Published in final edited form as:

Clin Pharmacol Ther. 2010 October ; 88(4): 499–505. doi:10.1038/clpt.2010.119.

Accurate Prediction of Dose-Dependent CYP3A4 Inhibition by Itraconazole and Its Metabolites From *In Vitro* Inhibition Data

I Templeton¹, C-C Peng¹, KE Thummel¹, C Davis², KL Kunze³, and N Isoherranen¹

¹Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington, USA

²Division of Nephrology, Department of Medicine, School of Medicine, University of Washington, Seattle, Washington, USA

³Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, Washington, USA.

Abstract

Inhibitory drug metabolites may contribute to drug–drug interactions (DDIs). The aim of this study was to determine the importance of inhibitory metabolites of itraconazole (ITZ) in *in vivo* cytochrome p450 (CYP) 3A4 inhibition. The pharmacokinetics of ITZ and midazolam (MDZ) were determined in six healthy volunteers in four sessions after administration of MDZ with and without oral ITZ. After doses of 50, 200, and 400 mg of ITZ, the clearance of orally administered MDZ decreased by 27, 74, and 83%, respectively. The *in vivo* half maximal inhibitory concentration (IC₅₀) for ITZ ranged from 5 to 132 nmol/l in the six subjects. The metabolites of ITZ were estimated to account for ~50% of the total CYP3A4 inhibition, with the relative contribution increasing with time after ITZ dosing. Of the total of 18 interactions observed, 15 (84%) could be predicted within a twofold error margin, with improved accuracy observed when ITZ metabolites were included in the predictions. This study shows that the metabolites of ITZ contribute to CYP3A4 inhibition and need to be accounted for in quantitative rationalization of ITZ-mediated DDIs.

Many clinically important cytochrome P450 (CYP) inhibitors have been shown to possess circulating metabolites,¹ and many of these metabolites are predicted to contribute to *in vivo* drug–drug interactions (DDIs).² Classic examples of inhibitors with circulating inhibitory metabolites include three potent CYP inhibitors: itraconazole (ITZ),³ fluoxetine,⁴ and bupropion.⁵ Norfluoxetine, the primary circulating metabolite of fluoxetine, has been shown to be responsible for the persistent CYP2D6 inhibition after administration of fluoxetine,⁶ although no quantitative prediction of CYP2D6 inhibition by norfluoxetine as compared with fluoxetine was shown. Using atomoxetine as an example, quantitative predictions of the contribution of metabolites to *in vivo* DDIs were performed, and, despite the overall weak *in vivo* inhibition of CYP3A4 and CYP2D6 by atomoxetine, the metabolites were predicted to be mainly responsible for the interaction.⁷

The metabolites of ITZ have been predicted to account for ~50% of the overall CYP3A4 inhibition *in vivo*. This prediction is based on *in vivo* disposition data of ITZ and its three

metabolites after a 100-mg dose of ITZ.⁸ However, the inhibitory metabolites of ITZ, namely hydroxy-ITZ (OH-ITZ), keto-ITZ (keto-ITZ), and N-desalkyl ITZ (ND-ITZ) have not been measured *in vivo* simultaneously with CYP3A4 inhibition. Given the partially dissociative sequential metabolism of ITZ by CYP3A4 *in vitro*³ and saturable *in vivo* clearance with escalating doses, it is likely that metabolite-to-parent ratios of ITZ decrease with increasing doses. Hence, if metabolites contribute significantly to *in vivo* CYP3A4 inhibition by ITZ, a dose-dependent underprediction of *in vivo* interactions would be expected when ITZ alone is considered in predictions. The aim of this study was to determine whether metabolites of ITZ contribute significantly to *in vivo* CYP3A4 inhibition and whether CYP3A4 inhibition by ITZ can be accurately predicted after administering escalating doses of ITZ. The concentrations of the inhibitors were measured in each subject, together with CYP3A4 activity. The individual *in vivo* half maximal inhibitory concentration (IC₅₀) values were calculated, and the predicted and observed magnitudes of interaction were compared in each of the six study subjects.

RESULTS

Six healthy volunteers (one woman and five men), in the age range of 22–42 (mean 34) years and within 20% of optimal body mass index, completed the study. An additional female subject was enrolled, but she withdrew after the midazolam (MDZ) control session because of nausea and vomiting. No other side effects or possible adverse effects were observed in any of the subjects. All subjects had normal liver and kidney function and were in good health. Each subject was homozygous for CYP3A5*3 and hence was expected to lack expression of functional CYP3A5 protein. Individuals with potential CYP3A5 expression based on genotype were excluded from the study because CYP3A5 has been shown not to metabolize ITZ.³

MDZ pharmacokinetics were measured after escalating doses of ITZ (50 mg, 200 mg, and 400 mg oral solution) and compared with corresponding values during a control session (in the absence of ITZ). In all sessions, MDZ was administered 4 h after the ITZ oral solution. The plasma concentration–time curves of MDZ are shown in Figure 1a. Table 1 summarizes the pharmacokinetic parameters of MDZ in the four study sessions. MDZ area under the plasma concentration–time curve (AUC)_{0–∞} increased and oral clearance decreased significantly ($P < 0.01$) with increasing ITZ doses (Figure 1b and Table 1). The MDZ AUC_{0–∞} increased on average 2.0-, 4.7-, and 5.4-fold compared with control after the 50-, 200-, and 400-mg ITZ doses, respectively (Table 1). The increase in MDZ AUC corresponded to a 50, 79, and 82% decrease in MDZ oral clearance after 50-, 200-, and 400-mg doses of ITZ, respectively. A significantly greater decrease ($P < 0.01$) in the oral clearance of MDZ was observed after 200 and 400 mg ITZ than after 50 mg ITZ. The decrease in MDZ oral clearance was accompanied by a significant increase in MDZ elimination half-life ($t_{1/2}$) (Table 1), suggesting inhibition of systemic clearance of MDZ. The $t_{1/2}$ increased 1.7-, 2.8-, and 3.2-fold from baseline after 50-, 200-, and 400-mg doses of ITZ, respectively.

The average plasma concentrations of ITZ, OH-ITZ, keto-ITZ, and ND-ITZ during MDZ elimination were determined from plasma concentration–time data (Table 2). As expected, greater-than-dose-proportional increases in ITZ and OH-ITZ concentrations were observed, but keto-ITZ and ND-ITZ plasma concentrations increased less than dose proportionally, leading to a decrease in the ratio of metabolite to ITZ concentration with increasing doses of ITZ. The ratio of OH-ITZ to ITZ concentration remained constant even with increasing doses of ITZ.

The measurement of inhibitor concentrations in each individual permitted the determination of apparent *in vivo* IC₅₀ values for ITZ, with the assumption that the CYP3A4 inhibition is entirely attributable to ITZ. Figure 2 shows the relationship between ITZ concentration and inhibition of clearance of oral MDZ in each of the six subjects, with the individual apparent IC₅₀ values also indicated. The mean *in vivo* IC₅₀ for ITZ was 32 nmol/l, which is equivalent to an unbound IC₅₀ value of 1.2 nmol/l. Subject 6 was determined to be an outlier with respect to the *in vivo* IC₅₀ value ($P < 0.01$). When this outlier in IC₅₀ values was excluded (*in vivo* IC₅₀ 132 nmol/l), the average *in vivo* IC₅₀ was 11.7 nmol/l and unbound IC₅₀ was 0.42 nmol/l. The maximum *in vivo* inhibition ($E_{\max}$) was in the range of 82–88% in the six subjects studied (Figure 2), corresponding to the reported f_m for MDZ by CYP3A4.

Because ITZ metabolites also exert an inhibitory effect, the relative contributions of the metabolites of ITZ to the decrease in the clearance of oral MDZ were predicted using the ratio of average unbound plasma concentrations to *in vitro* unbound IC₅₀ values, as reported by Isoherranen *et al.*³ The observed unbound plasma [I]/*in vitro* IC₅₀ ratios for ITZ and its metabolites are shown in Table 3. Supplementary Figure S1 online depicts the predicted percentage contribution by each inhibitor to the overall observed CYP3A4 inhibition after the administration of escalating doses of ITZ. On average, ITZ was predicted to be responsible for 50–60% of the overall CYP3A4 inhibition, and this predicted contribution of ITZ did not change with increasing doses. On the basis of unbound values, the sum of the effects of the three metabolites was approximately equivalent to that of ITZ in CYP3A4 inhibition. Despite the low circulating concentrations of ND-ITZ, the [I]_u/IC_{50,u} ratios suggested that this metabolite contributes 10–20% to the total CYP3A4 inhibition.

The time course of CYP3A4 inhibition was also predicted using the [I]_u/IC_{50,u} values for ITZ alone and for ITZ with its metabolites throughout the ITZ administration period (Supplementary Figure S2 online). The contribution of the metabolites increased with time, leading to a greater contribution of the metabolites to CYP3A4 inhibition 12–24 h after dosing. This resulted in a more persistent inhibition (up to 48 h) after the 400-mg ITZ dose than would be predicted from the effect of ITZ alone. In the case of a substrate with a long half-life, this persistent inhibitory effect of the metabolites may be more significant and contribute to greater underpredictions than with a probe such as MDZ, which has a relatively short half-life.

The impact of ITZ and its metabolites to the overall clearance of oral MDZ was predicted using unbound plasma concentrations in each subject and pharmacokinetic models of CYP3A4 inhibition in the gut and in the liver. The predicted inhibition of intestinal and hepatic CYP3A4 by ITZ and its metabolites after each ITZ dose is shown in Table 4. The gut bioavailability (F_g^{1/F_g}) after the 50-, 200-, and 400-mg ITZ doses was predicted to increase to 1.1, 1.5, and 1.6 times the baseline value of F_g (55%), respectively, when the combined effects of ITZ and its metabolites were considered. The predicted maximum value of F_g after the highest ITZ dose was 87%. The predicted percentage decrease in the hepatic intrinsic clearance is shown in Table 4. A maximum of 76% decrease in the systemic clearance of MDZ was predicted after the 400-mg dose of ITZ. Taking into consideration both gut and hepatic inhibition of CYP3A4, a maximum overall decrease of 85% in the clearance of oral MDZ was predicted (Table 4), which agrees well with the observed 82–88% maximum decrease (Figure 2).

On the basis of the plasma concentrations of ITZ and its metabolites in each subject, the overall increase in MDZ AUC was predicted for each study subject, using the static model described in Equation 5. This predicted overall increase was compared with the observed interactions after escalating ITZ doses. On average, the predicted increase in the AUCs of

oral MDZ after 50-, 200-, and 400-mg doses of ITZ were 1.4 ± 0.2 , 4.5 ± 0.6 , and 6.8 ± 0.8 fold, respectively, when the effects of ITZ and its metabolites were considered, and 1.2 ± 0.1 , 2.9 ± 0.5 , and 4.7 ± 0.7 , respectively, after each of the three doses when the effect of ITZ alone was considered. There was no significant difference between the observed and predicted AUCs after each ITZ dose ($P = 0.07$, 0.3 , and 0.4 for the 50-, 200-, and 400-mg ITZ doses, respectively). The comparison between predicted and observed interactions is shown in Figure 3. When the effect of ITZ alone was considered in the predictions, 78% of all the interactions were underpredicted, and three of these (26% of the total) individual interactions were underpredicted by more than twofold. When the effects of the ITZ metabolites were also taken into account, 50% of the interactions were underpredicted, with only one interaction being underpredicted by more than twofold. An overall improvement in the prediction accuracy was observed.

In order to test whether prediction accuracy was affected by ITZ dose, the predicted interactions using ITZ data alone were compared with those of ITZ and its three metabolites after each dose level. With data related to ITZ alone, 40, 38, and 13% underpredictions in the magnitude of interactions were observed after 50-, 200-, and 400-mg doses, respectively, and the accuracy of prediction improved with increasing ITZ doses. When the data related to the metabolites were also accounted for in predictions, a 22% underprediction was observed after the 50-mg ITZ dose, an 8% overprediction after the 200-mg dose, and 40% overprediction after the 400-mg dose, on average. Although all these average predictions are within a twofold range of the observed interaction, a dose-dependent effect on prediction accuracy was observed, and, as would be expected given the nonlinear (capacity limited) formation of ITZ metabolites, prediction accuracy improved with increasing doses when data for ITZ alone were analyzed.

DISCUSSION

The objective of this study was to determine the relative importance of ITZ metabolites to CYP3A4 inhibition after the administration of escalating doses of ITZ. It also aimed to establish whether the magnitude of CYP3A4 inhibition after ITZ administration could be rationalized using *in vitro* values for CYP3A4 inhibition by ITZ and its metabolites and the measured plasma concentrations of all the potential inhibitors. The data presented show that accurate interaction predictions could be reached after each dose level and that the metabolites contribute to ~50% of the overall CYP3A4 inhibition.

Previous studies have determined variable magnitudes of inhibition of MDZ clearance by ITZ.⁹⁻¹¹ The 4.7-fold increase in MDZ AUC observed in this study after 200 mg ITZ is in good agreement with the previously observed 3.4-fold increase when MDZ was administered 2 h after a single 200-mg dose of ITZ and the 6.6-fold increase when MDZ was administered after 200 mg ITZ daily for 6 days.⁹ However, the maximum inhibition observed in this study (5.4-fold increase in MDZ AUC) after a 400-mg dose of ITZ is less than the maximum 10.8-fold increase observed in exposure ($AUC_{0-\infty}$) when oral MDZ was administered after four daily doses of 200 mg of ITZ, the latter being administered 1 h before MDZ.⁹

The maximum extent of inhibition observed in this study was lower than would be expected on the basis of previous studies. This difference may be due to differences in the staggering of MDZ and ITZ doses. In previous studies, MDZ was administered 1 or 2 h after ITZ (at ITZ's t_{max}), whereas in this study ITZ was given 4 h prior to MDZ administration so as to ensure complete ITZ absorption at the time of MDZ administration and to provide for more accurate testing of *in vitro* to *in vivo* extrapolation. It is expected that if MDZ is administered at the time point of t_{max} of ITZ, a greater effect on F_g and F_h will be observed

than with a dose-staggered design. However, in a study that investigated the effect of ingestion time interval on the interaction between ITZ and triazolam, a larger magnitude of inhibition was observed when ITZ and triazolam doses were separated by 3 or 12 h as compared with administering them simultaneously,¹² thereby suggesting that ITZ causes a persistent inhibition of CYP3A4, perhaps because of its circulating metabolites. Unfortunately, in these studies involving dose staggering and timing of administration, the concentrations of the circulating metabolites were not available, and therefore the relative contributions of the metabolites cannot be characterized.

Persistent inhibition of CYP3A4-mediated MDZ clearance after four doses of ITZ (200 mg daily) has been previously reported.¹³ Four days after cessation of ITZ dosing, a 2.6-fold increase in MDZ AUC was still observed relative to the control. An evaluation of the time course of inhibition by all circulating inhibitors in this study indicates that the persistent inhibition of CYP3A4 after ITZ dosing is due, at least in part, to the effects of inhibitory metabolites with long half-lives. The concentrations and relative exposures to ITZ and its metabolites observed in this study were in excellent agreement with those observed previously, and the predicted time course of inhibition agrees with that predicted previously.⁸ After single doses of ITZ, the exposure to OH-ITZ was, on average, twofold greater than the exposure to ITZ, whereas keto-ITZ and ND-ITZ exposures were ~10% of the ITZ exposure. Extrapolation of these exposures of ITZ metabolites to multiple dosing situations will require additional mechanistic *in vitro* studies of the sequential metabolism of ITZ and the elimination pathways of the metabolites. In addition, complex models will be required to simulate the time- and dose-dependent disposition of ITZ and its metabolites.

On the basis of data related to plasma concentrations of ITZ and predictions of F_g , it can be inferred from this study that gut metabolism of MDZ was not completely inhibited, even after the 400-mg dose of ITZ. Assuming that circulating plasma concentrations are indicative of inhibitor concentration in the gut wall 4 h after ITZ administration, only a small change (6% increase) in gut availability of MDZ is predicted after the 50-mg ITZ dose; after the 400-mg dose of ITZ, the gut availability of MDZ was predicted to increase to 87% (1.6-fold increase) suggesting that with increase in ITZ doses further inhibition of gut CYP3A4 would be possible. Indeed, the plateau maximum inhibition predicted from the *in vivo* IC_{50} fits was a 7.7-fold increase in MDZ AUC as compared with the observed 5.4-fold increase after the 400-mg dose.

The literature indicates the existence of a relationship between inhibitor dose and observed magnitude of interaction.¹⁴ However, rarely is a plateau of inhibition clearly demonstrated in such studies, and *in vivo* IC_{50} values are usually not calculated. An inhibition plateau with increasing ITZ doses has previously been observed with triazolam when triazolam was administered 3 h after a 200- or 400-mg oral dose of ITZ. Only a marginal (1.1-fold) increase in the inhibition was observed between the two doses.¹² The fact that a plateau is observed is most likely a result of the fact that ITZ inhibits only CYP3A and no alternative elimination pathways of MDZ or triazolam.

The use of *in vivo* K_i has been proposed as a method for comparing an inhibitor's potency *in vitro* and *in vivo*.¹⁵ A ratio greater than 1 between the *in vitro* and *in vivo* K_i (RR_{K_i}) suggests that factors such as inhibitory metabolites and uptake transport may contribute to *in vivo* interaction, making the inhibitor more potent *in vivo* than *in vitro*. The same principle can be applied to IC_{50} values for reversible inhibitors such as ITZ. An apparent *in vivo* IC_{50} for ITZ in all six study subjects was determined, and the ratio between the *in vitro* and *in vivo* unbound IC_{50} values for ITZ was 5.1 (including data from all subjects) or 14.5 (with data from one outlier excluded), suggesting that ITZ is a more potent inhibitor *in vivo* than *in vitro*. One plausible explanation for this difference is that the metabolites of ITZ contribute

to the observed *in vivo* CYP3A4 inhibition, although the possibility of active hepatocellular uptake of ITZ as a factor cannot be excluded.

A comparison of the $[I]/IC_{50}$ ratios for ITZ and its metabolites supports the hypothesis that the metabolites have a distinct role in CYP3A4 inhibition. On average, the predictions based on ITZ alone underpredicted the observed change in MDZ AUC, although most static predictions were within a twofold margin of error. The static prediction was improved when the inhibitory contribution of the metabolites was accounted for, again suggesting that metabolites are quantitatively important in CYP3A4 inhibition caused by ITZ. The contribution of metabolites to perceived risks of DDIs was also important. When ITZ alone was considered after the 400-mg dose of ITZ, it was predicted to result in moderate CYP3A4 interactions. However, when metabolites were included in the predictions, ITZ was predicted to be a potent inhibitor.

The average magnitude of interaction between ITZ and MDZ after the three different ITZ doses could be accurately predicted despite the large interindividual variability in the magnitude of interaction. Even when the concentrations of all four circulating inhibitors were known and used in the prediction of the interaction in the same individual, the prediction accuracy in some individuals was outside the twofold range. This suggests that factors other than the absolute exposure to the inhibitor contribute to an individual's sensitivity to DDIs. For example, variability in the individual f_m for CYP3A4, determined partially by the expression levels of other enzymes contributing to MDZ clearance (UDP-glucuronosyltransferases), may be responsible for this interindividual variability.

In conclusion, this study was conducted to determine whether the metabolites of ITZ play a quantitatively important role in *in vivo* CYP3A4 inhibition. All the results obtained support the fact that approximately half of the CYP3A4 inhibition observed *in vivo* after ITZ dosing is caused by circulating metabolites of ITZ. The fivefold difference between the *in vitro* and *in vivo* IC_{50} values for ITZ may be rationalized by accounting for the inhibitory metabolites. When metabolites were accounted for, accurate predictions of the *in vivo* interaction were obtained after all three escalating doses of ITZ. These results demonstrate the usefulness of the *in vivo* IC_{50} (or K_i) in determining additional factors that contribute to *in vivo* interactions and the importance of accounting for inhibitory metabolites when quantitative predictions of interactions are conducted.

METHODS

Clinical protocol

The University of Washington Institutional Review Board approved this protocol. Six healthy volunteers, one woman and five men in the age range of 22–42 (mean 34) years and within 20% of optimal body mass index, participated in the study after giving their written informed consent. None of the subjects had a history of systemic disease. A standard clinical renal and hepatic function panel of laboratory test values was obtained from each subject, and all values were within the normal ranges. Tobacco smoking and a history of allergy to antifungal drugs were also part of the exclusion criteria. Each subject was genotyped for CYP3A5, and only subjects homozygous for CYP3A5*3 were enrolled. In brief, a buccal cell sample from each subject was collected using Cyto-pak cytosoft brushes (Medical Packing, Camarillo, CA), and genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen Science, Germantown, MD) in accordance with the manufacturer's instructions. The CYP3A5*3 status of the subjects was determined using Stepone Plus real-time PCR and TaqMan genotyping assay (assay ID C26201809_30) in accordance with the manufacturer's recommendations (Applied Biosystems, Foster City, CA).

Subjects were asked to abstain from over-the-counter and prescription medications from 1 week before the administration of study medications to 3 weeks after the completion of the study. They were also asked to abstain from eating grapefruit and grapefruit products for the duration of the study and to avoid alcohol and caffeine from 1 day before through the end of each urine-collection period. The study was divided into four sessions: a control session to measure baseline oral MDZ elimination, followed by three successive sessions with escalating doses (50, 200, and 400 mg) of ITZ. Each session was separated from the subsequent one by a washout period of at least 2 weeks. In each session, the subjects received 2 mg MDZ in oral solution. In the sessions involving ITZ dosing, the MDZ was administered 4 h after ITZ. A standard lunch was served 30 min after the MDZ dose. For the determination of MDZ concentrations, blood samples were collected through indwelling venous catheters into EDTA-containing tubes (BD Vacutainer; BD Biosciences, Franklin Lakes, NJ) before MDZ administration and at 0.5, 1, 2, 3, 4, 6, 8, and 12 h after administration (during the control session, no samples were collected at the 12-h time point). An additional blood sample was drawn by venous puncture 20 h after MDZ administration. Plasma was isolated from blood by centrifugation and stored at -20°C before analysis.

Measurements of plasma concentrations of MDZ and ITZ

Concentrations of MDZ and ITZ in the plasma samples were measured using high-performance liquid chromatography with mass-spectrometry detection as described previously⁸ except that a Waters Micromass (Manchester, UK) LC-MS platform mass spectrometer was used to analyze the samples. A calibration curve for MDZ was prepared using pooled blank human plasma separated from whole blood acquired from the Northwest Blood Bank. Calibration standards were prepared at concentrations between 1 and 150 nmol/l (from 100 μl plasma). The limit of quantification for MDZ was 1 nmol/l. Quality-control samples containing 3, 31, and 77 nmol/l MDZ were included with each analysis. The interday variability for MDZ was $<3\%$ at 77 nmol/l, $<2\%$ at 31 nmol/l, and $<14\%$ at 3 nmol/l.

Calibration curves for ITZ, OH-ITZ, keto-ITZ, and ND-ITZ were also prepared in pooled blank human plasma prepared from Northwest Blood Bank whole blood. Calibration standards were prepared at concentrations ranging from 4 to 1,500 nmol/l (from 0.1 ml plasma). The limit of detection for ITZ and metabolites was 1 nmol/l, and the limit of quantification was 5 nmol/l. Quality-control samples containing ITZ and metabolites at 50, 500, and 1,000 nmol/l were included with each analysis. The interday variability for ITZ and its metabolites was $<10\%$ at 1,000 nmol/l, $<10\%$ at 500 nmol/l, and $<20\%$ at 50 nmol/l.

Pharmacokinetic calculations

The AUC after each oral MDZ dose was calculated using the trapezoidal method (WinNonlin; Pharsight, Mountain View, CA). The MDZ dose was divided by the value of $\text{AUC}_{0-\infty}$ to calculate the clearance of oral MDZ. Elimination half-life was obtained from the linear terminal slope of the MDZ plasma concentration-time curve. The AUCs of ITZ, OH-ITZ, keto-ITZ, and ND-ITZ were calculated from 4.5 to 24 h after ITZ administration, to correspond to the interval of MDZ exposure. The AUC of the inhibitor was divided by this time interval (19.5 h) to calculate average inhibitor concentration (C_{ave}) during MDZ administration. The increase in MDZ AUC after each ITZ dose was calculated as the ratio of inhibited AUC to control-session AUC.

Determination of *in vivo* IC_{50}

The *in vivo* IC_{50} for ITZ was calculated using GraphPad Prism (GraphPad Software, La Jolla, CA). The ratio of clearance of oral MDZ under inhibited conditions (CL_i) and control conditions (CL) was calculated for each of the subjects for all four sessions. IC_{50} and

maximum inhibition ($E_{\max}$) were then obtained by fitting the dose–response (inhibition) model to the data according to Equation 1, in which CL_I/CL is modeled as a function of the total ITZ C_{ave} .

$$CL_I/CL = (CL_I/CL)_{\min} + \frac{1 - (CL_I/CL)_{\min}}{1 + 10^{(\log C_{ave} - \log IC_{50})}} \quad (1)$$

$(CL_I/CL)_{\min}$ refers to the fitted minimum value for the clearance ratio, i.e., the maximum inhibition obtained.

Statistical analysis

Data are expressed as mean values $\pm$ SD. Statistical analysis was performed using Excel (Microsoft Office 2007; Microsoft, Redmond, WA). A paired t -test was used to evaluate the significance of the average change in MDZ AUC between the four study sessions. The same test was used to test the significance of the difference between predicted and observed changes in MDZ AUC after each ITZ dose. The presence of outliers in the *in vivo* IC_{50} data was tested for, using the Grubbs' test for outliers in GraphPad Prism. A two-tailed P value of <0.0167 was considered significant after a Bonferroni correction to adjust the P value of <0.05 for multiple comparisons.

In vitro–in vivo predictions

The effect of ITZ and its metabolites on the CYP3A4-mediated intrinsic clearance of MDZ was predicted using the relationship:

$$\frac{Cl_{int}}{Cl} = 1 + \frac{[I]}{K_i} \quad (2)$$

in which the value of IC_{50} reported by Isoherranen *et al.*³ was used for K_i . Because ITZ and its metabolites are competitive inhibitors, and the IC_{50} values used were determined at $[S] \ll K_m$, these IC_{50} values are equal to the K_i values.³ The general equation (Equation 3)

$$\frac{AUC_i}{AUC} = \left(\frac{F_{g,i}}{F_g} \right) \cdot \left(\frac{1}{(1 - f_m) + \frac{f_m}{1 + [I]_u/K_{i,u}}} \right) \quad (3)$$

in which F_g indicates the fraction of the dose escaping metabolism in the gut wall,¹⁶ was used to predict the effect of ITZ on the AUC of oral MDZ, with adjustment for multiple inhibitors as described by Equation 5. The ratio of inhibited to uninhibited F_g was calculated using Equation 4 (ref. 16):

$$\frac{F_{g,i}}{F_g} = \frac{1}{F_g + (1 - F_g) \cdot \frac{1}{1 + [I]_g/K_{i,g}}} \quad (4)$$

with adjustment for multiple inhibitors. The inhibitory potency (K_i) of ITZ and its metabolites was assumed to be the same in the liver and gut, and the human liver microsomal IC_{50} values were used for predictions related to both gut and liver. Because MDZ was administered 4 h after ITZ (which is ~ 3 h after ITZ $t_{\max}$ in our previous study

with the same formulation of ITZ8), we took the value of the systemic concentration of ITZ and its metabolites as $[I]_g$.

For all predictions, the average unbound plasma concentration of ITZ alone or the sum of the average concentrations of ITZ and its metabolites were analyzed for $[I]$. The contribution of ITZ alone and of the circulating inhibitory metabolites to overall inhibition was

$$\frac{AUC_i}{AUC} = \left(\frac{1}{F_g + (1-F_g) \cdot \frac{1}{1 + \sum [I]_{u,g}/K_{i,u,g}}} \right) \cdot \left(\frac{1}{(1-f_m) + \frac{f_m}{1 + \sum [I]_{u,u}/K_{i,u}}} \right) \quad (5)$$

determined using the sum of $[I]/K_i$ values for ITZ and its metabolites according to Equation 5.

The fraction of MDZ elimination that occurs through CYP3A4 ($f_{m,CYP3A4}$) activity in the liver was assumed to be 93%.¹⁶ Absorption of MDZ was assumed complete and an F_g value of 0.55 was used to determine intestinal first-pass metabolism of MDZ.¹⁷

The accuracy of the predictions was evaluated for all interactions and for each dose level by comparing the magnitude of the observed interaction to the interactions predicted on the basis of considering ITZ alone or ITZ and its metabolites. A less-than-twofold error in predictions was considered accurate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by National Institutes of Health grants P01GM32165 and t32GM007750 and Clinical and Translational Science Award UL1 RR025014. We thank Angela Hein for her assistance in study coordination and Wendel Nelson for helpful discussions regarding ITZ metabolism.

References

1. Isoherranen N, Hachad H, Yeung CK, Levy RH. Qualitative analysis of the role of metabolites in inhibitory drug-drug interactions: literature evaluation based on the metabolism and transport drug interaction database. *Chem. Res. Toxicol* 2009;22:294–298. [PubMed: 19216580]
2. Fujioka Y, Yeung CK, Hachad H, Levy RH, Isoherranen N. Analysis of the contribution of circulating metabolites to inhibitory drug-drug interactions: literature evaluation based on the metabolism and transport drug interaction database. *Drug Metab Rev* 2009;41(suppl. 3):69.
3. Isoherranen N, Kunze KL, Allen KE, Nelson WL, Thummel KE. Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metab. Dispos* 2004;32:1121–1131. [PubMed: 15242978]
4. Stevens JC, Wrighton SA. Interaction of the enantiomers of fluoxetine and norfluoxetine with human liver cytochromes P450. *J. Pharmacol. Exp. Ther* 1993;266:964–971. [PubMed: 8355218]
5. Reese MJ, Wurm RM, Muir KT, Generaux GT, St John-Williams L, Mcconn DJ. An *in vitro* mechanistic study to elucidate the desipramine/bupropion clinical drug-drug interaction. *Drug Metab. Dispos* 2008;36:1198–1201. [PubMed: 18420781]
6. Preskorn SH, Alderman J, Chung M, Harrison W, Messig M, Harris S. Pharmacokinetics of desipramine coadministered with sertraline or fluoxetine. *J. Clin. Psychopharmacol* 1994;14:90–98. [PubMed: 8195463]

7. Sauer JM, et al. Atomoxetine hydrochloride: clinical drug-drug interaction prediction and outcome. *J. Pharmacol. Exp. Ther* 2004;308:410–418. [PubMed: 14610241]
8. Templeton IE, et al. Contribution of itraconazole metabolites to inhibition of CYP3A4 *in vivo*. *Clin. Pharmacol. Ther* 2008;83:77–85. [PubMed: 17495874]
9. Olkkola KT, Ahonen J, Neuvonen PJ. The effect of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesth Analg (Baltimore)* 1996;82:511–516.
10. Olkkola KT, Brunetto AV, Mattila MJ. Pharmacokinetics of oxicam nonsteroidal anti-inflammatory agents. *Clin. Pharmacokinet* 1994;26:107–120. [PubMed: 8162655]
11. Ahonen J, Olkkola KT, Neuvonen PJ. Effect of itraconazole and terbinafine on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers. *Br. J. Clin. Pharmacol* 1995;40:270–272. [PubMed: 8527290]
12. Neuvonen PJ, Varhe A, Olkkola KT. The effect of ingestion time interval on the interaction between itraconazole and triazolam. *Clin. Pharmacol. Ther* 1996;60:326–331. [PubMed: 8841155]
13. Backman JT, Kivistö KT, Olkkola KT, Neuvonen PJ. The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. *Eur. J. Clin. Pharmacol* 1998;54:53–58. [PubMed: 9591931]
14. Levy RH, Hachad H, Yao C, Ragueneau-Majlessi I. Relationship between extent of inhibition and inhibitor dose: literature evaluation based on the metabolism and transport drug interaction database. *Curr. Drug Metab* 2003;4:371–380. [PubMed: 14529369]
15. Yao C, Kunze KL, Trager WF, Kharasch ED, Levy RH. Comparison of *in vitro* and *in vivo* inhibition potencies of fluvoxamine toward CYP2C19. *Drug Metab. Dispos* 2003;31:565–571. [PubMed: 12695344]
16. Obach RS, Walsky RL, Venkatakrishnan K, Gaman EA, Houston JB, Tremaine LM. The utility of *in vitro* cytochrome P450 inhibition data in the prediction of drug-drug interactions. *J. Pharmacol. Exp. Ther* 2006;316:336–348. [PubMed: 16192315]
17. Paine MF, et al. First-pass metabolism of midazolam by the human intestine. *Clin. Pharmacol. Ther* 1996;60:14–24. [PubMed: 8689807]

Figure 1.

Effect of escalating doses of itraconazole (ITZ) on disposition of oral midazolam (MDZ). Panel **a** shows the plasma concentration–time curves for midazolam in the control session and three ITZ sessions. MDZ was administered 4 h after the oral dose of ITZ. Panel **b** depicts the AUC values of MDZ in the six study subjects, in the control session and in the sessions involving escalating doses of ITZ.

Figure 2.

In vivo inhibitory potency (IC₅₀ values) for itraconazole (ITZ) in the six study subjects. The subjects are labeled “S” along with a study number, and the fitted IC₅₀ value is given in the inset of each panel. The maximum percent inhibition is shown as the E_{max} value in the insets.

Figure 3.

Predicted vs. observed interactions between itraconazole (ITZ) and midazolam after escalating doses of ITZ. The circles represent data from each individual subject after a 50-mg dose of ITZ; the boxes represent the data from each subject after the 200-mg dose; and the triangles represent the data from each subject after the 400-mg dose. Each interaction was predicted using the measured unbound concentrations of either (a) ITZ alone, or (b) ITZ and its metabolites. The unbound IC_{50} values were measured in human liver microsomes. AUC, area under the curve.

Table 1

Average pharmacokinetic parameters of MDZ determined after each session

ITZ dose	MDZ AUC (h·nmol/l)	MDZ <i>t</i> 1/2 (h)	MDZ Cl/F (L/h) (% of 0 mg ITZ)	AUC _I /AUC ^a
0	63 ± 39	2.5 ± 0.9	122 ± 49	
50 mg	114 ± 41 *	4.4 ± 2.1	61 ± 28* (50%)	2.0 ± 0.6
200 mg	256 ± 75 **,##	7.1 ± 2.7 *	26 ± 9 *,# (21%)	4.7 ± 1.4
400 mg	289 ± 57 **,##	8.1 ± 3.8 *	22 ± 4 *,# (18%)	5.4 ± 1.5

AUC, area under the midazolam plasma concentration–time curve; Cl/F, clearance of oral midazolam; ITZ, itraconazole; MDZ, midazolam; *t*1/2, half-life.

^a AUC_I/AUC was calculated in each individual, and the average increase in this ratio was calculated.

* $P < 0.01$,

** $P < 0.001$ as compared with control;

$P < 0.01$,

$P < 0.001$ as compared with the 50-mg ITZ dose.

Table 2

Average plasma concentrations (AUC/ τ , $\tau = 19.5$ h, the MDZ sampling interval) of ITZ and its metabolites during MDZ sampling period

ITZ dose	ITZ (nmol/l)	OH-ITZ (nmol/l)	Keto-ITZ (nmol/l)	ND-ITZ (nmol/l)
50 mg	24 $\pm$ 10	41 $\pm$ 11	5 $\pm$ 3	2 $\pm$ 1
200 mg	220 $\pm$ 55 [*]	549 $\pm$ 124 [*]	24 $\pm$ 6 [*]	16 $\pm$ 5 [*]
400 mg	450 $\pm$ 100 ^{*,##}	969 $\pm$ 157 ^{*,#}	34 $\pm$ 8 ^{*,#}	20 $\pm$ 4 [*]

ITZ, itraconazole; MDZ, midazolam; ND-ITZ, N-desalkyl-ITZ; OH-ITZ, hydroxy-ITZ.

^{*} $P < 0.001$ when compared with the 50-mg ITZ dose;

[#] $P < 0.01$,

^{##} $P < 0.001$ when compared with the 200-mg ITZ dose.

Table 3

Relative inhibitory potency of ITZ and its metabolites as calculated using the $[I]/IC_{50}$ ratio of each compound after escalating doses of ITZ

Dose	$(C_{ave,u}/IC_{50,u})$			
	ITZ	OH-ITZ	Keto-ITZ	ND-ITZ
50 mg	0.2 ± 0.06	0.05 ± 0.01	0.04 ± 0.03	0.07 ± 0.04
200 mg	1.4 ± 0.3	0.6 ± 0.1	0.2 ± 0.05	0.4 ± 0.1
400 mg	2.8 ± 0.6	1.1 ± 0.2	0.3 ± 0.07	0.5 ± 0.1

IC_{50} values are those from Isoherranen *et al.*,³ and unbound fraction values are those from Templeton *et al.*⁸

ITZ, itraconazole; ND-ITZ, N-desalkyl ITZ; OH-ITZ, hydroxy-ITZ.

Table 4

Predicted gut availability (F_g) and average changes in systemic clearance (Cl_{systemic}), and clearance (Cl/F) of oral midazolam calculated using Eqs. 2, 4, and 6, considering ITZ alone or both ITZ and its metabolites

Parameter			
<i>ITZ alone</i>			
ITZ dose	50 mg ITZ	200 mg ITZ	400 mg ITZ
F_g (%)	58 ± 1	74 ± 3	82 ± 2
Cl_{systemic} decrease (%)	12 ± 4	53 ± 6	68 ± 4
Cl/F decrease (%)	17 ± 6	65 ± 6	78 ± 3
<i>ITZ and its metabolites</i>			
ITZ dose	50 mg ITZ	200 mg ITZ	400 mg ITZ
F_g increase (%)	62 ± 4	81 ± 4	87 ± 3
Cl_{systemic} decrease (%)	21 ± 6	67 ± 4	76 ± 2
Cl/F decrease (%)	30 ± 8	77 ± 3	85 ± 2

All predictions were carried out using unbound $[I]$ and K_1 values.

ITZ, itraconazole.