INHIBITORY EFFECTS OF AZELASTINE AND ITS METABOLITES ON DRUG OXIDATION CATALYZED BY HUMAN CYTOCHROME P-450 ENZYMES

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(Received January 21, 1999; accepted March 30, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:

Azelastine, an antiallergy and antiasthmatic drug, has been reported to be metabolized mainly to desmethylazelastine and 6-hydroxyazelastine in mammals. In the present study, the inhibitory effects of azelastine and its two metabolites on human cytochrome P-450 (CYP) isoform-dependent reactions were investigated to predict the drug interactions of azelastine using microsomes from human B-lymphoblast cells expressing CYP. The specific activities for human CYP isoforms included: 7-ethoxyresorufin O-deethylation (CYP1A1), phenacetin O-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), 7-benzoylxyresorufin O-dealkylation (CYP2B6), S-warfarin 7-hydroxylation (CYP2C9), S-mephenytoin 4'-hydroxylation (CYP2C19), bufuralol 1'-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), and testosterone 6β-hydroxylation (CYP3A4). In almost all the activities, desmethylazelastine exhibited stronger inhibition than azelastine and 6-hydroxyazelastine. Desmethylazelastine, but not azelastine and 6-hydroxyazelastine, uncompetitively inhibited CYP2B6 activity ($K_i = 32.6 \pm 4.8 \mu M$). Azelastine, desmethylazelastine, and 6-hydroxyazelastine competitively inhibited CYP2C9 activity ($K_i = 13.9 \pm 1.8, 15.0 \pm 3.1$, and $17.0 \pm 4.1 \mu M$, respectively), CYP2C19 activity ($K_i = 21.9 \pm 2.2, 7.3 \pm 1.6$, and $9.3 \pm 1.6 \mu M$, respectively), and CYP2D6 activity ($K_i = 1.2 \pm 0.1, 1.5 \pm 0.2$, and $3.0 \pm 0.5 \mu M$, respectively). Azelastine and desmethylazelastine competitively inhibited CYP3A4 activity ($K_i = 23.7 \pm 4.6$ and $13.2 \pm 2.3 \mu M$). 6-Hydroxyazelastine interfered with the determination of testosterone 6β-hydroxylation by HPLC. CYP1A2, CYP2A6, and CYP2E1 activities were not significantly inhibited by azelastine and the two metabolites. Among the human CYPs tested, the inhibitory effects of azelastine and its two metabolites were the most potent on human CYP2D6. In consideration of the $K_i$ values and the concentration of azelastine and desmethylazelastine in human livers after chronic oral administration of azelastine, the possibility of in vivo drug interaction of azelastine and other drugs that are mainly metabolized by CYP2D6 was suggested although it might not cause critical side effects. The inhibition of CYP2C9, CYP2C19, and CYP3A4 activity by azelastine and its two metabolites might be clinically insignificant.

Drug interactions can cause severe complications from medications. Clinically relevant drug interactions are often the result of the effects of cytochrome P-450 (CYP) enzymes during biotransformation (Muck, 1994). CYP comprises a superfamily of enzymes that have long been recognized as the primary enzymes responsible for human drug metabolism. Although the number of individual isoforms that have been identified and characterized is increasing (Nelson et al., 1996), the metabolism of xenobiotics in humans is handled mainly by enzymes from three families: CYP1, CYP2, and CYP3 (Spatzenegger and Jaeger, 1995).

Azelastine is a long-acting antiallergy and antiasthmatic drug that possesses properties beyond histamine H1 receptor-blocking activity.

1 Abbreviations used are: CYP, cytochrome P-450; BFOH, bufuralol 1'-hydroxylation activity; BROD, 7-benzoyloxysorufin O-dealkylation activity; COH, coumarin 7-hydroxylation activity; CZXOH, chlorzoxazone 6-hydroxylation activity; EROD, 7-ethoxycoumarin O-deethylation activity; POD, phenacetin O-deethylation activity; S-MPOH, S-mephenytoin 4'-hydroxylation activity; S-WFOH, S-warfarin 7-hydroxylation activity; TESOH, testosterone 6β-hydroxylation activity.

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These include antagonism of the chemical mediators adenosine, LTC4, LTD4, endothelin-1, and platelet activation factor, and inhibition of the generation and/or release of histamine, interleukin-1β, leukotrienes, and superoxide-free radicals (Perhach et al., 1989; Szelenyi, 1989). Azelastine has been reported to be metabolized to desmethylazelastine and 6-hydroxyazelastine (Fig. 1) in mammals (Tatsumi et al., 1984). 6-Hydroxyazelastine is a major metabolite of azelastine in rats and guinea pigs (Tatsumi et al., 1984). In humans, desmethylazelastine is detected in plasma after the administration of azelastine (Pivonka et al., 1987). It has also been established that desmethylazelastine has pharmacologic activity equivalent to the parent drug (Perhach et al., 1989; Szelenyi, 1989). Recently, it has been reported that azelastine and desmethylazelastine inhibited the CYP2C19 and CYP2D6 activities in human liver microsomes (Morganroth et al., 1997). Furthermore, in our previous study (M.N., S.N., S. Tokudome, N.S., H.Y. and T.Y., submitted), we clarified that azelastine N-demethylation is catalyzed by CYP3A4, CYP2D6, and CYP1A2 in different human liver microsomes. To further characterize the inhibitory effects of azelastine and its metabolites on human CYP activities, we investigated the specific activities for human CYP isoforms using microsomes from human B-lymphoblast cells expressing human CYP in the presence of azelastine, desmethylazelastine, and 6-hydroxyazelastine.
Materials and Methods

Chemicals. Azelastine hydrochloride [4-(p-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone hydrochloride] was provided by Eisai (Tokyo, Japan). Desmethylazelastine hydrobromide [4-(p-chlorobenzyl)-2-(hexahydro-1H-azepin-4-yl)-1(2H)-phthalazinone hydrobromide] and 6-hydroxylazelastine hydrochloride [6-hydroxy-4-(p-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone hydrochloride] were provided by Degussa Japan (Tokyo, Japan). Phenacetin, acetaminophen, coumarin, and 7-hydroxycoumarin were purchased from Wako Pure Chemical Industries (Osaka, Japan). Testosterone, 6-butyrolactone, testosterone, and 11β-hydroxysterosterone were purchased from Steraloids (Wilton, NH). NADP+, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Oriental Yeast (Tokyo, Japan). Other chemicals were of the highest grade commercially available.

Enzyme Preparations. Microsomes from human B-lymphoblast cells expressing CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9 (Arg), CYP2C19, CYP2D6 (Val), CYP2E1, and CYP3A4 were obtained from Gentest (Woburn, MA). Except for CYP1A2, CYP2B6, or CYP2C19, these were coexpressed with NADPH-CYP reductase. The CYP contents of these microsomes were provided in the data sheets by the manufacturer.

Enzyme Assays. 7-Ethoxyresorufin and 7-benzyloxyresorufin O-dealkylase activity (EROD and BROD) in microsomes from B-lymphoblast cells expressing CYP1A1 and CYP2B6, respectively, were determined as described previously (Nakajima et al., 1998b). The concentrations of microsomal protein were 0.08 mg/ml. The incubation mixture including 25 μM phenacetin was incubated for 30 min at 37°C. The concentrations of microsomal protein were 0.5 mg/ml. The incubation mixture including 10 μM phenacetin was incubated for 30 min at 37°C. The incubation mixture including 1 μM coumarin 7-hydroxylase activity (COH) in microsomes from B-lymphoblast cells expressing CYP2A6 was determined as described previously (Pearce et al., 1992). The concentrations of microsomal protein were 0.08 mg/ml. The incubation mixture including 1 μM coumarin was incubated for 10 min at 37°C. The concentrations of microsomal protein were 0.5 mg/ml. The incubation mixture including 25 μM S-naphthoflavone was incubated for 30 min at 37°C. The concentrations of microsomal protein were 0.2 mg/ml. The incubation mixture including 0.2 mg/ml phenacetin was incubated for 30 min at 37°C. The concentrations of microsomal protein were 0.5 mg/ml. The incubation mixture including 25 μM S-naphthoflavone was incubated for 30 min at 37°C. The concentrations of microsomal protein were 0.2 mg/ml. The incubation mixture including 0.2 mg/ml phenacetin was incubated for 30 min at 37°C. The concentrations of microsomal protein were 0.5 mg/ml. The incubation mixture including 2 mg/ml bufuralol was incubated for 10 min at 37°C. The concentrations of microsomal protein were 0.05 mg/ml. The incubation mixture including 2 mg/ml bufuralol was incubated for 10 min at 37°C. The concentrations of microsomal protein were 0.5 mg/ml. The incubation mixture including 100 μM testosterone was incubated for 5 min at 37°C. The S-9 fraction of human liver microsomes was used as a marker activity of human CYP1A2 (Tassaneeyakul et al., 1993). However, it has been reported that the Eadie-Hofstee plot of the POD activity in human liver microsomes is biphasic (Boobis et al., 2002).
suggesting that at least two enzymes are involved. In these previous reports, the \( K_M \) values for the high- and low-affinity components of the POD in human liver microsomes were 6 to 9 and 250 to 540 \( \mu M \), respectively. In our preliminary study, the \( K_M \) value for POD catalyzed by recombinant CYP1A2 of B-lymphoblast cells was determined to be 15.9 ± 1.5 \( \mu M \). The control activity was 0.3 pmol/min/pmol CYP. G, BFOH by recombinant CYP2D6 was determined at a bufuralol concentration of 2 \( \mu M \). The control activity was 1.5 pmol/min/pmol CYP. H, CXZOH by recombinant CYP2E1 was determined at a chlorzoxazone concentration of 50 \( \mu M \). The control activity was 1.5 pmol/min/pmol CYP. I, TESOH by recombinant CYP3A4 was determined at a testosterone concentration of 100 \( \mu M \). The control activity was 12.6 pmol/min/pmol CYP. 6-Hydroxyazelastine interfered with the quantification of 6-\( \beta \)-hydroxytestosterone. Each data point represents the mean of duplicate determinations. The IC\textsubscript{50} values of azelastine (AZ), desmethylazelastine (DAZ), and 6-hydroxyazelastine (6OH) are shown as \( \mu M \).

\[ \text{Fig. 2. Inhibitory effects of azelastine (○), desmethylazelastine (●), and 6-hydroxyazelastine (▲) on human CYP activities.} \]

A, EROD by recombinant CYP1A1 was determined at a 7-ethoxyresorufin concentration of 0.1 \( \mu M \). The control activity was 6.2 pmol/min/pmol CYP. B, POD by recombinant CYP1A2 was determined at a phenacetin concentration of 10 \( \mu M \). The control activity was 0.9 pmol/min/pmol CYP. C, COH by recombinant CYP2A6 was determined at a coumarin concentration of 1 \( \mu M \). The control activity was 1.4 pmol/min/pmol CYP. D, BROD by recombinant CYP2B6 was determined at a 7-benzoxylresorufin concentration of 2 \( \mu M \). The control activity was 0.3 pmol/min/pmol CYP. E, S-WFOH by recombinant CYP2C9 was determined at a S-warfarin concentration of 25 \( \mu M \). The control activity was 0.3 pmol/min/pmol CYP. G, BFOH by recombinant CYP2D6 was determined at a bufuralol concentration of 2 \( \mu M \). The control activity was 1.5 pmol/min/pmol CYP. H, CXZOH by recombinant CYP2E1 was determined at a chlorzoxazone concentration of 50 \( \mu M \). The control activity was 1.5 pmol/min/pmol CYP. I, TESOH by recombinant CYP3A4 was determined at a testosterone concentration of 100 \( \mu M \). The control activity was 12.6 pmol/min/pmol CYP. 6-Hydroxyazelastine interfered with the quantification of 6-\( \beta \)-hydroxytestosterone. Each data point represents the mean of duplicate determinations. The IC\textsubscript{50} values of azelastine (AZ), desmethylazelastine (DAZ), and 6-hydroxyazelastine (6OH) are shown as \( \mu M \).
droxyazelastine inhibited S-WFOH by CYP2C9 to a similar extent (Fig. 2E). The IC_{50} values were 17.9, 16.9, and 15.3 μM by azelastine, desmethylazelastine, and 6-hydroxyazelastine, respectively. S-MPOH is catalyzed by CYP2C19 in humans (Chiba et al., 1993). In our preparatory experiment, the K_{M} value for S-MPOH catalyzed by recombinant CYP2C19 of B-lymphoblast cells was determined to be 25.2 ± 8.2 μM. Therefore, S-MPOH by CYP2C19 at substrate concentration of 25 μM was examined for the specific activity of CYP2C19. Azelastine, desmethylazelastine, and 6-hydroxyazelastine, respectively.

In our previous study (M.N., S.N., S. Tokudome, N.S., H.Y. and T.Y., 1992), the role of CYP1A2, CYP2A6, CYP2C9, CYP2E1, and CYP3A4 activities using human liver microsomes was investigated for the specific activity of CYP2D6. Azelastine, desmethylazelastine, and 6-hydroxyazelastine exhibited potent inhibition of BFOH by CYP2D6 (IC_{50} values were 2.6, 2.7, and 6.6 μM, respectively; Fig. 2G).

CZXOH is catalyzed by CYP2E1 and CYP1A1 in humans (Carriere et al., 1993). In our preparatory experiment, the K_{M} value for CZXOH catalyzed by recombinant CYP2D6 of B-lymphoblast cells was determined to be 2.6 ± 0.1 μM. Therefore, BFOH by CYP2D6 at a substrate concentration of 2 μM was investigated for the specific activity of CYP2D6. Azelastine, desmethylazelastine, and 6-hydroxyazelastine inhibited the interaction of an inhibitor with a particular enzyme, as the IC_{50} value varies with the substrate concentration. Thus, the K_{i} values and inhibitory patterns (competitive, noncompetitive, or uncompetitive) were investigated for the activities that were inhibited by azelastine, desmethylazelastine, and 6-hydroxyazelastine (Table 1). BROD catalyzed by CYP2B6 was uncompetitively inhibited by desmethylazelastine (K_{i} = 32.6 ± 4.8 μM). The K_{i} values of azelastine, desmethylazelastine, and 6-hydroxyazelastine for S-WFOH catalyzed by CYP2C9 were 13.9 ± 1.8, 15.0 ± 3.1, and 17.0 ± 4.1 μM, respectively. The inhibitory pattern was competitive for azelastine, and mixed-type of competitive and noncompetitive for desmethylazelastine and 6-hydroxyazelastine. S-MPOH catalyzed by CYP2C19 was competitively inhibited by azelastine, desmethylazelastine, and 6-hydroxyazelastine (K_{i} = 21.9 ± 2.2, 7.3 ± 1.6, and 9.3 ± 1.6 μM, respectively). TESOH catalyzed by CYP3A4 was competitively inhibited by azelastine and desmethylazelastine (K_{i} = 23.7 ± 4.6 and 13.2 ± 2.3 μM). Finally, BFOH catalyzed by CYP2D6 was competitively inhibited by azelastine, desmethylazelastine, and 6-hydroxyazelastine (K_{i} = 1.2 ± 0.1, 1.5 ± 0.2, and 3.0 ± 0.5 μM, respectively).

In our previous study (M.N., S.N., S. Tokudome, N.S., H.Y. and T.Y., submitted), we confirmed that the transformation of azelastine to desmethylazelastine in human liver microsomes is catalyzed mainly by CYP3A4 and CYP2D6 and to a smaller extent, CYP1A2. The K_{M} value of CYP2D6 for azelastine N-demethylation was 1.4 μM. It is reasonable that the K_{i} value of azelastine for BFOH would be close to the K_{M} value of CYP2D6 for azelastine N-demethylation.

Recently, Morganroth et al. (1997) reported the inhibitory effects of azelastine and desmethylazelastine on CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 activities using human liver microsomes as follows: 1) S-MPOH (CYP2C19) in human liver microsomes was inhibited by azelastine and desmethylazelastine (the K_{i} values were 7.0–9.1 and 3.4–8.2 μM, respectively), 2) the metabolism of S-metoprolol (CYP2D6) in human liver microsomes was inhibited by azelastine (the K_{i} value was 1.7–12.1 μM) but not by desmethylazelastine, and 3) no inhibitory effects of azelastine and desmethylazelastine on CYP1A2, CYP2A6, CYP2C9, CYP2E1, and CYP3A4 activities were reported. The reason for the contradiction between the previous report (Morganroth et al., 1997) and the present study in which the inhibition of CYP2C9 and CYP3A4 by azelastine and desmethylazelastine was indicated is unknown. However, it might due to the difference of substrate, because carbamazepine and R-warfarin were utilized as marker probes for CYP3A4 in the previous report. Concerning CYP2C9, a marker probe used is same, i.e., S-warfarin. Therefore, the contradiction might be due to the differences of substrate concentrations and/or in vitro techniques. Furthermore, the difference in the source of enzymes, i.e., human liver

**TABLE 1**

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Activity</th>
<th>Azelastine IC_{50}</th>
<th>Desmethylazelastine</th>
<th>6-Hydroxyazelastine</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2B6</td>
<td>BROD</td>
<td>ND</td>
<td>32.6 ± 4.8, competitive</td>
<td>ND</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>S-WFOH</td>
<td>13.9 ± 1.8, competitive</td>
<td>15.0 ± 3.1, mixed</td>
<td>17.0 ± 4.1, mixed</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-MPOH</td>
<td>21.9 ± 2.2, competitive</td>
<td>7.3 ± 1.6, competitive</td>
<td>9.3 ± 1.6, competitive</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>BFOH</td>
<td>1.2 ± 0.1, competitive</td>
<td>1.5 ± 0.2, competitive</td>
<td>3.0 ± 0.5, competitive</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>TESOH</td>
<td>23.7 ± 4.6, competitive</td>
<td>13.2 ± 2.3, competitive</td>
<td></td>
</tr>
</tbody>
</table>

*For BROD, desmethylazelastine ranged from 10 to 50 μM.
For S-WFOH, azelastine and desmethylazelastine ranged 10 to 50 μM and 6-hydroxyazelastine ranged 20 to 100 μM.
For S-MPOH, azelastine, desmethylazelastine, and 6-hydroxyazelastine ranged from 10 to 50 μM, respectively.
For BFOH, all inhibitors ranged from 2 to 10 μM.
For TESOH, two inhibitors ranged from 10 to 50 μM.
For all IC_{50} determination, both the numbers of substrate and inhibitor concentrations were 4.
Data are expressed as mean ± S.E.
ND, not determined.
*6-Hydroxyazelastine interfered with the quantification of 6β-hydroxytestosterone.
micsomes or recombinant CYPs might possibly cause the contra-
diction.

If an enzyme reaction proceeds with a single enzyme and is inhibited competitively by other drugs, velocity (V) is expressed by eq. 1.

$$V = V_{\text{max}} \cdot S / (K_M + S)$$

(1)

where $I$ and $S$ indicate the concentrations of inhibitor and substrate, respectively. In addition, the inhibition type is noncompetitive, $V$ is expressed by eq. 2.

$$V = V_{\text{max}} / (K_M + S)$$

(2)

A percent inhibition in velocity is calculated as follows:

$$\text{% inhibition} = \left( \frac{V_o - V_i}{V_o} \right) \times 100$$

(3)

When we discuss the drug-drug interaction via inhibition of CYPs, it is important that the concentration of the inhibitor should be the concentration of the drug around the CYP. It is difficult to know the actual concentrations of drugs at the active site of CYP. However, in general, the concentration of the inhibitor can be approximated by the concentration in the liver. It has been reported that the $C_{\text{max}}$ values of azelastine and desmethylazelastine are 6.3 ± 3.5 and 3.4 ± 1.3 ng/ml, respectively, after repetitive oral administration of azelastine hydrochloride (4.4 mg every 12 h; Morganroth et al., 1997). These plasma concentrations of azelastine and desmethylazelastine are approximately 16.5 and 9.3 nM, respectively. 6-Hydroxyazelastine has been reported to be a main metabolite in rats and guinea pigs (Tatsumi et al., 1984). However, in humans, the concentration of 6-hydroxyazelastine in plasma is unknown. It has been reported that azelastine and its metabolites accumulate in the liver to a level almost 50-fold higher than the plasma concentration in experimental animals (Tatsumi et al., 1980, 1984). Accordingly, the concentrations of azelastine and desmethylazelastine in human livers could be projected to accumulate up to 0.8 and 0.5 μM, respectively. By using eq. 4, the percent inhibition of CYP2D6 can be estimated to be 40 and 25% with azelastine and desmethylazelastine, respectively. These results suggested the possibility of an interaction between azelastine and other drugs that are mainly metabolized by CYP2D6. Similarly, the percent inhibition of CYP2C9 can be estimated to be 5.4 and 3.2% with azelastine and desmethylazelastine, respectively. These values suggest the possibility that there might be no interaction between azelastine and warfarin or other drugs that are mainly metabolized by CYP2C9. Likewise, the interaction of azelastine and desmethylazelastine with drugs metabolized by CYP3A4 or CYP2C19 can be ruled out.

The results obtained in the present study that the inhibitory effects of azelastine on CYP2D6 activity were the most potent among the CYP isoforms tested were consistent with those of our previous study (M.N., S.N., S. Tokudome, N.S., H.Y. and T.Y., submitted) in which the $K_M$ value of CYP2D6 for azelastine $N$-demethylation was smaller than those of CYP3A4 and CYP1A2. Although the percent contribution of CYP2D6 for azelastine $N$-demethylation was estimated to be 1.4 to 45.1% (M.N., S.N., S. Tokudome, N.S., H.Y., and T.Y., submitted), the affinity would be high enough to cause drug interaction. Thus, even if the contribution of a CYP isoform to certain types of drug metabolism is relatively low, it is important to define the affinity of the CYP for the drug metabolism to predict the possibility of drug interaction.

In conclusion, the present study suggested that azelastine and its metabolites have the strong competitive inhibitory effects on CYP2D6. The possibility of an interaction of azelastine and other drugs that are mainly metabolized by CYP2D6 in humans should be considered, although it might not cause critical side effects in clinical use.

Acknowledgments. We thank Mikie Suzuki for the skillful technical assistance and Brent Bell for reviewing the manuscript.

References


