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Effects of Single and Repeated Treatment with Itraconazole on the Pharmacokinetics of Midazolam in Rats

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Summary: To estimate the influence of repeated administration of drug metabolism inhibitors on the extent of drug interaction, we investigated the effects of single intravenous or repeated oral administration of itraconazole on the pharmacokinetics of midazolam in rats.

In the single administration study, the plasma concentration of itraconazole was maintained by intravenous infusion, and midazolam was administered into the portal vein to investigate its kinetics. In the repeated administration study, the kinetics of midazolam was investigated after seven-day oral treatment with itraconazole. The in vitro metabolism of midazolam and the contents of cytochrome P450 were investigated using liver microsomes from the itraconazole-treated rats.

The area under the curve (AUC) of midazolam was increased by 1.45- or 1.44-fold after single or repeated itraconazole treatment, respectively. Meanwhile, the liver concentrations of itraconazole after single administration and repeated administration were 38.2 and 20.3 (nmol/g), respectively. In vitro maximum metabolic reaction velocity (Vmax) and Michaelis-Menten constant (Km) of midazolam were increased from 2.26 to 3.84 (nmol/min/mg protein) and from 8.28 to 13.0 (μM) by single itraconazole treatment, respectively, and decreased from 2.23 to 1.17 (nmol/min/mg protein) and from 7.86 to 4.47 (μM) by repeated treatment, respectively. Correspondingly, the content of CYP3A2 was significantly altered by single or repeated itraconazole administration. The increases in AUC could be predicted only when the changes in Vmax and Km were taken into consideration, in addition to the hepatic unbound concentration of itraconazole.

In conclusion, changes in enzyme kinetics should be taken into account to predict the extent of drug interaction after repeated treatment with inhibitors.

Key words: itraconazole; midazolam; drug interaction; cytochrome P450; pharmacokinetics

Introduction

We have previously attempted to predict quantitatively the extent of drug interaction resulting from the competitive inhibition of hepatic drug metabolism, and demonstrated that the increase of blood concentration of a substrate in the presence of a metabolism inhibitor can be estimated quantitatively from the inhibitory constant (Ki value) and the unbound concentration of inhibitor in the liver.1,2 Moreover, we have also demonstrated that the extent of “mechanism-based” drug interaction, resulting from irreversible inactivation of the metabolizing enzyme by inhibitors such as erythromycin, can be well predicted when the formation of inactive intermediate complex in the liver is taken into account.3

However, various metabolism inhibitors are chronically administered in the clinical setting. In such cases, the content of metabolizing enzyme in the liver may be affected. Indeed, the content of cytochrome P450 (CYP) has been reported to be increased after chronic administration of not only well-known inducers such as rifampi-
cin, phenobarbital and phenytoin, but also nifedipine, nicardipine and clotrimazole (an antifungal imidazole derivative). Moreover, grapefruit juice has been demonstrated to alter the content of CYP3A4 in the human intestine. Since other potent CYP inhibitors such as cimetidine, itraconazole or erythromycin are also likely to be administered chronically in the clinical setting, the content of CYP may be altered by these drugs.

If the content of metabolizing enzyme in the liver is affected by chronic treatment with metabolism inhibitors, our conventional model may incorrectly predict the increase of blood concentration of a substrate in the presence of the inhibitor. The aim of this study is to investigate the effects of single and repeated treatment with itraconazole, a typical potent inhibitor of CYP, on the content of CYP in the liver in rats. Moreover, we attempted to predict quantitatively the extent of in vivo drug interaction from the in vitro enzyme kinetics, taking into account the change in the content of CYP. We employed midazolam, a sleep inducer, as a model substrate.

Materials and Methods

Reagents: Itraconazole was a gift from Janssen-Kyowa Co., Ltd. (Tokyo, Japan). Midazolam injection (Dormicum Injection 10 mg/2 mL) was purchased from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). Hydroxypropyl-β-cyclodextrin was obtained from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Anti-rat CYP1A1, 2B1, 2C6 and 3A2 antibodies were purchased from Daiichi Pure Chemical Co., Ltd. (Tokyo, Japan). All other reagents were of reagent grade or high-performance liquid chromatography (HPLC) grade.

Animals: Male Wistar rats (7–8 weeks) were purchased from Seac Yoshitomi Co. (Fukuoka, Japan). Rats were allowed free access to water and food pellets.

Preparation of drug solutions: Itraconazole solution for intravenous administration was prepared by the method previously described by us. Itraconazole solution for oral administration was prepared by the method of Hostetler et al. using hydroxypropyl-β-cyclodextrin. Dormicum Injection was used for the pharmacokinetic study of midazolam. The solution of midazolam used for the in vitro study was the Dormicum Injection diluted with purified water.

The effects of itraconazole on the pharmacokinetics of midazolam: In the single itraconazole administration study, rats anesthetized with diethyl ether were cannulated in the femoral artery, femoral vein and portal vein. After recovery from anesthesia, itraconazole was administered through the femoral vein at a dose of 2 mg, followed by continuous intravenous injection at a rate of 0.31 mg/hr for 5 hours. At 120 min after the beginning of injection, midazolam was administered into the portal vein at a dose of 10 mg/kg. In the repeated itraconazole administration study, itraconazole was orally administered twice a day at a dose of 20 mg/kg for 7 days. At 2 hours after the last administration of itraconazole, midazolam was administered into the portal vein at a dose of 10 mg/kg. Preliminary experiments confirmed that the plasma concentrations of itraconazole at 2, 4 and 8 hours after a single oral administration of itraconazole (20 mg/kg) were within a twofold range, and that the plasma itraconazole concentration reached stable within 10 minutes after intravenous administration of 2 mg itraconazole followed by continuous intravenous injection at a rate of 0.31 mg/hr (data not shown).

In each study, blood samples were collected at 2, 5, 10, 30, 60, 120 and 180 min after the administration of midazolam, and centrifuged at 1,000×g for 5 min to obtain plasma. The liver was removed at the sampling point. Plasma and liver were stored at −20°C until analyzed.

Determination of itraconazole and midazolam: Itraconazole and midazolam were analyzed by using an HPLC system consisting of a pump (LC-9A, Shimadzu, Kyoto, Japan) and a spectrophotometer with UV detection (SPD-10AV, Shimadzu).

For the determination of itraconazole concentration in plasma, 0.1 mL of plasma was spiked with 0.2 mL of internal standard solution (1 mg/L β-naphthoflavone in methanol), mixed and centrifuged at 1,000×g for 10 min. The supernatant was filtered and injected into the HPLC system. For the determination of itraconazole concentration in the liver, 0.5 mL of 20% liver homogenate, 0.1 mL of methanol, 0.5 mL of 1 N NaOH and 5 mL of a mixture of n-hexane and isoamyl alcohol (98:2, v/v) were mixed and shaken for 5 min and centrifuged at 1,000×g for 5 min. An aliquot of 4 mL of the organic layer was separated and spiked with 3 mL of 0.1 N HCl. The mixture was shaken for 5 min and centrifuged at 1,000×g for 5 min. To 2 mL of the aqueous phase, 0.5 mL of 1 N NaOH and 5 mL of the mixture of n-hexane and isoamyl alcohol (98:2, v/v) were added and extraction was carried out in the same manner. The organic layer (2 mL) was transferred to another glass tube and evaporated under nitrogen stream. The residue was reconstituted with 0.2 mL of the mobile phase and injected into an HPLC system.

The column used for separation was a reversed-phase YMC-Pack ODS-A (6.0 mm I.D. × 150 mm) kept at 40°C. The mobile phase consisted of acetonitrile and 10 mM phosphate buffer (pH=7.0) (80:20, v/v) and was pumped at a constant rate of 1.2 mL/min. The detection wavelength was set at 263 nm.

For the determination of midazolam in plasma, 0.1 mL of plasma, 0.5 mL of NaOH and 3 mL of n-hexane were mixed and shaken for 5 min, and then cen-
trifuged at 1,000 \times g for 5 min. An aliquot of 2 mL of upper organic layer was transferred to another glass tube and dried under nitrogen stream. The residue was dissolved in 0.2 mL of the mobile phase and injected into the HPLC system. A reversed phase column, Inert-sil ODS (4.6 mm I.D. \times 250 mm; GL Sciences Inc., Tokyo, Japan) equipped with a guard column, YMC Guardpack ODS-AM (YMC Co., Ltd., Kyoto, Japan), was kept at 40°C and used for separation. The mobile phase consisted of acetonitrile and 10 mM phosphate buffer (pH = 6.5) (80:20, v/v) and was pumped at a constant rate of 1.0 mL/min. The detection wavelength was set at 245 nm.

**Preparation of liver microsomes:** Rat liver microsomes were prepared from rats treated with single intravenous itraconazole, repeated oral itraconazole, or the respective vehicles by the method of Kremers et al.\textsuperscript{10} Briefly, the liver from each rat was minced and homogenized on ice with three volumes of 1.15% KCl. The homogenate was centrifuged at 9,900 \times g, 4°C for 20 min, and the supernatant was centrifuged at 105,000 \times g, 4°C for 60 min. The microsomal pellets were further homogenized with 1.15% KCl and centrifuged again at 105,000 \times g, 4°C for 60 min. The obtained microsomal pellets were suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 20% glycerin. The content of protein was determined by the method of Lowry et al.\textsuperscript{11} The microsomal suspension was diluted with 50 mM Tris-HCl buffer (pH 7.4) to give a final protein content of 10 mg/mL and stored at −80°C until used for experiments.

**Metabolism of midazolam in liver microsomes:** The kinetics of midazolam metabolism was investigated by using liver microsomes prepared from rats treated with single intravenous itraconazole, repeated oral itraconazole, or the respective vehicles. Liver microsomes were prepared from different animals than those used in the in vivo study. The incubation mixture contained liver microsomes (0.4 mg protein/mL), 5 mM glucose-6-phosphate, 1 mM nicotinamide adenine dinucleotide phosphate, 1 unit/mL glucose-6-phosphate dehydrogenase, 0.1 mM di-sodium dihydrogen ethylenediamine, 5 mM MgCl\textsubscript{2}, 120 mM sodium-potassium phosphate buffer (pH 7.4) and midazolam in a volume of 0.4 mL. Midazolam was added after preincubation of the rest of the mixture for 2 min to give final midazolam concentration of 1, 2, 5, 10, 20 or 50 \mu M. As the binding of midazolam to a microsomal preparation (1 mg protein/mL) was reported to be only 12%,\textsuperscript{12} unbound midazolam concentration is virtually equal to the total concentration. The whole mixture was incubated at 37°C for 4 min. A preliminary experiment confirmed that the decrease in midazolam concentration was proportional to the elapse of time up to 4 min. The reaction was terminated by adding 0.4 mL of ice-cold acetonitrile and the mixture was centrifuged at 1,000 \times g for 2 min. The supernatant (0.4 mL) was submitted to analysis as described above. The metabolic velocity was estimated from the decrease in midazolam concentration, but not the formation of midazolam metabolite, to estimate overall metabolic velocity of midazolam in rat liver microsomes.

The relationship between the metabolic velocity (V) and the concentration of midazolam (C) was fitted to equation (1) by using the nonlinear least-squares program MULTI\textsuperscript{13} to obtain the maximum metabolic velocity (V\textsubscript{max}) and Michaelis-Menten constant (K\textsubscript{m}).

\[
V = \frac{V_{\text{max}} \cdot C}{(K_m + C)}
\]  
(1)

Quantitative determination of CYP1A1, 2B1, 2C6 and 3A2 in microsomes.

Contents of CYP1A1, 2B1, 2C6 and 3A2 in liver microsomes prepared from rats after single or repeated administration of itraconazole were analyzed by Western blotting using anti-rat CYP1A1, 2B1, 2C6 and 3A2 antibodies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli\textsuperscript{14} with minor modifications. Liver microsomal samples prepared from rats after itraconazole treatment were suspended in sample buffer and applied to the gel. The proteins were transferred electrophoretically onto a Clear Blot Membrane-P (Atto Corp., Tokyo, Japan). The membrane was blocked with 5% non-fat powdered milk phosphate-buffered saline (PBS)(−)-Triton at ambient temperature for 1 hour, then incubated overnight with an anti-rat CYP antibody at 4°C. The membrane was washed six times with PBS(−)-Triton and incubated with secondary antibody at ambient temperature for 1 hour. Horseradish peroxidase-conjugated anti-rat IgG was used as the secondary antibody. Detection was done with DAB solution consisting of 6 mg of diaminobenzene, 0.18 mL of 10% H\textsubscript{2}O\textsubscript{2}, 30 mL of 0.1 M phosphate buffer and 300 mL of H\textsubscript{2}O. The reaction was terminated by incubating the mixture in ice-cold water. The membrane was washed eight times with water and dried. The content of CYP was quantified, by using each CYP protein as a standard for calibration, by analyzing the band intensity using ImageNoFPU software (NSSDC, MD, USA).

**Analysis of data:** Since non-linear kinetics was not observed for midazolam, the intrinsic clearance of midazolam (CL\textsubscript{int}) in the absence of itraconazole can be expressed by the following equation.

\[
CL_{\text{int}} = \frac{V_{\text{max}}}{K_m}
\]  
(2)

where V\textsubscript{max} and K\textsubscript{m} represent the maximum metabolic velocity and Michaelis-Menten constant in the untreated
Fig. 1. Effect of itraconazole treatment on the pharmacokinetics of midazolam in rats.
A. Itraconazole was administered through the femoral vein at a dose of 2 mg/rat followed by continuous infusion at a constant rate of 0.31 mg/hr/rat. Midazolam was administered through the portal vein at a dose of 10 mg/kg, at 120 min after the beginning of the itraconazole infusion.

Each point represents the mean ± SD (n = 11). Significant differences were determined by Student’s t-test (*, p < 0.05, **, p < 0.01). ○, plasma concentration of midazolam in the absence of itraconazole; ●, plasma concentration of midazolam in the presence of itraconazole.

B. Itraconazole was administered twice a day at a dose of 20 mg/kg for seven days. Midazolam was administered through the portal vein, at a dose of 10 mg/kg, at 120 min after the final administration of itraconazole. Each point represents the mean ± SD (n = 8). ○, plasma concentration of midazolam in the absence of itraconazole; ●, plasma concentration of midazolam in the presence of itraconazole.

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After single or repeated administration of itraconazole, the intrinsic clearance of midazolam (CL\textsubscript{int'}) can be expressed by equation (3).

\[
\text{CL}_{\text{int'}} = \frac{V_{\text{max}'} K_m'}{K_m' + 1/K_i}
\]  

(3)

where I, K\textsubscript{i}, V\textsubscript{max}' and K\textsubscript{m}' represent the concentration of itraconazole, inhibitory constant of itraconazole for the metabolism of midazolam, maximum metabolic velocity and Michaelis-Menten constant in the itraconazole-treated rat liver, respectively.

The AUC (area under the concentration-time curve) after intra-portal venous administration, which is inversely proportional to CL\textsubscript{int}, was calculated by fitting a conventional two-compartment open model to the time course of plasma midazolam concentration after intra-portal venous administration by using the nonlinear least-squares program MULTI.\textsuperscript{13}

The increase of AUC (R\textsubscript{h}) after intra-portal venous administration of midazolam by itraconazole can be estimated by means of equation (4) when the hepatic unbound concentration (C\textsubscript{Hf}) is used as I.

\[
R_h = \frac{\text{AUC}'}{\text{AUC}} = \frac{V_{\text{max}'} K_m'}{V_{\text{max}}' K_m} \left( 1 + \frac{C_{\text{Hf}}}{K_i} \right)
\]  

(4)

where AUC and AUC' represent the areas under the concentration curve of midazolam after intra-portal venous administration in the absence and presence of itraconazole, respectively. C\textsubscript{Hf} can be estimated from the total liver concentration of itraconazole (C\textsubscript{H}) and the unbound fraction of itraconazole in the liver (f\textsubscript{H}; 0.0024 (2)) by means of the following equation.

\[
C_{\text{Hf}} = C_H \cdot f_H
\]  

(5)

When plasma unbound concentration (C\textsubscript{pf}) was used as I, the increase of AUC (R\textsubscript{p}) can be estimated by means of equation (6).

\[
R_p = \frac{\text{AUC}'}{\text{AUC}} = \frac{V_{\text{max}'} K_m'}{V_{\text{max}}' K_m} \left( 1 + \frac{C_{\text{pf}}}{K_i} \right)
\]  

(6)

C\textsubscript{pf} can be estimated from the plasma concentration of itraconazole (C\textsubscript{p}) and the unbound fraction of itraconazole in the plasma (f\textsubscript{p}; 0.0034 (2)) by mean of the following equation.

\[
C_{\text{pf}} = C_p \cdot f_p
\]  

(7)

Statistics: Each value is presented as the mean ± S.D. Statistical differences were analyzed using Mann-Whitney test or F-test followed by Student’s t-test and regarded as statistically significant when the p value was less than 0.05.

Results

The effects of itraconazole on the pharmacokinetics of midazolam: Fig. 1 shows the time profiles of plasma midazolam concentration after intra-portal venous administration in rats treated with single intravenous or
repeated oral administration of itraconazole. Regardless of the presence or absence of itraconazole, midazolam did not exhibited non-linear kinetics, although possible saturation of hepatic first-pass metabolism remains.

**Table 1** shows the pharmacokinetic parameters of midazolam in each group. Single intravenous administration of itraconazole increased the AUC of midazolam by 1.45-fold (4.27 μg·hr/mL to 6.18 μg·hr/mL; \( p < 0.05 \)) with a tendency for the half-life of the elimination phase to increase. Repeated oral administration also increased the AUC by 1.44-fold (4.85 μg·hr/mL to 6.97 μg·hr/mL) without obviously affecting the half-life of the elimination phase.

**Metabolism of midazolam in liver microsomes**

**Fig. 2** represents the enzymatic kinetics of midazolam metabolism in rat liver microsomes prepared from each group of rats. \( V_{\text{max}} \) was increased in the liver microsomes prepared from rats treated with single intravenous itraconazole (2.26 to 3.84 nmol/min/mg protein). In contrast, it was decreased in rats treated with oral repeated itraconazole (2.23 to 1.17 nmol/min/mg protein). **Table 2** summarizes the effect of itraconazole on the enzymatic kinetics of midazolam metabolism. Although \( K_m \) value was increased or decreased along with the increase or decrease in \( V_{\text{max}} \) value after single intravenous or repeated oral itraconazole, respectively, the overall intrinsic clearance (\( V_{\text{max}}/K_m \)) was increased after single intravenous itraconazole and decreased after oral repeated itraconazole.

**Quantitative determination of CYP1A1, 2B1, 2C6 and 3A2 in microsomes:** **Fig. 3** shows the contents of four major isoforms, CYP1A1, 2B1, 2C6 and 3A2, in the microsomes from each group assessed by means of Western blotting. Itraconazole treatment did not affect the contents of CYP1A1, 2B1 and 2C6 (Fig. 3B, C, D). However, the content of CYP3A2 increased after single intravenous itraconazole and decreased after repeated oral itraconazole treatment (Fig. 3A).

The above changes in the contents of CYP3A2 correlated well with the changes in the \( V_{\text{max}} \) value obtained from the enzymatic study (Fig. 4; \( r^2 = 0.878, p < 0.05 \)).

**Concentration of itraconazole in plasma and liver:** **Table 3** shows the concentrations of itraconazole in the

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**Fig. 2.** The effects of itraconazole treatments on the metabolism of midazolam in rat liver microsomes. The fitting lines to the equation (1) are superimposed.

A. Enzymatic kinetics of midazolam metabolism in liver microsomes of rats treated with single intravenous itraconazole. Before preparing liver microsomes, itraconazole was administered to rats through the femoral vein at a dose of 2 mg/rat followed by continuous intravenous infusion at a rate of 0.31 mg/hr/rat for 360 min. Liver microsomes were incubated with 1, 2, 5, 10, 20 and 50 μM midazolam. Each point represents the mean ± SD (n = 3). ○, microsomes from rats treated with vehicle; ●, microsomes from rats treated with intravenous itraconazole.

B. Enzymatic kinetics of midazolam metabolism in liver microsomes of rats repeatedly treated with oral itraconazole. Before the preparation of liver microsomes, itraconazole was administered twice a day for seven days at a dose of 20 mg/kg. Liver microsomes were incubated with 1, 2, 5, 10, 20 and 50 μM midazolam. Each point represents the mean ± SD (n = 3). ○, microsomes from rats treated with vehicle; ●, microsomes from rats repeatedly treated with itraconazole.
plasma and liver, along with the liver-to-plasma concentration ratio ($K_p$ value), after single intravenous or repeated oral administration of itraconazole. There are no differences in $K_p$ value between the groups given the two administration regimens of itraconazole.

**Discussion**

The liver is the major organ of elimination for xenobiotics, and also is the primary site of drug interaction. We have aimed to predict quantitatively the extent of drug interaction occurring in the liver. There are various drug metabolism inhibitors which are concentratively taken up into the liver,\textsuperscript{15-17} and metabolizing enzymes such as CYP are located in the hepatocytes. Therefore, it is indispensable for accurate prediction of the extent of hepatic drug interaction to estimate the liver concentration of inhibitors. Indeed, the increase rate of the AUC of midazolam caused by coadministration of metabolism inhibitors can be predicted quantitatively by using the unbound concentration of inhibitors in the liver ($C_{Hf}$).\textsuperscript{1,2,18} Moreover, the extent of drug interaction resulting from irreversible enzyme inactivation by inhibitors, such as erythromycin, can be predicted quantitatively when the formation of inactive intermediate complex in the liver is taken into account.\textsuperscript{3,19,20} However, many drugs are repeatedly administered to patients in the clinical setting. Therefore, it is important to investigate changes in enzymatic kinetics after chronic treatment with inhibitors.

Table 2. Kinetic parameters for metabolism of midazolam in rat liver microsomes prepared from itraconazole-treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vehicle for single IV</th>
<th>Itraconazole single IV</th>
<th>Vehicle for repeated PO</th>
<th>Itraconazole repeated PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ ($\mu$M)</td>
<td>8.28±1.44</td>
<td>13.0±2.00</td>
<td>7.86±1.96</td>
<td>4.47±1.08</td>
</tr>
<tr>
<td>$V_{max}$ (nmol/min/mg protein)</td>
<td>2.26±0.17</td>
<td>3.84±0.29</td>
<td>2.23±0.24</td>
<td>1.17±0.11</td>
</tr>
<tr>
<td>$V_{max}/K_m$</td>
<td>0.273</td>
<td>0.295</td>
<td>0.284</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Each value represents the estimate±S.D. of estimation (n=3).

Fig. 3. Quantification of rat CYP3A2(A), 1A1(B), 2B1(C) and 2C6(D) after single or repeated treatment with itraconazole. Data represent the means±SE (n=3). Significant differences were determined by Student’s t-test (*, p<0.05). a: control (untreated), b: vehicle-treated (solvent for IV), c: itraconazole-treated (single IV), d: vehicle-treated (solvent for PO), e: itraconazole-treated (repeated PO for 7 days).
the increase and decrease in the contents of CYP3A2 (Fig. 3). Since there was a good correlation between \( V_{\text{max}} \) values and the contents of CYP3A2 (Fig. 4), itraconazole may have altered the metabolic activity by affecting the content of CYP3A2. Indeed, the increase of the AUC of midazolam after single intravenous administration of itraconazole was identical to that after repeated oral administration, although the plasma and the liver concentrations of itraconazole were twofold lower after repeated oral administration than after single intravenous administration.

The mechanisms underlying the changes in the content of CYP3A are unclear. However, the changes are observed only in the case of CYP3A (Fig. 3). Itraconazole is known to be metabolized by hepatic CYP3A with higher affinity than other CYP isoforms. In addition to its non-specific binding to the heme, itraconazole also binds to the apoprotein of CYP3A. CYP3A has been reported to be degraded by 20S proteasome directly, and/or by 26S proteasome in a ubiquitin-dependent manner. Therefore, a possible explanation for the itraconazole-induced decrease in the content of CYP3A is that repeated itraconazole treatment enhances the degradation of CYP3A via pathways such as ubiquitination.

On the other hand, single intravenous itraconazole increased the content of CYP3A. This may have resulted from an increase of CYP3A synthesis at the transcriptional and/or translational level. Maurice et al. reported that clotrimazole, an antifungal imidazole derivative, induces CYP3A in a primary culture of human hepatocytes. Higashikawa et al. demonstrated that the hepatic extraction ratio of midazolam is increased by 12% in clotrimazole-pretreated rats, and similar results were obtained in an in vitro study. Therefore, the most likely explanation for the increase in the content of CYP3A in this study seems to be that itraconazole induced CYP3A in the same manner as clotrimazole.

As well as the above-mentioned change in the \( V_{\text{max}} \) value, the \( K_m \) value also seems to be affected by itraconazole. Although the mechanisms remain unclear, it has been reported that \( K_m \) can be increased or decreased by binding of modulators to the hypothetical “effector” site of CYP. Therefore, a speculative explanation for the difference in \( K_m \) observed in this study is that the effector site was covalently or irreversibly modulated by itraconazole.

To predict the extent of drug interaction, hepatic distribution of itraconazole should be taken into account. However, since the liver-to-plasma concentration ratio (\( K_p \)) remained unchanged after repeated administration (Table 3), hepatic distribution of itraconazole was not altered after chronic treatment. The unbound fraction of itraconazole in the liver (\( f_{\text{Hl}} \)) may possibly depend on the regimen of itraconazole, because it is possible that metabolites of itraconazole accumulate in the liver after repeated oral administration and affect the binding of itraconazole. However, it is difficult to determine in ex vivo experiments whether \( f_{\text{Hl}} \) is altered after repeated oral administration in vivo, because several compounds, such as metabolites of itraconazole, may be accumulated in the liver and affect \( f_{\text{Hl}} \).

Since hydroxyitraconazole, a major metabolite of itraconazole, has been reported to inhibit the hydroxylation of midazolam, it is possible that this metabolite accumulated and inhibited the elimination of midazol-

### Table 3. Quantitative prediction of the effects of single intravenous and repeated oral treatment with itraconazole on the AUC of midazolam based on the unbound concentration of itraconazole (IV: \( n = 11 \), PO: \( n = 8 \))

<table>
<thead>
<tr>
<th></th>
<th>Single IV</th>
<th>Repeated PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_p (\mu M) )</td>
<td>3.89 ± 1.52</td>
<td>1.98 ± 0.56</td>
</tr>
<tr>
<td>( C_H (\text{mmol/g}) )</td>
<td>38.2 ± 14.9</td>
<td>20.3 ± 7.7</td>
</tr>
<tr>
<td>( K_p (\text{mL/g}) )</td>
<td>10.4 ± 3.4</td>
<td>10.4 ± 2.7</td>
</tr>
<tr>
<td>( f_{\text{H}} (g/mL) )</td>
<td>0.0034</td>
<td>0.0024</td>
</tr>
<tr>
<td>( K_{\text{H}} (\mu M) )</td>
<td>13.2</td>
<td>6.73</td>
</tr>
<tr>
<td>( C_{\text{H}} (nM) )</td>
<td>91.7</td>
<td>48.7</td>
</tr>
<tr>
<td>( R_{\text{H}} )</td>
<td>0.98</td>
<td>1.12</td>
</tr>
<tr>
<td>( R_{\text{P}} )</td>
<td>1.30</td>
<td>1.32</td>
</tr>
<tr>
<td>( R_{\text{CH}} )</td>
<td>1.40</td>
<td>1.21</td>
</tr>
<tr>
<td>( R_{\text{Ob}} )</td>
<td>1.45</td>
<td>1.44</td>
</tr>
</tbody>
</table>

\( C_p \) and \( C_H \) were determined 180 min after the intraportal venous administration of midazolam.

\( a \) Calculated from equation (6).

\( b \) Calculated from equation (4).

\( c \) Calculated without taking the changes in \( V_{\text{max}} \) and \( K_m \) into consideration (i.e. \( R_{\text{CH}} = 1 + C_{\text{H}}/K_p \)).

\( d \) Observed increase rate (\( = \text{AUC}^{\text{IV}} / \text{AUC}^{\text{PO}} \)).
am in vivo after repeated oral administration. However, it is not conceivable that this metabolite remained in the purified microsomal preparation enough to affect the enzymatic kinetics. Therefore, the metabolite cannot fully account for the difference in the effects of itraconazole.

The increase of the AUC of midazolam could be quantitatively estimated by use of the hepatic unbound concentration of itraconazole (C_H), whereas it could not be well predicted by use of the plasma unbound concentration (C_U). This result is consistent with our previous reports. However, the observed increases in the concentration (C_P) are similar between single intravenous and repeated oral group, despite the twofold differences in itraconazole concentration. This discrepancy was resolved only when the changes in V_{max} and K_m were taken into consideration, in addition to the hepatic unbound concentration of itraconazole (Table 3). However, it may be practically infeasible to evaluate the individual enzymatic kinetics in the current clinical settings.

In conclusion, it was demonstrated that single intravenous treatment with itraconazole increases the maximum velocity of midazolam metabolism in the liver, as well as the content of hepatic CYP3A, while repeated oral treatment decreases the maximum velocity of midazolam metabolism and the content of CYP3A in the liver. Therefore, to predict quantitatively the extent of drug interaction after chronic treatment with inhibitors, changes in the enzymatic kinetics should be taken into account.

References


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