MULTIPLE CYTOCHROME P450 ENZYMES RESPONSIBLE FOR THE OXIDATIVE METABOLISM OF THE SUBSTITUTED (S)-3-PHENYLPIPERIDINE, (S,S)-3-[3-(METHYLSULFONYL)PHENYL]-1-PROPYLPIPERIDINE HYDROCHLORIDE, IN HUMAN LIVER MICROSOMES

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ABSTRACT:

(S,S)-3-[3-(Methylsulfonyl)phenyl]-1-propylpiperidine hydrochloride [(−)-OSU6162] is a weak dopamine D2 receptor modulator that possesses potential for the treatment of levodopa (L-DOPA)-induced dyskinesias in patients with Parkinson’s disease. In this report, incubations with human liver microsomes revealed that (−)-OSU6162 is selectively metabolized via N-dealkylation to yield N-depropyl (−)-OSU6162. Kinetics evidence is presented that the N-depropylation of (−)-OSU6162 in human hepatic microsomes is mediated by multiple cytochrome P450 (P450) enzymes, in particular CYP2D6. This hypothesis is borne out by several lines of in vitro evidence; 1) incubations of (−)-OSU6162 (5 μM) with hepatic microsomes from a panel of human donors showed that (−)-OSU6162 N-depropylase activity correlated well with CYP2D6-catalyzed dextromethorphan O-demethylase activity but not with other P450 enzyme-specific activities; 2) quinidine, a CYP2D6-specific inhibitor, inhibited (−)-OSU6162 N-depropylation, whereas other P450 enzyme-specific substrates/inhibitors did not significantly inhibit this activity; 3) CYP2D6 possessed highest intrinsic (−)-OSU6162 N-depropylase activity when compared with a battery of recombinant heterologously expressed human P450 enzymes. In addition, the selectivity of (−)-OSU6162 to inhibit six human P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP3A1, CYP2D6 and CYP3A4) was evaluated using an in vitro inhibition screen. Of the enzymes examined, only the activity of CYP2D6 was inhibited by coincubation with (−)-OSU6162. Thus, it is concluded that (−)-OSU6162 is metabolized by several P450 enzymes and that CYP2D6 accounts for the majority of the observed P450 N-depropylase activity in vitro.

1 Abbreviations used are: (−)-OSU6162, (S,S)-3-[3-(methylsulfonyl)phenyl]-1-propylpiperidine hydrochloride; L-DOPA, levodopa; P450, cytochrome P450; HPLC, high performance liquid chromatography; BROP, bropirimine; CPMB, 3-cyclopentyloxy-N-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide; COUM, coumarin; SULF, sulfaphenazole; NTRO, para-nitrophenol; QUIN, quinidine; KETO, ketoconazole; Meph, (S)-mephentoin; ACN, acetonitrile; LC/MS, liquid chromatography mass spectrometry; APCI, atmospheric pressure chemical ionization.

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HUMAN LIVER MICROSOMAL METABOLISM OF (−)-OSU6162

Analytical separation of (−)-OSU6162 and its metabolite was accomplished using the HPLC conditions described above. Under these conditions, authentic standards of (−)-OSU6162 and the corresponding N-despropyl derivative were characterized by retention time, molecular ion, and fragmentation pattern. The collision energy used was 1.5 and 1.6 V for (−)-OSU6162 and the metabolite, respectively.

Kinetic Analysis. Rates of formation of the N-despropyl metabolite of (−)-OSU6162 were determined in a panel of liver microsomes prepared from 12 different human organ donors and compared with the catalytic activities previously characterized for specific P450 substrates (Wienkers et al., 1996). Incubations and sample work-up were carried out as described above. Coefficient of determination ($r^2$) for enzyme activities was determined by linear regression analysis using the graphical/statistical program Prism 2.01 (GraphPad Software Inc., San Diego, CA).

Chemical Inhibition Experiments. (−)-OSU6162 was incubated at a single concentration in pooled human liver microsomes in the presence of a panel of compounds, which interacted selectively with various cytochrome P450 enzymes. The following P450 enzyme substrates/inhibitors were examined for their ability to inhibit the microsomal metabolism of (−)-OSU6162: bropanine/CYP1A2, BROB (200 μM); coumarin/CYP2A6, COUM (20 μM); 3-cyclopropylxoxy-N-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide/CYP2B6, CPMB (20 μM); sulfaphenazole/CYP2C9, SULF (5 μM); (S)-mephenytoin/CYP2C19, MEPH (200 μM); quinidine/CYP2D6, QUIN (5 μM); para-nitrophenol/CYP2E1, NTRO (50 μM); ketoconazole/CYP3A4, KETO (5 nM). The inhibitors were dissolved in ACN and were added to the incubations such that the final amount of solvent was 1%. Control incubations (minus inhibitor) also contained 1% ACN.

LC/APCI/MS and Metabolite Confirmation. The identity of the primary in vitro (−)-OSU6162 metabolite was confirmed using a Finnigan LCQ ion-trap (Thermo Finnigan MAT, San Jose, CA) operated in positive-ion atmospheric pressure chemical ionization APCI mode. The APCI vaporizer temperature was 450°C, and the discharge current and spray voltage were set at 5 μA and 4.5 kV, respectively. Nitrogen (99.9% pure; AGA Gas Inc., Maumee, OH) was employed as a drying gas at a sheath pressure of 80 psi and auxiliary flow rate of 20 ml/min, and the heated capillary was set at 250°C. Analytical separation of (−)-OSU6162 and its metabolite was accomplished using the HPLC conditions described above. Under these conditions, authentic standards of (−)-OSU6162 and the corresponding N-despropyl derivative were characterized by retention time, molecular ion, and fragmentation pattern. The collision energy used was 1.5 and 1.6 V for (−)-OSU6162 and the metabolite, respectively.

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The involvement of CYP2D6 in the in vitro metabolism of (−)-OSU6162 involves a transformation of (−)-OSU6162 (e.g., 5 μM) to N-despropyl(−)-OSU6162 formation correlated with CYP2D6-catalyzed dextromethorphan-O-demethylase activity ($r^2 = 0.908$). However, as depicted in Fig. 3B, at relatively high concentrations of (−)-OSU6162 (e.g., 200 μM) N-despropylase activity did not correlate with CYP2D6 activity ($r^2 = 0.201$). Moreover, the correlation of N-despropyl (−)-OSU6162 formation tested against activities selective for other P450 forms (e.g., 7-ethoxyresorufin O-dealkylation, CYP1A2; tolbutamide hydroxylation, CYP2C9; (S)-mephentoin 4'-hydroxylation, CYP2C19; chlorzoxazone 6-hydroxylation, CYP2E1; and testosterone 6β-hydroxylation, CYP3A4) was not significant at either concentration of (−)-OSU6162 tested (Table 1).

**Chemical Inhibition.** In addition to the correlation data, (−)-OSU6162 was co-inhibited with the following P450 enzyme-specific substrate/inhibitors: bropririnine (CYP1A2), coumarin (CYP2A6), 3-cyclopropenyl-N-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (CYP2B6), ketoconazole (CYP3A4), (S)-mephenytoin (CYP2C19), sulfaphenazole (CYP2C9), and quinidine (CYP2D6). The agents were examined for their ability to inhibit N-despropyl metabolite formation at a substrate concentration of 20 μM. As described in Fig. 4, only quinidine (a selective inhibitor for CYP2D6) strongly inhibited the formation of N-despropyl(−)-OSU6162, whereas the other inhibitors did not show any marked effects on metabolite generation (inhibition <25%).

**Incubations with Recombinant Human P450s.** Of the 10 human baculovirus-insect cell expressing P450 isoenzymes investigated, CYP1A1, CYP1A2, CYP2C19, CYP2D6, and CYP3A4 were able to catalyze the formation of N-despropyl (−)-OSU6162. Enzyme kinetic experiments revealed that apparent $K_m$ values for (−)-OSU6162 metabolite formation by the recombinant P450s ranged from 4 to 591 μM (Table 2), which was consistent with the previous multienzyme kinetic data obtained from human liver microsomes (Fig. 2). The most efficient enzyme ($V_{max}/K_m$) for total (−)-OSU6162 metabolite formation was CYP2D6, followed by CYP2C19 and then equally by CYP3A4 and CYP1A2 (Table 2). In addition to the enzymes mentioned above, CYP1A1 also exhibited (−)-OSU6162 N-despropylase activity, however, since this enzyme is expressed extra-hepatically (Wrighton and Stevens, 1992), its overall contribution to (−)-OSU6162 in vivo is difficult to predict. Incubations conducted using S-9 prepared from human lung and kidney were found to posses (−)-OSU6162 N-despropylase activity albeit at a reduced rate compared with S-9 prepared from liver (data not shown).

**Effect of (−)-OSU6162 on P450 Marker Substrates.** The selectivity of (−)-OSU6162 to inhibit six human P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2E1, and CYP3A4) was evaluated using a simple in vitro inhibition screen (Wynalda and Wienkers, 1997). Of the P450 enzymes tested, only CYP2D6 catalytic activity was markedly inhibited (~30% inhibition at 10 μM inhibitor concentration) by (−)-OSU6162 (Table 3).

**Discussion.** In the present study, it has been established that the hepatic biotransformation of (−)-OSU6162 involves a N-despropylation reaction, which is mediated by several P450 enzymes including CYP2D6. The involvement of CYP2D6 in the in vitro metabolism of (−)-OSU6162 is supported by several lines of evidence: 1) a good correlation between the rate of N-despropyl(−)-OSU6162 formation and dextromethorphan-O-demethylase activity in a panel of human liver microsomes, 2) marked inhibition of (−)-OSU6162 metabolism by quinidine (a selective inhibitor of CYP2D6), and 3) a severalfold
toward a lesser extent CYP1A1, CYP1A2, and CYP3A4, also contribute additional human liver microsomal P450s, in particular CYP2C19 and to metabolism is predicted to be mediated by CYP2D6 and that addition of the human hepatic P450s has been determined as CYP1A2. Typically substrates of CYP2D6 share common structural characteristics such as the presence of at least one basic nitrogen atom, a distance of 5 or 7 Å between the basic nitrogen atom and the site of oxidation, a flat hydrophobic area near the site of oxidation, and a greater in vitro intrinsic clearance for (−)-OSU6162 for CYP2D6 than other P450 enzymes tested.

While inspection of the data with respect to the in vitro intrinsic clearance revealed that the majority of the N-despropylation activity on a per picomole P450 scale was largely attributed to CYP2D6, an equally important consideration (i.e., the overall expression of each P450 enzyme in human liver) is required to predict the importance of each enzyme to (−)-OSU6162 in vivo clearance. The relative abundance of the human hepatic P450s has been determined as CYP1A2 (13%), 2A6 (4%), 2B6 (<1%), 2C (20%), 2D6 (2%), 2E1 (7%), and 3A4 (30%) (Shimada et al., 1994; Rendic and Carlo, 1997). Using these values to reexamine the intrinsic clearances normalized to reflect relative abundance/activities of each form in vivo narrows the (−)-OSU6162 total hepatic -despropylase activity on P450 enzyme-selective compounds were used to study their inhibitory effects on the oxidation of (−)-OSU6162 (20 μM) in human liver microsomes. The P450-selective inhibitors and/or substrates used in this experiment were 200 μM Bropridine (CYP1A2; Wynaeda et al., 1998); 50 μM coumarin (CYP2A6; Pearce et al., 1992); 50 μM 3-cyclopentylloxoy-N-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (CYP2B6; Stevens et al., 1997); 10 μM sulfaphenazole (CYP2C9; Newton et al., 1995); 200 μM (5)-mephenytoin (CYP2C19; Wrighton et al., 1993); 5 μM quinidine (CYP2D6; Guengerich et al., 1986); 100 μM para-nitrophenol (CYP2E1; Tassaneeyakul et al., 1993); 5 μM ketoconazole (CYP3A4; Gibbs et al., 1999). Incubation conditions were carried out as described under Experimental Procedures. Each data point represents the mean (± S.D.) of triplicate determinations.

| TABLE 1 |
| Correlation between rate of (−)-OSU6162 N-despropylation and standard cytochrome P450 enzyme-specific activities in a panel of human liver microsomal preparations* |
| P450 Enzyme | Coefficient of Determination (r²) with [5 μM] (−)-OSU6162 N-Despropylation | Coefficient of Determination (r²) with [200 μM] (−)-OSU6162 N-Despropylation |
| CYP1A2 | 0.125 | 0.454 |
| CYP2C9 | 0.014 | 0.311 |
| CYP2C19 | 0.376 | 0.528 |
| CYP2D6 | 0.908 | 0.201 |
| CYP2E1 | 0.016 | 0.034 |
| CYP3A4 | 0.005 | 0.031 |

* (−)-OSU6162 (5 or 200 μM) was incubated with human liver microsomes from 12 individual donors (0.3 mg/ml), in triplicate. Activities were normalized to P450 content and correlated with enzyme-specific marker activities using standard methods.

![FIG. 3. Correlation between dextromethorphan O-demethylation and (−)-OSU6162 N-despropylase activity in a panel of human liver microsomes.](Image)

The dashed line (—) represents the line of unity. (−)-OSU6162 (A, 5 μM or B, 200 μM) was incubated with human liver microsomes from 10 individual donors. Incubation conditions were carried out as described under Experimental Procedures. Each data point represents the mean (± S.D.) of triplicate determinations.

![FIG. 4. Percentage of control activities for N-despropylation of (−)-OSU6162 in the presence of various substrates and inhibitors selective for individual P450 enzymes.](Image)

TABLE 2

| TABLE 2 |
| Individual enzyme kinetic parameters for (−)-OSU6162 N-despropylation |

Michaels-Menten parameters (V_max and K_m) and intrinsic clearance (V_max/K_m) were determined from recombinant baculovirus-insect cell microsomes expressing human P450 enzymes.

<table>
<thead>
<tr>
<th>P450 Enzymes</th>
<th>K_m μM</th>
<th>V_max pmol/min</th>
<th>V_max/K_m</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>78.9 ± 8.7</td>
<td>7.23 ± 0.21</td>
<td>0.091</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>591.4 ± 146.7</td>
<td>2.58 ± 0.38</td>
<td>0.0043</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>194.7 ± 7.8</td>
<td>10.60 ± 0.14</td>
<td>0.054</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>4.0 ± 0.3</td>
<td>1.41 ± 0.04</td>
<td>0.351</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>439.6 ± 44.7</td>
<td>1.06 ± 0.051</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

It is interesting that (−)-OSU6162 is a relatively good substrate for CYP2D6. Typically substrates of CYP2D6 share common structural characteristics such as the presence of at least one basic nitrogen atom, a distance of 5 or 7 Å between the basic nitrogen atom and the site of oxidation, a flat hydrophobic area near the site of oxidation, and a...
negative molecular electrostatic potential above the planar part of the molecule (de Groot et al., 1996; Ellis et al., 1996; Lewis et al., 1997). However, for phenotyping of CYP2D6 substrate pharmacokinetics is observed between the common antidepressant fluoxetine, as well as the site of carbon-14 label.

In conclusion, the current in vitro findings show that the dopamine D2 receptor modulator, (−)-OSU6162, appears to be N-depropylated primarily by CYP2D6. However, given that the drug is oxidized by additional hepatic P450 enzymes (e.g., CYP2C19, CYP3A4, and CYP3A4), as well as an extra-hepatic P450 (e.g., CYP1A1), extrapolation of these in vitro results to predict the magnitude to which CYP2D6 contributes to the overall clearance of (−)-OSU6162 in vivo remains to be established. Lastly, coinubcation of (−)-OSU6162 did not inhibit the metabolic activity of the P450 enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2E1, and CYP3A4, and only moderately inhibited CYP2D6. Therefore, in terms of predicting potential drug-drug interactions, given the predicted low dose of this agent and the poor affinity toward the human hepatic cytochrome P450 enzymes tested, clinically important interactions between (−)-OSU6162 and coadministered drugs that are metabolized by these enzymes appears unlikely.

References


