

CYP2J2 and CYP4F12 Are Active for the Metabolism of Non-sedating Antihistamines: Terfenadine and Astemizole

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Abstract

Terfenadine and astemizole are non-sedating antihistamine prodrugs subject to extensive first pass metabolism in the liver and intestine. CYP3A4 has been identified as the principal enzyme for hepatic metabolism of terfenadine, while the enzymes participating in intestinal microsomes are less clear. The P450 enzymes involved in the formation of the major astemizole metabolite (desmethyl-astemizole) have not been clearly identified, but CYP2J2 has been implicated. Ebastine, a structurally related antihistamine drug, has been shown to be a substrate for both CYP2J2 and 4F12 in human intestinal microsomes. In the current study, CYP2J2 and 4F12 were expressed at high levels using the baculovirus/insect cell expression system. Both recombinant enzymes were active for terfenadine hydroxylation. CYP4F12 had similar activity as CYP3A4, while CYP2J2 was roughly 6-fold more active. CYP2J2 and 4F12 demonstrated low K_m values for terfenadine hydroxylation: 0.4 μM and 0.8 μM , respectively. Astemizole was O-demethylated to desmethyl-astemizole by both CYP2J2 and 4F12, with CYP4F12 showing about 4-fold greater activity. Other astemizole metabolites were not detected. Ketoconazole, a potent inhibitor of CYP3A4, was found to inhibit both CYP2J2 and 4F12 terfenadine hydroxylation (IC_{50} values of 5 μM and 0.7 μM , respectively). The results demonstrate that CYP2J2 and 4F12 are active for the metabolism of astemizole and terfenadine, and may contribute to their first pass metabolism in intestinal microsomes.

Introduction

Terfenadine and astemizole are non-sedating antihistamine prodrugs that undergo complete first pass-metabolism to their active, therapeutic metabolites. Both parent compounds (not the metabolites) are known cardiotoxic, due primarily to interference with certain ion-channels in the heart, in particular the HERG channel.¹ In the liver terfenadine undergoes P450-dependent metabolism via two routes: N-dealkylation to azacyclonol, and t-butyl methyl hydroxylation to the terfenadine alcohol metabolite, which is further oxidized to the active carboxy terfenadine metabolite. CYP3A4 is the major enzyme involved in both pathways.^{2,3} When terfenadine is taken with drugs that interfere with CYP3A4 metabolism (e.g. ketoconazole and erythromycin) the result can be increased plasma concentrations of the parent terfenadine, and the onset of life-threatening cardiac arrhythmias.⁴ As a result of CYP3A4 interactions, terfenadine, (marketed as Seldane®), has been replaced as an antihistamine drug by the active metabolite, fexofenadine (marketed as Allegra®), which is a safer, non-cardio toxic compound.

Astemizole undergoes metabolism to several products (desmethylastemizole, 6-hydroxyastemizole and norastemizole, along with other secondary metabolites). Both CYP3A4 and CYP2D6 are involved in the formation of 6-hydroxy and norastemizole. CYP2J2 was shown to be responsible for the first-pass formation of desmethylastemizole in the intestine. The O-demethylation product is considered the major metabolite, and the pharmaceutically active form of the drug.⁵ Ebastine, an antihistamine drug structurally similar to terfenadine, can undergo methyl hydroxylation by both intestinal and hepatic microsomes. In the liver the enzymes responsible are yet unknown, while in the intestine the major enzymes were shown to be CYP2J2 and CYP4F12.⁶

In the current study we show that cDNA-expressed CYP2J2 and CYP4F12 are both active for the hydroxylation of terfenadine, with rates comparable to (in the case of rCYP4F12) or exceeding (in the case of rCYP2J2) the rates obtained with recombinant CYP3A4 (+b5). Astemizole O-demethylation is shown to be carried out by CYP4F12, 2J2, and 3A4; with CYP4F12 having the highest activity. The results support a role for CYP2J2 and CYP4F12 in the intestinal, first-pass metabolism of terfenadine/astemizole prodrugs.

Methods

Enzyme Preparations

BD Supersomes™ Enzymes membrane fractions from insect cells expressing P450 isoforms 2D6 (P217), 2J2, 4F2 (P272), 4F3B (P274), 4F12, 3A4 (P202, +b5), and pooled human liver microsomes (HLM) (H161); were from BD Biosciences Discovery Labware (Woburn, MA).

Terfenadine and Astemizole Assay Conditions

Incubations consisted of 100 mM potassium phosphate buffer (pH 7.4), and NADPH generating system (consisting of 1.3 mM NADP, 3.3 mM MgCl_2 , and 3.3 mM glucose-6-phosphate), BD Supersomes Enzymes protein or HLM and astemizole or terfenadine substrates in a final volume of 0.2 ml. Incubations were carried out for 5 to 15 minutes at 37°C, depending on the enzyme source. Incubations were terminated with 50 μl of acetonitrile. Incubations were centrifuged at 14,000 rpm for three minutes and supernatants were removed for HPLC analysis. For kinetic analysis (K_m and V_{max}) the final terfenadine concentrations ranged between 0.2 μM and 75 μM (total of nine concentrations). Chemical inhibitor concentrations were 0, 0.1, 1, 10, and 100 μM . The concentration of terfenadine for determining IC_{50} values for the chemical inhibitors was 1.5 μM .

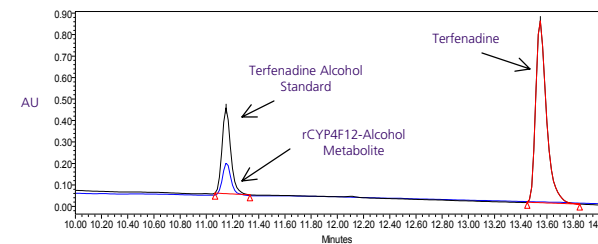
HPLC Analysis

The HPLC system was a Waters Alliance 2690 W2487 UV detector (Milford, MA). The HPLC column was a Zorbax C-18, 5 μ , 4.6 x 250 mm; the column temperature was constant at 45°C. The alcohol metabolite of terfenadine was separated using HPLC mobile phases consisting of 0.1% trifluoroacetic acid (TFA) in H_2O (Solvent A), and 0.1% TFA in acetonitrile (Solvent B). Initial HPLC solvent conditions were 90% Solvent A and 10% Solvent B. Metabolites were eluted with a linear increase in Solvent B to 100% over a 16 minute period, before returning to initial conditions. The flow rate of the HPLC run was 1 ml/min. HPLC detection was by UV-absorbance monitored at 212 nm. Metabolite peaks were quantitated by comparison to authentic standards (terfenadine alcohol and desmethylastemizole metabolites).

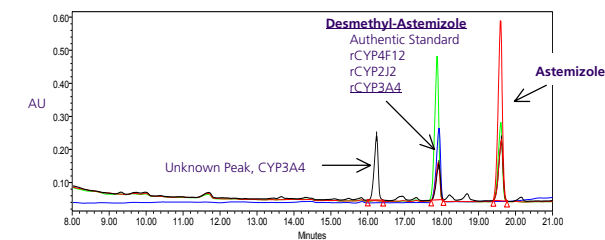
Statistical Analysis

IC_{50} values were calculated by linear interpolation. Kinetic analysis was by linear and non-regression using Sigma Plot software.

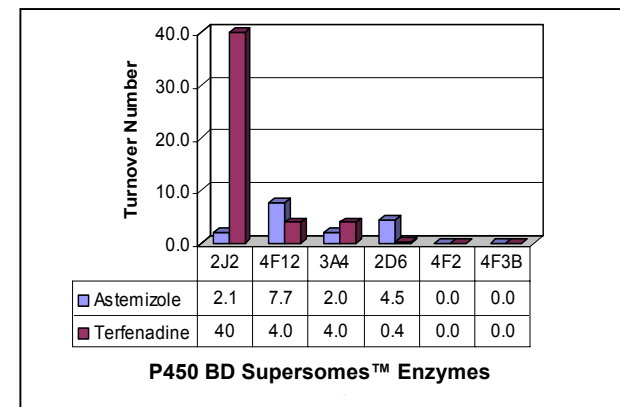
1 HPLC Chromatogram of Terfenadine and Alcohol Metabolite



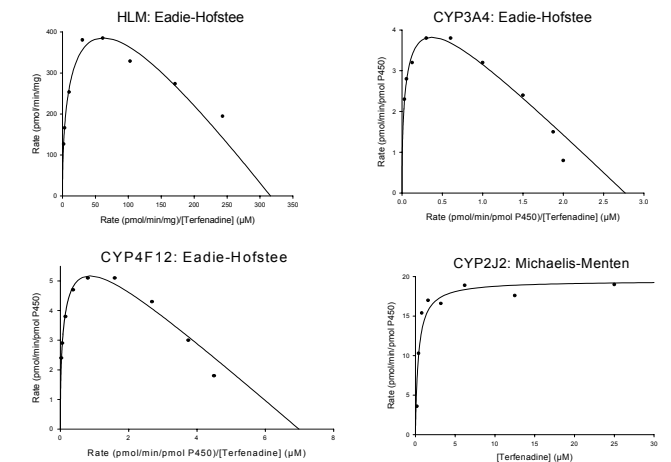
2 HPLC Chromatogram of Astemizole and Desmethyl Metabolite



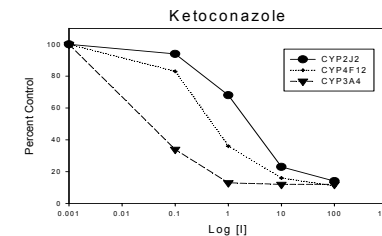
3 Metabolism of Terfenadine (Alcohol Metabolite) and Astemizole (Desmethyl Metabolite) by BD Supersomes™ Enzymes (Astemizole and Terfenadine concentrations: 50 μM)



4 Substrate-Dependence of Terfenadine Hydroxylation by HLM and BD Supersomes™ Enzymes



6 Inhibition of rP450-Dependent Terfenadine Hydroxylation by CYP3A4 Chemical Inhibitors: Ketoconazole, Erythromycin and Azamulin



Enzyme	IC_{50} Values (μM)		
	Ketoconazole	Erythromycin	Azamulin
CYP2J2	4.6	100	6.6
CYP4F12	0.7	100	29
CYP3A4	0.08	20	0.12

5 Kinetic Parameters Table

Enzyme	K_m (μM)	V_{max}^1	V_{max}/K_m (Intrinsic Clearance)	Substrate Inhibition K_i (μM)
HLM	2	634	317	19
rCYP3A4	1.9	5.2	2.7	57
rCYP4F12	1	6.9	6.9	36
rCYP2J2	0.4	20	53	Not Observed

¹For HLM, activity units = pmol/min/mg. For BD Supersomes™ Enzymes, activity units = pmol/min/pmol P450

Conclusions

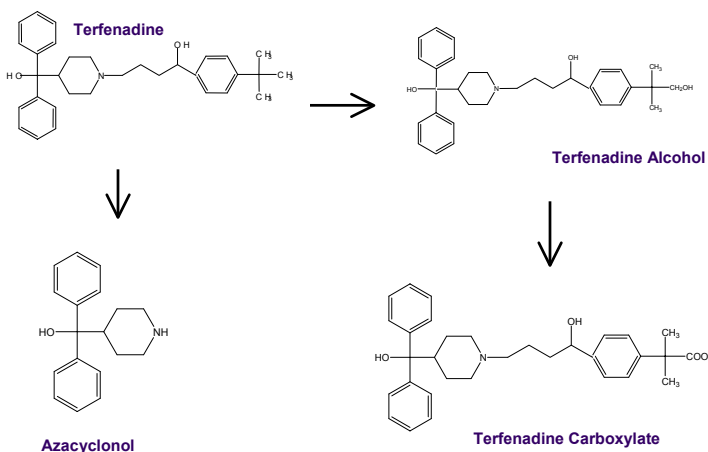
- BD Supersomes™ Enzymes CYP2J2 and CYP4F12 were active for the hydroxylation of terfenadine. CYP2J2 showed the highest activity, which was several fold higher than CYP3A4 (+b5). CYP2D6 showed minor activity, consistent with previous results.³
- BD Supersomes Enzymes CYP4F12 showed the highest activity for the O-demethylation of astemizole to desmethylastemizole. CYP2D6, CYP2J2 and CYP3A4 also carried out this reaction.
- Apparent K_m values for terfenadine hydroxylation by HLM and BD Supersomes Enzymes were similar (~1 μM). Substrate inhibition was apparent for HLM, CYP4F12, and CYP3A4, while CYP2J2 followed typical Michaelis-Menten kinetics.
- The intrinsic clearance for terfenadine hydroxylation by CYP2J2 was 8- and 20-fold greater than CYP4F12 and CYP3A4, respectively.
- As expected, ketoconazole and azamulin were potent inhibitors of CYP3A4-dependent terfenadine hydroxylation. Ketoconazole and azamulin also inhibited CYP2J2 (azamulin) and CYP4F12 (ketoconazole) activity at concentrations typically used to inhibit CYP3A4 (e.g. 1 μM in the case of ketoconazole, and 5 μM in the case of azamulin).
- In conclusion, the data supports a role for CYP2J2 and CYP4F12 in the hydroxylation of terfenadine and astemizole. The contribution may be most significant in the intestine (first-pass effect), where these P450s are known to be important for the hydroxylation of other structurally related drugs (e.g. ebastine). The results suggest the possibility for drug interactions involving terfenadine/astemizole and CYP4F12 and/or CYP2J2.

References

- Paakkari, I., Toxicol. Lett., **127**:279 (2002)
- Yun, C.H., et al., Drug Metab. Dispos., **21**:403 (1993).
- Jones, B.C., et al., Drug Metab. Dispos., **26**:875 (1998).
- Boxenbaum, H., J. Pharm. Pharmaceut. Sci., **2**:45 (1999).
- Matsumoto, S., et al., Drug Metab. Dispos., **30**:1240 (2002).
- Hashizume, T., et al., JPET, **300**:298 (2002).

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Terfenadine Metabolic Pathway



Astemizole Metabolic Pathway

