Oxidation of Histamine H1 Antagonist Mequitazine is Catalyzed by Cytochrome P450 2D6 in Human Liver Microsomes

KATSUNORI NAKAMURA, TSUYOSHI YOKOI, TAKAO KODAMA, KAZUAI INOUE, KAZUO NAGASHIMA, NORIYAKI SHIMADA, TOSHIKAZU SHIMIZU and TETSUYA KAMATAKI

Division of Drug Metabolism (K. Nakamura, T.Y., T. Kamataki), Faculty of Pharmaceutical Sciences and Department of Pathology (K. Nagashima) Faculty of Medicine, Hokkaido University, Sapporo 060, Japan; Department of Pathology (K.I.), Hokkaido University Medical Hospital, Sapporo 060, Japan; Hokkaido Association of Medical Service for Workers (T. Kodama), Sapporo 060, Japan; Daiichi Pure Chemicals Co., Ltd., Tokai Research Laboratories (N.S.), Ibaraki 319–11, Japan; Asahi Chemical Industry Co., Ltd. (T.S.), Tokyo 108, Japan

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ABSTRACT

Mequitazine [10-(3-quinuclidinylmethyl) phenothiazine] is a long-acting and selective histamine H1-receptor antagonist that is mainly biotransformed by human liver microsomes to yield hydroxylated and S-oxidized metabolites. Mequitazine hydroxylase was inhibited by propranolol and quinidine. Lineweaver-Burk plots for the hydroxylation and the S-oxidation indicated that the hydroxylation occurred with a low \( K_m \) (0.72 \( \pm \) 0.26 \( \mu \)M) in human liver microsomes. Microsomes from genetically engineered human B-lymphoblastoid cells expressing cytochrome P450 2D6 (CYP2D6) efficiently metabolized mequitazine to the hydroxylated and S-oxidized metabolites. The results indicated that CYP2D6 isozyme is a major form of CYP responsible for the metabolism of mequitazine in human liver microsomes.

The intensity and duration of drug effects depend on the individual variability of drug metabolism caused, in part, by genetic polymorphism of CYPs including CYP2D6, known as the debrisoquine/sparteine-type genetic polymorphism (Eichelbaum and Gross, 1990). CYP2D6 has a role in the metabolism of a wide range of drugs, particularly antiarrhythmic agents, \( \beta \)-blocking agents, antidepressants and other clinically useful drugs (Eichelbaum and Gross, 1990). In poor metabolizers, the low capacity to metabolize drugs results in a crucial role in the incidence and spectrum of side effects seen in some patients. If poor metabolizers are treated with a drug having a narrow therapeutic index, that is a substrate for CYP2D6, an exaggerated response and/or serious and toxic effects are expected to occur at therapeutic doses as a result of increased plasma concentration of the parental active form. The large interindividual variability in plasma concentrations of several tricyclic antidepressants seen in patients treated with similar doses has been attributed mainly to impaired metabolic capacity of CYP2D6 (Braun et al., 1986, Dahl and Bertilsson, 1993). Similar responses occur in extensive metabolizers if they are simultaneously treated with a potent inhibitor that competes with a substrate for the active site of CYP2D6. For example, during treatment with quinidine, EMs show the phenotype of PMs (Braun et al., 1987). Similarly, coadministration of imipramine and chlorpheniramine enhances the effect of imipramine in vivo in rats by the same mechanism (Alhaide and Mustafa, 1989).

Mequitazine is a potent, non-sedative and long-acting histamine H1 antagonist proven to be a better therapeutic drug than other conventional antihistamines (Fujimura et al., 1987). Adequate metabolic studies to determine which CYP isozyme mainly catalyzes this drug have not been reported in humans. The oxidative metabolism of mequitazine was extensively studied in rats and dogs (Uzan et al., 1976). Its

ABBREVIATIONS: CYP, cytochrome P450; FMO, flavin-containing monooxygenase; HPLC, high-performance liquid chromatography; PM, poor metabolizer; EM, extensive metabolizer.
primary metabolic pathways in rats consist of the aromatic hydroxylation of the phenothiazine structure, S-oxidation and N-oxidation of a side chain (Hojo et al., 1981, Soda et al., 1981). The oxidation of drugs having phenothiazine structure is generally known to be catalyzed by CYP and/or FMO (Cashman et al., 1993).

We investigated the effects of CYP inhibitors and anti-CYP antisera on the mequitazine metabolism to determine whether CYP is responsible for the metabolism of mequitazine in human liver microsomes. In addition, we have assessed the inhibitory effects of histamine H1 antagonists, including mequitazine, on the 1'-hydroxylation activity of midazolam in human liver microsomes. We found mequitazine as a novel substrate for CYP2D6 and exerting an inhibitory effect of CYP3A-mediated drug metabolism in vitro.

Methods

Materials. NADP+, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan), quinidine from Tokyo Kasei Kogyo (Tokyo, Japan), propranolol and 7-ethoxycoumarin from Aldrich Chemical (Milwaukee, WI), terfenadine from Sigma Chemical (St. Louis, MO), promethazine, phenacetin, coumarin and tolbutamide from Wako Pure Chemicals (Osaka, Japan). Sulfaphenazole was a kind gift from Ciba-Geigy Japan (Takarazuka, Japan), cyclosporine A from Sandoz Pharmaceuticals (Tokyo, Japan) and Azelastine, mequitazine and mequitazine metabolites from Asahi Chemical Industry (Tokyo, Japan), respectively. All other chemicals and solvents were of the highest grade commercially available.

Human liver microsomes. Human livers were obtained from patients after pathological examination of specimens isolated after death or during hepatic surgery. Surgeries were performed for the removal of metastatic liver tumors. The use of the human liver for these studies was approved by the Ethics Committee of Hokkaido University. Liver samples were stored at -80°C until use. Microsomes were prepared as described previously (Kamataki and Kitagawa, 1973). The microsomal protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Analytical procedures. Metabolites of mequitazine were determined by HPLC. A typical reaction mixture consisted of 50 μM mequitazine, 100 mM potassium-phosphate buffer (pH 7.4), 0.05 mM EDTA, an NADPH-generating system (0.5 mM NADP+, 5 mM MgCl2, 5 mM glucose 6-phosphate and 1 U/ml of glucose 6-phosphate dehydrogenase) and human liver microsome (0.5 mg/ml) in a final volume of 0.25 ml. The sample was subjected to the same HPLC systems as described above. Inhibition studies. Incubation of mequitazine with human liver microsomes. The proposed in vitro metabolic pathways of mequitazine in human liver microsomes are illustrated in figure 1. Mequitazine and its metabolites are well separated with HPLC. Typical HPLC chromatograms are illustrated in figure 2. Incubation of mequitazine with human liver microsomes yielded two major metabolites, both of which were produced linearly with an incubation time of up to 60 min.

Results

Metabolism of mequitazine in human liver microsomes. The proposed in vitro metabolic pathways of mequitazine in human liver microsomes are illustrated in figure 1. Mequitazine and its metabolites are well separated with HPLC. Typical HPLC chromatograms are illustrated in figure 2. Incubation of mequitazine with human liver microsomes yielded two major metabolites, both of which were produced linearly with an incubation time of up to 60 min.

Inhibition studies. To examine the possibility that the hydroxylation of mequitazine is catalyzed by a specific isozyme of CYP, the effects of inhibitors of CYP on the production of the hydroxylated metabolite were studied. A typical incubation mixture contained 100 mM potassium-phosphate (pH 7.4), 0.05 mM EDTA, an NADPH-generating system and 0.2 mg/ml microsomal protein. Phenacetin, caffeine, coumarin, 7-ethoxycoumarin, sulfaphenazole, tolbutamide, propranolol, chloroxazone or cyclosporine A was added to the incubation mixture as the relatively specific inhibitor of a CYP isozyme at a concentration of 100 μM. An inhibitor was added to the incubation mixture and preincubated for 5 min before the reaction was started with an NADPH-generating system. All inhibitors were dissolved in methanol and added to the incubation mixture at a final methanol concentration of 1%.

A typical incubation mixture for the assay of midazolam metabolism contained 100 mM potassium-phosphate (pH 7.4), 50 μM EDTA, 12.5 μM midazolam, an NADPH-generating system and 0.1 mg/ml microsomal protein in a final volume of 0.25 ml. Azelastine, mequitazine, promethazine or terfenadine was added to the incubation mixture as an inhibitor of a CYP at a concentration of 12.5, 25 or 50 μM. The sample was subjected to the same HPLC systems as described above, except that an analytical column TSK-GER ODS-120T (120 A, 5 μm, 4.6 mm i.d x 150 mm; Tosoh, Tokyo, Japan) was used. The mobile phase consisting of 10 mM sodium acetate (pH 7.8) acetonitrile/methanol (53:29:18, v/v/v) was delivered at a flow rate of 1.0 ml/min. The elution of midazolam and its metabolites was monitored at 240 nm. Other methods were the same as described above. For immuno-inhibition studies, the preparations of rabbit anti-human CYP2D6 serum and rabbit anti-rat CYP3A2 serum from Daiichi Pure Chemicals (Tokyo, Japan) were used. Rabbit anti-human CYP2D6 recognized CYP2D6 and did not cross-react with human CYP1A1, 1A2, 2A6, 2B6, 2C9, 2C19, 2E1 and 3A4 (N Shimada, unpublished data). The immuno-inhibition of mequitazine metabolism was examined by preincubation of human liver microsomes with various concentrations of anti-human CYP2D6 serum at room temperature for 30 min. Incubations were carried out as described above, except that mescaline protein 0.125 mg was added in a final volume of 0.25 ml.

Metabolism of mequitazine by human CYPs expressed in human B lymphoblastoid cells. Microsomes from human B-lymphoblastoid cells expressing human CYP1A1, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4 were obtained from Daiichi Pure Chemicals (Tokyo, Japan). As a negative control, microsomes from cells carrying the expression vector without CYP cDNA were used. Incubations were carried out as described above, except that microsomal protein (0.125 mg) was added in a final volume of 0.2 ml. Other methods were the same as described in the analytical procedure.
and with the amounts of microsomal protein of up to 1.0 mg per incubation. Typical Lineweaver-Burk plots are shown in figure 3a. Thus, the apparent CL\text{int} value of the hydroxylation and the S-oxidation were estimated to be 38 and 4.5 ml/min/nmol P450 in human liver microsomes (HL12), respectively. Eadie-Hofstee plots for the hydroxylation of mequitazine by human liver microsomes (HL12, HL29 and HL35) are shown in figure 3b. A biphasic pattern in the hydroxylation was seen. These results suggest that at least two enzymes are involved in the hydroxylation. The kinetic parameters of hydroxylation for high- (\(K_m^1, V_{\text{max}}^1\)) and low- (\(K_m^2, V_{\text{max}}^2\)) affinity enzymes were calculated. Thus, apparent \(K_m^1\) was estimated to be .72 ± .26 \(\mu\)M with \(V_{\text{max}}^1\) of 17 ± 6.6 pmol/min/mg protein for the high-affinity enzyme. The apparent \(K_m^2\) was 4.7 ± 2.2 \(\mu\)M and \(V_{\text{max}}^2\) was 15 ± 8.8 pmol/min/mg protein for the low-affinity enzyme (mean ± S.D., \(n = 3\)).

**Effects of CYP inhibitors on the hydroxylation of mequitazine.** To clarify the forms of CYP responsible for the hydroxylation of mequitazine, the effects of inhibitors on the activity of mequitazine hydroxylase were examined (fig. 4). Specific inhibitors of CYPs employed in the present study included phenacetin (an inhibitor of CYP1A1 and CYP1A2) (Tassaneeyakul et al., 1993), caffeine (CYP1A2) (Tassaneeyakul et al., 1994), coumarin (CYP2A6) (Yamano et al., 1990), 7-ethoxycoumarin (CYP2B6) (Baker et al., 1995), sulfaphenazole, tolbutamide (CYP2C8, CYP2C9) (Rettie et al., 1992), propranolol (CYP2D6) (Distlerath et al., 1985), chlorozoxazine (CYP2E1) (Hyland et al., 1992) and cyclosporin A (CYP3A) (Kronbach et al., 1988; Fischer et al., 1994). Among these inhibitors, propranolol inhibited the mequitazine hydroxylase potently (about 80% inhibition), although cyclosporin A inhibited the hydroxylase activity slightly (about 20% inhibition).
rin A inhibited weakly (about 20% inhibition). These results suggest that the mequitazine hydroxylation was mainly catalyzed by CYP2D6.

To further confirm the involvement of CYP2D6 in the mequitazine hydroxylation, quinidine, a known typical and potent inhibitor of CYP2D6 (Otton et al., 1984; Rodrigues 1994) was added to the reaction mixture (fig. 5). As a result, mequitazine hydroxylation was suppressed by the inhibitor in a concentration-dependent manner. These results further support the idea that CYP2D6 is the major enzyme that catalyzes the hydroxylation of mequitazine in human livers.

**Immuno-inhibition by rabbit anti-human CYP2D6 serum of the metabolism of mequitazine in human liver microsomes.** To confirm the involvement of CYP2D6 in the hydroxylation of mequitazine, an immuno-inhibition study was performed using rabbit anti-human CYP2D6 antibodies (fig. 6). Rabbit anti-human CYP2D6 antibodies inhibited the mequitazine hydroxylase in liver microsomes from the human subject of HL12. At the highest concentration of the antibodies, the hydroxylation of mequitazine was inhibited by 90%. This result is consistent with the results of the effects of inhibitors and lends support to the idea that CYP2D6 is mainly involved in the hydroxylation of mequitazine in human liver microsomes.

**Metabolism of mequitazine by CYPs expressed in microsomes of human B-lymphoblastoid cells.** Metabolism of mequitazine by microsomes from human B lymphoblastoid cells expressing human CYPs (CYP1A1, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4) was investigated (fig. 7). The hydroxylation was exclusively catalyzed by CYP2D6. These results further support the notion that CYP2D6 is the key enzyme that catalyzes the metabolism of mequitazine in human livers.

**Effects of histamine H1 antagonists on the catalytic activities of CYP3A4.** Biotransformation of the histamine H1 antagonist terfenadine is well known to be mediated specifically by CYP3A4 in human liver microsomes. Thus, terfenadine was expected to be an inhibitor of CYP3A. Together with this possibility, it was examined whether or not other histamine H1 antagonists, including mequitazine, were capable of inhibiting the CYP3A-mediated drug metabolism, adopting midazolam as a substrate. Midazolam 1'-hydroxylation is a highly selective CYP3A reaction (Gorski et al., 1994). As shown in figure 8, midazolam 1'-hydroxylase was inhibited by histamine H1 antagonists used in our study. The kinetic values for the inhibition by the histamine H1 antagonists in human liver microsomes are shown in Table 1. The *K*<sub>i</sub> values of these
drugs showing potent inhibition were estimated by Lineeweaver-Burk plots (Cornish-Bowden and Eisenthal, 1978). The $K_i$ values (mean ± SD, $n = 5$) of mequitazine, azelastine, and terfenadine for the midazolam 1'-hydroxylase were 45.9 ± 19.6, 14.5 ± 2.7 and 14.6 ± 4.2 μM, respectively.

### Discussion

This study was originated with a sparteine PM subject who exhibited serious sedation by taking histamine H1 antagonist. This PM subject had a new mutation in the CYP2D6 (data not shown). Judging from the fact that the plasma concentration of mequitazine in PM subjects might reach a level higher than was expected even after administration of therapeutic doses.

### Acknowledgment

We thank Dr. Hiroko Sato, Hoffmann-La Roche, Nutley, USA, for providing Midazolam and its metabolites.

### References


Nelson DR, Roymans L, Kamataki T, Stegeman JJ, Fryerisens R, Waxman DJ, Waterman MR, Getoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW (1986) three orders lower than the inhibition constants for CYP3A-mediated midazolam 1'-hydroxylation by histamine H1 antagonists, azelastine, mequitazine and terfenadine. This makes it unlikely that histamine H1 antagonist mequitazine would cause drug interactions on CYP3A-metabolizing drugs, since plasma concentrations of mequitazine are quite low. Because CYP2D6 was proven in our study to play a major role in mequitazine metabolism, the plasma concentration of mequitazine in CYP2D6 PM subjects might reach a level higher than was expected even after administration of therapeutic doses.

### Table 1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Type of Inhibition</th>
<th>Apparent $K_i$ (μM) (n = 5, mean ± S.E.)</th>
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<tr>
<td>Mequitazine</td>
<td>Mixed</td>
<td>45.9 ± 19.6</td>
</tr>
<tr>
<td>Azelastine</td>
<td>Mixed</td>
<td>14.5 ± 2.7</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>Mixed</td>
<td>14.6 ± 4.2</td>
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TABLE 1 $K_i$ values for the inhibition of midazolam 1'-hydroxylase in human liver microsomes by three histamine H1 antagonists.


Send reprint requests to: Dr. Tetsuya Kamataki, Division of Drug Metabolism, Faculty of Pharmaceutical Sciences, Hokkaido University, N12W6, Sapporo 060 Japan.