Terfenadine–antidepressant interactions: an *in vitro* inhibition study using human liver microsomes

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Aims Inhibition of the metabolism of terfenadine has been associated with *torsades de pointes* ventricular arrhythmias. The aim of this study was to assess *in vitro* the potency of the antidepressants nefazodone, sertraline and fluoxetine in inhibiting terfenadine biotransformation.

Methods Human liver microsomes were incubated with terfenadine and the antidepressants at various concentrations. Formation of the two major metabolites of terfenadine was determined by h.p.l.c.

Results The apparent K_m for microsomes from four human livers was 11 ± 5 and $18 \pm 3 \mu\text{M}$ (mean $\pm$ s.e. mean) for the *N*-dealkylation and *C*-hydroxylation pathways, respectively. Nefazodone, sertraline and fluoxetine inhibited terfenadine *N*-dealkylation with K_i values of 10 ± 4 , 10 ± 3 and $68 \pm 15 \mu\text{M}$ respectively. Inhibition of the *C*-hydroxylation pathway yielded noncompetitive K_i values of 41 ± 4 , 67 ± 13 and $310 \pm 40 \mu\text{M}$ respectively.

Conclusions Nefazodone and sertraline were moderately weak *in vitro* inhibitors of terfenadine metabolism while fluoxetine was a very weak inhibitor. Clinically significant interaction of terfenadine is more likely with nefazodone than sertraline or fluoxetine since therapeutic plasma levels of nefazodone are comparatively higher.

Keywords: terfenadine, nefazodone, sertraline, fluoxetine, CYP3A4, human liver microsomes

Introduction

The nonsedating antihistamine, terfenadine, has been associated with prolongation of the QT interval, and a rare occurrence of *torsades de pointes*, during concomitant administration of inhibitors of CYP3A4, such as ketoconazole and erythromycin [1–3]. Because of the cardiotoxicity risk, the United States Food and Drug Administration has recently announced its intention to withdraw approval of terfenadine [4]. Terfenadine undergoes rapid and extensive first-pass CYP3A4-mediated biotransformation involving *C*-hydroxylation to an alcohol intermediate which is further oxidized to an active carboxylic acid metabolite, and *N*-dealkylation to an inactive metabolite, azacyclonol [5, 6]. Cardiotoxicity has been attributed to the parent drug whereas the acid metabolite is free of this untoward effect [7]. In previous studies using human liver microsomes, we demonstrated potent inhibition of terfenadine *C*-hydroxylation and *N*-dealkylation by azole antifungals, macrolide antibiotics and a few other CYP3A4 substrates [8], supporting the concept of inhibition of terfenadine biotransformation as the underlying mechanism for the adverse drug interactions.

Many of the newer antidepressants, the selective serotonin reuptake inhibitors (SSRIs) and related compounds, have the property of inhibiting CYP enzymes, and as a result, are involved in clinically significant drug interactions [9]. In a recent study, several SSRIs were evaluated for their effects on terfenadine metabolism by human liver microsomes, and

were found to be much weaker inhibitors than the prototype CYP3A4 inhibitor, ketoconazole [10]. Nefazodone is a new antidepressant which acts as a 5-HT receptor antagonist and serotonin reuptake inhibitor. It exhibits nonlinear kinetics and is extensively metabolized to several active metabolites [11]. *In vivo* studies demonstrated significant inhibition of the clearance of alprazolam and triazolam by coadministration of nefazodone [12, 13]. As alprazolam and triazolam are both predominantly metabolized by CYP3A4, these findings suggest that nefazodone is a CYP3A4 inhibitor. *In vitro* inhibition studies indicated relatively weaker inhibition of dextromethorphan *N*-demethylation and alprazolam 4-hydroxylation (reactions proposed to reflect CYP3A4 activity) by nefazodone and its metabolites compared to ketoconazole (K_i values 25 to 100-fold higher than K_i for ketoconazole) [14, 15]. As the effect of nefazodone on terfenadine metabolism had not been previously evaluated, and the potency of nefazodone as a CYP3A4 inhibitor has not been clearly established, accordingly, we investigated *in vitro* the inhibition of terfenadine metabolism by nefazodone and for comparison, by two SSRIs, fluoxetine and sertraline, using human liver microsomes.

Methods

Materials

Nefazodone HCl was a gift from Bristol-Myers Squibb Canada Inc. (Montréal, Québec) and sertraline HCl was donated by Pfizer Canada Inc. (Montréal, Québec). Fluoxetine and norfluoxetine were provided by Eli Lilly and Co. (Indianapolis, Indiana). Terfenadine was purchased from

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Sigma Chemical Co. (St Louis, Missouri). Analytical reference standards of terfenadine metabolites were donated by Hoechst Marion Roussel (Cincinnati, Ohio). Human liver tissue was obtained from the tissue bank of the Department of Pharmacology, University of Toronto.

Microsome preparation

Microsomes were prepared from human liver tissue as previously described [8]. Protein concentration was determined by the Bradford method [16].

Incubation conditions

Incubation mixtures contained 0.1 M potassium phosphate buffer (pH 7.4), 0.5 mg ml⁻¹ microsomal protein and 1 mM NADPH, in a final volume of 2 ml. In preliminary incubations without inhibitors, terfenadine (dissolved in methanol) was added at 5, 10, 20, 40, 80 and 160 µM, to determine enzyme kinetic parameters for microsomes prepared from different livers. For inhibition studies, terfenadine was added at 5 and 30 µM, and nefazodone, sertraline, fluoxetine and norfluoxetine were dissolved in methanol and individually added at various concentrations. All reactions had a total of 20 µl of methanol. Mixtures were shaken vigorously at 37°C for 20 min. Reaction rates were linear with respect to protein concentration and time. Reactions were stopped by adding 2 ml of cold methanol.

H.p.l.c. analysis

Samples were centrifuged at 2000g for 12 min to separate the protein. The supernatant was diluted with an equivalent volume of cold deionized water and applied to a Bakerbond 3-ml C18 solid phase extraction column (J. T. Baker, Phillipsburg, New Jersey), which was then rinsed with 2 × 1 ml water, followed by 1 ml methanol: water (1:3, v/v), and the sample eluted with 8 × 1 ml 50 mM triethylamine in methanol. The mean recoveries (±s.d., *n*=4) of azacyclonol, acid metabolite, alcohol metabolite and unchanged terfenadine were 81.5 ± 0.4, 94.0 ± 4.0, 83.5 ± 0.4 and 79.6 ± 2.9%, respectively. The eluate was analyzed by h.p.l.c. as previously described [8], except that the mobile phase, delivered at a flow rate of 1 ml min⁻¹, consisted of 12 mM ammonium acetate buffer (pH 4.5), acetonitrile and methanol (55: 20: 25, by volume) for the first 10 min, followed by the same buffer, acetonitrile and methanol in a composition of 40: 28: 32 by volume, for the next 20 min. Under these conditions, the internal standard

(metoprolol), azacyclonol, acid metabolite, alcohol metabolite and unchanged terfenadine eluted at 7.3, 11.4, 14.7, 19.6 and 28.0 min, respectively.

Data analysis

The kinetic parameters, K_m and V_{max} , for terfenadine C-hydroxylation to alcohol metabolite and N-dealkylation to azacyclonol, were estimated from Eadie-Hofstee plots (Quattro Pro version 6.0). Inhibition data were analyzed by Dixon and Cornish-Bowden plots to determine the mode of inhibition. The apparent K_i values were calculated from Dixon plots. For estimating inhibition *in vivo*, the equation % inhibition = $100 \cdot [I] / \{ [I] + K_i \}$ was applied, where [I] is the approximate steady state total plasma concentration of the inhibitor *in vivo*, and assuming the substrate (terfenadine) concentration to be $\ll K_m$.

Results

Terfenadine biotransformation was inhibited by nefazodone, sertraline and fluoxetine (Table 1). In all cases, the N-dealkylation pathway was more potently inhibited (lower K_i) than the C-hydroxylation pathway. Inhibition by fluoxetine was much weaker than inhibition by nefazodone or sertraline. The metabolite of fluoxetine, norfluoxetine, was a more potent inhibitor of azacyclonol formation than the parent drug, with a K_i value of 14 µM. However, the effect of norfluoxetine on alcohol metabolite formation could not be determined due to chromatographic interference by the norfluoxetine peak.

Representative Dixon plots obtained with microsomes from one human liver are shown in Figure 1. The pattern of inhibition demonstrated by Dixon plots and confirmed by Cornish-Bowden plots (not shown) was consistent with noncompetitive inhibition, except for inhibition of terfenadine N-dealkylation by fluoxetine which was consistent with competitive inhibition.

Discussion

The present study demonstrated that nefazodone and sertraline were capable of inhibiting terfenadine biotransformation *in vitro*, whereas fluoxetine was a very weak inhibitor. The K_i values for inhibition of terfenadine N-dealkylation by sertraline, fluoxetine and norfluoxetine, and of the C-hydroxylation pathway by sertraline, were similar within a two-fold range to values determined in an earlier study by von Moltke *et al.* [10]. In the latter study, a

Table 1 Terfenadine metabolism by human liver microsomes: kinetic parameters and inhibition by three antidepressant drugs.

Metabolic pathway	K_m^a (µM)	V_{max}^a (pmol min ⁻¹ mg ⁻¹ protein)	K_i values ^b (µM)		
			Nefazodone	Sertraline	Fluoxetine
N-dealkylation	11 ± 5 (3–25)	240 ± 60 (140–360)	10 ± 4 (3–16)	10 ± 3 (2–18)	68 ± 15 (42–95)
C-hydroxylation	18 ± 3 (13–25)	410 ± 40 (320–480)	41 ± 4 (34–52)	67 ± 13 (43–85)	310 ± 40 (240–360)

^aMean ± s.e.mean of *n* = 4 separate determinations using microsomes from different human livers (range in parentheses).

^bMean ± s.e.mean of *n* = 3–4 separate determinations using microsomes from different human livers (range in parentheses).

Figure 1 Representative Dixon plots of inhibition of terfenadine N-dealkylation (left panel) and C-hydroxylation (right panel) by nefazodone, sertraline and fluoxetine, using human liver microsomes. Terfenadine concentrations were 5 μM ($\square$) and 30 μM ($\blacktriangle$).

harmonic mean K_i value of 66 μM for fluoxetine inhibition of terfenadine C-hydroxylation was reported, however, microsomes from some liver samples apparently gave very large K_i values. In our study, the K_i values for this interaction were large, $>200 \mu\text{M}$, for all samples.

To predict *in vivo* pharmacokinetic drug interactions, the plasma drug levels and the liver/plasma partition ratio need to be considered. The combined plasma levels of fluoxetine

and norfluoxetine at steady-state are several fold higher than plasma levels of other SSRIs. For example, approximately 1 μM for fluoxetine plus norfluoxetine compared with approximately 0.2 μM for sertraline plus desmethylsertraline at their usually effective minimum doses [9]. Therapeutic plasma levels of nefazodone plus its major active metabolite, hydroxynefazodone, are considerably higher than those of SSRIs, reaching 3–5 μM at steady-state with a 200 mg twice

daily dose [11, 17]. Hydroxynefazodone has been reported to have an *in vitro* inhibitory potency similar to nefazodone of reactions purported to be catalyzed by CYP3A4 [14, 15]. Thus, the potential for inhibition of terfenadine metabolism *in vivo* is greater for nefazodone than sertraline or fluoxetine, assuming similar liver uptake. For lipophilic drugs such as the SSRIs and nefazodone, the intrahepatic concentrations are expected to exceed those in plasma. On the other hand, extensive plasma protein binding that occurs with nefazodone, fluoxetine and sertraline might be expected to restrict hepatic uptake, in which case the use of total (free plus bound) plasma concentrations would overestimate the percentage inhibition. Liver/plasma partitioning data for SSRIs and other lipophilic drugs indicate that despite a high degree of plasma protein binding, such drugs partition extensively from plasma into liver [18]. Although the liver/plasma partition ratio of nefazodone and its metabolites is not known, if a partition ratio similar to that of SSRIs, approximately 12, is assumed [19], then the percentage inhibition of terfenadine C-hydroxylation at therapeutic dosages of nefazodone is estimated to be 45–55%, and of terfenadine N-dealkylation, 75–85%. Plasma terfenadine levels are normally less than 5 ng ml⁻¹ [2, 3]. Assuming that the C-hydroxylation pathway accounts for approximately 75% and the N-dealkylation pathway for 25% of terfenadine clearance [5], this degree of inhibition would be expected to increase the plasma AUC by about 2.5-fold [10]. Plasma concentrations of parent terfenadine would not be expected to exceed 10 ng ml⁻¹, and therefore nefazodone inhibition of terfenadine metabolism should not cause QT interval prolongation in most individuals, however, some individuals may be unusually susceptible.

To date, there have not been any published reports of adverse interactions of terfenadine and nefazodone. Current labelling for nefazodone advises that this drug should not be used in combination with terfenadine, and such warning probably plays an important role in reducing the likelihood of a drug interaction. On the other hand, two published case reports describing possible terfenadine–fluoxetine interaction [20, 21], suggest that even when extrapolation of *in vitro* findings appears to rule out a pharmacokinetic interaction [10], in rare cases, a clinically significant interaction may occur. We conclude that the demonstration of inhibition of *in vitro* terfenadine metabolism by nefazodone supports a label precaution against the concomitant use of these two drugs.

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