Role of Flavin-Containing Monoxygenase in Oxidative Metabolism of Voriconazole by Human Liver Microsomes

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ABSTRACT:
Voriconazole is a potent second-generation triazole antifungal agent with broad-spectrum activity against clinically important fungi. It is cleared predominantly via metabolism in all species tested including humans. N-Oxidation of the fluoropyrimidine ring, its hydroxylation, and hydroxylation of the adjacent methyl group are the known pathways of voriconazole oxidative metabolism, with the N-oxide being the major circulating metabolite in humans. In vitro studies have shown that CYP2C9, CYP3A4, and to a lesser extent CYP2C9 contribute to the oxidative metabolism of voriconazole. When cytochrome P450 (P450)-specific inhibitors and antibodies were used to evaluate the oxidative metabolism of voriconazole by human liver microsomes, the results suggested that P450-mediated metabolism accounted for ~75% of the total oxidative metabolism. The studies presented here provide evidence that the remaining ~25% of the metabolic transformations are catalyzed by flavin-containing monoxygenase (FMO). This conclusion was based on the evidence that the NADPH-dependent metabolism of voriconazole was sensitive to heat (45°C for 5 min), a condition known to selectively inactivate FMO without affecting P450 activity. The role of FMO in the metabolic formation of voriconazole N-oxide was confirmed by the use of recombinant FMO enzymes. Kinetic analysis of voriconazole metabolism by FMO1 and FMO3 yielded $K_m$ values of 3.0 and 3.4 mM and $V_{max}$ values of 0.025 and 0.044 pmol/min/pmol, respectively. FMO5 did not metabolize voriconazole effectively. This is the first report of the role of FMO in the oxidative metabolism of voriconazole.

Voriconazole (Vfend), a second-generation triazole, is a potent antifungal agent with activity against a broad spectrum of fungal pathogens (Patterson, 2002; Boucher et al., 2004). Its pharmacokinetic properties after i.v. and oral administration have been thoroughly investigated in healthy volunteers (Putkins et al., 2002, 2003a,b) and in patients at risk of fungal infections (Lazarus et al., 2002); the pharmacokinetic/pharmacodynamic profile has been reviewed recently (Theuretzbacher et al., 2006). These studies show that voriconazole is readily absorbed upon oral administration (oral bioavailability >90%) and that it is eliminated with a terminal elimination half-life of approximately 6 h. Studies with radioisotope-labeled voriconazole have demonstrated that it is cleared via extensive hepatic metabolism in preclinical species and in humans; less than 2% of the administered dose is excreted as the parent drug in humans and a slightly higher percentage of the dose (<10%) appears as voriconazole in the excreta of other preclinical species—mouse, rat, guinea pig, and dog (Roffey et al., 2003). After either i.v. or oral administration to humans, nearly 80% of the voriconazole dose is excreted renally, mostly as metabolites (Purkins et al., 2003a). Multiple dose studies have revealed nonlinear pharmacokinetics with both $C_{max}$ and area under the plasma concentration-time curve during dosage intervals $\tau$ (AUC$_{\tau}$) increasing more than dose proportionately. For example, a 2-fold increase in oral dose (200–400 mg) caused 2.8- and 3.9-fold increases in $C_{max}$ and AUC$_{\tau}$, respectively (Putkins et al., 2002). Because voriconazole is cleared predominantly by metabolism, it is reasonable to conclude that the nonlinear pharmacokinetics is probably due to saturation of metabolism.

Voriconazole is metabolized to several oxidative metabolites, with N-oxidation of the fluoropyrimidine ring and hydroxylation of the adjacent methyl group being the major pathways in humans (Roffey et al., 2003; Murayama et al., 2007). Of note, the $N$-oxide (Fig. 1) is a major circulating metabolite in humans and in preclinical species, such as rat and dog (Roffey et al., 2003). In vitro studies with human liver microsomes (HLM) and expressed enzymes have indicated that the $N$-oxide metabolite is formed predominantly by CYP3A4, CYP2C19, and to a smaller extent by CYP2C9 (Hyland et al., 2003). These studies suggested that at low micromolar concentrations of voriconazole, CYP2C19 plays a major role in the formation of the $N$-oxide, whereas at low millimolar concentrations, CYP3A4 appears to be the major contributor to the metabolism of voriconazole.

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ABBREVIATIONS: AUC, area under the plasma concentration-time curve during dosage intervals $\tau$; HLM, human liver microsomes; P450, cytochrome P450; FMO, flavin-containing monoxygenase; WT, wild-type; HPLC, high-performance liquid chromatography; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance.
to be the major contributor. Recently, in vitro studies have shown that oxidative metabolism of voriconazole to the hydroxymethyl metabolite (Fig. 1) by human and rat liver microsomes is catalyzed exclusively by CYP3A (Murayama et al., 2007). Significant alteration of voriconazole clearance in CYP2C19 poor metabolizers, resulting in approximately 4-fold greater exposure to voriconazole, provides evidence for a substantial contribution of CYP2C19 to the clearance of voriconazole in vivo (Theuretzbacher et al., 2006). Because two key P450 enzymes, CYP3A4 and CYP2C19, play a pivotal role in its metabolic clearance, significant drug interactions involving voriconazole are likely when it is coadministered with inducers or inhibitors of CYP3A4 and CYP2C19 or with drugs that are predominantly cleared by these enzymes (reviewed by Theuretzbacher et al., 2006). The NADPH-dependent oxygenation of functional groups containing soft nucloephiles is often catalyzed by another class of oxidative enzymes, FMO (Rodriguez et al., 1999; Krueger and Williams, 2005). In this study, we demonstrate that, in addition to P450 enzymes, certain FMO isoforms can metabolize voriconazole to N-oxide; we further show that these FMO isoforms contribute significantly to the formation of the N-oxide by HLM, a finding that may significantly affect our understanding of the drug interactions involving voriconazole metabolism.

Materials and Methods

Chemicals and Reagents. Voriconazole was generously supplied by Pfizer Central Research (Groton, CT). Testosterone and 6β-hydroxytestosterone were purchased from Steraloids (Newport, RI). P450 substrates and their respective hydroxylated metabolites, mephenytoin, 4-hydroxymephenytoin, diclofenac, and 4-hydroxydiclofenac were purchased from BD Gentest (Woburn, MA). Purity of the N-oxide was determined by HPLC. Voriconazole (2 μM) with HLM (1.0 mg/ml) were carried out in phosphate buffer (0.1 M, pH 7.4) containing MgCl2 (3 mM) at 37°C. The reactions were initiated by addition of NADPH (2 mM); 200-μl aliquots were removed at defined time points and mixed with 600 μl of ice-cold methanol (containing internal standard-1 μM diclofenac) to terminate the reactions. After centrifugation at 10,000g for 10 min, the supernatants were evaporated to dryness under a stream of nitrogen gas, and the dry residues were dissolved in 10% acetonitrile in water. Quantitative determination of voriconazole was achieved using HPLC with UV detection (see Analytical Methods). The conditions for initial velocity with respect to time and protein (<20% depletion of initial voriconazole concentration) were determined, and voriconazole metabolism rate and half-life (t½) were then calculated as described by Obach and Reed-Hagen (2002).

To assess the role of the three putative P450 enzymes in voriconazole metabolism, incubations of voriconazole with HLM were carried out as described above in the presence of selective chemical inhibitors of CYP3A4 (3 μM ketoconazole), CYP2C9 (30 μM sulfaphenazole), CYP2C19 (3 μM fluvoxamine), or a mixture of all three inhibitors to assess the role of the three P450 enzymes, individually and collectively, in its metabolism. The role of P450 enzymes in the metabolism of voriconazole by HLM was also assessed by selective inhibition of P450-mediated voriconazole metabolism using P450-specific inhibitory antibodies. Aliquots of HLM (0.1 mg) were preincubated at 4°C with the inhibitory antibody against CYP2C19 (10 μl), CYP3A4 (20 μl), or CYP2C9 (20 μl), or with a mixture of the three antibodies. Preincubation of HLM with nonspecific rabbit IgG (20 μl) for 15 min was performed to serve as a “no inhibition” control. The ratio of microsomal protein to antibody volume was chosen to give a maximal inhibitory effect. Voriconazole (2 μM) was then added and mixed with other reaction constituents as described above, and incubations were carried out for 20 min.

To investigate the potential role of FMO in voriconazole metabolism, thermal inactivation conditions that selectively diminish FMO activity and not P450 activity were applied by preheating HLM (20 mg/ml) at 45°C for 5 min (without NADPH; HLM (20 mg/ml) preincubated at 37°C for 5 min served as controls. Aliquots of preheated control HLM were then incubated with voriconazole and NADPH, with or without a cocktail of P450 chemical inhibitors to determine the contribution of FMO, P450, or both in voriconazole metabolism by HLM. Effect of thermal inactivation condition on FMO activity was confirmed by the incubation of benzydamine (a selective FMO substrate) with preheated HLM and determining the attenuation of its metabolism by the heat treatment.

Metabolism of Voriconazole with Recombinant Human Flavin-Containing Monoxygenase. To determine the metabolic products formed by metabolism of voriconazole by FMO enzymes, FM01 (60 pmol/ml), FM03 (60 pmol/ml), or FM05 (150 pmol/ml) was incubated with voriconazole (100 μM) in the presence of NADPH (2 mM) for 60 min. The voriconazole N-oxide formed in the incubation samples was measured by HPLC-MS/MS (see Analytical Methods) against a calibration curve of authentic N-oxide standard. The rates of voriconazole N-oxide formed were calculated and normalized to the protein concentration in the samples. For kinetic analysis, voriconazole was incubated with 60 pmol/ml of FMO1 or FMO3 at several concentrations ranging from 1 to 8000 μM. Reactions were initiated by addition of NADPH and terminated after 30 min by addition of ice-cold methanol-containing internal standard and centrifuged, and supernatants were analyzed by HPLC-MS/MS for the formed N-oxide. The rates of N-oxide formation, expressed as picomoles of N-oxide formed per minute per milligram of protein or as
pico
time was 6.9 min for N-oxide and 8.2 min for voriconazole. The HPLC-
separation was achieved on an Aquasil C18 analytical column (50 × 2.1, 5 μm; Thermo
Scientific, Waltham, MA) using gradient elution of mobile phases A-B (v/v), where mobile phase A was 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid. The starting condition of 90:10 (A-B) was held as the mobile phase composition changed linearly to 5:95 (A-B). The flow rate was 0.7 ml/min, and the injection volume was 5 μl. Multiple reaction monitoring was used to monitor voriconazole (m/z transition 350→127), the N-oxide (m/z transition 366→224), and the internal standard (diclofenac, m/z transition 296→215) in positive-ion mode. The retention times for the N-oxide and voriconazole were 2.5 and 2.8 min, respectively. For quantitative determination of the N-oxide formed, a calibration curve was constructed using the peak area ratio of an analyte to its internal standard over a concentration range of 1 nM to 5 μM (R² >0.99).

The quantitative determination of hydroxy metabolites of P450 probe sub-
strates was achieved by a simultaneous HPLC-MS/MS method with HPLC conditions similar to those used in voriconazole separation and monitored at m/z transitions of 235→150, 312→230, and 305→269 by positive-ion mode multiple reaction monitoring for 4-hydroxyphenytoin, 4-hydroxydiclofenac, and 6-hydroxytestosterone, respectively. Quantitative determination was based on a constructed calibration curve of corresponding hydroxyl metabolite standards as reported previously (Yanni, 2004).

Statistical Analysis. All data represent a minimum of three experimental determ
inations and are expressed as mean ± S.D. The statistical significance for the difference of mean values of treated and control was evaluated by unpaired t test and was also evaluated using a nonparametric method as Wilcoxon rank-sum test with * , p < 0.05; **, p < 0.01; and *** , p < 0.001 compared with the control.

Results

Metabolism of Voriconazole by Human Liver Microsomes. Voriconazole (2 μM) was metabolized by HLM in the presence of NADPH as determined by the loss of the substrate as a function of time (Fig. 2); no loss of the substrate was observed in the absence of NADPH (data not shown). The rate of metabolism over the linear range (up to 20 min of incubation and 20% loss of voriconazole) was 54 pmol/min/mg of protein and t1/2 was estimated to be 57 min.

Metabolism of voriconazole (2 μM) by HLM was measured in the presence of ketconazole (3 μM), sulfaphenazole (30 μM), or fluvoxamine (3 μM), which selectively inhibit CYP3A4, CYP2C9, and CYP2C19, respectively, to determine the relative contributions of these enzymes toward voriconazole metabolism. Approximately 35, <5, and 50% of voriconazole metabolism was inhibited by ketcon-
azole, sulfaphenazole, and fluvoxamine, respectively. A mixture of the three P450 inhibitors (at respective concentrations indicated above) inhibited the metabolism by 75% (Fig. 3A); thus, approxi-
mately 25% of the oxidative metabolism of voriconazole by HLM persisted after chemical inhibition of CYP3A4, CYP2C9, and CYP2C19. The P450-selective inhibitors, at the concentrations used, nearly completely inhibited the respective P450 enzyme activities: CYP3A4 (testosterone 6-β hydroxylase), CYP2C9 (diclofenac 4-hy-
droxylase), and CYP2C19 (S-mephenytoin 4-hydroxylase) activities in HLM were inhibited by 100, 93, and 93% by ketconazole (3 μM), sulfaphenazole (30 μM), and fluvoxamine (3 μM), respectively (Fig. 3A).

The contributions of CYP3A4, CYP2C9, and CYP2C19 to voricon-
azole metabolism by HLM were also assessed using commercially available selective inhibitory antibodies against CYP3A4, CYP2C9, and CYP2C19. As shown in Fig. 3B, inhibitory antibodies against CYP3A4, CYP2C9, and CYP2C19 inhibited voriconazole metabolism by 30, 20, and 52%, respectively. Similar to the chemical inhibitors, the three inhibitory antibodies together caused approximately 70% reduction in voriconazole metabolism (Fig. 3B). Under the experimental conditions used, the respective P450 inhibitory antibo-
dies inhibited CYP3A4, CYP2C9, and CYP2C19 activities (determined using their probe substrates) by 90, 70, and 78%. Evaluation of the cross-reactivity of the three inhibitory antibodies showed that anti-CYP2C9 inhibited CYP2C19 and CYP3A4 by 40 and 45%, respectively, anti-CYP2C19 inhibited CYP3A4 by 17%, and anti-
CYP3A4 inhibited CYP2C9 by 20%. The cross-reactivity of anti-
CYP2C9 for CYP2C19 could explain the difference between the estimated contribution of CYP2C9 to voriconazole metabolism determined by the chemical inhibitor, sulfaphenazole (5%) (Fig. 3A) and by anti-CYP2C9 (20%) (Fig. 3B). The information supplied by XenoTech in the package insert for anti-CYP2C9 indicated that it inhibited 20% of CYP2C9 activity.

Evidence for Voriconazole Metabolism by Human Liver Flav
-Containing Monoxygenase. The results in Fig. 3 indicate that 25 to 30% of the oxidative metabolism of voriconazole by HLM persisted after inhibition of CYP3A4, CYP2C9, and CYP2C19, thus suggesting a role for another P450 enzyme(s) or for a different (non-P450) oxidative enzyme family. Other P450 enzymes were not considered because Hyland et al. (2003) have previously reported that none of the other P450 enzymes, e.g., CYP1A2, CYP2D6, CYP2B6, and CYP2A6, played a role in voriconazole metabolism. A role for FMO enzymes was anticipated on the basis of their known ability to
Materials and Methods, where the no inhibition control was the metabolism result-
ate oxidation of hetero atoms (Ziegler, 1993) including N-oxidation (Cashman, 1995), and on previous identification of an N-oxide (Fig. 1) as the major microsomal metabolite of voriconazole (Hyland et al., 2003; Roffey et al., 2003; Murayama et al., 2007). This hypothesis was first investigated using the lability of FMO toward mild heat, a condition that does not affect P450 enzymes, mostly CYP3A4 and CYP2C19 toward voriconazole can be catalyzed by FMO with the remaining 70 to 75% of the metabolism catalyzed by P450 enzymes, mostly CYP3A4 and CYP2C19.

**Metabolism of Voriconazole to N-Oxide by Recombinant Human Flavin Monooxygenase.** To confirm the role of FMO in the metabolism of voriconazole, its metabolism by recombinant FMO enzymes (FM01, FM03, and FM05) was examined. The HPLC-MS/MS chromatogram of the products generated from the incubation of voriconazole with recombinant FMO enzymes showed that voriconazole was metabolized to a product that coeluted with the authentic N-oxide (N\(^{1}\)-oxide of the 5-fluoropyrimidine moiety) (Fig. 5, A and B); ion trap mass spectrometry analysis of this metabolite further confirmed its identity. Interestingly, the HPLC-MS/MS chromatogram of voriconazole metabolites produced by HLM showed two metabolites—the N-oxide and a more polar metabolite (Fig. 5C), presumably hydroxymethyl voriconazole, reported as a CYP3A4-specific metabolite by Murayama et al. (2007). The formation of the more polar (hydroxymethyl) metabolite was abolished by ketoconazole (3 \(\mu\)M) treatment of the HLM (Fig. 5D), confirming the finding of Murayama et al. (2007) that it is selectively formed by CYP3A4. Evaluation of the cross-reactivity of P450 inhibitors, ketoconazole (3 \(\mu\)M) and fluvoxamine (3 \(\mu\)M), for voriconazole N-oxidation by FM01 and FM03 indicated that these inhibitors diminished FM03-catalyzed voriconazole N-oxidation by 25 and 40%, respectively, but had negligible effect on the FM01 activity. The rate at which voriconazole (100 \(\mu\)M) was metabolized by the three commercially available recombinant FM01, FM03, or FM05 enzymes is shown in Fig. 6A. FM03 metabolized voriconazole at a greater velocity than did FM01, and metabolism by FM05 was too low to detect any product. The rate of N-oxide formation by either FM01 or FM03 as a function of voriconazole concentrations (1–8000 \(\mu\)M) followed Michaelis-Menten kinetics (Fig. 6B), yielding \(K_m\) values of 3.0 and 3.3 mM and \(V_{max}\) values of 0.025 and 0.044 pmol/min/pmol, respectively (Table 1).

**Metabolism of Voriconazole to N-Oxide by Liver Microsomes from CYP2C19 Poor Metabolizers.** If CYP3A4 and FMO are the two most important contributors, next to CYP2C19, toward voriconazole N-oxidation, it is important to assess their relative role in CYP2C19 poor metabolizers. Thus, inhibition of voriconazole N-
oxidation by ketoconazole (3 μM) and by heat-inactivation (45°C for 5 min) was assessed using two samples of HLM obtained from CYP2C19-competent HLM (WT). As expected, the total voriconazole N-oxidation by CYP2C19-deficient HLM (~20 pmol/min/mg) was less than 50% of that by CYP2C19-competent HLM (>40 pmol/min/mg) (Fig. 7A). Interestingly, FMO activity (as determined by metabolism of the probe substrate, benzylamine) appeared to be lower (<50%) in CYP2C19-deficient HLM than in CYP2C19-competent HLM (Fig. 7A). CYP3A4 contributed to more than 60% of the total voriconazole N-oxidation in CYP2C19-deficient HLM, whereas the contribution of FMO was <20%. This may reflect lower FMO expression in CYP2C19-deficient individuals. In contrast, FMO appeared to contribute as much as or more than CYP3A4 to voriconazole N-oxidation by CYP2C19-competent HLM, with the two enzymes together contributing to >50% of the total voriconazole N-oxidation.

Discussion

Voriconazole is an antifungal agent that is known to be cleared predominantly via metabolism in all species tested; for example, only 2% of the administrated dose is recovered in excreta as the parent drug in humans. Its elimination is capacity-limited in preclinical species (Roffey et al., 2003) and in humans (Purkins et al., 2002), presumably due to saturation of metabolic transformations. Thus, in humans, with a 2-fold increase in the oral dose (200–400 mg) of voriconazole, a 2.8-fold increase in $C_{\text{max}}$ and a 3.9-fold increase in $AUC_{\text{c}}$ was observed (Purkins et al., 2002). The major metabolic pathways in humans (Roffey et al., 2003) appear to involve N-oxidation of the fluoropyrimidine ring, hydroxylation of the methyl group, and glucuronidation of the hydroxylated metabolites. An interesting metabolic step that appears to be stimulated by the formation of the N-oxide is the cleavage of voriconazole, resulting in the loss of the fluoropyrimidine ring (Roffey et al., 2003). Thus, the N-oxide may contribute to an even greater percentage of the total metabolites than would be apparent from the percentages of the isolable oxide. The N-oxide is also the major circulating metabolite in humans, rats, and dogs. In vitro studies have implicated CYP2C19, CYP3A4, and to a lesser extent CYP2C9 in the oxidative metabolism of voriconazole (Hyland et al., 2003; Murayama et al., 2007), and their role in the formation of N-oxide seems to depend on the concentration of voriconazole in the reaction (Hyland et al., 2003).
The results reported here provide unequivocal evidence that, in addition to the P450 enzymes, FMO can metabolize voriconazole to its major circulating metabolite, the N-oxide. Studies with chemical inhibitors and with P450-specific antibodies clearly established that CYP3A4, CYP2C9, and CYP2C19 could not account for more than approximately 70% of voriconazole metabolism by HLM.

Because of the known selectivity of this enzyme family to oxidize compounds at the hetero atoms, such as sulfur and nitrogen (reviewed in Krueger and Williams, 2005), the role of FMO in the oxidation of voriconazole at the ring nitrogen of the fluoropyrimidine ring was investigated. Initial evidence for the involvement of FMO in the oxidation of voriconazole was obtained when the results showed that approximately 20% of the total enzyme activity contributing to oxidative metabolism of voriconazole was heat-labile; it was established previously that short incubation (2–5 min) at 45–50°C (without NADPH) destroys FMO activity without significantly affecting P450 activity (Ziegler, 1980; Krueger and Williams, 2005). Further studies with recombinant enzymes (FMO1, FMO3, and FMO5) definitively established that enzymes of the FMO family oxidized voriconazole to the N-oxide metabolite. The kinetic analysis yielded $K_{\text{m}}$ values in the low millimolar range for FMO1 and FMO3, whereas FMO5 appeared to exhibit only a very low level of catalytic activity toward voriconazole. Taken together, these results suggest that certain FMO isoforms contribute significantly to oxidative metabolism of voriconazole. We recognize that the estimation of the relative contribution of FMO toward voriconazole metabolism by HLM is based solely on the heat sensitivity of a fraction of the metabolism. Unfortunately, the lack of inhibitory antibodies and FMO-selective inhibitors does not allow us to estimate the contribution of individual FMO enzymes in the overall metabolism of voriconazole. When the kinetic parameters for voriconazole metabolism by FMO1/FMO3 (Table 1) versus CYP2C19 (Hyland et al., 2003) are scaled to a typical human liver, containing 80 (FMO3), 15 (CYP2C9), and 100 (CYP3A4) pmol of enzyme/mg of microsomal protein (Fisher et al., 2002; Koukouritaki et al., 2004; Hines 2006), it is expected that FMO would contribute to less than 2% of the voriconazole metabolic clearance by CYP3A4 and an even smaller percentage of the metabolic clearance by CYP2C19.
However, the in vitro (heat inactivation) studies with human liver microsomes (Fig. 4) suggest a much greater (~20%) contribution of FMO toward voriconazole N-oxidation. This is not surprising because a similar scaling exercise suggested a much smaller contribution of CYP3A4 (<4%) compared with that of CYP2C19 toward voriconazole metabolic clearance than has been found (~35%) in clinical studies (Mikus et al., 2006).

It is important to note that both CYP3A4 and FMO play a lesser role compared with that of CYP2C19 toward the metabolic clearance of voriconazole. In two separate studies, inhibitors of CYP3A4, erythromycin (1 g twice daily for 7 days) (Purkins et al., 2003c) and indinavir (800 mg three times daily for 7 days) (Purkins et al., 2003d), did not alter Cmax or AUC of coadministered voriconazole (200 mg twice daily). These studies led to the conclusion that although CYP3A4 might contribute to metabolic clearance, it did not govern the pharmacokinetics of voriconazole in humans. However, this conclusion should be considered with caution because these studies did not include appropriate positive controls. In fact, Mikus et al. (2006) contradicted this conclusion and reported that the CYP3A4 inhibitor, ritonavir (300 mg twice daily), reduced the oral clearance of voriconazole (400 mg) by ~35%. Not surprisingly, the effect of ritonavir in reducing the oral clearance of voriconazole was much larger (>80%) in CYP2C19 poor metabolizers. Thus, it appears that CYP3A4 plays a much more significant role in CYP2C19 poor metabolizers than in normal subjects; the in vitro data in Fig. 7, in fact, confirm this.

Further clinical and in vitro studies should be conducted to investigate the role of FMO in CYP2C19 poor metabolizers and in normal subjects.

This is the first report of the role of FMO in the metabolism of voriconazole in an in vitro system. Clearly, it would be important to assess the role of FMO in the metabolic clearance of this antifungal agent in preclinical species and in humans. There is compelling evidence suggesting that the metabolic clearance of voriconazole is saturated over the therapeutic dose range of this drug, as doubling the dose leads to an almost 4-fold increase in the systemic exposure of the parent drug in adult patients. Interestingly, the metabolic clearance does not show evidence of saturation in children (Walsh et al., 2004). Whether this difference in dose dependence observed in adults versus children is due to different relative contributions of FMO, CYP3A4, and CYP2C19 in these two patient populations remains to be determined.

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References


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