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 it was 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 Ki for terfenadine against bufuralol 1’-hydroxylase activity in four human livers. Terfenadine inhibited bufuralol 1’-hydroxylase activity with a Ki 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 a 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 (Jurrima-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 isozyme. 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)-mephenytoin, 4-hydroxymephenytoin, bufuralol, 1’-hydroxybufuralol, hydroxyterfenadine, and sulfaphenazole were obtained from Salford Ultrafine Chemicals.
Terfenadine and its metabolites were incubated in a concentration range of 0 – 1000 μM (Jones et al., 1997), diclofenac 4'-hydroxylase activity (CYP2C9) was determined at a substrate concentration of 40 μM (Jones et al., 1997), (S)-mephenytoin 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 (Punan and Imaoka, 1987).

Chlorozoxazolone 6-hydroxylase activity (CYP2E1) was determined with 33 μM chlorozoxazone 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 (zoxazolamine, 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)/acetonitrile (70:30, v/v), at a flow rate of 1 ml/min. Detection was by UV absorbance at 295 nm. Under these conditions, 6-hydroxychlorozoxazone had a retention time of 3 min, zoxazolamine had a retention time of 5 min, and chlorozoxazone 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'-hydroxylase as the CYP2D6-selective reaction. The Ki was estimated from the IC50 value using the following equation (Segel, 1993):

\[ K_i = IC_{50}(1 + (S/K_M)) \]

where [S] is the bufuralol concentration (10 μM) and K_M is the Km for bufuralol in human liver microsomes (10 μM). Based on this estimated Ki value, the incubations were carried out at terfenadine concentrations equal to \( \frac{1}{2} K_i \), K_i, 2K_i, and 4K_i, and bufuralol concentrations equal to \( \frac{1}{2} K_M \), K_M, 2K_M, and 4K_M.

**Assay for Terfenadine and Hydroxyterfenadine Metabolism.** The metabolism of terfenadine and hydroxyterfenadine by human liver microsomes or expressed recombinant P450s was assessed by the following method. Each incubation (final volume, 120 μl) was composed of microsomal protein, 50 mM Tris-HCl (pH 7.4), 5 mM MgCl_2, and 5 μM MnCl_2. 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 hydroxyterfenadine 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)/acetonitrile/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-Hitchachi FI800) 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)/acetonitrile/methanol (70:15:15, v/v), at a flow rate of 0.7 ml/min. Quantitation was achieved using a fluorescence detector (Merck-Hitchachi FI800) 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$_{50}$). Terfenadine was an inhibitor of testosterone 6β-hydroxylase activity (a substrate probe activity for CYP3A4), with an IC$_{50}$ 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$_{50}$ 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$_{50}$ 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 $K_v$ 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 $K_v$ 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
of CYP2D6 (IC$_{50}$ values of $\approx$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.

**TABLE 1**

<table>
<thead>
<tr>
<th>Liver Sample</th>
<th>$K_i$ (μM)</th>
</tr>
</thead>
<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>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD of triplicate determinations.
enzymes involved, with similar \( K_M \) values (\( K_M \) for CYP2D6, 13 \( \mu M \); \( K_M \) for CYP3A4, 9 \( \mu M \)).

The mean apparent \( V_{\text{max}} \) for the hydroxylation was 273 pmol/min/mg, and the mean apparent \( K_M \) was 14 \( \mu M \) (table 2). The apparent \( K_M \) value is similar to the value of 9.6 \( \mu M \) observed for hydroxyterfenadine formation in human liver microsomes by Rodrigues et al. (1995)

**Inhibition of Terfenadine Hydroxylase Activity by P450 Enzyme-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), \((\beta)\)-mephénytoin (CYP2C19), and chlorzoxazone (CYP2E1). Compounds found to inhibit terfenadine hydroxylation by \( > 10\% \) were quinidine (which inhibited the activity by 17 and 33\% at 2.5 and 25 \( \mu M \), respectively), chlorzoxazone (which inhibited the activity by 22\% at 500 \( \mu M \), and ketoconazole (which inhibited the activity by 52 and 78\% at 2.5 and 25 \( \mu M \), 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 concentration 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 quinidine and ketoconazole on terfenadine hydroxylation at a terfenadine concentration of 25 \( \mu M \) yielded an IC\(_{50}\) value of 4 \( \mu M \) for inhibition by ketoconazole (fig. 9). Quinidine did not inhibit the reaction completely in the concentration range studied (fig. 9). However, approximately 50\% inhibition of the control (no inhibitor) rate was achieved at a quinidine concentration of 100 \( \mu M \).

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 \( \mu M \)), terfenadine hydroxylase activity was inhibited to a small degree (\( \sim 10\% \)). This concentration of quinidine had no effect on CYP3A4, as measured by testosterone 6\( \beta\)-hydroxylase activity, but had a significant inhibitory effect on CYP2D6 activity, as measured by bufuralol 1\'-hydroxylase activity (\( > 80\% \) inhibition) (data not shown). This suggests that there is a small component of terfenadine hydroxylase activity in human liver microsomes that is mediated by CYP2D6.

**Discussion**

The SSARs for the major human drug-metabolizing P450s predict that the \( \mathrm{H}_1 \) 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...
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 should 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 6ß-hydroxylase activity, which is selective for CYP3A4. This was expected, because terfenadine was previously shown to be a poor substrate for this enzyme (Yun et al., 1993; Jurima-Romet et al., 1994; Rodrigues et al., 1995).

All of the P450-derived metabolites of terfenadine retain the basic center (piperidine nitrogen). In terms of interaction with CYP2D6, only hydroxyterfenadine inhibited the enzyme with a potency approximately equal to that of terfenadine; however, there was no detectable metabolism of hydroxyterfenadine to carboxyterfenadine by CYP2D6. This was not the case for CYP3A4, which metabolized hydroxyterfenadine to carboxyterfenadine and azacyclonal.

These studies show that terfenadine is one of a number of H$_1$ antagonists, including loratadine (Yumibe et al., 1996) and promethazine (Nakamura et al., 1996), that interact with CYP2D6. The SSARs of the H$_1$ receptor and CYP2D6 show a degree of commonality, in that both proteins bind molecules via an aspartic acid residue (ter Laak et al., 1995). This residue is proposed to be Asp-301 in the case of CYP2D6 (Ellis et al., 1995), whereas it is Asp-116 in the H$_1$ receptor (ter Laak et al., 1995). In terms of other interactions with the proteins, it has been demonstrated that the diphenyl-4-piperidinomethanol group is critical for interaction with the H$_1$ receptor (Zhang et al., 1993). This may explain why carboxyterfenadine is still an H$_1$ antagonist, because the area in which the molecule undergoes metabolism appears to be less important, in terms of binding to the receptor. This is not the case for CYP2D6, where the change from terfenadine to carboxyterfenadine results in a >20-fold reduction in apparent affinity for the enzyme.

Terfenadine is metabolized to hydroxyterfenadine and azacyclonal in human liver microsomes. It was shown previously that the hydroxylation is the major route of metabolism. The kinetics of the hydroxylation reaction in four human livers suggest that only one enzyme mediates the process, with a mean apparent $K_m$ of 14 $\mu$M. The hydroxylation of terfenadine is inhibited by the CYP3A4-selective inhibitor ketoconazole and to a lesser extent by the CYP2D6-selective inhibitor quinidine.

By comparison, terfenadine metabolism by microsomes from B lymphoblastoid cells expressing single P450 enzymes showed the formation of hydroxyterfenadine to be mediated by CYP3A4 and to a lesser extent by CYP2D6, with little or no metabolism being observed with the other enzymes investigated. Additional studies indicated that CYP2D6 and CYP3A4 have similar $K_m$ values (13 $\mu$M for CYP2D6 and 9 $\mu$M for CYP3A4) with respect to the formation of hydroxyterfenadine, but the $V_{max}$ value for CYP3A4 is approximately 6-fold greater than that for CYP2D6.

These observations group terfenadine with compounds such as the antiarrhythmic agent quinidine and the antimalarial drug halofantrine, which are both inhibitors of, but poor substrates for, CYP2D6 (Holland et al., 1995). The $K_i$ value for terfenadine against CYP2D6 is comparable to that of halofantrine, but terfenadine is approximately 100-fold less potent as an inhibitor of CYP2D6, compared with quinidine (Holland et al., 1995). Clinically, it has been shown that small doses of quinidine can produce phenocopying. That is, quinidine can alter the apparent phenotype of an extensive metabolizer for CYP2D6 to that of a poor metabolizer (Brosen et al., 1987), with obvious implications. This is unlikely to be the case with terfenadine, because it is rapidly converted to hydroxyterfenadine, which is in turn rapidly converted to carboxyterfenadine, a much less potent inhibitor of CYP2D6.

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 azacyclonal 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 azacyclonal.

**References**


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