Sertraline N-Demethylation Is Catalyzed by Multiple Isoforms of Human Cytochrome P-450 In Vitro

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ABSTRACT:

Sertraline, a new antidepressant of the selective serotonin re-uptake inhibitor class, is extensively metabolized to desmethylsertraline in humans. We identified the cytochrome P-450 (CYP) isoforms involved in sertraline N-demethylation using pooled human liver microsomes and cDNA-expressed CYP isoforms. Eadie-Hofstee plots for the sertraline N-demethylation in human liver microsomes were monophasic. The estimated Michaelis-Menten kinetic parameters were: $K_M = 18.1 \pm 2.0 \mu M$, $V_{max} = 0.45 \pm 0.03 \text{nmol/min/mg}$ of protein, and $V_{max}/K_M = 25.2 \pm 4.3 \mu M\text{min/mg}$ of protein. At the substrate concentration of 20 $\mu M$, which approximated the apparent $K_M$ value, sulfaphenazole (CYP2C9 inhibitor) and triazolam (CYP3A substrate) reduced the N-demethylation activities by 20 to 35% in human liver microsomes, whereas the inhibition induced by mephentoin (CYP2C19 substrate) or quinidine (CYP2D6 inhibitor) was marginal. The anti-CYP2B6 antibody inhibited the sertraline N-demethylation activities by 35%. Sertraline N-demethylation activities were detected in all CYP isoforms studied. In particular, CYP2C19, CYP2B6, CYP2C9-Arg, CYP2D6-Val, and CYP3A4 all showed relatively high activity. When the contributions of CYP2D6, CYP2C9, CYP2B6, CYP2C19, and CYP3A4 were estimated from the $V_{max}/K_M$ of cDNA-expressed CYP isoforms and from their contents in pooled human liver microsomes, the values were found to be 35, 29, 14, 13, and 9%, respectively. The results suggest that at least five isoforms of CYP (CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4) are involved in the sertraline N-demethylation in human liver microsomes and that the contribution of any individual isofrom does not exceed 40% of overall metabolism. Therefore, concurrent administration of a drug that inhibits a specific CYP isofrom is unlikely to cause a marked increase in the plasma concentration of sertraline.
Sertraline, in a final volume of 250 μl. The immunoinhibition of sertraline CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 (product reactions catalyzed by CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C8, mephenytoin (CYP2C19; Küpper and Preisig, 1984), 10 μm for 5 min and 50 μl of supernatant was analyzed by HPLC as described below.

HPLC Conditions. The determination of desmethylsertraline was carried out using the HPLC-UV assay method. The HPLC system consisted of a model L-6000 pump (Hitachi Ltd., Tokyo, Japan), a model L-4000 UV detector (Hitachi), a model AS-2000 autosampler (Hitachi), a model D-2500 integrator (Hitachi), and a 4.6 × 250-mm CAPCELL PAK C18 UG120 column (Shiseido Co. Ltd., Tokyo, Japan). The mobile phase consisted of 0.05 M potassium dihydrogen phosphate/acetonitrile (62:38, v/v) delivered at a flow rate of 1.0 ml/min. The eluate was monitored at a wavelength of 204 nm. Under these chromatographic conditions, the internal standard (phenobarbital), desmethylsertraline, and sertraline were eluted at 6.9, 10.3, and 11.3 min, respectively. Calibration curves were generated from 0.03 to 0.6 nmol/ml by processing the authentic standard substance through the entire procedure. Desmethylsertraline was quantified by comparison with the standard curves using the peak-height ratio method. The detection limit for desmethylsertraline was 0.5 ng.

Kinetics in Human Liver Microsomes. Kinetic studies were performed using pooled human liver microsomes (lot 1). The N-demethylation activities of sertraline in human liver microsomes were determined at sertraline concentrations ranging from 2.5 to 100 μM. All reactions were performed in a linear range with respect to protein concentration and incubation time: 0.1 mg/ml microsomal protein and 20-min incubation time. The kinetic parameters (Km, Vmax, and Vmax/Km) were estimated by graphic analysis of Eadie-Hofstee plots. These values were subsequently used as initial estimates for the nonlinear least-squares regression analysis, MULTI (Yamaoka et al., 1981).

Inhibition Study. CYP isoform-selective xenobiotic probes were used to study their inhibitory effects on the N-demethylease activities of sertraline (15 μM in human liver microsomes. The isoform-selective inhibitors and alternative substrates used in this part of the study were 10 μM α-naphthoflavone (CYP1A1; Tassaneeyakul et al., 1993), 100 μM coumarin (CYP2A6; Yun et al., 1991), 100 μM sulfaphenazole (CYP2C9; Newton et al., 1995), 100 μM mephenytoin (CYP2C19; Küpper and Preisig, 1984), 10 μM quinidine (CYP2D6; Guengerich et al., 1986), 100 μM chlorozoxazone (CYP2E1; Peter et al., 1990), and 100 μM triazolam (CYP3A; Kronbach et al., 1989). The monoclonal antibody raised against human CYP2B6 used in the present study inhibited 7-ethoxy-4-trifluoromethylcoumarin deethylation in cDNA-expressed CYP2B6 by more than 90%, whereas it did not inhibit specific reactions catalyzed by CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 (product information of Gentest). The immunoinhibition of sertraline N-demethylation was examined by preincubating human liver microsomal samples (25 μg microsomal protein) with various amounts of anti-CYP2B6 antibodies (0–100 μg/mg microsomal protein) in 25 mM Tris buffer (pH 7.5) for 15 min on ice. Sertraline (20 μM) and the other components of the incubation medium were then added and the above mentioned reaction occurred.

Assay with cDNA-Expressed CYP Isoforms. Microsomes from human B-lymphoblastoid cell lines expressing human CYP1A1 (lot 21), CYP1A2 (lot 52), CYP2A6 (lot 32), CYP2B6 (lot 40), CYP2C8 (lot 18), CYP2C9-Arg (lot 15), CYP2C19 (lot 12), CYP2D6-Val (lot 13), CYP2E1 (lot 31), or CYP3A4 (lot 49) and control microsomes (lot 17) were used. cDNA-expressed CYP2A6, CYP2C8, CYP2C9-Arg, CYP2D6-Val, CYP2E1, and CYP3A4 were coexpressed with NADPH-CYP reductase in human B-lymphoblastoid cell lines. To examine the roles of individual CYP isoforms involved in sertraline N-demethylation, control microsomes and each of the ten cDNA-expressed CYPs listed above (each at a protein concentration of 0.5 mg/ml) were first incubated with 100 μM sertraline for 120 min at 37°C, according to the procedure recommended by the supplier (Gentest).

Kinetics in cDNA-Expressed CYP Isoforms. Kinetic studies were performed using microsomes from human B-lymphoblastoid cell lines expressing CYP2B6 (lot 40), CYP2C9-Arg (lot 26), CYP2C19 (lot 12), CYP2D6-Val (lot 29), or CYP3A4 (lot 49). The activities of sertraline N-demethylation in CYP2B6 and CYP2C19 were determined at sertraline concentrations ranging from 2.5 to 100 μM. The concentrations of sertraline used for determining the activities in CYP2D6-Val and CYP3A4 ranged from 1 to 40 μM and from 10 to 200 μM, respectively. The protein concentration of each cDNA-expressed CYP isoform was 0.25 mg/ml. Incubation with cDNA-expressed CYP2B6 or CYP3A4 was carried out for 30 min and with CYP2C9-Arg, CYP2C19, or CYP2D6-Val for 60 min. All reactions were performed in a linear range with respect to protein concentration and incubation time. The kinetic parameters (Km, Vmax, and Vmax/Km) were estimated as described above.

Results and Discussion

The Eadie-Hofstee plot for sertraline N-demethylation in human liver microsomes was linear (data not shown). Thus, we were able to estimate the kinetic parameters by fitting them to a simple Michaelis-Menten equation. The mean (±S.D.) kinetic parameters estimated from three independently undertaken experiments were: apparent Km = 18.1 ± 2.0 μM, Vmax = 0.45 ± 0.03 nmol/min/mg of protein, and Vmax/Km = 25.2 ± 4.3 μmol/min/mg of protein. At a substrate concentration of sertraline (i.e., 20 μM), which approximated the apparent Km value of sertraline N-demethylation in human liver microsomes, sulfaphenazole (CYP2C9; Newton et al., 1995), α-naphthoflavone (CYP1A1; Tassaneeyakul et al., 1993), and triazolam (CYP3A; Kronbach et al., 1989) were seen to inhibit the N-demethylation activities of sertraline in human liver microsomes by 35.8, 22.1, and 21.7%, respectively. In addition, the anti-CYP2B6 antibody reduced the N-demethylation activity of sertraline by around 35%. These results suggest that, at a 20 μM substrate concentration of sertraline, CYP2C9, CYP2B6, CYP1A, and CYP3A are involved in the sertraline N-demethylation in human liver microsomes. On the other hand, me-
Among the cDNA-expressed CYP isoforms studied, CYP2D6-Val
approximately 30 $V_{\text{max}}/K_M$ because apparent $K_M$ only slightly reduced, the results that indicated that coumarin did not inhibit, and that chlorzoxazone (CYP2E1) only marginal (Fig. 1). This finding is consistent with the previous formation of desmethylsertraline by CYP2A6 and CYP2E1 was also to be due to the inhibition of CYP2C9 rather than of CYP1A. The CYP2C9, as well as CYP1A enzymes (Chang et al., 1994), the sertraline Arg, CYP2D6-Val, and CYP3A4 extensively catalyzed the sertraline isoforms studied herein. In particular, CYP2C19, CYP2D6, and CYP3A4), but that CYP1A, CYP2A6, and CYP3A4 play a negligible role in the N-demethylation of sertraline in human liver microsomes. Microsomes from human B-lymphoblastoid cell lines expressing each of the ten human CYP isoforms were examined to clarify the role of individual CYP isoforms involved in sertraline N-demethylation. As shown in Fig. 1, the formation of desmethylsertraline at sertraline concentration of 100 $\mu$M was found in all the cDNA-expressed CYP isoforms studied herein. In particular, CYP2C19, CYP2B6, CYP2C9-Arg, CYP2D6-Val, and CYP3A4 extensively catalyzed the sertraline N-demethylation (133.3, 111.8, 76.4, 52.3, and 28.4 pmol/120 min/ pmol CYP, respectively). Accordingly, kinetic parameters for the sertraline N-demethylation were estimated for five cDNA-expressed CYP isoforms (CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). As listed in Table 1, CYP2D6-Val showed the lowest apparent $K_M$ value (2.6 $\mu$M), followed by CYP2C19 (9.0 $\mu$M). The apparent $K_M$ values for CYP2B6 and CYP2C9-Arg were approximately 30 $\mu$M; for CYP3A4, the apparent $K_M$ value was 45.3 $\mu$M. Among the cDNA-expressed CYP isoforms studied, CYP2D6-Val showed the greatest $V_{\text{max}}/K_M$ value (0.309 $\mu$min/ pmol CYP). The $V_{\text{max}}/K_M$ values of CYP2C19, CYP2B6, CYP2C9-Arg, and CYP3A4 were 40.1, 21.7, 18.4, and 4.2% of the CYP2D6-Val, respectively. In this study using cDNA-expressed CYP isoforms, the formation of desmethylsertraline by CYP1A1 and CYP1A2 was found to be only marginal (Fig. 1). This contradicts the previous finding that the N-demethylation activity of sertraline in human liver microsomes was inhibited by 22% in the presence of $\alpha$-naphthoflavone. However, because $\alpha$-naphthoflavone is an effective inhibitor of CYP2C8 and CYP2C9, as well as CYP1A enzymes (Chang et al., 1994), the inhibition of sertraline N-demethylation by $\alpha$-naphthoflavone appears to be due to the inhibition of CYP2C9 rather than of CYP1A. The formation of desmethylsertraline by CYP2A6 and CYP2E1 was also only marginal (Fig. 1). This finding is consistent with the previous results that indicated that coumarin did not inhibit, and that chlorzoxazone only slightly reduced, the N-demethylation activity of sertraline in human liver microsomes. Taken together, the results of the present study suggest that the sertraline N-demethylation could be catalyzed by at least five CYP isoforms (i.e., CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4), but that CYP1A, CYP2A6, and CYP2E1 play only a negligible role in the N-demethylation of sertraline in human liver microsomes.

According to the data sheets provided by the manufacturer, the CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 contents in the human liver microsomes used in this study were 41, 96, 19, 22, and 142 pmol CYP/mg of protein, respectively (Table 1). The contribution of each CYP isoform to the overall N-demethylation activities of sertraline in human liver microsomes is thought to depend on the amount of each CYP isoform present in the microsomes. Therefore, the $V_{\text{max}}/K_M$ values of five CYP isoforms in pooled human liver microsomes (CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) in the human liver microsomes were estimated from those of the cDNA-expressed CYP isoforms and from the specific contents of corresponding CYP isoforms in the human liver microsomes (i.e., estimated $V_{\text{max}}/K_M = V_{\text{max}}/K_M$ of cDNA-expressed CYP $\times$ specific content of each CYP isoform in the pooled microsomes). Among these five CYP isoforms, CYP2D6 showed the highest value for estimated $V_{\text{max}}/K_M$, followed by CYP2C9, CYP2B6, CYP2C19, and CYP3A4 and the percent contributions of these isoforms to the sum of $V_{\text{max}}/K_M$ values were 35, 29, 14, 13, and 9%, respectively. These findings suggest that CYP2D6 is one of the predominant enzymes involved in the in vivo metabolism of sertraline in humans. However, this is inconsistent with the earlier finding that there is no significant difference in the clearance of sertraline between poor and extensive metabolizers of debrisoquine (Hamelin et al., 1996). The reason for this discrepancy is unknown. However, the estimated contribution of CYP2D6 is at most 35%, which might not be enough to produce a significant difference in the clearance of sertraline between poor and extensive metabolizers of debrisoquine with a limited numbers of subjects. Thus, a larger number of phenotyped subjects may be required to clarify the involvement of CYP2D6 in the metabolism of sertraline in vivo.

The results of the present study showed that at least five CYP isoforms are involved in the N-demethylation of sertraline and that the contribution of any individual isoform does not exceed 40% of overall metabolism (Table 1). These findings suggest that concurrent administration of a drug that may inhibit one of these CYP isoforms does not cause a marked increase in the plasma concentration of sertraline. Moreover, sertraline has a wide therapeutic window and causes fewer side effects than tricyclic antidepressants (Murdoch and McTavish, 1992). Therefore, serious interaction does not appear to occur even when a drug that inhibits specific CYP isoforms is administered concurrently with sertraline. In contrast, four (CYP2B6, CYP2C9, CYP2C19, and CYP3A4) and three (CYP2C9, CYP2C19, and CYP3A4) of the five isoforms involved in the N-demethylation of sertraline have been reported to be induced by the administration of anticonvulsants (Serlin and Breckenridge, 1983; Jones et al., 1992; Backman et al., 1996) and rifampicin (Zilly et al., 1975; Kay et al., 1985; Heimark et al., 1987; Zhou et al., 1990; Villikka et al., 1997), respectively. This suggests that sertraline is rapidly metabolized and

### Table 1

<table>
<thead>
<tr>
<th>Microsomes</th>
<th>$K_M$ (µM)</th>
<th>$V_{\text{max}}$ (pmol/min/µmol CYP)</th>
<th>$V_{\text{max}}/K_M$ (µmin/µmol CYP)</th>
<th>Contents of CYP in Human Liver Microsomes</th>
<th>Estimated $V_{\text{max}}/K_M$ in Human Liver Microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2B6</td>
<td>30.7 ± 1.2</td>
<td>2.04 ± 0.36</td>
<td>0.067 ± 0.012</td>
<td>41</td>
<td>2.7 (14%)†</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>30.3 ± 5.3</td>
<td>1.73 ± 0.28</td>
<td>0.057 ± 0.006</td>
<td>96</td>
<td>5.5 (29%)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>9.0 ± 1.7</td>
<td>1.11 ± 0.22</td>
<td>0.124 ± 0.016</td>
<td>19</td>
<td>2.4 (13%)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>2.6 ± 0.3</td>
<td>0.79 ± 0.05</td>
<td>0.309 ± 0.022</td>
<td>22</td>
<td>6.8 (35%)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>45.3 ± 8.5</td>
<td>0.57 ± 0.06</td>
<td>0.013 ± 0.002</td>
<td>142</td>
<td>1.8 (9%)</td>
</tr>
</tbody>
</table>

*The values are expressed as the mean ± S.D. from three different experiments.
†Estimated from the $V_{\text{max}}/K_M$ values of cDNA-expressed CYP isoforms and their contents in pooled human liver microsomes.
‡Numbers in parentheses represent the estimated $V_{\text{max}}/K_M$ as a percentage of the total $V_{\text{max}}/K_M$ values of five CYP isoforms (CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4).
that its pharmacological effect is reduced in patients taking anticonvulsants or rifampicin.

To our knowledge, this is the first study to identify the CYP isoforms responsible for the N-demethylation of sertraline in human liver microsomes. The results of the present study using human liver microsomes and cDNA-expressed CYP isoforms suggest that at least five isoforms of CYP (CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4) are involved in the sertraline N-demethylation in human liver microsomes. This characteristic of sertraline metabolism (i.e., multiple forms of CYP being involved in the metabolism of sertraline) may lead to fewer occurrences of serious interaction caused by the inhibition of specific CYP isoforms.

References
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