# Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: Effect of ketoconazole

*Background:* **The cytochrome P450 3A (CYP3A) isoforms are responsible for the metabolism of a majority of therapeutic compounds, and they are abundant in the intestine and liver. CYP3A activity is highly variable, causing difficulty in the therapeutic use of CYP3A substrates. A practical in vivo probe method that characterizes both intestinal and hepatic CYP3A activity would be useful.**

*Objectives:* **To determine the intestinal and hepatic contribution to the bioavailability of midazolam with use of the CYP3A inhibitor ketoconazole.**

*Methods:* **The pharmacokinetics of midazolam was assessed in nine (six men and three women) healthy individuals after single doses of 2 mg intravenous and 6 mg oral midazolam (phase I). These pharmacokinetic values were compared with those obtained after single doses of 2 mg intravenous and 6 mg oral midazolam and three doses of 200 mg oral ketoconazole (phase II).**

*Results:* **After ketoconazole therapy, area under the concentration versus time curve of midazolam increased 5-fold after intravenous midazolam administration ($P \leq .001$) and 16-fold after oral midazolam administration ($P \leq .001$). Intrinsic clearance decreased by 84% ($P = .003$). Total bioavailability increased from 25% to 80% ($P < .001$). The intestinal component of midazolam bioavailability increased to a greater extent than the hepatic component (2.3-fold [$P = .003$] and 1.5-fold [$P \leq .001$], respectively). In the control phase, female subjects had greater midazolam clearance values than the male subjects.**

*Conclusions:* **Ketoconazole caused marked inhibition of CYP3A activity that was greater in the intestine than the liver. Administration of single doses of oral and intravenous midazolam with and without oral ketoconazole exemplifies a practical method for differentiating intestinal and hepatic CYP3A activity. (Clin Pharmacol Ther 1999;66:461-71.)**

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The cytochrome P450 3A (CYP3A) subfamily is the most important of the cytochrome P450 superfamily because of its abundance in the liver and intestine and its ability to metabolize a wide variety of therapeutic compounds. The activity of CYP3A is highly variable both between and within individuals, which makes the dosing and therapeutic use of many CYP3A substrates difficult, especially those with narrow therapeutic ranges. The CYP3A subfamily consists of three known isoforms in humans—CYP3A4, CYP3A5, and CYP3A7. The CYP3A4 isoform is thought to be the dominant form in humans[1]; however, recent evidence reports the presence of CYP3A5 in 10% to 25% of adult human livers[2] and CYP3A7 in 50% of adult liver samples.[3] It has not been established whether the presence or absence of CYP3A5 and/or CYP3A7 in the intestine or liver contributes to a significant difference in metabolic capacity.[4-8] Therefore, to maintain consistency, we will use the general term "CYP3A" to refer to the

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subfamily of enzymes when appropriate. The CYP3A activity of an individual is probably determined both by genetics and by environmental factors that can modulate the activity. Many factors may potentially influence the activity of CYP3A, including age,[9] gender,[10] menopausal status,[11] hormones,[12] and numerous xenobiotics.[13] However, in general these factors do not have a predictable effect on CYP3A activity. In addition, the CYP3A activities of an individual in the intestine and the liver do not appear to be coordinately regulated[14,15]; therefore it is likely that these many factors may differentially modulate intestinal and hepatic activity.

Because of the importance of the CYP3A subfamily and because of the many factors that influence CYP3A activity, a practical method to determine the CYP3A activity of an individual is desirable. There have been many attempts to use various CYP3A substrates as probe compounds to predict CYP3A activity, including dapsone, 6-β-hydroxylation of testosterone, nifedipine, 6-β-hydroxycortisol, and the erythromycin breath test. Although the erythromycin breath test has shown correlations with cyclosporine (INN, ciclosporin) clearance,[16] OG 37-325,[17] and midazolam clearance,[18] there are limitations to this test, including the administration of radioactivity, the variability in correlating radioactive exhaled carbon dioxide and *N*-demethylation of erythromycin, the role of CYP3A in erythromycin metabolism,[1] and the lack of prediction of intestinal CYP3A activity. Therefore an optimal CYP3A probe has yet to be identified.

Midazolam is a benzodiazepine that is used clinically for conscious sedation. It has pharmacokinetic properties that make it an attractive in vivo probe: it is specifically metabolized by CYP3A[19] to one predominant metabolite (1′-hydroxymidazolam); it has a short half-life ($t_{½}$), so estimation of total area under the concentration versus time curve (AUC) is easily measurable and it can be given intravenously and orally (with the injection preparation). In addition, hepatic CYP3A content measured in vitro has been shown to be highly correlated with midazolam intravenous clearance ($r$ = 0.93; $P < .001$),[20] and plasma 1′-hydroxymidazolam/midazolam ratio.[21]

Ketoconazole is one of the most potent inhibitors of CYP3A in clinical use. Its estimated in vitro inhibition constant ($K_i$) with use of human liver microsomes for 1-hydroxymidazolam formation is less than 0.01 μmol/L (1 μmol/L = 0.53 μg/mL).[22] Administration of the CYP3A substrate midazolam intravenously (when the contribution of intestinal CYP3A activity is assumed to be negligible) and orally both in the absence and presence of the CYP3A inhibitor ketoconazole allows the contributions of intestinal and hepatic CYP3A to the bioavailability of midazolam to be estimated. Our study population consisted of healthy individuals; therefore potential confounding variables such as abnormal liver or intestinal function and concomitant medications were minimized.

## METHODS

Nine healthy nonsmoking human subjects (six men and three women) were enrolled in the study after each gave written informed consent. Their mean age was 26 years (age range, 19 to 41 years). The mean body weight of male subjects was 77.5 kg, and the mean body weight of female subjects was 59.7 kg. All subjects were white. The study was approved by the Human Investigation Review Committee at New England Medical Center and Tufts University School of Medicine and by the Institutional Review Board at Northeastern University. The study was conducted in the General Clinical Research Center at New England Medical Center. All subjects were healthy adults with no evidence of medical disease. No subjects took any medications. Female subjects were not taking oral contraceptives.

The study was conducted in two phases. In phase I, baseline oral and intravenous midazolam pharmacokinetic parameters were established. Subjects received a single dose of 2 mg intravenous midazolam (Versed, 2 mg/mL for intravenous injection, Hoffmann-LaRoche Inc, Nutley, NJ) or 6 mg oral midazolam as 3 mL of the parenteral preparation diluted with 30 mL water. Although midazolam bioavailability has been estimated to average 41%,[9] we chose the conservative estimate of 33% to ensure adequate measurement of plasma concentrations. In phase II, subjects received single doses of 2 mg intravenous or 6 mg oral midazolam with 200 mg ketoconazole (Nizoral, Janssen Pharmaceutica, Titusville, NJ). The first dose was administered 12 hours before the midazolam dose and subsequent doses were administered every 12 hours for a total of three doses to maintain plasma ketoconazole levels in excess of the $K_i$. The order of treatments was randomized; however, treatments remained constant between phase I and II for each subject. Before each study day, subjects were instructed to fast from midnight until they were given a standardized light breakfast approximately 90 minutes before midazolam administration. All subsequent meals during each study day were standardized. Alcohol, caffeine, and grapefruit juice were prohibited during each study day. Blood samples were collected before and at 15, 30, and 45 minutes and 1, 1½, 2, 2½, 3, 4, 5, 6, and 8 hours after midazolam dosing during

**Table I.** Intravenous and oral midazolam pharmacokinetic parameters: Noncompartmental analysis

| | *Control* | | *With ketoconazole* | |
|---|---|---|---|---|
| | *Intravenous* | *Oral* | *Intravenous* | *Oral* |
| *Pharmacokinetic parameters* | | | | |
| AUC (ng · h/mL) | 70.2 ± 25.8 | 54.2 ± 28.0 | 354 ± 185* | 738 ± 191* |
| CL (mL/min/kg) | 7.6 ± 2.9 | 34.2 ± 18.7 | 1.6 ± 0.8* | 2.1 ± 0.6* |
| $CL_{int}$ (mL/min/kg) | 13.3 ± 8.9 | | 1.8 ± 1.0† | |
| $V_{area}$ (L/kg) | 1.9 ± 0.8 | | 1.6 ± 0.5 | |
| Elimination $t_{½}$ (h) | 3.4 ± 2.0 | — | 14.0 ± 8.2† | — |
| $C_{max}$ (ng/mL) | — | 19.1 ± 8.1 | — | 81.0 ± 28.9* |
| $t_{max}$ (h) | — | 0.8 ± 0.2 | — | 0.8 ± 0.6 |
| F (%) | — | 25.3 ± 9.6 | — | 80.0 ± 31.6* |
| $F_H$ (%) | — | 64.6 ± 13.4 | — | 92.4 ± 3.7* |
| $F_{ABS} \cdot F_G$ (%) | — | 40.1 ± 14.7 | — | 87.8 ± 38.5† |
| *Ratios (ketoconazole/control)* | | | | |
| AUC | | | 5.1 ± 1.9 | 16.0 ± 6.1 |
| $CL_{int}$ | | | 0.16 ± 0.06 | — |
| F | | | | 3.4 ± 1.7 |
| $F_H$ | | | | 1.5 ± 0.3 |
| $F_{ABS} \cdot F_G$ | | | | 2.3 ± 0.8 |

Data are mean values ± SD.
AUC, Area under the concentration versus time curve; CL, clearance; $CL_{int}$, intrinsic clearance; $V_{area}$, volume of distribution; $t_{½}$, half-life; $C_{max}$, maximum concentration; $t_{max}$, time to reach $C_{max}$; F, total bioavailability; $F_H$, hepatic bioavailability; $F_{ABS} \cdot F_G$, intestinal bioavailability.
*$P \leq .001$.
†$P = .003$.

phase I, with additional samples taken at 12 and 24 hours after midazolam dosing for phase II.

Plasma was analyzed for midazolam concentrations by electron capture gas chromatography according to a previously published method.[23] In addition, plasma ketoconazole concentrations were determined by HPLC.[24]

Pharmacokinetic analysis was conducted by use of two methods. The first method used noncompartmental methods in which midazolam plasma AUC values were calculated by use of the log-linear and linear trapezoidal methods for decreasing and increasing concentrations, respectively, with extrapolation from the last measured concentration to infinity. Estimation of the terminal elimination rate constant was performed by linear regression of at least the last three concentration–time points. Oral and intravenous clearance rates ($CL_{oral}$ and $CL_{IV}$, respectively) were calculated by dividing the respective dose by the AUC after oral and intravenous administration, respectively. Intrinsic clearance ($CL_{int}$), defined as the clearance attributed solely to hepatic metabolism, was calculated with the equation:

$$(CL_{IV} \cdot Q_H)/(Q_H - CL_{IV})$$

in which $Q_H$ is the liver blood flow. Liver blood flow was assumed to be 1500 mL/min/70 kg, normalized for body weight of each individual subject. Volume of distribution ($V_{area}$) was calculated with use of the area method. The second method used nonlinear regression with a linear sum of two exponential terms to analyze plasma midazolam concentrations after intravenous administration. Coefficients and exponents from the fitted function were used to estimate total AUC, $V_{area}$, elimination $t_{½}$, and total clearance. After oral administration, AUC until the last detectable concentration was calculated by use of the linear trapezoidal method and extrapolated to infinity by addition of the final concentration divided by β. The maximum concentration ($C_{max}$) and the time to reach $C_{max}$ ($t_{max}$) were determined by the highest measured concentration and respective time.

Bioavailability (F) was determined from dose-corrected AUC values, assuming dose-independent pharmacokinetics. The components of bioavailability were further predicted on the basis of the equation:

$$F = F_{ABS} \cdot F_G \cdot F_H$$

in which $F_{ABS}$ is the fraction of the dose absorbed from the gut lumen, $F_G$ is the fraction of the dose not metabolized by intestinal metabolic enzymes, and $F_H$ is the fraction of the dose absorbed into the hepatic portal vein that escapes first-pass liver metabolism. $F_H$ was defined as follows:

$$1 - ER_H$$

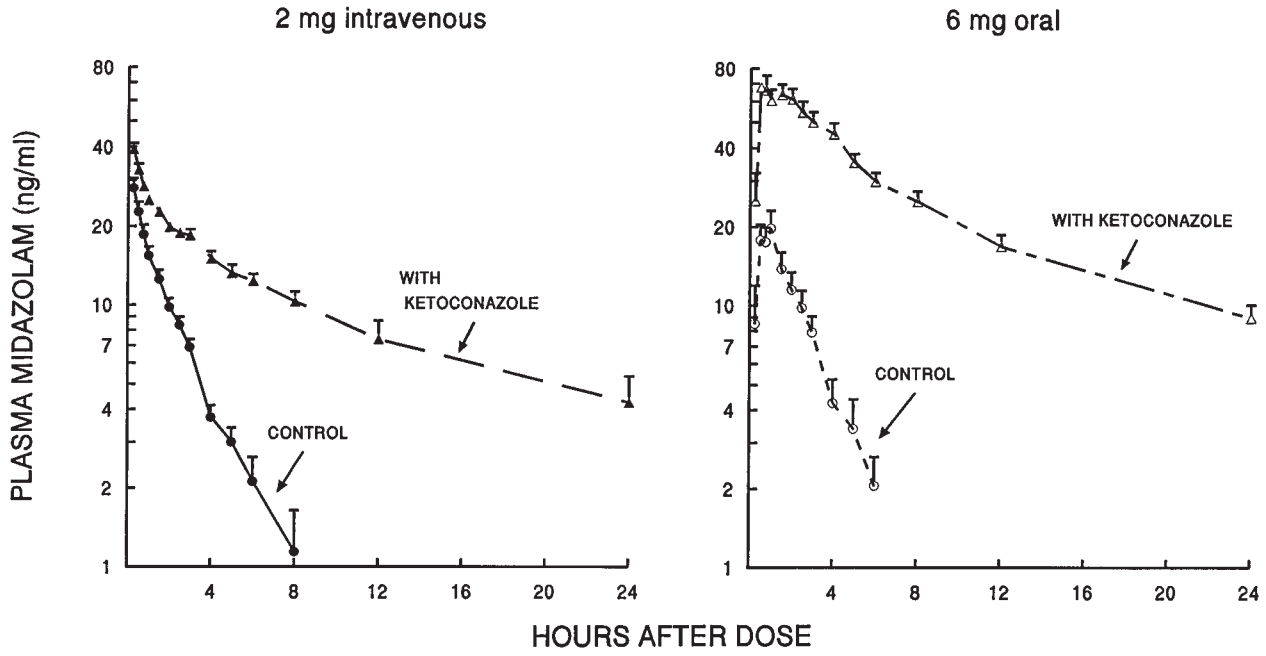


**Fig 1.** Mean ± SEM plasma concentration versus time curves after intravenous and oral midazolam in the control phase versus with ketoconazole.

**Table II.** Intravenous and oral midazolam pharmacokinetic parameters: Nonlinear regression with a linear sum of two exponential terms

| | *Control* | | *With ketoconazole* | |
|---|---|---|---|---|
| | *Intravenous* | *Oral* | *Intravenous* | *Oral* |
| *Pharmacokinetic parameters* | | | | |
| AUC (ng · h/mL) | 63.4 ± 18.7 | 58.2 ± 31.1 | 300 ± 104* | 719 ± 181* |
| CL (mL/min/kg) | 8.1 ± 2.8 | 32.7 ± 19 | 1.7 ± 0.5* | 2.1 ± 0.6* |
| $CL_{int}$ (mL/min/kg) | 14.6 ± 9.4 | | 1.9 ± 0.6* | |
| $V_{area}$ (L/kg) | 1.3 ± 0.4 | | 1.3 ± 0.4 | |
| Elimination $t_{½}$ (h) | 1.9 ± 0.5 | 1.7 ± 0.4 | 9.7 ± 4.1* | 9.9 ± 3.3* |
| $C_{max}$ (ng/mL) | — | 19.1 ± 8.1 | — | 81.0 ± 28.9* |
| $t_{max}$ (h) | — | 0.8 ± 0.2 | — | 0.8 ± 0.5 |
| F (%) | — | 29.3 ± 11.3 | — | 83.8 ± 21.0* |
| $F_H$ (%) | — | 62.4 ± 13.0 | — | 92.0 ± 2.4* |
| $F_{ABS} \cdot F_G$ (%) | — | 47.5 ± 16.6 | — | 91.4 ± 24.4* |
| *Ratios (ketoconazole/control)* | | | | |
| AUC | | | 4.8 ± 1.4 | 15.1 ± 6.7 |
| $CL_{int}$ | | | 0.15 ± 0.06 | — |
| F | | | | 3.2 ± 1.3 |
| $F_H$ | | | | 1.5 ± 0.4 |
| $F_{ABS} \cdot F_G$ | | | | 2.1 ± 0.7 |

Data are mean values ± SD.
*$P \leq .001$.

in which $ER_H$ is the hepatic extraction ratio defined as clearance of intravenously administered midazolam divided by liver blood flow: $CL_{IV}/Q_H$. Nonhepatic contributions to clearance were assumed to be negligible. Therefore, assuming linear pharmacokinetics, the product $F_{ABS} \cdot F_G$ was determined and compared with and without the presence of the enzyme inhibitor ketoconazole. It was assumed that

ketoconazole does not significantly affect liver blood flow or $F_{ABS}$.

Statistical analysis was conducted by use of the paired Student *t* test, with $\alpha = .05$.

## RESULTS

Midazolam pharmacokinetic parameters before (control) and with ketoconazole calculated by use of noncompartmental analysis are shown in Table I; parameters calculated by use of nonlinear regression with a linear sum of two exponential terms are shown in Table II. In the presence of ketoconazole, intravenous midazolam AUC increased 5-fold and oral midazolam AUC increased 16-fold ($P < .001$). The comparatively larger increase in oral midazolam AUC versus intravenous midazolam AUC is shown graphically in Fig 1. Accordingly, midazolam clearance after intravenous and oral administration decreased from 7.6 ± 2.9 to 1.6 ± 0.8 ($P < .001$) and 34.2 ± 18.7 to 2.1 ± 0.6 mL/min/kg ($P < .001$) for control versus administration with ketoconazole, respectively. Consistent with the presumed effect of ketoconazole on CYP3A metabolism, the $CL_{int}$ decreased dramatically after ketoconazole administration: 13.3 ± 8.9 versus 1.8 ± 1.0 mL/min/kg ($P = .003$; Tables I and II). The comparative declines in all three clearance values are shown in Fig 2.

Ketoconazole caused total measured bioavailability to increase from 25% to 80% ($P < .001$). Ketoconazole also caused both the hepatic and intestinal components of total bioavailability to be increased, although the intestinal component was increased to a greater extent (Tables I and II). There was a lack of correlation between $F_H$ and $F_{ABS} \cdot F_G$ ($r^2 = 0.13$; Fig 3, *A*).

The $C_{max}$ of oral midazolam increased 5-fold with ketoconazole, consistent with the large gut effect. The $t_{max}$ was not changed with ketoconazole (0.78 ± 0.21 versus 0.82 ± 0.57 hour, control versus with ketoconazole, respectively; $P$ = NS). The $t_{½}$ increased approximately 5-fold in the presence of ketoconazole ($P = .003$; Fig 1).

Interestingly, in the control phase, the three female subjects had a mean midazolam clearance that was greater than the six male subjects when corrected for weight (10.2 ± 3.2 versus 6.3 ± 1.8 mL/min/kg; $P = .047$). However, this difference disappeared during the ketoconazole phase (1.8 ± 1.3 versus 1.5 ± 0.5 mL/min/kg; $P = .64$). The $F_H$ was significantly lower in the women compared with the men in the control phase only (52.6% ± 14.8% versus 70.7% ± 8.4%; $P = .047$), which was consistent with the change in clearance. Women also had a larger weight-adjusted $V_{area}$ than men that reached statistical significance during the ketoconazole phase (2.17 ± 0.25 versus 1.32 ± 0.34 L/kg; $P = .007$), when calculated with noncompartmental methods. It should be noted that there were no changes in weight between the control and ketoconazole phases. However, using the two-compartment method, females had larger weight-adjusted $V_{area}$ compared to males in both the control (1.65 ± 0.49 versus 1.08 ± 0.20 L/kg, $P = .03$) and ketoconazole (1.70 ± 0.68 versus 1.10 ± 0.23 L/kg, $P = .025$) phases. In addition, there were no gender-related differences in clearance or the elimination rate constant during the ketoconazole phase. There were no other gender-related differences in any other pharmacokinetic parameter.

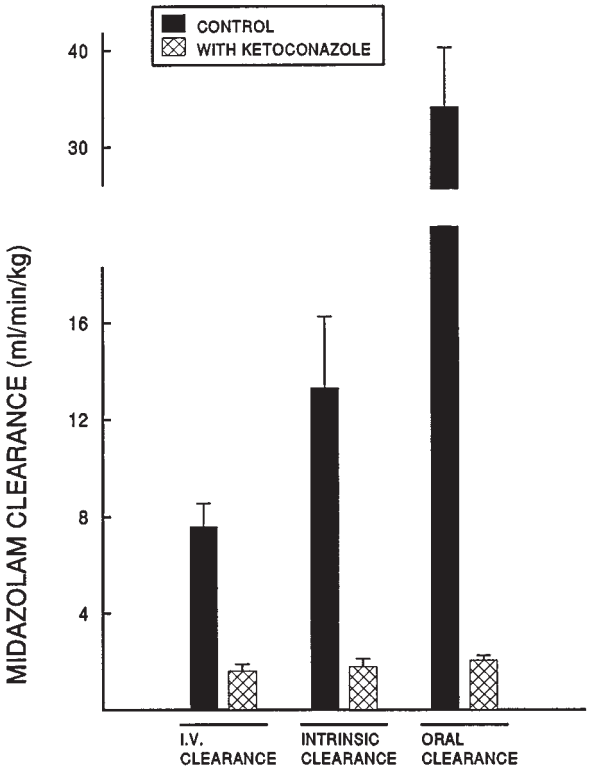


**Fig 2.** Mean ± SEM plasma clearance rates in the control phase versus with ketoconazole. Intravenous and oral clearance values were measured. Intrinsic clearance was calculated as described in the text.

Ketoconazole plasma concentrations ranged from 0.077 to 4.852 μg/mL, thereby exceeding the in vitro $K_i$ at most time points (Fig 4).

## DISCUSSION

The CYP3A subfamily is clearly important in determining the pharmacokinetics and subsequent pharmaco-

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