INTERACTION OF TERFENADINE AND ITS PRIMARY METABOLITES WITH CYTOCHROME P450 2D6

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ABSTRACT:
The substrate structure-activity relationships described for the major human drug-metabolizing cytochrome P450 (P450 or CYP) enzymes suggest that the H1 receptor antagonist terfenadine could interact with CYP2D6 either as a substrate or as an inhibitor, in addition to its known ability to act as a substrate for CYP3A4. Based on this substrate structure-activity relationship, computer modeling studies were undertaken to explore the likely interactions of terfenadine with CYP2D6. An overlay of terfenadine and dextromethorphan, a known substrate of CYP2D6, showed that terfenadine would be possible to superimpose the site of hydroxylation (t-butyl group) and the nitrogen atom of terfenadine with similar regions in dextromethorphan. These observations were substantiated by the ease of docking of terfenadine into a protein model of CYP2D6. Experimentally, terfenadine inhibited CYP2D6 activity in human liver microsomes with an IC50 of 14–27 μM, depending on the CYP2D6 substrate used. The inhibition of CYP2D6 was further defined by determining the Kι for terfenadine against bufuralol 1′-hydroxylase activity in four human livers. Terfenadine inhibited bufuralol 1′-hydroxylase activity with a Kι of approximately 3.6 μM. The formation of the hydroxylated metabolite (hydroxyterfenadine) in microsomes prepared from human liver and specific P450 cDNA-transfected B lymphoblastoid cells indicated that only CYP2D6 and CYP3A4 were involved in this transformation. As expected, the rate of formation was greatest with CYP3A4 (Vmax = 1257 pmol/min/nmol of P450), with CYP2D6 forming the metabolite at 6-fold lower rate (Vmax = 206 pmol/min/nmol of P450). The two enzymes had similar Km values (9 and 13 μM, respectively). These data indicate that, as predicted from modeling studies, terfenadine has the structural features necessary for interaction with CYP2D6.

TERFENADINE, a nonsedating H1 receptor antagonist, is used in the treatment of histamine-mediated allergic conditions such as allergic rhinitis (Woodward and Munro, 1982). In humans, terfenadine is well absorbed but undergoes extensive first-pass metabolism. It is metabolized by two routes, i.e. N-dealkylation to azacyclonol and hydroxylation of the t-butyl group to form hydroxyterfenadine (Garteiz et al., 1982). Hydroxyterfenadine undergoes subsequent oxidation to the corresponding carboxylic acid, which is thought to be the biologically active antihistamine (von Moltke et al., 1994). CYP3A4 has been shown to be the principal P450 enzyme involved in the metabolism of terfenadine to both azacyclonol and hydroxyterfenadine (Yun et al., 1993) (fig. 1).

Terfenadine exhibits adverse clinical drug interactions, which have been related to inhibition of its CYP3A4-mediated metabolism (Jumr-Romet et al., 1994). These adverse interactions are manifested as torsade de pointes, brought about by QT prolongation and ventricular arrhythmias in susceptible individuals (Kivisto et al., 1994). This toxicity has been related to an increase in terfenadine plasma levels as a result of administration of known CYP3A4 inhibitors such as ketoconazole (Honig et al., 1993).

In terms of chemical structure, terfenadine can be considered to be a lipophilic arylalkylamine. The SSARs described for the major human drug-metabolizing P450s suggest that terfenadine should be a CYP3A4 substrate, because it is lipophilic and contains a basic nitrogen atom (Smith and Jones, 1992). However, these same physicochemical features also suggest that terfenadine could interact with CYP2D6, either as a substrate or as an inhibitor (Smith and Jones, 1992). The important feature that determines whether a compound is a CYP2D6 substrate is the distance between the site of metabolism and the basic nitrogen atom. Template models developed for CYP2D6, using substrate/inhibitor overlays, have determined this distance to be 5–7 Å (Koymans et al., 1992; Strobl et al., 1993). The butyl chain in the center of the terfenadine molecule has sufficient flexibility to allow terfenadine to exist in conformations that could satisfy the CYP2D6 substrate/inhibitor criteria. The compound therefore is a useful probe, because it is not a member of the recognized families of CYP2D6 inhibitors, such as cyclic antidepressants.

We are engaged in an ongoing program of research to validate the use of P450 models in the prediction of drug metabolism. The CYP2D6 models are most advanced in their development, and it should be possible in the near future to use them either for drug designing or for screening to select compounds that interact with this isoform. The purpose of this study was therefore to investigate whether, as the SSAR predicts, terfenadine and its metabolites interact with CYP2D6 as either substrates or inhibitors.

Materials and Methods

Chemicals and Reagents. Furafylline, 4′-hydroxydiclofenac, (S)-mephénytoin, 4-hydroxymephenytoin, bufuralol, 1′-hydroxybufuralol, hydroxyterfenadine, and sulfaphenazole were obtained from Salford Ultrafine Chemicals.

1 Abbreviations used are: CYP or P450, cytochrome P450; SSAR, substrate structure-activity relationship.

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and its metabolites. Liver microsomes prepared from a pool of equal amounts of four human livers.

Substrates by terfenadine and its metabolites was investigated using human

Sato (1964), and protein concentrations were determined using the method of

potassium phosphate (pH 7.4) and stored at

contaminating hemoglobin. The final pellet was resuspended in 100 mM

g to pellet the microsomal fraction. This pellet was washed with 100 mM

for 60 min, to remove any

were obtained from Gentest Corp. (Exton, PA). Hepatic microsomes were prepared from individual human livers

obtained from the International Institute for the Advancement of Medicine

and Research Ltd. (Manchester, UK). Specific P450-cDNA-transfected human

B lymphoblastoid cell-derived microsomes were obtained from Gentest Corp.

(Woburn MA). All other reagents were obtained from Sigma Chemical Co.

(Dorset UK) and were of the highest grade available.

Computer Modeling. Computer modeling studies were undertaken using Quanta (Molecular Simulations, Burlington, MA) and Sybyl (Tripos Associates, St. Louis, MO) software. Protein homology models of CYP2D6 were obtained from the report of Modi et al. (1996) and were used without modification. Dextromethorphan was used in its X-ray crystal structure conformation, whereas reasonable low-energy conformers of terfenadine were modeled and energy-minimized using Sybyl. Terfenadine was manually docked into the CYP2D6 active site using Quanta.

Preparation of Microsomes. Transplant-quality human liver tissue was obtained from the International Institute for the Advancement of Medicine (Exton, PA). Hepatic microsomes were prepared from individual human livers by the process of differential centrifugation. The liver tissue was homogenized in 50 mM Tris-HCl (pH 7.4) containing 250 mM sucrose and was then centrifuged at 9000g for 20 min, to remove cell debris and the nuclear fraction. The supernatant was removed and further centrifuged at 105,000g for 60 min, to pellet the microsomal fraction. This pellet was washed with 100 mM Tris-HCl (pH 7.4) and centrifuged at 105,000g for 60 min, to remove any contaminating hemoglobin. The final pellet was resuspended in 100 mM potassium phosphate (pH 7.4) and stored at −80°C until use. For the chemical inhibition experiments, a separate batch of microsomes was produced from a combination of equal amounts of four human livers (HM-3, HM-6, HM-14, and HM-16). P450 contents were determined using the method of Omura and Sato (1964), and protein concentrations were determined using the method of Lowry et al. (1951), with bovine serum albumin as the protein standard.

Inhibition of Metabolism of P450-Selective Substrates by Terfenadine and Its Metabolites. The inhibition of the metabolism of P450-selective substrates by terfenadine and its metabolites was investigated using human liver microsomes prepared from a pool of equal amounts of four human livers. Terfenadine and its metabolites were incubated in a concentration range of 0–1000 μM, in the presence of P450-selective substrates at concentrations equal to their previously determined Km values. The inhibitory effects of terfenadine and its metabolites were assessed by determining IC50 values.

The methods for determining the P450-selective enzyme activities were as described previously. Phenacetin O-deethylation activity (CYP1A2) was determined at a substrate concentration of 10 μM (Jones et al., 1997), diclofenac 4'-hydroxylase activity (CYP2C9) was determined at a substrate concentration of 40 μM (Jones et al., 1997), (S)-mephénytoin 4'-hydroxylase activity (CYP2C19) was determined at a substrate concentration of 130 μM (Meier et al., 1985), bufuralol 1'-hydroxylase activity (CYP2D6) was determined at a substrate concentration of 10 μM (Kronbach et al., 1987), debrisoquine 4'-hydroxylase activity (CYP2D6) was determined at a substrate concentration of 100 μM (Kronbach et al., 1987), dextromethorphan O-demethylase activity (CYP2D6) was determined at a substrate concentration of 10 μM (Kronbach et al., 1987), and testosterone 6β-hydroxylase activity (CYP3A4) was determined at a substrate concentration of 150 μM (Punne and Imaoka, 1987). Chlorzoxazone 6-hydroxylase activity (CYP2E1) was determined with 33 μM chlorzoxazone and 0.5 mg/ml microsomal protein for 15 min, in a total incubation volume of 120 μl. The incubations were terminated by the addition of 10 μl of perchloric acid (600 mM), followed by 10 μl of the internal standard (oxazolamine, 10 μg/ml). The precipitated microsomal protein was pelleted by centrifugation at 3000 rpm for 5 min. The supernatant was removed, and 80 μl was injected into the HPLC system. The samples were chromatographed on a 15-cm Spherisorb-5-ODS2 column with a mobile phase of 20 mM sodium perchlorate (pH 2.5)/acetoni trile (70:30, v/v), at a flow rate of 1 ml/min. Detection was by UV absorbance (Shimadzu SPD-6A) at 295 nm. Under these conditions, 6-hydroxychlorzoxazone had a retention time of 3 min, oxazolamine had a retention time of 5 min, and chlorzoxazone had a retention time of 9 min.

The Ki for terfenadine against CYP2D6 was determined in hepatic microsomes prepared from four individual human livers, using bufuralol 1'-hydroxy-lyse as the CYP2D6-selective reaction. The Ki was estimated from the IC50 value using the following equation (Segel, 1993): 

Ki = IC50/[1 + ([S]/Km)], where [S] is the bufuralol concentration (10 μM) and Km is the Km for bufuralol in human liver microsomes (10 μM). Based on this estimated Ki, the incubations were carried out at terfenadine concentrations equal to 1/5Km, 1/2Km, 2Km, and 4Km, and bufuralol concentrations equal to 1/5Km, 1/2Km, 2Km, and 4Km.

Assay for Terfenadine and Hydroxysterfenadine Metabolism. The metabolism of terfenadine and hydroxysterfenadine by human liver microsomes or expressed recombinant P450s was assessed according to the following method. Each incubation (final volume, 120 μl) was composed of microsomal protein, 50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, and 5 μM MnCl2. Reducing equivalents required for P450 metabolism were provided by NADPH, which was regenerated in situ by an isocitric acid/isocitric acid dehydrogenase system. The incubation mixture was preincubated at 37°C, in the presence of substrate, before the addition of NADPH to initiate the reaction.

At the end of each incubation, the reaction was terminated by the addition of 10 μl of 600 mM perchloric acid to precipitate the microsomal protein. The precipitated microsomal protein was pelleted by centrifugation at 3000 rpm for 5 min. The supernatant was removed, and 80 μl was subjected to HPLC analysis.

HPLC Analysis. The formation of hydroxysterfenadine was determined using the method of Yun et al. (1993). Samples were chromatographed on a Spherisorb-5-CN column (150 × 4.6 mm i.d.; Hichrom), with elution with 10 mM ammonium acetate (pH 4)/acetoni trile/methanol (55:22.5:22.5, v/v), at a flow rate of 1.3 ml/min. Quantitation was achieved using a fluorescence detector (Merck-Hitachi F1080) operating with an excitation wavelength of 230 nm and an emission wavelength of 270 nm. Under these conditions, azacyclonol had a retention time of 4 min, hydroxyterfenadine had a retention time of 6 min, and terfenadine had a retention time of 8 min. Quantitation of hydroxyterfenadine was achieved by interpolation from standard curves constructed with microsomes using authentic hydroxyterfenadine (1–1000 ng).

For the metabolism of hydroxyterfenadine, samples were chromatographed on a Spherisorb-5-CN column (150 × 4.6 mm i.d; Hichrom), with elution with 10 mM ammonium acetate (pH 4)/acetoni trile/methanol (75:22.5:22.5, v/v), at a flow rate of 0.7 ml/min. Quantitation was achieved using a fluorescence detector (Merck-Hitachi F1080) operating with an excitation wavelength of 230 nm and an emission wavelength of 270 nm. Under these conditions, azacyclonol had a retention time of 10 min, carboxyterfenadine had a retention time of 15 min, and hydroxyterfenadine had a retention time of 19 min.
Metabolism by Expressed Recombinant P450s. The incubations with microsomes derived from specific P450 cDNA-transfected human B lymphoblastoid cells were conducted as described above, with terfenadine and hydroxyterfenadine concentrations of 5 μM, incubation times of 30 min, and protein concentrations of 1 mg/ml. The kinetic constants (V_max and K_M) for the formation of hydroxyterfenadine from terfenadine in microsomes derived from B lymphoblastoid cells expressing only CYP2D6 or CYP3A4 were determined under conditions of linearity with respect to time and protein concentration (1 mg/ml microsomal protein, for 60 min), with terfenadine concentrations of 0.5–100 μM.

Metabolism by Human Liver Microsomes. The kinetic constants (V_max and K_M) for hydroxyterfenadine formation from terfenadine in human liver microsomes were determined under conditions of linearity with respect to protein concentration and time (0.5 mg/ml microsomal protein, for 30 min), with terfenadine concentrations of 1–100 μM.

Chemical Inhibition of Terfenadine Metabolism by Specific P450 Inhibitors. In the chemical inhibition experiments, methanolic stock solutions of each inhibitor were added to the incubation mixtures before initiation of the reaction. In all cases, the methanol concentration did not exceed 0.1% (v/v). This resulted in a minimal inhibitory effect (<10%) on the rate of terfenadine hydroxylation. The concentration of terfenadine used in the chemical inhibition experiments was 50 μM. Each of the specific inhibitors was used at two concentrations, which were found to yield 50 and 90% inhibition of the relevant P450 enzyme activity, as determined using an appropriate probe substrate.

Analysis of Results. All results are presented as the mean ± SD. Determination of apparent K_M, V_max, K_i, and IC_50 values were carried out using GraFit version 3 (Leatherbarrow, 1992).

Results

Computer Modeling. Computer modeling studies were undertaken to explore the likely binding conformation and interactions of terfenadine with CYP2D6. An overlay between terfenadine (fig. 2, magenta) and dextromethorphan (fig. 2, yellow), a known substrate of CYP2D6 (Koymans et al., 1992; Guengerich, 1995), showed that it was possible to overlay the site of oxidation, the phenyl group, and the nitrogen atom with similar regions in dextromethorphan (fig. 2), suggesting that the compound was a potential CYP2D6 substrate and/or inhibitor.

To investigate this further, terfenadine was manually docked into the active site of a protein homology model of CYP2D6 (Modi et al., 1996), which had been developed by a direct study of the interaction of codeine with the enzyme using paramagnetic relaxation effects on the NMR spectrum of the substrate (fig. 3). Positioning of the t-butyl group of terfenadine 4.5 Å from the catalytic surface of the heme group allowed placement of the protonated nitrogen in the region of Asp-301 (fig. 3), the amino acid responsible for binding (Ellis et al., 1995). Consequently, the bulky diphenyl-4-piperidinomethanol group was orientated in a lipophilic pocket bounded by sections of the F...
Amino acids that appeared to be in direct contact with the diphenyl-4-piperidinomethanol group in this model included Ala-300, Leu-248, Phe-247, Leu-208, Gly-113, and Pro-114.

**Effect of Terfenadine on P450 Enzyme-Selective Activities.** The ability of terfenadine to inhibit the metabolism of P450 enzyme-selective substrate probes was investigated with microsomes prepared from equal amounts of four different human livers. In the concentration range used in this study, terfenadine did not significantly inhibit the activity of substrate probes for CYP1A2, CYP2C9, CYP2C19, or CYP2E1 (IC<sub>50</sub> > 100 μM). Terfenadine was an inhibitor of testosterone 6β-hydroxylase activity (a substrate probe activity for CYP3A4), with an IC<sub>50</sub> of 62 μM. Terfenadine was previously shown to be a substrate for CYP3A4 (Yun et al., 1993); hence, the inhibition of testosterone 6β-hydroxylase activity was expected. In addition, terfenadine was also found to inhibit bufuralol 1'-hydroxylase activity (an assay that represents CYP2D6 activity), with an IC<sub>50</sub> of 15 μM.

It has been reported that CYP1A2 also contributes to bufuralol 1'-hydroxylase activity in human liver microsomes (Yamazaki et al., 1994), albeit to a lesser extent than CYP2D6. However, the lack of inhibition of phenacetin O-deethylase activity by terfenadine suggests that the inhibition of bufuralol 1'-hydroxylase is the result of inhibition of CYP2D6. To further investigate the inhibition of CYP2D6 by terfenadine, two additional CYP2D6-selective substrates, i.e. debrisoquine and dextromethorphan, were used. Terfenadine inhibited the CYP2D6-mediated 4'-hydroxylation of debrisoquine and O-demethylation of dextromethorphan with IC<sub>50</sub> values of 27 and 14 μM, respectively, supporting the contention that terfenadine inhibited CYP2D6 activity.

The inhibition of CYP2D6 activity by terfenadine was further characterized by the determination of K<sub>i</sub> values against bufuralol 1'-hydroxylase activity in human liver microsomes prepared from four separate individuals, possessing a range of CYP2D6 activities. Terfenadine was a competitive inhibitor of bufuralol 1'-hydroxylase activity in all four livers, with a mean K<sub>i</sub> of 4.6 μM (table 1). A Dixon plot illustrating the results obtained from the liver designated HM-3 is shown in fig. 4.

Terfenadine is metabolized in human liver microsomes by two routes, i.e. N-dealkylation to azacyclonol and hydroxylation of the t-butyl group to form hydroxyterfenadine, which is subsequently metabolized to carboxyterfenadine. All of these metabolites retain the protonated basic center, which is critical for binding to CYP2D6. Therefore, each metabolite was investigated with respect to its ability to inhibit CYP2D6-mediated 1'-hydroxylation of bufuralol.

Both azacyclonol and carboxyterfenadine showed weak inhibition...
TABLE 1

Inhibitory constant values for the inhibition of bufuralol 1'-hydroxylase activity by terfenadine, determined in microsomes prepared from four individual human livers

<table>
<thead>
<tr>
<th>Liver Sample</th>
<th>$K_i$ (µM)</th>
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<tbody>
<tr>
<td>HM-3</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>HM-6</td>
<td>5.8 ± 1.5</td>
</tr>
<tr>
<td>HM-14</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>HM-16</td>
<td>6.5 ± 1.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.6 ± 1.9</td>
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</table>

Results are expressed as the mean ± SD of triplicate determinations.

![Graph](image.png)

**Fig. 4.** Dixon plot of the effect of terfenadine on bufuralol 1'-hydroxylase activity in human liver microsomes derived from the human liver designated HM-3.

Each data point represents the mean ± SD of triplicate determinations.

of CYP2D6 ($IC_{50}$ values of ≈100 and >300 µM, respectively), whereas hydroxyterfenadine inhibited bufuralol 1'-hydroxylase activity with an $IC_{50}$ of 18 µM (fig. 5). The inhibition by hydroxyterfenadine was comparable to that observed with terfenadine ($IC_{50} = 15$ µM).

**Metabolism by Expressed Recombinant P450s.** The oxidation of terfenadine (5 µM) to hydroxyterfenadine was investigated in microsomes derived from B lymphoblastoid cells expressing human CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 (fig. 6). Under these conditions, only CYP2D6 and CYP3A4 formed hydroxyterfenadine. In addition to hydroxyterfenadine, the CYP3A4-expressing cell line also formed significant amounts of azacyclonol.

Because hydroxyterfenadine had the same inhibitory potency as terfenadine for CYP2D6, the conversion of hydroxyterfenadine to carboxyterfenadine was also investigated in microsomes derived from B lymphoblastoid cells expressing human CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. Under these conditions, only CYP3A4 metabolized hydroxyterfenadine, producing azacyclonol and carboxyterfenadine (data not shown).

The combination of experiments described above indicate that CYP2D6 does have the capacity to hydroxylate terfenadine but does not metabolize hydroxyterfenadine to carboxyterfenadine. Therefore, the kinetics of formation of hydroxyterfenadine from terfenadine were determined in microsomes derived from B lymphoblastoid cells expressing CYP2D6 or CYP3A4.

Under conditions of linearity with respect to time and protein concentration (1 mg/ml microsomal protein, for 60 min), B lymphoblastoid cells expressing CYP2D6 showed Michaelis-Menten saturation kinetics with increasing substrate concentrations up to 100 µM. These data are shown in fig. 7A. The apparent $K_M$ for the CYP2D6-mediated hydroxylation of terfenadine was 13 µM.

![Graph](image.png)

**Fig. 5.** Effects of carboxyterfenadine, hydroxyterfenadine, and azacyclonol on bufuralol hydroxylation in human liver microsomes.

Each data point represents the mean ± SD of triplicate determinations.

![Graph](image.png)

**Fig. 6.** Rates of hydroxyterfenadine formation in microsomes from a panel of B lymphoblastoid cell lines expressing different human P450s.

Each data point represents the mean ± SD of triplicate determinations.

Similar experiments were conducted with microsomes derived from B lymphoblastoid cells expressing CYP3A4. In this case, the kinetics were also determined with 1 mg/ml microsomal protein for 60 min, with terfenadine concentrations of 1–100 µM. As with CYP2D6, CYP3A4 showed Michaelis-Menten saturation kinetics (fig. 7B). The apparent $K_M$ for the CYP3A4-mediated hydroxylation of terfenadine was 9 µM.

Thus, the $K_M$ values for CYP2D6 and CYP3A4 were similar. However, the $V_{max}$ values differed by a factor of approximately 6 ($V_{max}$ for CYP2D6, 206 pmol/min/nmol of P450; $V_{max}$ for CYP3A4, 1257 pmol/min/nmol of P450). Calculation of the $V_{max}/K_M$ ratio (a measure of catalytic efficiency) for each enzyme yielded values of 16 µl/min/nmol of P450 for CYP2D6 and 140 µl/min/nmol of P450 for CYP3A4. Thus, although CYP2D6 is capable of hydroxylation terfenadine, it does so approximately 8 times less efficiently than does CYP3A4.

**Enzyme Kinetics in Human Liver Microsomes.** Terfenadine is metabolized to hydroxyterfenadine in human liver microsomes. The hydroxylation was linear with time up to 45 min and with protein concentration up to 1 mg/ml. The apparent kinetic constants for this conversion were estimated using terfenadine concentrations up to 100 µM. The conversion followed Michaelis-Menten kinetics in the four human livers investigated. This is illustrated for HM-16 in fig. 8, with the corresponding Eadie-Hofstee plot (fig. 8, inset), which suggests either that a single enzyme is responsible for this transformation or that multiple enzymes with similar $K_M$ values mediate the transformation. The results obtained with microsomes from B lymphoblastoid cells expressing CYP2D6 and CYP3A4 suggest that there are two
enrich formation in human liver microsomes by Rodrigues et al.
using furafylline (CYP1A2), sulfaphenazole (CYP2C9), quinidine
zyme-Selective Inhibitors/Substrates.

Typical Michaelis-Menten plots describing the hydroxylation of
terfenadine in microsomes derived from B lymphoblastoid cells expressing
CYP2D6 (A) and microsomes derived from B lymphoblastoid cells expressing
CYP3A4 (B). Each data point represents the mean ± SD of triplicate determinations.

Inhibition of Terfenadine Hydroxylase Activity by P450 En-
zyme-Selective Inhibitors/Substrates. The effect of specific P450
inhibitors on the rate of terfenadine hydroxylation was investigated
using furafylline (CYP1A2), sulfaphenazole (CYP2C9), quinidine
(CYP2D6), and ketoconazole (CYP3A4) as inhibitors. For enzymes
for which there are no identified specific inhibitors, specific substrates
were used as competitive inhibitors. These were coumarin (CYP2A6),
(5)-mephenytoin (CYP2C19), and chlorzoxazone (CYP2E1). Com-
pounds found to inhibit terfenadine hydroxylation by >10% were
quinidine (which inhibited the activity by 17 and 33% at 2.5 and 25
μM, respectively), chlorzoxazone (which inhibited the activity by
22% at 500 μM), and ketoconazole (which inhibited the activity by 52
and 78% at 2.5 and 25 μM, respectively). The remaining compounds
had no effect on terfenadine hydroxylation. Recent studies have
shown that chlorzoxazone is metabolized by CYP3A4 in addition to
CYP2E1 (Gorski et al., 1997). Therefore, the inhibition of terfenadine
hydroxylase activity observed at the highest chlorzoxazone concen-
tration might be a result of chlorzoxazone inhibition of CYP3A4.

Inhibition by quinidine and ketoconazole suggests CYP2D6 and
CYP3A4 involvement. Further investigations of the effects of quini-
dine and ketoconazole on terfenadine hydroxylation at a terfenadine
concentration of 25 μM yielded an IC50 value of 4 μM for inhibition
by ketoconazole (fig. 9). Quinidine did not inhibit the reaction com-
pletely in the concentration range studied (fig. 9). However, approx-
imately 50% inhibition of the control (no inhibitor) rate was achieved
at a quinidine concentration of 100 μM.

At low concentrations, quinidine and ketoconazole are specific
inhibitors of CYP2D6 and CYP3A4, respectively (Bourrie et al.,
1996). However, because quinidine has also been shown to be a
substrate for CYP3A4 (Guengerich et al., 1986), this inhibitory effect
on terfenadine hydroxylation might have arisen as a result of the two
compounds competing for CYP3A4. At low quinidine concentrations (1
μM), terfenadine hydroxylase activity was inhibited to a small
degree (~10%). This concentration of quinidine had no effect on
CYP3A4, as measured by testosterone 6β-hydroxylase activity, but
had a significant inhibitory effect on CYP2D6 activity, as measured
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CYP2D6.

Discussion

The SSARs for the major human drug-metabolizing P450s predict
that the H1 receptor antagonist terfenadine should interact with both
CYP3A4 and CYP2D6 (Smith and Jones, 1992). A number of reports
have detailed the CYP3A4-related metabolism of terfenadine (Yun et
al., 1993; Jurima-Romet et al., 1994; Rodrigues et al., 1995), but the
role of CYP2D6 in this metabolism, if any, has not been determined.

The CYP2D6 SSAR requires a site of metabolism 5–7 Å from a
basic nitrogen (Smith and Jones, 1992). In the case of terfenadine,
these requirements are met by the nitrogen atom of the piperidine
moiety and the t-butyl group, the site of hydroxylation. Indeed,
substrate overlays indicate that terfenadine has the basic nitrogen
atom and the site of metabolism in a spatial orientation that would
facilitate binding to CYP2D6, by comparison with the prototypical

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Table 2: Apparent kinetic parameters for terfenadine hydroxylation obtained from four individual human livers

<table>
<thead>
<tr>
<th>Liver Sample</th>
<th>Apparent Vmax (pmol/min/mg)</th>
<th>Apparent Km (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM-3</td>
<td>181 ± 6</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>HM-6</td>
<td>367 ± 19</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>HM-14</td>
<td>157 ± 7</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>HM-16</td>
<td>386 ± 15</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>273 ± 104</td>
<td>14 ± 5</td>
</tr>
</tbody>
</table>
CYP2D substrate dextromethorphan. When terfenadine is docked into a protein model of CYP2D6 with the t-butyl group orientated close to the heme to allow metabolism, the basic nitrogen is able to interact with Asp-301, the amino acid residue identified as being key in the ion-pair interaction that is characteristic of this enzyme (Ellis et al., 1995). Furthermore, this binding orientation identified other potential interactions between terfenadine and CYP2D6.

These findings confirm the SSAR-based observations that terfenadine would interact with CYP2D6. However, it is not clear whether terfenadine would interact as a substrate or as an inhibitor. The inhibition of CYP2D6 by terfenadine is competitive and is characterized by a relatively low mean \( K_i \) of 4.6 \( \mu \)M against bufuralol 1'-hydroxylase in four individual human livers. The only other P450 enzyme-selective reaction to be inhibited by terfenadine was testosterone 6β-hydroxylase activity, which is selective for CYP3A4. This was expected, because terfenadine was previously shown to be a poor substrate for this enzyme. However, it is not clear whether terfenadine would interact as a substrate or as an inhibitor. The inhibition of CYP2D6 by terfenadine was expected, because terfenadine was previously shown to be a poor substrate for this enzyme. However, it is not clear whether terfenadine would interact as a substrate or as an inhibitor. The inhibition of CYP2D6 by terfenadine was competitive and is characterized by a relatively low mean \( K_i \) of 4.6 \( \mu \)M against bufuralol 1'-hydroxylase in four individual human livers. The only other P450 enzyme-selective reaction to be inhibited by terfenadine was testosterone 6β-hydroxylase activity, which is selective for CYP3A4. This was expected, because terfenadine was previously shown to be a poor substrate for this enzyme.

In conclusion, SSAR and modeling studies predicted that terfenadine would interact with CYP2D6. Further in vitro studies using human liver microsomes and expressed recombinant P450s demonstrated that terfenadine is a relatively potent inhibitor of CYP2D6 but a poor substrate for this enzyme. Terfenadine is metabolized to hydroxyterfenadine, carboxyterfenadine, and azacyclonol by human liver microsomes. Of these metabolites, only hydroxyterfenadine produced significant inhibition of CYP2D6 (approximately equal to that by terfenadine). However, CYP2D6 did not further metabolize hydroxyterfenadine to either carboxyterfenadine or azacyclonol.

**References**


chromatographic assays for bufuralol 1'-hydroxylase, debrisoquine 4-hydroxylase and dextromethorphan O-demethylase in microsomes and purified cytochrome P-450 isozymes of human liver. Anal Biochem 162:24–32.


