IN VITRO CHARACTERIZATION OF THE METABOLISM OF HALOPERIDOL USING RECOMBINANT CYTOCHROME P450 ENZYMES AND HUMAN LIVER MICROSONES

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ABSTRACT:

A systematic in vitro study was carried out to elucidate the enzymes responsible for the metabolism of haloperidol (HAL) using human liver microsomes and recombinant human cytochrome P450 isoenzymes. In the first series of experiments, recombinant cytochrome P450 (P450) isoenzymes were used to evaluate their catalytic involvement in the metabolic pathways of HAL. Recombinant CYP3A4, CYP3A5, and CYP1A1 were shown to be able to catalyze the metabolism of HAL to its pyridinium analog (HP−) and the oxidation of reduced HAL (RH) back to HAL; Recombinant CYP3A4, CYP3A5, CYP1A1, CYP2C19, CYP2C8, CYP2C9, and CYP2D6 were able to catalyze the dealkylation of HAL to 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP). CYP3A4 was capable of metabolizing HAL to its tetrahydropyridine analog 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridine and metabolizing to CPHP; CYP3A4 and CYP3A5 were able to metabolize RH to its pyridinium analog (RHP+); CYP1A1, CYP1A2, and CYP3A4 were able to catalyze the oxidation of RHP+ to HP+. In the second series of experiments, the metabolic activities of human liver microsomes from 12 donors were correlated with catalytic activities of selective substrates of different P450 isoenzymes and immuno-reactivities toward different P450 isoenzymes. CYP3A4 activities were found to correlate to all the seven metabolic pathways of HAL mentioned above. This suggests a prominent role for CYP3A4 in the metabolism of HAL. Interestingly, it was found that recombinant CYP1A1 has the highest activity for oxidizing RHP+ to HP+. The activity of recombinant CYP1A1 was 50 times higher than CYP1A2 and 220 times higher than CYP3A4.

Haloperidol (HAL1; 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol) is one of the most widely used antipsychotic drugs (Wysowski and Baum, 1989; Shander, 1994; Lohse et al., 1996). HAL is extensively metabolized with only about 1% of the administered dose excreted unchanged in urine (Forsman and Larsson, 1978). The metabolism of HAL can be summarized as shown in Fig. 1 and consists of the following pathways: 1) N-dealkylation, which leads to the formation of 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) (Soudijn et al., 1967; Fang and Gorrod, 1993; Gorrod and Fang, 1993; Fang et al., 1996a); 2) reduction of the ketone group, which leads to the formation of reduced HAL (RH) (Forsman and Larsson, 1978); 3) the reverse oxidation of RH back to HAL (Forsman and Larsson, 1978; Midha et al., 1988; Someya et al., 1992); 4) N-dealkylation of RH leading to the formation of CPHP (Fang et al., 1996a); 5) a pyridinium analog of HAL (HP−) (Subramanyam et al., 1990; Fang and Gorrod, 1991; 1993; Gorrod and Fang, 1993); 6) the dehydration product of HAL leading to the formation of haloperidol 1,2,3,6-tetrahydropyridine (HTTP), which was proposed to be an intermediate product for the formation of HP+ from HAL (Fang and Gorrod, 1991; 1993; Gorrod and Fang, 1993); and 7) the pyridinium analog of RH (RHP+) (Eyles et al., 1994; Van der Schyf et al., 1994). RHP+ is reported to be produced either by reduction of HP− or by oxidation from RH (Eyles et al., 1996). Both HP+ and RHP+ have been shown to be present in plasma and postmortem brain of patients administered HAL (Eyles et al., 1994; 1997); 8) oxidation of RHP+ back to HP+; 9) a few unknown metabolites were also identified (Fang and Gorrod, 1991; 1993; Gorrod and Fang, 1993). The major phase II metabolite of HAL is reported to be the glucuronidation product of HAL.

Dose individualization is required for the use of HAL because of a narrow therapeutic window (Ulrich et al., 1998) and a large interindividual variability with the plasma levels of HAL. The interindividual variability may be related to enzyme polymorphism (Llerena et al., 1992a,b; Lane et al., 1997), interethnic differences (Potkin et al., 1984; Chang et al., 1987; Jann et al., 1989; Someya et al., 1990), and smoking status (Perry et al., 1993; Shimoda et al., 1999). HAL is reported to interact with a number of other drugs through inhibition or induction of cytochrome P450 (P450) enzymes (Kudo and Ishizaki, 1984; Chang et al., 1987; Jann et al., 1989; Someya et al., 1990), and smoking status (Perry et al., 1993; Shimoda et al., 1999). A good understanding of the enzymes responsible for the
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metabolism of HAL would assist in rationalizing the interindividual variations and drug-drug interactions involving HAL.

There has not been a comprehensive study on the enzyme(s) responsible for the metabolism of HAL covering most of the metabolic pathways and known isoenzymes. In this study, a thorough in vitro study was carried out to study the P450 isoenzymes responsible for the metabolism of HAL and the secondary metabolism of its metabolites.

Experimental Procedures

Materials. Haloperidol was purchased from Sigma (St. Louis, MO). CPHP and CPTP were purchased from Aldrich Chemical Company (Milwaukee, WI). HTP, HP, and RH were synthesized as previously described (Gorrod and Fang, 1993). RHP was a kind gift from Dr. Neal Castagnoli (Virginia Tech, Blacksburg, VA). All other chemicals were of analytical grades.

Recombinant human P450 enzymes were purchased from GENTEST (Woburn, MA). These enzymes were CYP1A1 Supersomes (1 nmol of P450/ml), CYP1A2 Supersomes (1 nmol of P450/ml), CYP2C8 Supersomes (1 nmol of P450/ml), CYP2C9 Supersomes (1 nmol of P450/ml), CYP2C19 Supersomes (1 nmol of P450/ml), CYP2D6 Supersomes (1 nmol of P450/ml), CYP2E1 Supersomes (1 nmol of P450/ml), CYP3A4 Supersomes (1 nmol of P450/ml), and CYP3A5 Supersomes (1 nmol of P450/ml). A panel of human liver microsomal preparations from 12 donors was obtained from GENTEST. For each microsomal preparation, catalytic activities toward selective substrates of the isoenzymes were correlated with the isoenzyme-specific activities of the microsomes (using selective substrates of the isoenzymes; Table 2). The metabolic activities toward different pathways were investigated using a panel of 12 human liver microsomal preparations (Table 2). The metabolic activities toward different pathways were correlated with the isoenzyme-specific activities of the microsomes (using selective substrates of the isoenzymes; Table 2) and the immuno-quantified amount of each isoenzyme in the microsomal preparations (Table 4).

Correlation studies. For correlation analysis, HAL, RH, HTP, or RHP (20 μM) was incubated with a bank of human liver microsomal preparations from 12 donors (10 μl/incubate) for 20 min. Sample preparation and HPLC analysis of the metabolites were carried out as described under Enzymatic Studies. The rates of formation of the HAL metabolites were correlated to the catalytic activities of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 in the microsomes (data supplied by GENTEST). The rates of formation of the HAL metabolites were also correlated to the immunoactivities of CYP1A2, CYP2C9, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 in the microsomes (data supplied by GENTEST). Correlations of metabolite formation velocities with enzyme activities were evaluated by simple linear regression (GraphPad Prism software; GraphPad Software, San Diego, CA). Whether the slope of the regression line is significantly different from zero is determined by an F-test.

cDNA-Expressed Enzymes. HAL, RH, and RHP (100 μM for all substrates) were incubated with recombinant CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. Among these isoenzymes, CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1). HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1). HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1).HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1).HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1).

Results

HAL, RH, and RHP+ were investigated as substrates of recombinant CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. Among these isoenzymes, CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1). HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1). HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1). HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1).HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1).HAL was shown to be metabolized to CHPH by CYP3A4, CYP3A5, CYP1A1, CYP2C8, CYP2C9, CYP2C19, and likely CYP2D6 showed some catalytic activity toward these substrates (Table 1).

In a second series of experiments, the above-mentioned metabolic pathways were investigated using a panel of 12 human liver microsomal preparations (Table 2). The metabolic activities toward different pathways were correlated with the isoenzyme-specific activities of the microsomes (using selective substrates of the isoenzymes; Table 3) and the immuno-quantified amount of each isoenzyme in the microsomal preparations (Table 4).

Discussion

The current study demonstrated that CYP3A4 is the most important P450 isoenzyme responsible for most of the metabolic pathways of HAL. These pathways include the dealkylation of HAL and RH, reverse oxidation of RH to HAL, aromatization of the pyridine ring of both HAL and RH, and metabolism of HAL to HTP. Recombinant CYP3A4 is shown to be capable of catalyzing the above-mentioned metabolic pathways investigated and had higher activities than other isoenzymes. The "panel study" showed that metabolic activities of all the pathways investigated correlate with the selective catalytic (Table 3) and immuno (Table 4) activities of CYP3A4. Recombinant
Metabolic conversion of HAL, RH, and RHP by CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5

HAL (100 μM), RH (100 μM), and RHP (100 μM) were incubated with the recombinant P450 isoenzyme preparations for 60 min. Data are means (±S.E.M.) of three independent experiments and are presented as pmol/pmol of P450/h. With the HPLC-UV method used in the present study, the detection limit for HAL, HTP, HP, and RHP (245 nm) was 0.1 μM, which enable the assay to detect a minimum enzyme activity of 0.5 pmol/pmol of P450/h. The detection limit for CPHP and RH (220 nm) was 0.2 μM, which enable the assay to detect a minimum enzyme activity of 1 pmol/pmol of P450/h.

**TABLE 1**

<table>
<thead>
<tr>
<th>Metabolic Pathways</th>
<th>CYP1A1</th>
<th>CYP1A2</th>
<th>CYP2C8</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
<th>CYP3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAL → HTP</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>3.18 ± 0.12</td>
<td>N.D.</td>
</tr>
<tr>
<td>HAL → HP⁺</td>
<td>8.38 ± 0.01</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.29 ± 0.13</td>
<td>4.10 ± 0.30</td>
<td>N.D.</td>
<td>23.78 ± 1.18</td>
<td>3.30 ± 0.25</td>
</tr>
<tr>
<td>HAL → CPHP</td>
<td>14.32 ± 0.47</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.44 ± 0.19</td>
<td>1.00*</td>
<td>N.D.</td>
<td>44.62 ± 1.99</td>
<td>16.50 ± 0.79</td>
</tr>
<tr>
<td>RH → HAL</td>
<td>7.87 ± 0.74</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>43.77 ± 2.14</td>
<td>7.01 ± 1.33</td>
<td></td>
</tr>
<tr>
<td>RH → CPHP</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>19.58 ± 4.12</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>RH → RHP⁺</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>16.92 ± 0.49</td>
<td>2.18 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>RHP⁺ → HP⁺</td>
<td>123.9 ± 3.1</td>
<td>2.55 ± 0.22</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.56 ± 0.12</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

N.D., not detected.

*Formation of CPHP from HAL was detected previously using human lymphoblast-expressed CYP2D6 and a more sensitive gas chromatographic method for CPHP (Fang et al., 1997).

Metabolic conversion of HAL, HTP, RH, and RHP by 12 human liver microsomal preparations

Data are from one duplicate experiment and are presented as picomoles per milligram of protein per minute.

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>CYP1A1</th>
<th>CYP1A2</th>
<th>CYP2C8</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
<th>CYP3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPHP</td>
<td>HAL</td>
<td>HTP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLM003</td>
<td>25.3</td>
<td>22.8</td>
<td>5.0</td>
<td>1.93</td>
<td>25.3</td>
<td>274</td>
<td>0.101</td>
<td>1.29</td>
</tr>
<tr>
<td>HLM006</td>
<td>20.0</td>
<td>13.1</td>
<td>3.8</td>
<td>1.53</td>
<td>20.0</td>
<td>286</td>
<td>0.023</td>
<td>0.95</td>
</tr>
<tr>
<td>HLM023</td>
<td>17.8</td>
<td>13.4</td>
<td>0.0</td>
<td>1.23</td>
<td>17.8</td>
<td>282</td>
<td>0.046</td>
<td>0.90</td>
</tr>
<tr>
<td>HLM030</td>
<td>47.6</td>
<td>34.5</td>
<td>6.1</td>
<td>2.93</td>
<td>47.6</td>
<td>270</td>
<td>0.277</td>
<td>1.87</td>
</tr>
<tr>
<td>HLM042</td>
<td>84.9</td>
<td>87.1</td>
<td>13.7</td>
<td>6.46</td>
<td>84.9</td>
<td>180</td>
<td>0.455</td>
<td>4.55</td>
</tr>
<tr>
<td>HLM043</td>
<td>16.1</td>
<td>16.5</td>
<td>0.0</td>
<td>2.32</td>
<td>16.1</td>
<td>291</td>
<td>0.032</td>
<td>1.18</td>
</tr>
<tr>
<td>HLM056</td>
<td>19.1</td>
<td>19.5</td>
<td>3.0</td>
<td>2.26</td>
<td>19.1</td>
<td>305</td>
<td>0.055</td>
<td>1.38</td>
</tr>
<tr>
<td>HLM066</td>
<td>30.1</td>
<td>30.3</td>
<td>6.7</td>
<td>3.70</td>
<td>30.1</td>
<td>279</td>
<td>0.103</td>
<td>1.68</td>
</tr>
<tr>
<td>HLM070</td>
<td>43.8</td>
<td>40.5</td>
<td>7.3</td>
<td>4.48</td>
<td>43.8</td>
<td>254</td>
<td>0.117</td>
<td>2.16</td>
</tr>
<tr>
<td>HLM089</td>
<td>55.8</td>
<td>49.9</td>
<td>9.0</td>
<td>5.89</td>
<td>55.8</td>
<td>230</td>
<td>0.269</td>
<td>2.86</td>
</tr>
<tr>
<td>HLM093</td>
<td>13.3</td>
<td>8.2</td>
<td>3.6</td>
<td>1.51</td>
<td>13.3</td>
<td>287</td>
<td>0.000</td>
<td>1.37</td>
</tr>
<tr>
<td>HLM112</td>
<td>109.0</td>
<td>104.3</td>
<td>24.0</td>
<td>12.47</td>
<td>109.0</td>
<td>153</td>
<td>0.286</td>
<td>6.49</td>
</tr>
<tr>
<td>Mean</td>
<td>40.2</td>
<td>36.7</td>
<td>6.9</td>
<td>3.89</td>
<td>40.2</td>
<td>258</td>
<td>0.146</td>
<td>2.22</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>8.7</td>
<td>8.8</td>
<td>1.9</td>
<td>0.92</td>
<td>8.7</td>
<td>14</td>
<td>0.041</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**TABLE 3**

Correlation coefficients (r) relating catalytic activities of P450 isof orm-selective substrate to HAL metabolite formation by a bank of 12 human liver microsomes

Data on the activities of the isoenzymes were supplied by GENTEST. The selective substrates used for each isoenzyme are listed under Experimental Procedures.

<table>
<thead>
<tr>
<th>Isoenzymes</th>
<th>CYP1A2</th>
<th>CYP2C8</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D</th>
<th>CYP2E</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>HAL</td>
<td>HTP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A2</td>
<td>0.10</td>
<td>0.16</td>
<td>0.20</td>
<td>0.20</td>
<td>0.10</td>
<td>0.26</td>
<td>0.13</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>0.42</td>
<td>0.39</td>
<td>0.33</td>
<td>0.20</td>
<td>0.42</td>
<td>0.32</td>
<td>0.61*</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>0.29</td>
<td>0.18</td>
<td>0.26</td>
<td>0.22</td>
<td>0.29</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>0.33</td>
<td>0.31</td>
<td>0.31</td>
<td>0.47</td>
<td>0.33</td>
<td>0.24</td>
<td>0.08</td>
</tr>
<tr>
<td>CYP2D</td>
<td>0.27</td>
<td>0.20</td>
<td>0.26</td>
<td>0.28</td>
<td>0.27</td>
<td>0.23</td>
<td>0.18</td>
</tr>
<tr>
<td>CYP2E</td>
<td>0.23</td>
<td>0.23</td>
<td>0.38</td>
<td>0.26</td>
<td>0.23</td>
<td>0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.97*</td>
<td>0.96</td>
<td>0.92</td>
<td>0.93</td>
<td>0.97*</td>
<td>0.92*</td>
<td>0.87*</td>
</tr>
</tbody>
</table>

*P < 0.05.  
**P < 0.01.

CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP2D6 were shown to catalyze one or more metabolic pathways; with human liver microsomes, however, these metabolic activities did not correlate with the isoenzyme-selective substrate activities and isoenzyme immuno-reactivities. Since CYP3A4 is the most abundant P450 isoenzyme expressed in human liver, CYP3A4 should play an important role in the in vivo metabolism of HAL as well.

The oxidation of RHP⁺ to HP⁺ can be catalyzed by recombinant CYP1A1, CYP1A2, and CYP3A4. It is interesting to note that recombinant CYP1A1 showed the highest activity for this reaction, which was about 50 times more active than CYP1A2 and 220 times more active than CYP3A4. In the panel study, the rate of oxidation of RHP⁺ to HP⁺ was shown to be correlated with CYP3A4 levels (Table 3 and Table 4). Correlation with CYP1A1 activities was not possible because there is currently a lack of a selective substrate for CYP1A1. In addition, CYP1A1 is not consistently detected in human liver, and it was proposed that CYP1A1 is not normally present in human liver unless induced. It is therefore not surprising that CYP3A4 was found to be responsible for the oxidation of RHP⁺ in liver microsomal preparations. RHP⁺ is among the best selective substrates for
CYP1A1 discovered so far. No good probe substrate is currently available for determination of CYP1A1 activity. Ethoxyresorufin O-deethylolation was found to be catalyzed by CYP1A1 and CYP1A2; the intrinsic clearance of recombinant CYP1A1 is 10 times higher than that of CYP1A2 (Penman et al., 1994). Verlukast epoxidation was another metabolic pathway reported to be selectively catalyzed by CYP1A1 in mice, rats, rhesus monkeys, and humans (Grossman et al., 1993). It was shown that lymphoblasts-expressed human CYP1A1 had high catalytic activity, whereas CYP1A2 exhibited activity similar to that of the uninfected lymphoblasts, which constitutively express low levels of P450s. However, the role of other P450 isoenzymes in the metabolism of verlukast is not known. A more thorough study is needed for verlukast to be accepted as a selective substrate of CYP1A1. Since CYP1A1 is not normally present in human liver, studies with other tissues, such as placenta or lung, is needed to establish whether RHP⁺ can be used as a selective probe substrate of CYP1A1. These studies are currently being conducted in this laboratory.

The findings from the current study correlate well with the evidence reported for individual metabolic pathways. The N-dealkylation of HAL was shown to be catalyzed by CYP3A4 by a number of experimental techniques, such as the use recombinant P450 isoenzymes (Fang et al., 1997; Pan et al., 1997; Kudo and Odomi, 1998), a correlation study with human liver microsomes prepared from nine donors, and inhibition by inhibitors and antibodies (Pan et al., 1998). 

Since CYP1A1 is not normally present in human liver, studies with other tissues, such as placenta or lung, is needed to establish whether RHP⁺ can be used as a selective probe substrate of CYP1A1. These studies are currently being conducted in this laboratory.

The current study further demonstrated that the activities for the oxidation of RH to HAL in the panel of human liver microsomes correlate with the catalytic (Table 3) and immuno (Table 4) activities of CYP3A4. This provided further evidence that CYP3A4 is primarily responsible for the oxidation of RH to HAL. The formation of HP⁺ from HAL was investigated using recombinant P450 isoenzymes (Fang et al., 1997), and it was demonstrated that recombinant CYP3A4 can catalyze this reaction. Using human liver microsomes, Usuki et al. (1996) demonstrated that CYP3A4 plays a major role in the formation of HP⁺ from HAL by a correlation study in a panel of human liver microsomes and an inhibition study using selective chemical and immunochemical inhibitors.

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The formation of RH from HAL was found to be enantio-selective and S(-)-RH is produced in higher quantities than its R(+) -enantioomer (Eyles and Pond, 1992; Eyles et al., 1998). This ketone reductase belongs to a group of enzymes called carbonyl reductases, which are found ubiquitously in mammalian tissue (Inaba and Kovacs, 1989). RH used in the current study is a racemic mixture, and further study using S(-)-RH and R(+) -RH is needed to establish whether the oxidation of RH back to HAL is stereoselective.

In a previous report, a small quantity of CPHP was detected in the microsomal incubation mixture of HAL with recombinant CYP2D6 (Fang et al., 1997) because a sensitive electron-capture gas chromatography method was used to measure the levels of CPHP. In the current study, a less sensitive HPLC-UV (220 nm) method was used to detect CPHP, and the level of CPHP was below the sensitivity limit in the incubation mixture of HAL with recombinant CYP2D6. CYP2D6 is generally considered as a high-affinity high-capacity enzyme, whereas CYP3A4 is a low-affinity high-capacity enzyme. Therefore, the contribution of CYP2D6 in vivo may be more significant than suggested by these in vitro studies in which a high substrate concentration was used. In fact, the contribution of CYP2D6 in the metabolism of HAL has been indicated by the observation that plasma concentrations of HAL correlate to the polymorphism of CYP2D6 (Llerena et al., 1992). CYP2D6 is also considered to be a factor causing the interethnic differences in the pharmacokinetics of HAL (Kudo and Ishizaki, 1999). Thus, a more detailed enzyme kinetic study is needed to clarify the role of CYP2D6 in the metabolism of HAL.

The mechanism of the dehydration of HAL to HTP is not clear. HTP was shown to be produced from HAL enzymatically in in vitro studies by several research groups (Gorrod and Fang, 1993; Tomlinson et al., 1993; Igarashi et al., 1995). There must be an intermediate oxidative step involved in this reaction because of the involvement of P450 enzymes. Castagnoli and coworkers (Usuki et al., 1998) proposed the possibility that the first step of the aromatization of the piperidine ring is α-carbon hydroxylation, which leads to the 2,3-dihydropiperidine analog of HAL (HDP⁺). HDP⁺ then chemically oxidized to HP⁺. This mechanism did not include the formation of HTP, which has been consistently detected in the incubation mixture of HAL. It is therefore proposed that HDP⁺ can be autooxidized by disproportionation to HTP and HP⁺ (Fig. 2). The current study used a less sensitive HPLC-UV (220 nm) method to measure the levels of CPHP. In the current study, a less sensitive HPLC-UV (220 nm) method was used to detect CPHP, and the level of CPHP was below the sensitivity limit in the incubation mixture of HAL with recombinant CYP2D6. CYP2D6 is generally considered as a high-affinity high-capacity enzyme, whereas CYP3A4 is a low-affinity high-capacity enzyme. Therefore, the contribution of CYP2D6 in vivo may be more significant than suggested by these in vitro studies in which a high substrate concentration was used. In fact, the contribution of CYP2D6 in the metabolism of HAL has been indicated by the observation that plasma concentrations of HAL correlate to the polymorphism of CYP2D6 (Llerena et al., 1992). CYP2D6 is also considered to be a factor causing the interethnic differences in the pharmacokinetics of HAL (Kudo and Ishizaki, 1999). Thus, a more detailed enzyme kinetic study is needed to clarify the role of CYP2D6 in the metabolism of HAL.

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Human liver microsomes had the lowest activities for the RH ⇒ RHP⁺ pathway (Table 2). Since relatively high plasma levels of RHP⁺ were detected in patients taking HAL (Eyles et al., 1994), RHP⁺ is unlikely to be produced from the oxidation of RH by liver microsomes. RHP⁺ is more likely produced from the reduction of HP⁺ by carbonyl reductase (Eyles et al., 1996).

Knowledge of the isoenzymes responsible for the metabolism of HAL is useful in rationalizing some of the known drug-drug interactions observed clinically. It has been reported that plasma concentrations of HAL can be elevated by coadministration of inhibitors of P450 enzymes (Kudo and Ishizaki, 1999), such as fluvoxamine and fluoxetine (Goff et al., 1991; Daniel et al., 1994). Inhibition of CYP3A4 seems to be the mechanism of these drug-drug interactions.

The clearance of HAL was shown to be enhanced by carbamazepine (Jann et al., 1985; Kidron et al., 1985), phenytoin and phenobarbital.

### Table 4

<table>
<thead>
<tr>
<th>Isoenzymes</th>
<th>Substrates</th>
<th>HAL</th>
<th>HTP</th>
<th>RH</th>
<th>RHP⁺</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CPHP</td>
<td>HP⁺</td>
<td>CTP</td>
<td>HP⁺</td>
</tr>
<tr>
<td>CYP1A2</td>
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<td>0.31</td>
<td>0.26</td>
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<tr>
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<td>0.28</td>
<td>0.31</td>
<td>0.33</td>
<td>0.35</td>
</tr>
<tr>
<td>CYP2D6</td>
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<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>CYP2E1</td>
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<td>0.08</td>
<td>0.23</td>
<td>0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.98⁺</td>
<td>0.99⁺</td>
<td>0.92⁺</td>
<td>0.92⁺</td>
<td>0.98⁺</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>0.02</td>
<td>0.02</td>
<td>0.09</td>
<td>0.08</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* P < 0.01.
phenyl-1,2,3,6-tetrahydropyridine, MPP metabolite of HAL was therefore proposed to be involved in some of the movement disorders induced by HAL. This was supported by the observations that HP⁺ was neurotoxic both in vitro (Fang et al., 1995, 1996b) and in vivo (Rollema et al., 1994).

In summary, the present study systematically investigated the cytochrome P450 isoenzymes responsible for the metabolism of HAL. CYP3A4 was demonstrated to be responsible for catalyzing all the metabolic pathways investigated, those include the dealkylation of HAL and RH, reverse oxidation of RH to HAL, aromatization of the pyridine ring of both HAL and RH, and dehydration of HAL to HTP. The oxidation of RHP⁺ to HP⁺ can be catalyzed by CYP1A1, CYP1A2, and CYP3A4. The recombinant CYP1A1 showed much higher activities than CYP1A2 and CYP3A4 in catalyzing this reaction. Further studies are currently being carried out to evaluate RHP⁺ as a selective substrate of CYP1A1.

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References


Fang J, Lai CT, and Yu PH (1996b) Neurotoxic effects of 1,4-4-(fluorophenyl)-1,4-oxazinyl-pyridinium (HP⁺), a major metabolite of haloperidol, on dopaminergic systems in vitro and in vivo. Biogenic Amines 12:125–134.


