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

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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 ± 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. Inhibition of CYP3A-catalyzed midazolam 1'-hydroxylase by various histamine H1 antagonists, including mequitazine, suggested that mequitazine and some other histamine H1 antagonists could also be inhibitors of CYP3A in human liver microsomes.

Many isozymes of CYP have been identified, purified, cloned and expressed (Nelson et al. 1996). CYP consists of a superfamily of mixed-function monoxygenases associated with the metabolism of a broad group of structurally unrelated compounds, including drugs, chemical carcinogens, environmental pollutants and endogenous substrates such as steroids, fatty acids and prostaglandins (Guengerich, 1991). The intensity and duration of drug effects depend on the activity of CYP and other drug metabolizing enzymes in the liver. It is widely recognized that the individual variability of drug metabolism is 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 (Brøsen 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, EMCs show the phenotype of PMs (Brøsen et al., 1987). Similarly, coadministration of imipramine and chlorpheniramine enhances the effect of imipramine \textit{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. 1981). 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

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ABBREVIATIONS: CYP, cytochrome P450; FMO, flavin-containing monoxygenase; 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'-hydroxylase 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.** NADPH, 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), cyclosporin 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 Kita-gawa, 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), 0.5 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 .25 ml. The reaction mixture was preincubated at 37°C for 5 min. Reaction was initiated by the addition of the NADPH-generating system and 0.1 mg/ml of microsomal protein (0.25 ml). The sample was subjected to the same HPLC systems as described above, except that an analytical column TSK-GEL ODS-120T (120 Å, 5 μm, 4.6 mm i.d.x 150 mm; Waters, 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.

**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 various concentrations of anti-human CYP2D6 serum and rabbit anti-rat CYP3A2 serum 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 0.25 ml.

**Results.**

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.

**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.
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- ($K_m^1$, $V_{\max}^1$) and low- ($K_m^2$, $V_{\max}^2$) affinity enzymes were calculated. Thus, apparent $K_m^1$ was estimated to be $0.72 \pm 0.26 \mu M$ with $V_{\max}^1$ of 17 $\pm$ 6.6 pmol/min/mg protein for the high-affinity enzyme. The apparent $K_m^2$ was 4.7 $\pm$ 2.2 $\mu M$ and $V_{\max}^2$ was 15 $\pm$ 8.8 pmol/min/mg protein for the low-affinity enzyme (mean $\pm$ 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 cyclospo-
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 CYP3A 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 $K_i$ values of these

![Fig. 5](image-url)  
Fig. 5. Inhibition by quinidine of the mequitazine metabolism by human liver microsomes. Mequitazine was used at a concentration of 10 μM. Liver microsomes from a human subject (HL12) were used for the mequitazine hydroxylation. The rate of the hydroxylation in the absence of an inhibitor was 44 pmol/min/mg prot. Incubations were performed in duplicate.

![Fig. 6](image-url)  
Fig. 6. Immuno-inhibition by anti-human CYP2D6 serum of the hydroxylation of mequitazine by human liver microsomes. Mequitazine was used at a concentration of 10 μM. Microsomes (HL12) were preincubated in the presence of indicated amounts of rabbit anti-human CYP2D6 serum (○) and rabbit preimmune serum (□) at room temperature for 30 min. Mequitazine was used at a concentration of 10 μM. Each datapoint represents the mean of duplicate determinations.

![Fig. 7](image-url)  
Fig. 7. Metabolism of mequitazine by microsomes from genetically engineered B-lymphoblastoid cells expressing a specific human CYP. Microsomes (HL12) were preincubated in the presence of an NADPH-generating system for 5 min. Mequitazine was used at 5 μM concentration. The hydroxylated metabolite of mequitazine was determined. Each datapoint represents the mean of duplicate determinations.
drugs showing potent inhibition were estimated by Line-weaver-Burk plots (Cornish-Bowden and Eisenhal, 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 gene designated as CYP2D6*18 (Yokoi et al., 1996). Phenotyping studies with debrisoquine, sparteine and metoprolol have shown that the expression of human CYP2D6 is polymorphic in a number of racial groups, and approximately 84% of Japanese are PMs (Nakamura et al., 1985; Ishizaki et al., 1987; Sohn et al., 1991). Mequitazine has been marketed in Japan and several European countries for the treatment of bronchial asthma and allergic manifestations. Mequitazine, as with other structurally related phenothiazines, is extensively metabolized in mammalian species. Studies conducted with human liver microsomes have shown that mequitazine was primarily converted to S-oxidized and hydroxylated compounds. This was in agreement with the results of in vivo metabolic studies performed by analysis of human urine (data not shown). Judging from the fact that the $V_{max}/K_m$ of mequitazine hydroxylation was higher than that of the S-oxidation, this pathway was assumed to play a major role in the metabolism of mequitazine. In our study, the in vitro formation of hydroxylated metabolites from mequitazine by human liver microsomes was strongly inhibited by a well-known CYP2D6 inhibitor, quinidine. Although the amount of CYP2D6 in human liver microsomes was estimated to be only about 5 pmol/mg of microsomal protein, or about 1.5% of total CYP (Shimada et al., 1994), CYP2D6 was found to be the enzyme mainly involved in the hydroxylation of mequitazine in our study. These findings suggest that mequitazine is likely to interact with human CYP2D6 in vivo with the potential of causing drug interaction. Among other histamine H1 receptor antagonists, mepyramine was reported to have a high affinity for human CYP2D6 (Hiroi et al., 1995) and the metabolism of loratadine was also partially inhibited by quinidine (Yumibe et al., 1995). CYP2D6 was proven in our study to play a major role in mequitazine metabolism, the plasma concentration of mequitazine in PM subjects might reach a level higher than was expected even after administration of therapeutic doses.

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### 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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<tbody>
<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>
</tr>
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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.

### Note

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.

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