotine patch alone or placebo (Jorenby et al., 1999). Since there are 50 million smokers in the United States and almost half are trying to quit each year, the use of bupropion as an anti-smoking agent is expected to increase (Jorenby et al., 1999).

Bupropion may induce seizures with high doses or in patients with a predisposition to seizures (Davidson, 1989). There is a 0.1% incidence of seizures with doses up to 300 mg per day of sustained release bupropion; this incidence increases to 0.4% with doses up to 450 mg per day of the immediate release formulation (Dunner et al., 1998). It has been hypothesized that the seizures may be due to high concentrations of bupropion or a metabolite (Preskorn, 1991).

In humans, bupropion is extensively metabolized to three principal metabolites (Fig. 1): hydroxybupropion (morphinol), erthyrohydrobupropion, and threo-hydrobupropion (Schroeder, 1983; Golden et al., 1988; Preskorn, 1991). The pharmacologically active metabolite hydroxybupropion appears to be the major metabolite, since plasma levels of hydroxybupropion greatly exceed those of the parent drug (Golden et al., 1988). Product labeling information and data reported in abstract form indicate that the cytochrome P450 (CYP) enzyme system, especially CYP2B6, has an important role in bupropion...
Bupropion inhibits CYP2D6.

The present study has examined the in vitro hydroxylation of bupropion by the CYP enzyme system. CYP2B6 is identified as having the major role in hydroxybupropion formation. In addition, we investigated the possibility of CYP2D6 inhibition by bupropion or hydroxybupropion. Finally, we studied the in vitro effects of several newer antidepressants on bupropion hydroxylation.

Experimental Procedures

Materials. Bupropion hydrochloride and hydroxybupropion were kindly provided by Glaxo-Wellcome (Research Triangle Park, NC), and trazodone was provided by Mead Johnson (Evansville, IN). S-Mephenytoin, nirvalan, inhibitory antibody to CYP2B6 (catalog no. A326), and the Western blotting inhibitory antibody to CYP2B6 (catalog no. A143) were purchased from Gentest Corp. (Woburn, MA). Chemical inhibitors, antidepressants and their metabolites, and other chemical reagents were provided by their manufacturers or purchased from commercial sources. NADP$^+$, isocitrate dehydrogenase, dl-isocitrate, and 50 mM potassium phosphate buffer (pH 7.5) were purchased from Sigma (St. Louis, MO).

Liver samples from donors with no known liver disease were obtained from either the National Disease Research Interchange (Philadelphia, PA) or the Liver Tissue Procurement and Distribution Service (Minneapolis, MN). The microsomes were prepared as previously described (von Moltke et al., 1993). Briefly, the tissue was partitioned and prepared by differential ultracentrifugation. Microsomal pellets were suspended in 0.1 M potassium phosphate buffer containing 20% glycerol and kept at $-70^\circ$C, an isocitrate/isocitric dehydrogenase regenerating system, and 5 mM MgCl$_2$. The samples were preincubated in a water bath at 37°C for 2 to 3 min. Reactions were initiated by the addition of microsomal protein, and the final volume was 0.25 ml. A protein concentration of 0.25 mg/ml was used for human liver microsomal incubations. Incubations were performed in a shaking water bath for 20 min at 37°C and terminated by addition of 50 l of 1 N HCl. Trazodone (10–25 l of 125 l M) was added as an internal standard. The mixture was vortex mixed and spun at 16,000g for 10 min. Supernatants were injected into the HPLC system for analysis.

Concentrations of hydroxybupropion were determined by HPLC using a method adapted from Cooper et al. (1984). A 300 $\times$ 3.9-mm Bondapak C$_{18}$ column (Waters Associates, Milford, MA) was used for separation with a flow rate of 2 ml/min and ultraviolet detection at 214 nm. The mobile phase consisted of 79% 50 mM KH$_2$PO$_4$ (adjusted to pH 3 with 1 N HCl) and 21% acetonitrile. Standard curves were prepared by adding known amounts of hydroxybupropion (50–2000 ng) to an incubation tube and evaporating off the solvent. The metabolite was reconstituted in 0.25 ml of incubation buffer. Fifty microliters of 1 N HCl and internal standard (trazodone) were added to the mix. Chromatograms were analyzed by measuring peak height using the internal standard method (Fig. 2). Formation of hydroxybupropion, the only metabolic product detected, was linear with respect to protein up to 0.3 mg/ml and with respect to time up to 25 min.

When the panel of 36 different human livers was screened for velocities of hydroxybupropion formation, incubations were performed as described above, with 250 l M bupropion (exceeding the reaction $K_m$) and 0.25 mg/ml microsomal protein for 20 min. In the chemical inhibitor screen, a specific concentration of inhibitor dissolved in methanol (omeprazole, CYP2C19 inhibitor (25 l M); quinidine, CYP2D6 inhibitor (5 l M); sulfaphenazole, CYP2C9 inhibitor (10 l M); ketocozol, CYP3A4 inhibitor (1.0 and 2.5 l M)) was added to bupropion (50 l M final concentration) in an incubation tube. The solvent was evaporated, and incubations were performed as described above. In the antidepressant inhibition study, varying concentrations ranging from 0 to 250 l M were added to incubation tubes with bupropion (final concentration 50 l M), and incubations were performed as described above.

To determine possible inhibition of CYP2D6 by bupropion or hydroxybupropion in human liver microsomes, the CYP2D6 index reaction of dextromethorphan was used (von Moltke et al., 1998). Varying concentrations of bupropion or hydroxybupropion in methanol were added to dextromethorphan (final concentration 25 l M) in methanol. The drug mix was dried in an incubation tube, and incubations were performed as described above. A protein concentration of 0.25 mg/ml was used. Samples were incubated for 20 min and reactions were stopped with 100 l M of acetonitrile. Pronethalol was used as the internal standard. Concentrations of dextromethorphan were determined by HPLC using previously described methodology (Schmider et al., 1996; von Moltke et al., 1998).

S-Mephenytoin was purified by HPLC as described by Ko et al. (1998). S-Mephenytoin N-demethylation activity, yielding nirvanol as the product, was determined by adapting an HPLC method previously described for measuring 4-hydroxy-S-mephenytoin (Schmider et al., 1996). Determination of nirvanol was performed using a 30-cm $\times$ 3.9-mm steel C$_{18}$ Bondapak column with ultraviolet detection at a wavelength of 204 nm. The flow rate was 1.5 ml/min, and the mobile phase consisted of 22% acetonitrile and 78% 50 mM potassium phosphate buffer (pH 6). The final concentration of S-mephenytoin was 250 l M with 0.5 mg/ml human microsomal protein. Samples were incubated for 120 min.

Incubations Using Microsomes Containing cDNA-Expressed CYPs. A screen of microsomes from human lymphoblastoid cells expressing CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, or transfected with vector, was performed using the incubation method described above. Bupropion concentrations were 50 and 500 l M, and cDNA-expressed microsomal protein concentration was 1 mg/ml. Hydroxybupropion formation rates were normalized to picomoles of CYP. The cDNA-expressed microsomal incubations were performed with little agitation and mild shaking, as recommended by the supplier. A kinetic curve was generated by incubating microsomes (1 mg/ml) containing CYP2B6 with varying concentrations of bupropion (0–1000 l M). CYP2B6 was also incubated with bupropion (50 l M) alone or with coaddition of ketocozol (2.5 l M).

Inhibition of Human Liver Microsomes with CYP2B6 Inhibitory Antibody. Inhibition studies with the CYP2B6 inhibitory antibody were performed by preincubating human liver microsomal protein (62–70 l g) with antibody (15–l g total volume) for 15 min on ice. To determine the optimal
amount of antibody to achieve maximal inhibition, the concentration of bupropion (100 μM) and the amount of protein (70 μg) were held constant while the amount of antibody was varied (0–20 μg). Twenty micrograms of CYP2B6 inhibitory antibody and a bupropion concentration of 50 μM were used in the subsequent CYP2B6 inhibitory antibody studies. The reaction was started with a 235-μl addition of a mixture of substrate, cofactor, buffer, and bupropion. The incubations were performed with minimal agitation for 20 min, and 50 μl 1 N HCl and internal standard (trazodone) were added. Samples were processed as before for HPLC analysis.

**Western Blotting.** Microsomal protein [10–50 μg of human liver microsomes, and 0.05–2.5 pmol of lymphoblast-expressed CYP2B6 (Gentest Corp.)] was denatured for 5 min in 100 mM Tris buffer containing 10% glycerol, 2% β-mercaptoethanol, 2% SDS, and 5 μg/ml pyronin Y (pH 6.8) at 100°C. Protein was separated by SDS-polyacrylamide gel electrophoresis in precast 7.5% polyacrylamide gels (Bio-Rad, Hercules, CA). Samples were run at 100 V for 1 h in 25 mM Tris buffer/0.192 M glycine/0.1% SDS buffer. Then samples were transferred to Immobilon-P (polyvinylidene difluoride membrane) (Millipore, Bedford, MA) for 1 h at 100 V in 25 mM Tris buffer/20% methanol. Blots were blocked with 0.5% powdered nonfat milk in TBS-Tween (0.15 M NaCl, 0.04 M Tris-HCl, pH 7.7, and 0.06% Tween 20) for 1 h at room temperature. Blots were then incubated with a 1:500 dilution of a polyclonal anti-peptide CYP2B6 antibody (Stresser and Kupfer, 1999) (Gentest Corp.) in TBS-Tween containing 0.1% BSA for 1 h at room temperature. Blots were rinsed with TBS-Tween, and the Super Signal Cl-HRP Substrate System (Pierce) was used for enhanced chemiluminescence detection. Blots were exposed to film (Fig. 6A). Quantitation of CYP2B6 content was completed via computer image analysis (NIH Image 1.62 software). A standard curve of pixel area × density versus pmol of CYP2B6 was created and fit to the equation $y = m \times \ln(x) + A$ using nonlinear least-squares regression (Fig. 6A).

**Data Analysis.** The formation of hydroxybupropion by human liver microsomes and cDNA-expressed CYP2B6 were consistent with a one-enzyme Michaelis-Menten model. Data points were fitted to this equation using nonlinear regression (Sigma Plot software; SPSS Inc., Chicago, IL), yielding values of $V_{\text{max}}$ and $K_{m}$.

In studies using a fixed concentration of substrate, $IC_{50}$ values for chemical inhibitors were determined by nonlinear regression analysis of data using the following equation (Venkatakrishnan et al., 1998):

$$R = 100 \left(1 - \frac{E_{\text{max}} C}{(IC_{50}^{\text{p}}} + C\right)$$

where $R$ is the percentage of the control (uninhibited) reaction velocity that is observed at an inhibitor concentration $C$; $E_{\text{max}}$ is a parameter that describes the extent of maximal inhibition. $A$ is an exponent reflecting the sigmoidicity of the equation. $IC_{50}$ is the apparent $IC_{50}$ (concentration of inhibitor at which $R$ equals 100 × [1 − $0.5E_{\text{max}}$]), from which is calculated the true $IC_{50}$ using the following equation (Venkatakrishnan et al., 1998):

$$IC_{50} = \frac{IC_{50}^{\text{p}}}{(2E_{\text{max}} - 1)^{\frac{1}{A}}}$$

**Results**

Estimated $V_{\text{max}}$ and $K_{m}$ values for hydroxybupropion formation by human liver microsomes (Fig. 3A) are shown in Table 1. Bupropion hydroxylation was reduced to 62% of control by 1.0 μM ketoconazole and 51% of control by 2.5 μM ketoconazole. Omeprazole, sulfaphenazole, and quinidine had minimal effect (Fig. 4A). Incubation of heterologously expressed CYP2B6 with 2.5 μM ketoconazole reduced reaction velocities to 68% of control at 50 μM bupropion. This indicates that the modest degree of inhibition by ketoconazole in liver microsomes is attributable to its effect on CYP2B6.

According to the supplier, the anti-CYP2B6 antibody does not inhibit human CYPs 1A1, 1A2, 1B1, 2A6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, or 3A. We determined the optimal amount of antibody to achieve maximal inhibition of CYP2B6 (Fig. 4B). The anti-CYP2B6 inhibitory antibody produced almost complete inhibition of hydroxybupropion formation (Fig. 4A).

Among cDNA-expressed Cyps, hydroxybupropion was formed only by CYP2B6 at 50 μM bupropion. At 500 μM bupropion, hydroxybupropion was formed by both CYP2B6 and CYP2E1, although the formation rate with CYP2B6 was nearly 80-fold greater than that with CYP2E1. CYP 1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4 or microsomes from vector-transfected cells showed no detectable activity with a limit of detection of 5 ng per sample. The formation of hydroxybupropion by heterologously expressed CYP2B6 was consistent with single-enzyme Michaelis-Menten kinetics (Fig. 3B; Table 1).

In a random sampling of 12 liver samples, CYP2B6 content ranged from 1.5 to 148.4 pmol of CYP2B6/mg of protein, with a median value of 44.6 pmol of CYP2B6/mg of protein (Fig. 5A). Velocities of bupropion hydroxylation in this subset were significantly correlated with immunochemically determined CYP2B6 content ($r = 0.96$) (Fig. 5B).
CYP2B6 mediates the in vitro hydroxylation of bupropion

Michaelis-Menten equation. See Table 1 for the kinetic parameters.

The velocity of formation of hydroxybupropion versus the concentration of bupropion (µM) for four different human livers (A) (velocity units = pmol of hydroxybupropion formed/min/mg of protein) and microsomes from lymphoblastoid cells expressing CYP2B6 (B) (velocity units = pmol of hydroxybupropion formed/min/pmol of CYP).

Symbols are experimental data points. Lines are fitted functions described by a Michaelis-Menten equation. See Table 1 for the kinetic parameters.

**TABLE 1**

The kinetics of bupropion hydroxylation in microsomes from four human livers and cDNA-expressed CYP2B6.

<table>
<thead>
<tr>
<th>Liver Sample</th>
<th>( V_{\text{max}} )^a</th>
<th>( K_m )^a</th>
<th>( V_{\text{max}}/K_m )^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM1</td>
<td>7675</td>
<td>127</td>
<td>60</td>
</tr>
<tr>
<td>HLM2</td>
<td>477</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>HLM3</td>
<td>2468</td>
<td>67</td>
<td>37</td>
</tr>
<tr>
<td>HLM4</td>
<td>3874</td>
<td>91</td>
<td>43</td>
</tr>
<tr>
<td>Mean^d</td>
<td>3623</td>
<td>89</td>
<td>37</td>
</tr>
<tr>
<td>S.E.^d</td>
<td>1520</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>7.4^f</td>
<td>85</td>
<td>86.3^e</td>
</tr>
</tbody>
</table>

\(^a\) \( V_{\text{max}} \) in units of picomoles per minute per milligram of protein.
\(^b\) \( K_m \) in units of micromolar.
\(^c\) \( V_{\text{max}}/K_m \) in intrinsic clearance, in units of microliters per minute per milligram of protein.
\(^d\) Mean = arithmetic mean of HLM1–4.
\(^e\) S.E. = standard error of HLM1–4.
\(^f\) \( V_{\text{max}} \) in units of picomoles per minute per pico mole of CYP.
\(^g\) \( V_{\text{max}}/K_m \) in intrinsic clearance, in units of nanoliters per minute per milligram of protein.

5B). Bupropion hydroxylation velocity among several different human livers samples (Fig. 6) was significantly correlated with velocities of N-demethylation of S-mephenytoin, a relatively specific CYP2B6-mediated reaction, in the same samples \( r = 0.94 \) (Fig. 7). With the high outlying value excluded, the correlation coefficient increases to 0.97.

Mean IC\(_{50}\) values for bupropion and hydroxybupropion versus dextromethorphan formation from dextromethorphan (25 µM) were 58 and 74 µM (Fig. 8; Table 2). Among antidepressants tested as potential inhibitors of bupropion hydroxylation, paroxetine was the most potent inhibitor (IC\(_{50}\) = 1.6 µM). Sertraline, norfluoxetine, and fluvoxamine also had significant inhibitory potency, while desmethylsertraline, fluoxetine, and nefazodone were less active as inhibitors (Fig. 9, A and B; Table 3). Other antidepressants tested at 100-µM concentrations were weak inhibitors. Bupropion hydroxylation velocity was reduced to 92 ± 2% of control by venlafaxine, 60 ± 5% of control by O-desmethylvenlafaxine, 81 ± 1% by citalopram, and 68 ± 1% by desmethylcitalopram.

Discussion

Bupropion hydroxylation among several different human livers samples was significantly correlated with velocities of N-demethylation of S-mephenytoin, a relatively specific CYP2B6-mediated reaction, in the same samples \( r = 0.94 \) (Fig. 7). With the high outlying value excluded, the correlation coefficient increases to 0.97.

Mean IC\(_{50}\) values for bupropion and hydroxybupropion versus dextromethorphan formation from dextromethorphan (25 µM) were 58 and 74 µM (Fig. 8; Table 2). Among antidepressants tested as potential inhibitors of bupropion hydroxylation, paroxetine was the most potent inhibitor (IC\(_{50}\) = 1.6 µM). Sertraline, norfluoxetine, and fluvoxamine also had significant inhibitory potency, while desmethylsertraline, fluoxetine, and nefazodone were less active as inhibitors (Fig. 9, A and B; Table 3). Other antidepressants tested at 100-µM concentrations were weak inhibitors. Bupropion hydroxylation velocity was reduced to 92 ± 2% of control by venlafaxine, 60 ± 5% of control by O-desmethylvenlafaxine, 81 ± 1% by citalopram, and 68 ± 1% by desmethylcitalopram.

CYP2B6 is the primary enzyme mediating the formation of hydroxybupropion from bupropion in human liver microsomes. CYP2E1 may make a very small contribution at high concentrations of bupropion, but this contribution is unlikely to be of clinical importance. A \( C_{\text{max}} \) of 0.6 µM was reported after a single 150-mg tablet of sustained-release bupropion hydrochloride (Hsyu et al., 1997), and at this concentration, CYP2B6 would be the dominant enzyme mediating hydroxylation. The mean \( K_m \) for hydroxybupropion formation in liver microsomes is 89 µM, which is close to the \( K_m \) for hydroxybupropion formation by cDNA-expressed CYP2B6 (85 µM). Bupropion hydroxylation and S-mephenytoin N-demethylation activities among individual liver samples were highly correlated, as were immunounquantified CYP2B6 in human livers and bupropion hydroxylation. Our results are consistent with results reported in abstract form, indicating that bupropion hydroxylation is a valid CYP2B6 probe (Lindley et al., 2000). These authors also found a high correlation between bupropion hydroxylation activity and CYP2B6 content \( r^2 = 0.99 \) and between bupropion hydroxylation and S-mephenytoin N-demethylation activities \( r^2 = 0.98 \).

The manufacturer of bupropion found cDNA-expressed CYP3A4 to form detectable levels of hydroxybupropion (Wurm et al., 1996). Since CYP3A4 is more abundant than CYP2B6 in human livers, a minor role of CYP3A4 in bupropion hydroxylation could be clinically significant. However, in agreement with our results, Fauci et al. (2000) found that CYP3A4 has no significant contribution to bupropion hydroxylation because of the poor correlation of hydroxylation activity with both immunounquantified CYP3A4 content and with testosterone 6β-hydroxylation activity.

Since the anti-CYP2B6 antibody inhibits bupropion hydroxylation almost completely at a substrate concentration less than the \( K_m \) use of this antibody probably represents the most valid and specific approach for studies requiring inhibition of CYP2B6 activity. Although orphenadrine has been proposed as a chemical inhibitor of CYP2B6, Guo et al. (1997) showed orphenadrine is nonspecific and also inhibits CYP2D6, CYP1A2, CYP2A6, CYP3A4, and CYP2C19 at high concentrations. Omeprazole, sulfaphenazole, and quinidine produced minimal inhibition of bupropion hydroxylation. Ketoconazole at 1.0 and 2.5 µM produced measurable inhibition of the reaction in both liver microsomes and heterologously expressed CYP2B6, confirming that ketoconazole is not fully specific for CYP3A.

There are many substrates biotransformed partially by CYP2B6 in vitro, but few relatively specific CYP2B6 substrates have been identified, since the role of this enzyme in drug metabolism is not fully characterized (Mimura et al., 1993; Ekins and Wrighton, 1999; Gervot et al., 1999; Hanna et al., 2000). Identified substrates include S-
mephobarbital (Kobayashi et al., 1999), S-mephenytoin (Ko et al., 1998), cyclophosphamide (Chang et al., 1993), and RP73401 (Stevens et al., 1997; Domanski et al., 1999). At the present time, it is not known whether CYP2B6 is involved in the biotransformation of these substrates in vivo. Heyn et al. (1996) identified N-demethylation of S-mephenytoin as a reasonable probe for CYP2B6 activity. Ko et al. (1998) showed this reaction also has a high-affinity/low-capacity component mediated by CYP2C9 and demonstrated it must be used at high concentrations to be used as a CYP2B6 probe. Recently, Kobayashi et al. (1999) showed that N-demethylation of S-mephobarbital is mediated mainly by CYP2B6, by using chemical inhibition and cDNA-expressed enzymes. They used 100 and 300 μM orphenadrine to inhibit CYP2B6 activity to 47 and 29% of control activity.

FIG. 4. A, the inhibition of bupropion hydroxylation activity in human liver microsomes from four different donors; B, percentage of control activity of bupropion hydroxylation versus amount of inhibitory antibody (μg) using 70 μg of human liver microsomal protein.

A, activity values from incubations with inhibitor were divided by activity values from control incubations (no inhibitor) and are represented as a percentage of control activity (mean ± S.E.). The bupropion concentration was 50 μM. [KET, ketoconazole, 1.0 μM (solid bar) and 2.5 μM (striped bar); QUIN, quinidine, 5 μM; OME, omeprazole, 25 μM; SPA, sulfaphenazole, 10 μM; and anti-2B6, inhibitory antibody to CYP2B6.] B, percentage of control activity of bupropion hydroxylation versus amount of inhibitory antibody (μg) using 70 μg of human liver microsomal protein. A bupropion concentration of 100 μM was used.

TABLE 2

IC₅₀ values for inhibition of CYP2D6 by bupropion and hydroxybupropion in four different human livers

<table>
<thead>
<tr>
<th>Liver Sample</th>
<th>IC₅₀ Bupropion</th>
<th>IC₅₀ Hydroxybupropion</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM1</td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>HLM2</td>
<td>80</td>
<td>94</td>
</tr>
<tr>
<td>HLM3</td>
<td>51</td>
<td>63</td>
</tr>
<tr>
<td>HLM4</td>
<td>52</td>
<td>72</td>
</tr>
<tr>
<td>Mean</td>
<td>58</td>
<td>74</td>
</tr>
<tr>
<td>S.E.</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

*IC₅₀ in units of micromolar.

FIG. 5. A, immunoquantification of CYP2B6 content (representative blots); B, correlation plot of the velocity of bupropion hydroxylation for 12 different human livers (250 μM bupropion) with the amount of CYP2B6 as determined by immunoquantification using the same 12 livers.

A, lanes 1 to 5 were loaded with 0.1, 0.25, 0.5, 1.0, and 2.5 pmol of CYP2B6, respectively. Lanes 6 to 9 were loaded with 10 μg of microsomal protein from four different human livers with relatively high bupropion hydroxylation activity. Lanes 10 to 19 were loaded with 2.5, 1.0, 0.5, 0.25, 0.1, and 0.05 pmol of CYP2B6, respectively. Lanes 10 to 13 were loaded with 50 μg of human microsomal protein from four different human livers with relatively low bupropion hydroxylation activity. The apparent altered mobility of CYP2B6 in microsomal samples in lanes 10 to 13 when compared with lanes 14 to 19 may be due to the larger amount of total protein loaded onto lanes 10 through 13. B, correlation plot of the velocity of bupropion hydroxylation for 12 different human livers (250 μM bupropion) with the amount of CYP2B6 as determined by immunoquantification using the same 12 livers.

FIG. 6. Rates of bupropion hydroxylation in vitro by human liver microsomes from 36 different donors at 250 μM bupropion.

Velocity units are picomoles of hydroxybupropion formed per minute per milligram of protein.
Earlier immunoquantification studies suggested that CYP2B6 comprised less than 1% of total hepatic P450, and CYP2B6 was concluded to be a minor CYP isoform (Shimada et al., 1994). Using more specific antibodies to CYP2B6, several research groups have immunoquantified CYP2B6 in panels of livers and observed highly variable expression levels. Code et al. (1997) detected CYP2B6 in 12 of 17 human livers, ranging from 0 to 74 pmol of CYP2B6/mg of protein. Ekins et al. (1998) detected 0.7 to 7.1 pmol of CYP2B6/mg of protein in a human liver panel. Stresser and Kupfer (1999) developed a polyclonal antibody that recognized 20 residues of human CYP2B6 and detected 2 to 82 pmol of CYP2B6/mg of protein in a human liver panel. Immunoquantification results from these groups suggest that CYP2B6 may not be a minor human hepatic enzyme. Highly variable expression levels suggest variability of bupropion metabolism. Since high concentrations of bupropion and its metabolites are associated with toxicity (Preskorn, 1991), a very low or very high amount of CYP2B6 may increase the risk of toxicity.

We used the same polyclonal antibody as did Stresser and Kupfer (1999) and have likewise found high variability of CYP2B6 content in 12 different human livers (2.5–148.4 pmol of CYP2B6/mg of protein). Previous results from a study of variability of propofol hydroxylation in our laboratory support data that CYP2B6 expression is highly variable (Court et al., 2000). CYP2B6 was found to have a significant role in propofol hydroxylation. Inhibition by anti-CYP2B6 antibody and good correlations between propofol hydroxylation and CYP2B6 marker activities indicate that CYP2B6 is responsible for the variability in propofol hydroxylation activity.

The interaction of other drugs with CYP2B6 has not been thoroughly investigated. Newer antidepressants, including SSRIs, may inhibit the activity of human cytochromes, but interactions with CYP2B6 are not established. We observed that several SSRIs (sertraline, paroxetine, norfluoxetine, and fluvoxamine) have low IC50 values for inhibition of bupropion metabolism (Table 3). Although the clinical significance of these IC50 values is not established, paroxetine appears to have the highest probability of interference with CYP2B6. Clinical monitoring during combined use of these SSRIs with bupropion is necessary, since elevated bupropion plasma levels may be associated with central nervous system toxicity.

Product labeling for bupropion indicates that CYP2D6 is inhibited by bupropion or hydroxybupropion. The present study indicates that bupropion and hydroxybupropion have relatively low inhibitory potential of CYP2D6 in vitro, with IC50 values of 58 and 74 μM, respectively. A case report has suggested the inhibition of CYP2D6 by bupropion in a patient whose desipramine levels were increased after combination therapy of imipramine and bupropion (Shad and Preskorn, 1997). Pollock et al. (1996) reported that debrisoquine metabolic ratios in three patients before and after at least 2 months of bupropion treatment did not change importantly. Furthermore, the plasma concentration/dose ratio for bupropion was not substantially different between CYP2D6 extensive and poor metabolizers. It was concluded that bupropion does not inhibit CYP2D6 in vivo and that bupropion itself is not likely to be a substrate for CYP2D6.

The present study has focused on the biotransformation of bupro-
pion to hydroxybupropion, but we did not detect formation of two other metabolites, threoxyhydrobupropion and erythrohydrobupropion. According to the manufacturer, threoxyhydrobupropion formation was detected in vitro using human liver microsomes, but no threoxyhydrobupropion was detected using individually expressed CYP isozymes (Wurm et al., 1996). Erythrohydrobupropion formation was not detected in vitro (Wurm et al., 1996), and we did not detect either of these metabolic products in the present study. Hydroxybupropion shows stronger anti-tetramine activity and has a lower LD50 value than the ethyro and three metabolites, suggesting that hydroxybupropion is the most important active metabolite in vivo (Scheroder, 1983). Since plasma hydroxybupropion levels are usually higher than bupropion, it has been suggested that this metabolite may be responsible for toxicity (Laizure et al., 1985). In vivo plasma levels of both erythrohydrobupropion and threoxyhydrobupropion also exceed those of bupropion itself. However, the mechanism of formation of these metabolites remains uncertain.

Since bupropion has been approved as an anti-smoking agent, its use is expected to increase. In addition, bupropion may be prescribed in place of other antidepressants since it has less likelihood of sexual side effects, and combination of bupropion with SSRIs is used to treat refractory depression. The risk of drug interactions or central nervous system toxicity associated with bupropion may be of clinical importance and may correlate with high bupropion or metabolite levels. Therefore, understanding the metabolism of bupropion and in vitro interactions with other xenobiotics may give insight into the risk of adverse effects.

### References


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**TABLE 3**

<table>
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<tr>
<th>Liver Sample</th>
<th>Sertraline</th>
<th>Desmethylsertraline</th>
<th>Fluoxetine</th>
<th>Norfluoxetine</th>
<th>Paroxetine</th>
<th>Nefazodone</th>
<th>Fluvoxamine</th>
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<td>HLM1</td>
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<td>20.8</td>
<td>90</td>
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<td>2.8</td>
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<td>0.1</td>
<td>0.1</td>
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</table>

**Notes:**

- $IC_{50}$ in units of micromolar.
- Mean = arithmetic mean.
- S.E. = standard error.