Potential impact of cytochrome P450 3A5 in human liver on drug interactions with triazoles

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Cytochrome P450 3A is the main enzyme subfamily involved in the metabolism of a variety of marketed medicines. It is generally believed that the substrate specificity of polymorphic P450 3A5 is similar to that of the predominant P450 3A4 isoform, although some differences in catalytic properties have been found. It has been hypothesized that individuals with CYP3A5*1 (P450 3A5 expresser) might clear the HIV protease inhibitor saquinavir, administered by mouth, more rapidly than subjects lacking functional CYP3A5 alleles. Enhanced midazolam hydroxylation and cyclosporin metabolism occur in an *in vitro* P450 3A5 system and in liver microsomes expressing P450 3A5 in the presence of thalidomide. However, inhibition constants (K_i) of three triazole anti-fungal drugs (itraconazole, fluconazole, and voriconazole) for liver microsomal P450 3A5 are higher than for liver microsomal P450 3A4. To predict drug interactions *in vivo*, we estimated increases of areas under the curves (AUC) dependent on polymorphic P450 3A5 expression, using both $1 + [\text{Inhibitor}] / K_i$ (recommended in US FDA guidance), and $1 + [\text{Inhibitor}]_{\text{unbound}} / K_i$ (as recommended by Japanese MHLW Notice). Voriconazole would be expected to cause approximately a three-fold higher increase in AUC in subjects with CYP3A5*3/*3 than in those with CYP3A5*1/*3, especially when estimated using the FDA guidance. We conclude that drug interactions between marketed drugs may differ substantially between individuals with genetically distinct P450 3A5 catalytic functions.

Introduction

Cytochrome P450 enzymes (P450s) are heme-thiolate mono-oxygenases involved in oxidation of many endogenous and exogenous substrates [1]. P450 3A is the most important human P450 subfamily due to its high relative abundance in the liver and broad substrate specificity [2, 3]. P450 3A4 is a predominant form generally expressed in the human liver and intestine, whereas P450 3A5, which is expressed polymorphically, might contribute as much as 50% of hepatic P450 3A in a third to one half of Caucasians and African-Americans [4]. We reported a high frequency of hepatic P450 3A5 expression in a Japanese population [5, 6]. Despite 83% homology between P450 3A5 and P450 3A4, differences in catalytic properties have been demonstrated [7, 8].

Triazole antifungal drugs are clinically important for serious, invasive fungal infections [9]. Itraconazole is a fat-soluble synthetic triazole antifungal drug and is a broad spectrum antifungal. Voriconazole was approved in Europe and the United States in 2002 for the treatment of invasive *Aspergillosis* and has an extremely broad spec-

trum. Voriconazole is structurally related to fluconazole, but its activity and handling by the body are completely different. These triazole antifungals cause clinically important drug interactions via their effects on human P450 3A enzymes. This has been studied *in vitro* and *in vivo* [10, 11]. However, few reports compare in detail their effects on the kinetics of drug oxidation by P450 3A4 and P450 3A5 or compare their potential for drug interactions resulting from their inhibition of P450 3A4 vs. P450 3A5 [12].

The present paper summarizes the roles of P450 3A enzymes in metabolic clearance and inhibition of triazole antifungal drugs, including voriconazole, emphasising polymorphic P450 3A5 expression in genotyped livers and the potential impact of this on drug interactions and activation (co-operativity).

Methods

The kinetic parameters used in this study were taken from our earlier reviews [12, 13] as well as from newer literature

Table 1

Reported inhibition constants of fluconazole and itraconazole toward recombinant P450 3A4- and P450 3A5-mediated midazolam 1'-hydroxylation activities

Triazole	Inhibition type	K_i (μM) P450 3A4	P450 3A5	References
Itraconazole	Competitive	0.0157 (0.0013)	–	[22]
Fluconazole	Noncompetitive	9.2 $\pm$ 0.5	85 $\pm$ 3	[17]
Fluconazole	Mixed-type (3A4) competitive (3A5)	7.4 $\pm$ 1.9	53 $\pm$ 4	[18]

The value in brackets shows $K_{i,fu,mic}$ where $f_{u,mic}$ is the unbound free fraction ($8.8 \pm 3.2\%$) in the microsomes expressing P450 3A4.

Table 2

Reported inhibition constants of fluconazole and itraconazole toward human liver microsomal midazolam 1'-hydroxylation activities

Inhibitor	Inhibition type	K_i (μM)	IC_{50} (μM)	References
Itraconazole	–	–	0.045 $\pm$ 0.007	[19]
Itraconazole	–	0.50 $\pm$ 0.04	–	[20]
Itraconazole	–	–	0.029 (0.0061)	[22]
Itraconazole	–	–	0.15	[21]
Fluconazole	Noncompetitive	15 $\pm$ 0.8	–	In P450 3A5 non-expressing liver microsomes [18].
Fluconazole	Noncompetitive	25 $\pm$ 15	–	In P450 3A5 expressing liver microsomes [18].
Fluconazole	–	–	>100	[23]
Fluconazole	–	–	19	[24]
Fluconazole	–	11.9 $\pm$ 0.9	–	[20]
Fluconazole	–	–	43 $\pm$ 6	[19]

The value in brackets shows $\text{IC}_{50,fu,mic}$ where $f_{u,mic}$ is the free fraction ($19.6 \pm 9.2\%$) in human liver microsomes.

and from some original data. Human P450 3A4 and 3A5 expressed in *Escherichia coli* membranes and liver microsomes genotyped for the CYP3A5*1 and 3A5*3 were used as enzyme sources [14]. Inhibition constants (K_i) of itraconazole, fluconazole, and voriconazole were determined against midazolam hydroxylation catalyzed by liver microsomal P450 3A enzymes [14]. Inhibitory effects of voriconazole on cyclosporin oxidation were also determined [14]. To predict the potential for drug interactions *in vivo*, increases of areas under the curves (AUC) dependent on polymorphic P450 3A5 expression were estimated both from $1 + [I] / K_i$ (as recommended in US FDA guidance) and from $1 + [I]_{\text{unbound}} / K_i$ (as recommended by Japanese MHLW Notice), where $[I]$ and $[I]_{\text{unbound}}$ are the total and unbound inhibitor concentrations, respectively, and K_i is the inhibition constant [11, 15]. Docking simulations of voriconazole and other substrates in a reported structure of P450 3A4 (PDB 1WOG) and a model of P450 3A5 were calculated using MOE software [14, 16].

Results of data review and discussion

Reported inhibition constants (K_i) of triazoles toward recombinant P450 3A4 and P450 3A5 are summarized in

Table 1. Inhibition by triazoles of 1'-hydroxylation of midazolam varied by a factor of 10 between the K_i values of fluconazole toward P450 3A4 and P450 3A5 [17, 18]. With human liver microsomes as the enzyme source (Table 2), reported IC_{50} or K_i values of itraconazole indicated 17-fold differences [19–22]. Similar results have been obtained with fluconazole [18–20, 23, 24]. Apparent differences in K_i of fluconazole were reported between liver microsomes that either do or do not express P450 3A5 [18].

To investigate the apparently low potential for inhibition of triazoles toward P450 3A5 compared with P450 3A4, we conducted inhibition studies with P450 3A5 and P450 3A4 under identical conditions using P450 isoforms expressed in bacterial membranes. Recombinant P450 3A5 catalyzed midazolam 1'-hydroxylation more efficiently than did P450 3A4 (Table 3). K_i values of these triazoles for midazolam hydroxylation catalyzed by recombinant P450 3A4 were lower than those catalyzed by P450 3A5. K_i values of each of the three triazoles determined using human liver microsomes genotyped for the CYP3A5*3/*3 (poor P450 3A5 expresser) were approximately half those determined using human liver microsomes from the CYP3A5*1/*3 genotype (P450 3A5 expressers) (Table 3). It should be mentioned that the apparent kinetic parameters for liver microsomes shown in Table 3 were affected by liver microsomal P450 3A4 and/or P450 3A5.

Table 3

Examined inhibition constant (K_i) of itraconazole, fluconazole, and voriconazole on midazolam 1'-hydroxylation activities catalyzed by P450 3A4 and P450 3A5 and human liver microsomes genotyped for the CYP3A5 gene

	$V_{\max}$	K_m (μM)	K_i (μM) Itraconazole	Fluconazole	Voriconazole
Recombinant					
P450 3A4	8 ± 1^a	2 ± 1	0.03 ± 0.01	1.9 ± 0.3	0.15 ± 0.02
P450 3A5	15 ± 1	3 ± 1	0.94 ± 0.16	21 ± 10	0.20 ± 0.09
Liver microsomes					
3A5*3/*3	2.0 ± 0.1^b	5 ± 2	0.13 ± 0.030	5.1 ± 1.1	0.20 ± 0.12
3A5*1/*3	1.5 ± 0.1	2 ± 1	0.26 ± 0.032	7.4 ± 4.2	0.45 ± 0.12

^anmol min⁻¹ nmol⁻¹ P450 3A, ^bnmol min⁻¹ mg⁻¹ protein. Kinetic parameters are mean and SE by nonlinear regression analysis. K_i values were determined in a competitive manner. The liver microsomal samples genotyped as 3A5*3/*3 and 3A5*1/*3 contained 18 pmol P450 3A4 and 6.1 pmol P450 3A5 mg⁻¹ protein and 17 pmol P450 3A4 and 24 pmol P450 3A5 mg⁻¹ protein, respectively [5, 6].

Table 4

Effects of voriconazole on cyclosporin A oxidation activities of recombinant P450 3A4 and P450 3A5 and human liver microsomes genotyped for the CYP3A5 gene

P450	Control	Plus voriconazole
Recombinant	(nmol min ⁻¹ nmol ⁻¹ P450)	
P450 3A4	0.47 (100)	0.18 (38)
P450 3A5	0.40 (100)	0.27 (68)
Liver microsomes	(nmol min ⁻¹ mg ⁻¹ protein)	
CYP3A5*3/*3	0.31 (100)	0.17 (55)
CYP3A5*1/*3	0.25 (100)	0.22 (88)

Cyclosporin A (10 μM) was incubated with voriconazole (0.20 μM). Data are average from duplicate determinations. Numbers in parentheses are % of the control. The P450 3A contents in human liver microsomes are shown in the legend of Table 3.

Voriconazole is of particular interest because of different metabolic pathways catalyzed by P450 2C19 and P450 3A4 [16]. Voriconazole *N*-oxidation and 4-hydroxylation are catalyzed more efficiently by P450 3A4 than by P450 3A5 [16]. Using voriconazole to inhibit cyclosporin oxidation, it was found that voriconazole was a weak inhibitor of P450 3A5. Liver microsomes expressing P450 3A5 were less inhibited than were microsomes expressing P450 3A4 (Table 4). $V_{\max}$ and K_m for tacrolimus metabolism have been reported to be 1.47 nmol min⁻¹ mg⁻¹ protein and 10.6 $\mu\text{mol l}^{-1}$ in the absence and 0.37 nmol min⁻¹ mg⁻¹ protein and 5.43 $\mu\text{mol l}^{-1}$ in the presence of voriconazole (200 $\mu\text{g ml}^{-1}$ = 573 $\mu\text{mol l}^{-1}$), respectively, suggesting that voriconazole inhibits both by competitive and noncompetitive mechanisms [25]. Docking simulation revealed that voriconazole can adopt an orientation suitable for *N*-oxidation over the P450 3A4 heme (Figure 1A). However, voriconazole could adopt far from the P450 3A5 heme (Figure 1B), resulting in a slower metabolic rate by P450 3A5 than P450 3A4 in a Michaelis-Menten kinetic manner.

Midazolam can adopt an orientation suitable for 1'-hydroxylation over the P450 3A5 heme (Figure 1C) as well as the P450 3A4 heme. It is noteworthy that thalidomide adapts closely to the P450 3A5 heme (Figure 1D) and enhances midazolam 1'-hydroxylation or cyclosporin A clearance of P450 3A5 [14] via an unexpected phenomenon, which is called activation or heterotropic co-operativity [13], in a mechanism distinct from that of voriconazole. In our preliminary experiments, thalidomide was hydroxylated in a sigmoidal velocity curve vs. substrate, presumably because of docking two substrate molecules into the P450 3A5 pocket, which is called as homotropic co-operativity [13]. Why the mechanism is different between voriconazole and thalidomide is not known but it might be due to substrate molecular size, polarity or affinity towards a P450 3A5 pocket.

It is generally believed that the substrate specificity of polymorphic P450 3A5 is similar to that of the predominant P450 isoform, P450 3A4, although some differences in catalytic properties have been found. It has been hypothesized that individuals with CYP3A5*1 (P450 3A5 expresser) might have a higher clearance of the HIV protease inhibitor saquinavir, administered by mouth, compared with subjects lacking functional CYP3A5 alleles [12]. This and the results summarized in Tables 3 and 4, suggest that inter-individual differences in drug clearances and drug-interactions of triazole antifungal drugs, including voriconazole, are appreciably affected by the CYP3A5 genotypes (Tables 2, 3). Polymorphic P450 3A5 expression in human livers could influence several clinically important drug interactions. To investigate this we estimated the potential for *in vivo* interaction from the $[I]/K_i$ or $[I]_{\text{unbound}} : K_i$ ratios, using blood concentration data in their package inserts. In order to calculate the $[I]_{\text{unbound}} : K_i$ ratios, the pharmacokinetic parameters of each drug were obtained from the literature, together with the K_i values from *in vitro* studies using human liver microsomes (Table 3). The findings indicated that the highest increased AUC caused by

Figure 1
Docking simulation of voriconazole into a reported structure of P450 3A4 (A) and a homology model of P450 3A5 (B) in comparison with the cases of midazolam (C) and thalidomide (D). Voriconazole adopted an orientation suitable for P450 3A4 (corresponding to 1WOG) ($U=21.4$) [16]. A homology model of P450 3A5 was constructed as reported previously [14]

Table 5
In vivo estimated fold increase in AUC in the presence of itraconazole, fluconazole, and voriconazole from $1 + [I]/K_i$ equation in *in vitro* experiments

Azoles, single dose ^a	Reported maximal plasma concentration (μM) ^a	f_u (%)	Fold increase in AUC ^b 3A5*3/*3	3A5*1/*3
Itraconazole 200 mg	0.31	0.2	3.4 (1.0)	2.2 (1.0)
Fluconazole 400 mg	26	88	6.1 (5.5)	4.5 (4.1)
Voriconazole 400 mg	8.3	42	43 (18)	19 (8.7)

^aTaken from data in package insert for each medicine. ^b K_i (μM) values were taken from Table 3 in human liver microsomes genotyped for the CYP3A5 gene. Numbers in parentheses indicate fold increase in AUC by $1 + [I]_{\text{unbound}}/K_i$ equation.

co-administered voriconazole would be expected to be low in expressors of P450 3A5 (Table 5). There are not any published data in our literature search so far suggesting/indicating the changes *in vivo* shown in Table 5 from the viewpoint of the CYP3A5 genotype. However, drug interactions via P450 3A inhibition should be anticipated especially in subjects of the CYP3A5*3/*3 genotype, who are expected to be poor expressors of P450 3A5.

In conclusion, CYP3A5 genotype may influence inter-individual differences in triazole antifungal drug clearance and the intensity of drug interactions with voriconazole. P450 3A5 also shows the heterotropic co-operativity

phenomenon in some cases. Increased AUC caused by co-administered voriconazole should be anticipated as a potential cause of toxicity, especially in subjects with the CYP3A5*3/*3 genotype.

Competing interests

There are no competing interests to declare.

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