Effect of Antifungal Drugs on Cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 Activities in Human Liver Microsomes

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The effects of five antifungal drugs, fluconazole, itraconazole, micafungin, miconazole, and voriconazole, on cytochrome P450 (CYP) 2C9-mediated tolbutamide hydroxylation, CYP2C19-mediated S-mephenytoin 4'-hydroxylation, and CYP3A4-mediated nifedipine oxidation activities in human liver microsomes were compared. In addition, the effects of preincubation were estimated to investigate the mechanism-based inhibition. The IC50 value against tolbutamide hydroxylation was the lowest for miconazole (2.0 μM), followed by voriconazole (8.4 μM) and fluconazole (30.3 μM). Similarly, the IC50 value against S-mephenytoin 4'-hydroxylation was the lowest for miconazole (0.33 μM), followed by voriconazole (8.7 μM) and fluconazole (12.3 μM). On the other hand, micafungin at a concentration of 10 or 25 μM neither inhibited nor stimulated tolbutamide hydroxylation and S-mephenytoin 4'-hydroxylation, and the IC50 values for itraconazole against these were greater than 10 μM. These results suggest that miconazole is the strongest inhibitor of CYP2C9 and CYP2C19, followed by voriconazole and fluconazole, whereas micafungin would not cause clinically significant interactions with other drugs that are metabolized by CYP2C9 or CYP2C19 via the inhibition of metabolism. The IC50 value of voriconazole against nifedipine oxidation was comparable with that of fluconazole and micafungin and higher than that of itraconazole and miconazole. The stimulation of the inhibition of CYP2C9-, CYP2C19-, or CYP3A4-mediated reactions by 15-min preincubation was not observed for any of the antifungal drugs, suggesting that these drugs are not mechanism-based inhibitors.

Key words antifungal drug; fluconazole; itraconazole; micafungin; miconazole; voriconazole

Cytochrome P450s (CYP) comprise a superfamily of enzymes that catalyzes the oxidation of a wide variety of xenobiotic chemicals, including drugs and carcinogens.3–5) Multiple-drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions, and many drug–drug interactions involving metabolic inhibition are reported.6,7)

Antifungal drugs, including fluconazole, itraconazole, micafungin, miconazole, and voriconazole (Fig. 1), are widely used in the treatment of systemic candidal infections and mycoses. The mechanism of action of these antifungal drugs is the inhibition of fungal CYP (14α-sterol demethylase), an enzyme responsible for the conversion of lanosterol to 14α-demethyllanosterol in the ergosterol biosynthetic pathway,8,9) except that micafungin inhibits 1,3-b-D-glucan synthase, leading to disruption of the growing fungal cell wall and death of the fungal cell.10,11) Because antibiotics, including antifungal drugs, are coadministered in most cases, the possibility of interactions between them and other drugs exists. Recently, we have investigated the effects of antifungal drugs excluding voriconazole on CYP3A4 activity and multidrug resistance protein 1 (MDR1), and found that itraconazole and miconazole, as well as ketoconazole, had greater inhibitory effects on both CYP3A4 metabolic and MDR1 transport activities than fluconazole and micafungin.12) In addition, it has been demonstrated that the K1 values of fluconazole and micafungin against nifedipine oxidation activity, a marker enzyme activity of CYP3A4, are 10.7 μM and 17.3 μM, respectively.11) Zhang et al.4) reported that miconazole competitively inhibits several CYPs, including CYP2C9, CYP2C19, and CYP3A4, with K1 values ranging from 0.01 to 7.3 μM. Furthermore, it is likely that fluconazole and voriconazole inhibit CYP2C9, CYP2C19, and CYP3A4.9,15–18) However, there are few studies comparing the effects of antifungal drugs on human hepatic CYP-mediated drug-metabolizing activity under the same experimental conditions.

Many inhibitors are known to be activated metabolically to a reactive intermediate(s) that, in turn, is irreversibly or quasi-irreversibly bound to the enzyme(s).19) Some acetylenes, including those synthetic steroids such as gesto-
dene and ethynyl estradiol, have been demonstrated to cause mechanism-based inactivation of CYP3A4. Sorivudine is converted by gut flora to (S)-5-(2-bromovinyl)uracil (BVU), which is metabolized to dihydro-BVU by dihydropyrimidine dehydrogenase (DPD), and the dihydro-BVU binds to DPD itself.21 It has been reported that these mechanism-based inhibitors exhibit preincubation time dependence of inhibition.18—31

In the present study, we compared the effects of five antifungal drugs on specific activities of CYP2C9, CYP2C19, and CYP3A4 in human liver microsomes under the same experimental conditions. In addition, the effects of preincubation were estimated to investigate whether these antifungal drugs are mechanism-based inhibitors.

MATERIALS AND METHODS

Materials Pooled human liver microsomes from 46 or 50 individuals (lot no. 0210171 or lot no. 0310241) were obtained from Xenotech (Lenexa, KS, U.S.A.). Micafungin and voriconazole were synethized in the Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. Miconazole and fluconazole were purchased from ICN Biomedicals, Inc. (Irvine, CA, U.S.A.) and itraconazole from Janssen-Kyowa (Tokyo, Japan). Tolbutamide and nifedipine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). S-Mephenytoin, 4′-hydroxymephenytoin, hydroxytolbutamide, and oxidized nifedipine were purchased from Ultrafine Chemicals (Manchester, U.K.). Phenobarbital sodium and p-hydroxybenzoic acid isopropyl were obtained from Wako Pure Chemicals (Osaka, Japan) and Tokyo Chemical Industry (Tokyo, Japan), respectively. All other reagents were of the highest purity commercially available.

Determination of Human CYP Activities Tolbutamide hydroxylation activity (CYP2C9), S-mephenytoin 4′-hydroxylaction activity (CYP2C19), and nifedipine oxidation activity (CYP3A4) in human liver microsomes in the presence or absence of antifungal drugs were determined as described previously.32,33 The incubation mixture consisted of human microsomes, 2 mM NADP⁺, 10 mM glucose-6-phosphate, 5 mM magnesium chloride, 1 unit/ml of glucose-6-phosphate dehydrogenase, 100 mM phosphate buffer (pH 7.4), and 5 µl of methanol or 0.001—5 mM antifungal drugs dissolved in methanol in a final volume of 500 µl.21,22 The maximum concentration of itraconazole in the incubation mixture was 10 µM, because itraconazole at a concentration greater than 1 mM was not dissolved in methanol. The microsomal protein concentration in the mixture was 0.05 (nifedipine oxidation) or 0.5 mg/ml (for tolbutamide hydroxylation and S-mephenytoin 4′-hydroxylation). Because the Kᵦᵢ values for tolbutamide hydroxylation, S-mephenytoin 4′-hydroxylation, and nifedipine oxidation by human liver microsomes were 150.8, 27.3, and 12.2 µM, respectively (data not shown),21,22 the concentrations of tolbutamide, S-mephenytoin, and nifedipine were 200, 30, and 10 µM, respectively, which are around the expected Kᵦᵢ values. Incubation was carried out at 37 °C for 10 min (for the determination of IC₅₀ against nifedipine oxidation), 15 min (for the determination of the effects of preincubation on nifedipine oxidation), or 30 min (for tolbutamide hydroxylation and S-mephenytoin 4′-hydroxylation).

Preincubation in many studies of the mechanism-based inhibition against CYP activities was conducted for 1—30 min, and obvious preincubation-dependent increases in the inhibition rates were observed in more than 5—10 min preincubation.20,22—30 In addition, Draper et al. examined the inhibitory effects of 5-min preincubation on coumarine 7-hydroxylase (CYP2A6) activity for 47 chemicals, including fluconazole and itraconazole.31 Therefore the incubation mixture without substrate was preincubated at 37 °C for 15 min. After preincubation, the substrate was added and then incubated at 37 °C as mentioned above.

Data Analysis All data were analyzed using the average of duplicate, triplicate, or quadruplicate determinations. In preliminary experiments, the linearity of reaction with incubation time and protein concentration was confirmed for each assay condition. IC₅₀ values were calculated using the WinNonlin Standard computer program (Version 4.1, Pharsight, CA, U.S.A.). Because the substrate concentrations are around the Kᵦᵢ values, Kᵦᵢ values are expected to be equal to IC₅₀/2 or IC₅₀ in competitive or noncompetitive inhibition, respectively.31 Statistical differences for the determination of the effect of preincubation were determined with Student’s t-test using the SAS program (SAS Institute Inc, Cary, NC, U.S.A.), and the significance level was set at p<0.05.

RESULTS

Inhibition of CYP Activities by Antifungal Drugs We have reported the inhibitory effects of antifungal drugs excluding voriconazole on CYP3A4 activity.12) Therefore the effects of voriconazole on CYP3A4 activity and the effects of five antifungal drugs on CYP2C9 and CYP2C19 activities were investigated in this paper (Fig. 2, Table 1). The IC₅₀ value against tolbutamide hydroxylation (CYP2C9) was the lowest for miconazole (2.0 µM), followed by voriconazole (8.4 µM) and fluconazole (30.3 µM). Similarly, the IC₅₀ value...
against S-mephenytoin 4'-hydroxylation (CYP2C19) was the lowest for miconazole (0.33 μM), followed by voriconazole (8.7 μM) and fluconazole (12.3 μM). IC₅₀ values of itraconazole against these metabolic activities were greater than 10 μM. The IC₅₀ value of voriconazole against nifedipine oxidation (CYP3A4) was comparable with that of fluconazole and micafungin and higher than that of itraconazole and miconazole. On the other hand, micafungin at a concentration of 10 or 25 μM neither inhibited nor stimulated CYP2C9 and CYP2C19 activities (Fig. 3).

**Effects of Preincubation on Inhibitory Effects of Antifungal Drugs** The effects of 15-min preincubation on the inhibitory effects of antifungal drugs are shown in Fig. 3. The stimulation of the inhibition of CYP2C9-, CYP2C19-, or CYP3A4-mediated reactions by 15-min preincubation was not observed for any of the antifungal drugs, suggesting that these drugs are not the mechanism-based inhibitors. On the other hand, a slightly decrease in the inhibition of the CYP2C19-mediated reaction after preincubation was observed for miconazole and voriconazole.

**DISCUSSION**

The IC₅₀ value of voriconazole against CYP3A4 activity (10.5 μM) was higher than that of itraconazole and miconazole, and comparable with that of fluconazole and micafungin (Table 1). Itraconazole and miconazole are potent clinically important inhibitors of the clearance of CYP3A4 substrates.15,34—38 In addition, it has been reported that fluconazole (≥200 mg/d) elevates the plasma/blood concentrations of midaolazam and cyclosporine,15,34—38 which are mainly metabolized by CYP3A4,39 and that voriconazole increases the blood concentrations of cyclosporin.17,40 However, the blood concentrations of tacrolimus and cyclosporin are not affected by coadministration of micafungin.41,42 Fluconazole and voriconazole are orally administered, whereas micafungin is given only by infusion. It has been reported that the maximum plasma concentration (Cmax) after an oral dosing of 100 mg of fluconazole is 1.88 μg/ml (6.1 μM),34 that Cmax after oral dosing with voriconazole (200 mg bid) is 1.89 μg/ml (5.4 μM),39 and that Cmax after 1-h infusion of 150 mg of micafungin is 14.30 μg/ml (11.3 μM).41 In addition, protein binding of fluconazole,34 voriconazole,9,17 and micafungin16,41 in human serum or plasma is reported to be 11—12%, 58%, and 99.8%, respectively. Therefore it is speculated that fluconazole and voriconazole, but not micafungin, might be sufficiently distributed to the liver to cause drug interactions, although they have similar inhibitory effects in vitro. In addition, because the intestinal first-pass metabolism mediated by CYP3A4 has been shown to be clinically relevant for several drugs,45 drug interactions with oral antifungal drugs might occur not only in the liver but also small intestine.

IC₅₀ values against tolbutamide hydroxylation (CYP2C9) and S-mephenytoin 4'-hydroxylation (CYP2C19) were the lowest for miconazole (0.33—2.0 μM), followed by voriconazole (8.4—8.7 μM) and fluconazole (12.3—30.3 μM). IC₅₀ values for itraconazole against these metabolic activities were greater than 10 μM (Table 1). On the other hand, micafungin at 10 or 25 μM neither inhibited nor stimulated CYP2C9 and CYP2C19 activities (Fig. 3). Although the IC₅₀ values of miconazole against CYP2C9 and CYP2C19 were higher than those against CYP3A4, the IC₅₀ values of fluconazole and voriconazole against CYP2C9 and CYP2C19 were comparable with those against CYP3A4. Therefore it is possible to speculate that fluconazole and voriconazole, but not micafungin, would clinically cause drug interactions with other drugs, which are metabolized by CYP2C9 or CYP2C19 as well as CYP3A4. It has been reported that the plasma concentrations of phenytoin and S-warfarin, which

<table>
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<th>Antifungal drug</th>
<th>IC₅₀ (μM)</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP3A4</th>
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<tr>
<td>Fluconazole</td>
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<td>12.3</td>
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<td>&gt;10</td>
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<tr>
<td>Micafungin</td>
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<td>No inhibition</td>
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<tr>
<td>Miconazole</td>
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<td>0.33</td>
<td>0.0742</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>8.4</td>
<td>8.7</td>
<td>10.5</td>
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* Sakaeda et al.12  
* No inhibition was observed at 10 and 25 μM concentrations.
are mainly metabolized by CYP2C9,\(^\text{39}\) are enhanced by coadministration of fluconazole,\(^{15,46,47}\) and that voriconazole increases the plasma concentrations of phenytoin.\(^{48}\)

Mechanism-based inactivators have been reported to exhibit preincubation time dependence of inhibition.\(^{20,29−31}\) However, the inhibition of CYP2C9-, CYP2C19-, or CYP3A4-mediated reactions after 15-min preincubation was not stimulated by any of the antifungal drugs investigated (Fig. 3), suggesting that these drugs are not mechanism-based inhibitors. It has been reported that itraconazole, micafungin, and voriconazole are biotransformed by drug-metabolizing enzymes including CYP2C9, CYP2C19, and/or CYP3A4,\(^{15,49}\) and that fluconazole, itraconazole, and micafungin, and voriconazole competitively inhibit CYP3A4 activities.\(^{14,49,50}\)

On the other hand, the slight decrease in the inhibition of CYP2C19-mediated reaction after preincubation was observed for miconazole and voriconazole (Fig. 3). A possible explanation for these phenomena is that these antifungal drugs are metabolized by microsomes during the preincubation period, thereby lowering the levels of inhibitors prior to assay.

In conclusion, the present study showed that CYP2C9 and CYP2C19 are inhibited most potently by miconazole, followed by voriconazole and fluconazole, and that itraconazole and miconazole inhibit CYP3A4 stronger than fluconazole, micafungin, and voriconazole. In addition, the antifungal drugs investigated are not suggested to be mechanism-based inhibitors.

REFERENCES AND NOTES

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