Prediction of In Vivo Interaction Between Triazolam and Erythromycin Based on In Vitro Studies Using Human Liver Microsomes and Recombinant Human CYP3A4

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Purpose. To quantitatively predict the in vivo interaction between triazolam and erythromycin, which involves mechanism-based inhibition of CYP3A4, from in vitro studies using human liver microsomes (HLM) and recombinant human CYP3A4 (REC).

Methods. HLM or REC was preincubated with erythromycin in the presence of NADPH and then triazolam was added. α- and 4-hydroxy (OH) triazolam were quantified after a 3 min incubation and the kinetic parameters for enzyme inactivation (k_{inact} and K'_{app}) were obtained. Drug-drug interaction in vivo was predicted based on a physiologically-based pharmacokinetic (PBPK) model, using triazolam and erythromycin pharmacokinetic parameters obtained from the literature and kinetic parameters for the enzyme inactivation obtained in the in vitro studies.

Results. Whichever enzyme was used, triazolam metabolism was not inhibited without preincubation, even if the erythromycin concentration was increased. The degree of inhibition depended on preincubation time and erythromycin concentration. The values obtained for k_{inact} and K'_{app} were 0.062 min^{-1} and 15.9 μM (α-OH, HLM), 0.055 min^{-1} and 17.4 μM (4-OH, HLM), 0.173 min^{-1} and 19.1 μM (α-OH, REC), and 0.097 min^{-1} and 18.9 μM (4-OH, REC). Based on the kinetic parameters obtained using HLM and REC, the AUCpo of triazolam was predicted to increase 2.0- and 2.6-fold, respectively, following oral administration of erythromycin (333 mg t.i.d. for 3 days), which agreed well with the reported data.

Conclusions. In vivo interaction between triazolam and erythromycin was successfully predicted from in vitro data based on a PBPK model involving a mechanism-based inhibition of CYP3A4.

KEY WORDS: drug interaction; mechanism-based inhibition; triazolam; erythromycin; physiologically-based pharmacokinetics.

INTRODUCTION

Multi-drug combination therapy is now common and drug-drug interactions have become a significant medical problem. The interaction between sorivudine, an antiviral drug, and 5-fluorouracil, an anticancer drug, caused one of the most serious cases of toxicity ever seen in Japan. In 1993, fifteen Japanese patients with cancer and herpes zoster died from 5-fluorouracil toxicity which was strongly suggested to be caused by high blood concentrations due to an interaction between 5-fluorouracil and sorivudine (1,2). The interaction between sorivudine and 5-fluorouracil is based on a "mechanism-based inhibition," which differs from competitive or non-competitive inhibition (2,3). A mechanism-based inhibitor is metabolized by an enzyme to form a metabolite which covalently binds to the same enzyme, leading to irreversible inactivation of the enzyme. This type of interaction deserves more attention than the more common type of inhibition, because the inhibitory effect remains after elimination of the inhibitor from blood and tissue and this can lead to serious side-effects. We have already succeeded in predicting quantitatively the interaction between sorivudine and 5-fluorouracil from in vitro data using rat and human liver cytosol and human recombinant enzyme using methodology we developed ourselves (4).

A similar inhibition mechanism is reported for macrolide antibiotics, such as erythromycin, in which cytochrome P450 demethylates the macrolide to a nitrosoalkane which forms a stable, inactive complex with P450 (5). Therefore, in the case of enzyme inhibition by erythromycin, in predicting in vivo interactions from in vitro studies, it is necessary to consider the exposure time of the enzyme to the inhibitor and enzyme turnover (4). Clinically, the area under the concentration-time curve after oral administration (AUCpo) of triazolam, which is metabolized by CYP3A4, is reported to increase by a factor of 2.1 following co-administration of erythromycin (6). In the present study, as a case of a drug-drug interaction involving mechanism-based inhibition, the interaction between triazolam and erythromycin in humans was investigated using our methodology for the quantitative prediction of in vivo interactions from in vitro data.

MATERIALS AND METHODS

Chemicals and Reagents

Triazolam, α-hydroxy (OH) triazolam and 4-OH triazolam were generously supplied by Upjohn Company (Kalamazoo, MI). Erythromycin, acetonitrile, methanol and other reagents of analytical grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Boehringer Mannheim (Mannheim, Germany). Microsomal preparations of recombinant human CYP3A4 and P450 reductase expressed by the human B lymphoblastoid cell line, M107r (recombinant microsomes) was a gift from Gentest Corp. (Woburn, MA). Three human liver microsomes (H-35, H-36 and H-66) with large variations in their CYP3A4 content were selected for the in vitro metabolism experiments out of 26 different microsomes prepared from human livers stored in the human liver bank of SRI International (Menlo Park, CA). Antiserum for human CYP3A4 was generously supplied by Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan).

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Triazolam Metabolism by Human Liver Microsomes or Recombinant Human CYP3A4

Triazolam was incubated with a reaction mixture (0.6 mL) consisting of 0.2 mg/mL human liver microsomal protein or 29 pmol/mL recombinant human CYP3A4 (the amount of CYP3A4 similar to human liver microsomes) and an NADPH-generating system (0.33 mM NADP, 8 mM glucose-6-phosphate, 0.1 U/mL glucose-6-phosphate dehydrogenase, 6 mM $MgCl_2$) in 100 mM potassium phosphate buffer (pH 7.4). Enzyme reactions were initiated by adding 60 μL triazolam in 50% acetone solution. After incubation at 37°C in a shaking water bath for 20 min, the reaction was terminated by addition of 800 μL acetonitrile to 400 μL of the aliquot. The final triazolam concentration ranged from 10 to 1000 μM.

Immunoinhibition Study

Human liver microsomes (H-66) at a final concentration of 0.2 mg/mL were preincubated for 30 min at room temperature with increasing amounts of antiserum for human CYP3A4 (from 40 to 200 μL/0.2 mg microsomal protein). Triazolam metabolism was then measured as described above. The final triazolam concentration was 100 μM.

Inhibition by Erythromycin

After preincubation of human liver microsomes (H-66) or recombinant human CYP3A4 with erythromycin and NADPH-generating system at 37°C for 0, 5, 10, 20, or 40 min, triazolam was added and incubated at 37°C for another 3 min. The final concentration of erythromycin ranged from 3 to 100 μM and the final triazolam concentration was 300 μM. The enzyme reaction was terminated as described above.

HPLC Assay

α-OH triazolam and 4-OH triazolam in the incubation mixture were determined by an HPLC-UV detection method. The incubation mixture was mixed with acetonitrile and centrifuged at 10,000 × g for 10 min and 1 mL supernatant was evaporated. The residues were reconstituted with 150 μL HPLC mobile phase as described below, and 50 μL was injected onto the HPLC system. The HPLC system consisted of a model L-7100 pump (Hitachi Ltd., Tokyo, Japan), a model L-7200 sample injector (Hitachi), a model L-4250 UV absorbance detector (Hitachi) set at 220 nm, and a TSKgel ODS-80Ts reversed-phase column (250 × 4.6 mm internal diameter, Tosoh, Tokyo, Japan). The mobile phase consisted of a 4/2/1 (v/v/v) mixture of 20 mM potassium phosphate buffer (pH 7.4), acetonitrile and methanol delivered at 1.0 mL/min. All chromatograms were recorded using a model D-7500 Chromato-Integrator (Hitachi) and quantitative measurements of α-OH triazolam and 4-OH triazolam were obtained from their peak areas.

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD).

Analysis of Enzyme Inactivation Kinetics

Kinetic parameters for enzyme inactivation were obtained as reported elsewhere (4). The logarithm of the remaining enzymatic activity (formation rate of α-OH triazolam or 4-OH triazolam) was plotted against the preincubation time. The apparent inactivation rate constant (k_{obs}) was determined from the slope of the initial linear phase. The value of k_{obs} was plotted against the erythromycin concentration and the parameters (k_{inact} and K'_{app}) were obtained by the nonlinear least-squares method (MULTI program (7)) according to the following equation (8,9):

$$k_{obs} = k_{inact} \cdot I_0/(K'_{app} + I_0) \quad (1)$$

where k_{obs}, k_{inact} and K'_{app} represents the apparent inactivation rate constant of the enzyme at the initial concentration of erythromycin (I_0), the maximum inactivation rate constant and the apparent dissociation constant between the enzyme and erythromycin, respectively.

Quantitative Prediction of the Triazolam/Erythromycin Interaction

The differential equations for active and inactive CYP3A4 for triazolam metabolism in the liver (E_{act} and E_{inact}, respectively) can be described as follows:

$$dE_{act}/dt = -(k_{inact} \cdot E_{act} \cdot f_b \cdot I_{liver}/Kp)/(K'_{app} + f_b \cdot I_{liver}/Kp) + k_{deg}(E_0 - E_{act}) \quad (2)$$

$$dE_{inact}/dt = (k_{inact} \cdot E_{act} \cdot f_b \cdot I_{liver}/Kp)/(K'_{app} + f_b \cdot I_{liver}/Kp) - k_{deg} \cdot E_{inact} \quad (3)$$

where k_{deg}, Kp, f_b, I_{liver} and E_0 represent the degradation rate constant (turnover rate constant) of CYP3A4, liver-to-blood concentration ratio of erythromycin, unbound fraction of erythromycin in blood, erythromycin concentration in the liver, and total concentration of CYP3A4, respectively. The initial conditions (at $t = 0$) are $E_{act} = E_0$ and $E_{inact} = 0$. In the absence of erythromycin, the CYP3A4 content in the liver is at steady-state and the degradation rate ($k_{deg} \cdot E_0$) is equal to the synthesis rate, which was assumed to be unaffected by erythromycin. It was also assumed in the above equations that k_{deg} for the inactive enzyme is the same as that for the active enzyme.

The differential equations for triazolam (C) and erythromycin (I) can be expressed as follows according to the perfusion model (Fig. 1):

For triazolam:

$$V_{liver} \cdot (dC_{liver}/dt) = Q \cdot C_{pv} - Q \cdot C_{liver}/Kp - f_b \cdot CL_{int} \cdot C_{liver}/Kp \quad (4)$$

$$CL_{int} = V_{max,1}/(K_{m,1} + f_b \cdot C_{liver}/Kp) + V_{max,2}/(K_{m,2} + f_b \cdot C_{liver}/Kp) \quad (5)$$

$$V_{max,1} = V_{max,1}(0) \cdot E_{act,1}/E_0 \quad (6)$$

$$V_{max,2} = V_{max,2}(0) \cdot E_{act,2}/E_0 \quad (7)$$

$$V_{pv} \cdot (dC_{pv}/dt) = Q \cdot C_{sys} + V_{abs} - Q \cdot C_{pv} \quad (8)$$

$$V_{abs} = k_a \cdot Dose \cdot F_a \cdot e^{-ka \cdot t} \quad (9)$$

$$V_{sys} \cdot (dC_{sys}/dt) = Q \cdot C_{liver}/Kp - Q \cdot C_{sys} \quad (10)$$

For erythromycin:

$$V_{liver} \cdot (dI_{liver}/dt) = Q \cdot I_{pv} - Q \cdot I_{liver}/Kp - f_b \cdot CL_{int} \cdot I_{liver}/Kp \quad (11)$$

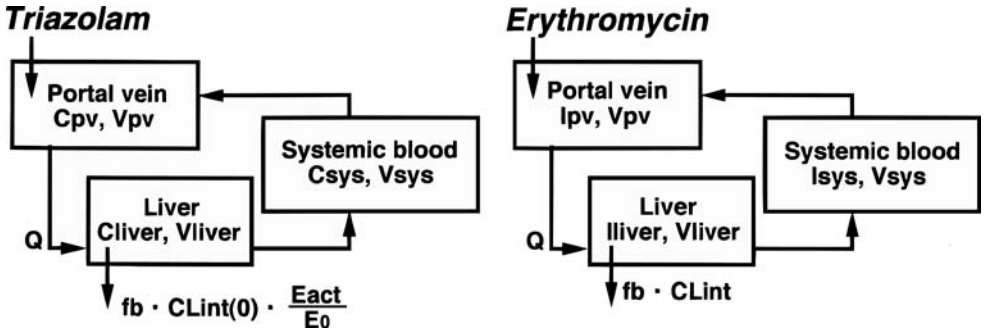

Fig. 1. Physiological model for the description of the time-profiles of triazolam and erythromycin concentrations. See MATERIALS AND METHODS for the abbreviations used.

$$CL_{int} = V_{max}/(K_m + f_b \cdot I_{liver}/Kp) \quad (12)$$

$$V_{pv} \cdot (dI_{pv}/dt) = Q \cdot I_{sys} + V_{abs} - Q \cdot I_{pv} \quad (13)$$

$$V_{abs} = k_a \cdot Dose \cdot F_a \cdot e^{-ka \cdot t} \quad (14)$$

$$V_{sys} \cdot (dI_{sys}/dt) = Q \cdot I_{liver}/Kp - Q \cdot I_{sys} \quad (15)$$

where V_{liver} and V_{pv} represent the volume of liver and portal vein, respectively; V_{sys} represents the volume of distribution in the central compartment; C_{liver} represents the concentration in the liver; C_{pv} and I_{pv} represent the concentration in the portal vein; C_{sys} and I_{sys} represent the concentration in the central compartment; Q represents the blood flow rate; CL_{int} represents the intrinsic metabolic clearance; K_m represents the Michaelis-Menten constant; $K_{m,1}$ and $K_{m,2}$ represent the K_m for α- and 4-hydroxylation of triazolam, respectively; V_{max} represents the maximum rate of metabolism; $V_{max,1}$ and $V_{max,2}$ represent the V_{max} for α- and 4-hydroxylation of triazolam, respectively; $E_{act,1}$ and $E_{act,2}$ represent the E_{act} for α- and 4-hydroxylation of triazolam, respectively; V_{abs} represents the absorption velocity; k_a represents the first-order absorption rate constant; and F_a represents the fraction absorbed from the gastrointestinal tract.

The following assumptions have been made in the above mass-balance equations:

1. Triazolam and erythromycin are administered orally.
2. Triazolam and erythromycin are eliminated only by the liver.
3. The distribution of triazolam and erythromycin in the liver rapidly reaches equilibrium and the unbound concentrations in the hepatic vein are equal to those in the liver at equilibrium (Well-stirred model).
4. Only the unbound molecule in the liver is subject to elimination.
5. The contribution of CYP3A4 to the total elimination of erythromycin in the liver is small (i.e., the elimination of erythromycin itself is not altered by inactivation of CYP3A4).
6. Gastrointestinal absorption can be described by a first-order rate constant.

The pharmacokinetic parameters of triazolam and erythromycin were determined from data in the literature (Table 1). Using the program STELLA II (High Performance Systems, Inc.) and kinetic parameters for CYP3A4 inactivation, the above differential equations were numerically solved to simulate the effects of erythromycin pre-treatment. Erythromycin (333 mg = 454000 nmol, t.i.d.) was assumed to be orally administered for 3 days before oral administration of triazolam (0.5 mg = 1460 nmol) and the time courses of the erythromycin blood concentration, active CYP3A4 content in the liver (E_{act}), and triazolam blood concentration were simulated.

RESULTS

Triazolam Metabolism In Vitro

Triazolam was biotransformed to α- and 4-hydroxy metabolites by human liver microsomes (H-66) in vitro (Fig. 2). Although the K_m value for the 4-hydroxylation was approximately 5-fold greater than for the α-hydroxylation, the V_{max} value for the 4-hydroxylation was also higher than for the α-hydroxylation, and the CL_{int} (V_{max}/K_m ratio) for the α- and 4-hydroxylation was comparable (54.2% and 45.8% of total, respectively). Similar results were also obtained with other human liver microsomes (H-35 and H-36) and recombinant human CYP3A4 (Table 2).

90.6% and 89.7% of the α- and 4-hydroxylation of triazolam were inhibited by 200 μL/0.2 mg of antiserum for human CYP3A4 (Fig. 3).

Inhibition of Triazolam Metabolism In Vitro by Erythromycin

When human liver microsomes were incubated for 40 min in the presence of NADPH-generating system and 120 μM erythromycin, the active CYP3A4 concentration obtained from the CO-binding spectrum of dithionite-reduced microsomes was reduced to about 40% of the control value (data not shown).

Figs. 4 and 5 show the effect of erythromycin concentration and preincubation time on triazolam metabolism by human liver microsomes and recombinant human CYP3A4, respectively. Whichever enzyme was used, triazolam metabolism was not inhibited without preincubation, even if the erythromycin concentration was increased. The degree of inhibition depended on the preincubation time and erythromycin concentration. α- and 4-hydroxylation of triazolam by human liver microsomes were reduced to approximately 33% and 40% of the control value, respectively, by 40 min preincubation in the presence of 100 μM erythromycin. Similarly, the α- and 4-hydroxylation of triazolam by recombinant human CYP3A4 were reduced to approximately 26% and 45% of the control value, respectively, under the same conditions.

Table 1. Pharmacokinetic Parameters Used in the Simulation

			Ref.
Triazolam			
Dose	1460 nmol		
F_a	0.82		10
f_b	0.11		11
Kp	1 (assumed)		
V_{liver}	2800 mL		
V_{pv}	70 mL		
Q	1610 mL/min		
CL_{int}	2090 mL/min	[a]	
$CL_{int,\ 1}$	1130 mL/min	α-hydroxylation, 54.2% of CL_{int}	
$CL_{int,2}$	960 mL/min	4-hydroxylation, 45.8% of CL_{int}	
$K_{m,1}$	200 μM		
$V_{max,\ 1}$	226000 nmol/min	calculated from $K_{m,1} \times CL_{int,1}$	
$K_{m,2}$	962 μM		
$V_{max,2}$	924000 nmol/min	calculated from $K_{m,2} \times CL_{int,2}$	
k_a	$0.017/min^{-1}$	[a]	
V_{sys}	70000 mL	[a]	
Enzyme			
k_{deg}	$0.00033/min^{-1}$	rat P450	12
E_0	5 nmol/g liver	CYP3A4 content in the liver	13
Erythromycin			
Dose	454000 nmol	t.i.d.	
F_a	0.58		Unpublished observation
f_b	0.16		14
K_p	1 (assumed)		
V_{liver}	2800 mL		
V_{pv}	70 mL		
Q	1610 mL/min		
CL_{int}	2940 mL/min	[b]	
K_m	20 μM (assumed)		
V_{max}	58800 nmol/min	calculated from $K_m \times CL_{int}$	
k_a	$0.011/min^{-1}$	[b]	
V_{sys}	18000 mL	[b]	
T_{lag}	96 min		15

[a] Calculated using eq. (4)–(10) to fit the reported blood concentration (6).
[b] Calculated using eq. (11)–(15) to fit the reported blood concentration (15).

Kinetic parameters for CYP3A4 inactivation, calculated from the data showing the initial velocity, are summarized in Table 3. The obtained values of K'_{app} were almost the same for both hydroxylation pathways of triazolam using either human liver microsomes or recombinant human CYP3A4 (16–19 μM). Although the values of k_{inact} obtained using recombinant human CYP3A4 were slightly higher than those obtained using human liver microsomes, the difference was less than 3-fold.

Quantitative Prediction of the Triazolam/ Erythromycin Interaction.

Fig. 6 shows the simulation based on the kinetic parameters for CYP3A4 inactivation obtained using human liver microsomes. It was predicted that active CYP3A4 concerned with α-hydroxylation of triazolam in the liver was gradually reduced following administration of erythromycin and that approximately 50% and 60% of the CYP3A4 was inactivated after 3 days of administration of erythromycin, using two different values of k_{deg}. Almost identical results were obtained in the simulation of the 4-hydroxylation pathway. The blood concentration of triazolam was predicted to increase following administration of erythromycin and the predicted AUC increase compared with control group was 1.6- and 2.0-fold when a k_{deg} value of 0.0005 and 0.00033 min^{-1}, respectively, was used in the simulation.

The simulated effects of the Kp of erythromycin are shown in Fig. 7. Whatever value of Kp was used, it was predicted that the active CYP3A4 concerned with the α-hydroxylation of triazolam in the liver was gradually reduced following administration of erythromycin and that approximately 60 or 80% of the CYP3A4 was inactivated after 3 days administration of erythromycin. Almost identical results were obtained in the simulation of the 4-hydroxylation pathway. The blood concentration of triazolam was predicted to increase following administration of erythromycin and the predicted AUC increase compared with the control group was 2.6-fold (Kp = 0.1) and 2.0-fold (Kp = 1 and 10).

The k_a of erythromycin was also changed from 0.0035 to 0.1 min^{-1} in the simulation study. Whatever value of k_a was used, it was predicted that active CYP3A4 in the liver was gradually reduced following administration of erythromycin and that approximately 60% of the CYP3A4 was inactivated after 3 days of administration of erythromycin (data not shown). The predicted increase in triazolam AUC compared with control

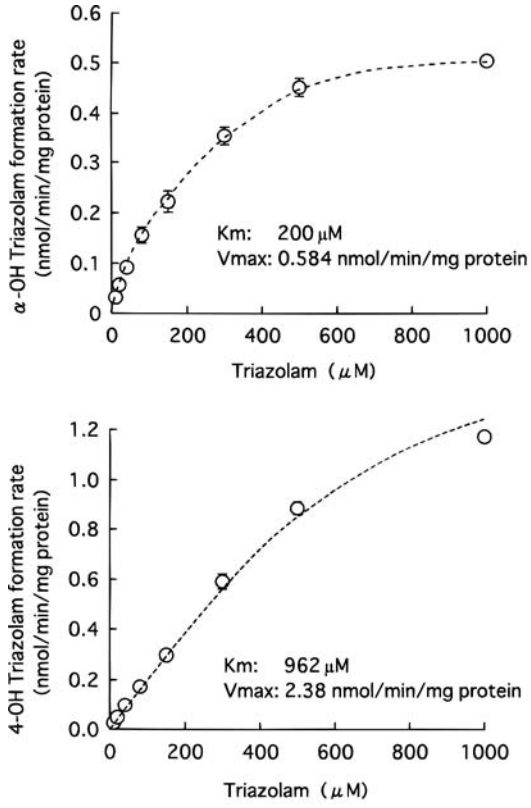

Fig. 2. In vitro metabolism of triazolam by human liver microsomes (H-66).

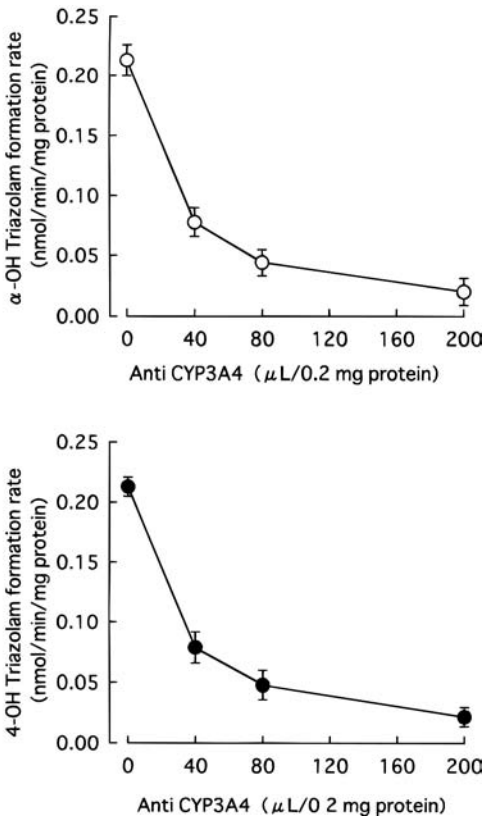

Fig. 3. Effect of anti-CYP3A4 antiserum on triazolam metabolism by human liver microsomes (H-66).

group was 2.0-fold (k_a = 0.0035 and 0.011 min^{-1}) and 1.9-fold (k_a = 0.1 min^{-1}).

Based on the kinetic parameters for CYP3A4 inactivation obtained using recombinant human CYP3A4, it was predicted that active CYP3A4 concerned with the α-hydroxylation of triazolam in the liver was gradually reduced following administration of erythromycin and that approximately 75% and 80% of the CYP3A4 was inactivated after 3 days of administration of erythromycin, using two different values of k_{deg} (data not shown). Under the same conditions, approximately 60% and 70% of the active CYP3A4 concerned with the 4-hydroxylation of triazolam in the liver was predicted to be inactivated. The predicted increase in triazolam AUC compared with the control group was 2.0- and 2.6-fold when a k_{deg} value of 0.0005 and 0.00033 min^{-1}, respectively, was used in the simulation.

DISCUSSION

Antiserum for human CYP3A4 inhibited approximately 90% of triazolam metabolism (Fig. 3) and 10 μM ketoconazole inhibited 70.0% of the α-hydroxylation and 82.1% of the 4-hydroxylation (data not shown) by human liver microsomes. Furthermore, metabolic activity of triazolam was observed by recombinant human CYP3A4 (Table 2) but not by recombinant human CYP1A1, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6-Val, 2D6-Met and 2E1 (data not shown). These results are consistent

Table 2. Kinetic Parameters of Triazolam Metabolism In Vitro by Human Liver Microsomes and Recombinant Human CYP3A4

	α-Hydroxylation				4-Hydroxylation			
Enzyme	K_m μM	V_{max} nmol/min/mg	CL_{int} μL/min/mg	% of CL_{int}	K_m μM	V_{max} nmol/min/mg	CL_{int} μL/min/mg	% of CL_{int}
H-66	200	0.58	2.92	54.2	962	2.38	2.47	45.8
H-35	255	1.67	6.55	46.6	851	6.39	7.51	53.4
H-36	384	0.33	0.85	43.0	1150	1.29	1.12	57.0
Mean	280	0.86	3.44	47.9	988	3.35	3.70	52.1
SD	94	0.71	2.89	5.7	151	2.69	3.37	5.7
recombinant human	μM nmol/min/nmol CYP3A4		μL/min/nmol CYP3A4		μM nmol/min/nmol CYP3A4		μL/min/nmol CYP3A4	
CYP3A4	176	3.84	21.8	41.2	652	20.3	31.1	58.8

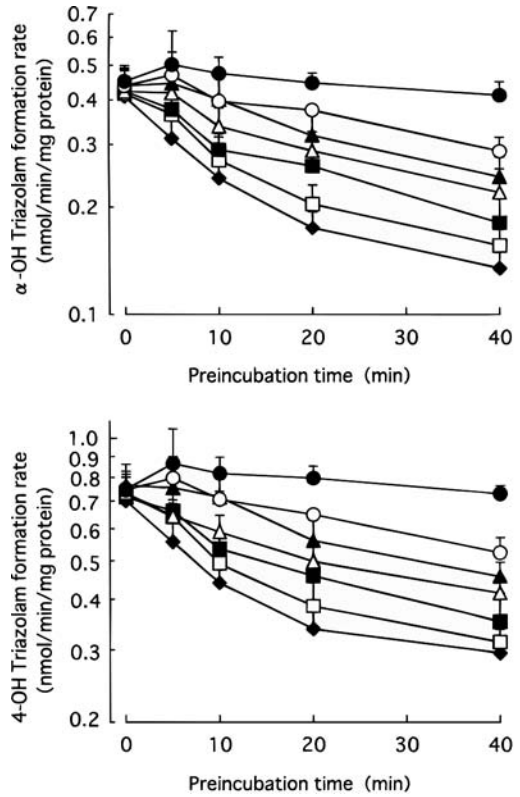

Fig. 4. Effect of erythromycin on triazolam metabolism by human liver microsomes (H-66). Erythromycin concentrations were ● 0, ○ 3, ▲ 6, △ 10, ■ 20, □ 40, and ◆ 100 μM.

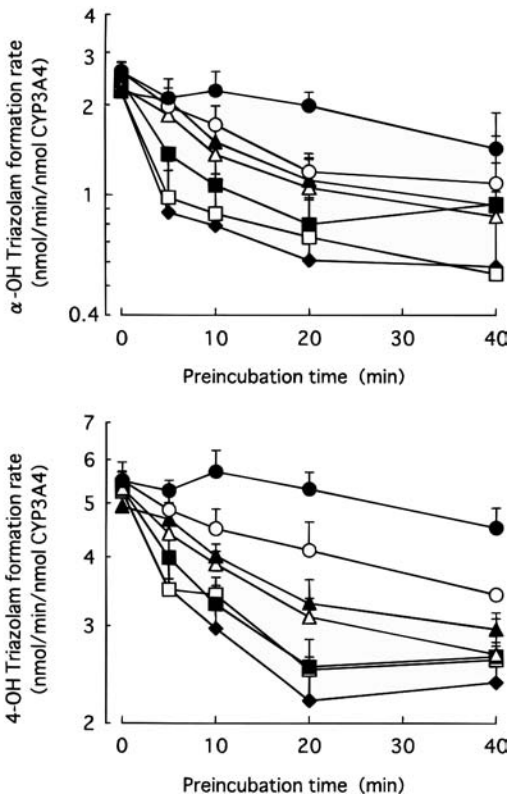

Fig. 5. Effect of erythromycin on triazolam metabolism by recombinant human CYP3A4. Erythromycin concentrations were ● 0, ○ 3, ▲ 6, △ 10, ■ 20, □ 40, and ◆ 100 μM.

with the previous reports that CYP3A4 makes the predominant contribution to triazolam α- and 4-hydroxylation in humans (16,17). The findings that K_m value for the 4-hydroxylation in human liver microsomes was greater than that for the α-hydroxylation and that the CL_{int} for the α- and 4-hydroxylation were similar are also in agreement with the report by von Moltke et al. (17) (Fig. 2, Table 2). Similar results were also obtained for recombinant human CYP3A4 (Table 2).

The inhibitory effect of erythromycin on CYP3A4 has been considered to be based on competitive or noncompetitive inhibition (17–20). On the other hand, complex formation with P450 is also reported to be involved in the inhibition by macrolides (5,21). Assuming a competitive inhibition of triazolam metabolism by erythromycin, the AUC of triazolam was predicted to increase only by 1% based on the ratio of erythromycin concentration and inhibition constant (Ki) (4,22), which could not explain the clinical report (6). In the present study, the active CYP3A4 concentration obtained from the CO-binding spectrum of dithionite-reduced microsomes was reduced when human liver microsomes were incubated in the presence of NADPH-generating system and erythromycin (data not shown). Furthermore, triazolam metabolism was not inhibited without preincubation, even if the erythromycin concentration was increased, and the degree of inhibition depended on the preincubation time and erythromycin concentration (Figs. 4 and 5). These findings indicate that the inhibitory effect of erythromycin on triazolam metabolism was predominantly caused by mechanism-based inhibition of CYP3A4 with little contribution from competitive inhibition. Therefore, as a case of drug-drug interaction involving mechanism-based inhibition, the interaction between triazolam and erythromycin in humans was investigated using our methodology for the quantitative prediction of in vivo interactions from in vitro data.

The simulated increase in triazolam AUC following pretreatment of erythromycin was 2.0-fold (from 61.0 to 119 nM · hr; Fig. 6) and 2.6-fold (from 61.0 to 156 nM · hr) based on the kinetic parameters for CYP3A4 inactivation obtained using human liver microsomes and recombinant human CYP3A4, respectively, and $k_{deg} = 0.00033\ min^{-1}$. These results are comparable with the reported in vivo increase of 2.1-fold (from 58.6 to 121 nM · hr)(6). Using similar methodology, we have already investigated the interaction between 5-fluorouracil and sorivudine and the predicted interaction was also comparable with the reported in vivo observation (23), suggesting the validity of the methodology used in the present study.

Table 3. Summary of Kinetic Parameters for the Inhibition of Triazolam Metabolism by Erythromycin

Enzyme	Pathway	k_{inact} (min^{-1})	K'_{app} (μM)
Human microsomes	α-hydroxylation	0.062 ± 0.009	15.9 ± 2.9
Recombinant human	4-hydroxylation	0.055 ± 0.012	17.4 ± 6.3
CYP3A4	α-hydroxylation	0.173 ± 0.026	19.1 ± 5.7
	4-hydroxylation	0.097 ± 0.029	18.9 ± 7.6

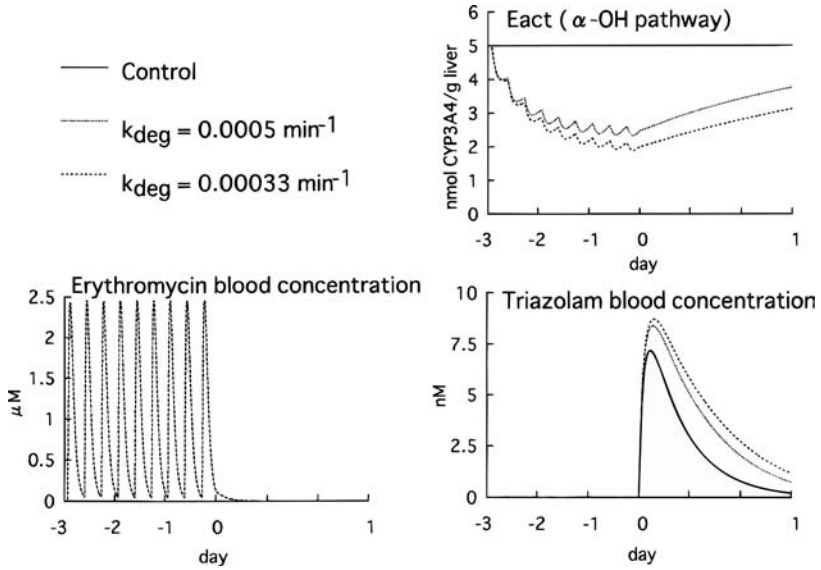

Fig. 6. Simulation of erythromycin effects on the hepatic CYP3A4 content and blood concentration of triazolam based on the parameters obtained using human liver microsomes.

The turnover rate constant (k_{deg}) of rat P450 was used in the present simulation because the corresponding value for human CYP3A4 has not been reported. Since the turnover rate of the enzyme is one of the most important parameters that affect the results of simulation, it is important to alter this parameter to some extent in the simulation in order to predict the range of the interaction.

It is also important in the present prediction to precisely estimate the unbound concentration of inhibitor in the liver, but this cannot be measured, especially in humans. However, some of the pharmacokinetic parameters can be determined to fit the blood concentration profile of the inhibitor, which can be measured in many cases. A change of about 30-fold in the k_a of erythromycin had little effect on the results of the simulation, while a change of 100-fold in the liver-to-blood concentration ratio (Kp) of erythromycin had some effect (Fig. 7). Such parameters which cannot be measured may also have to be altered to some extent in the simulation study to predict the range in E_{act} and delay in substrate elimination.

Furthermore, it is reported that CYP3A4 exists not only in the liver but also in the small intestine, playing an important role in the first-pass metabolism after oral administration of its substrates (24,25). Therefore, the predictability of in vivo interactions may be improved if the inhibitory effect of erythromycin on triazolam metabolism in the small intestine could be incorporated into the model.

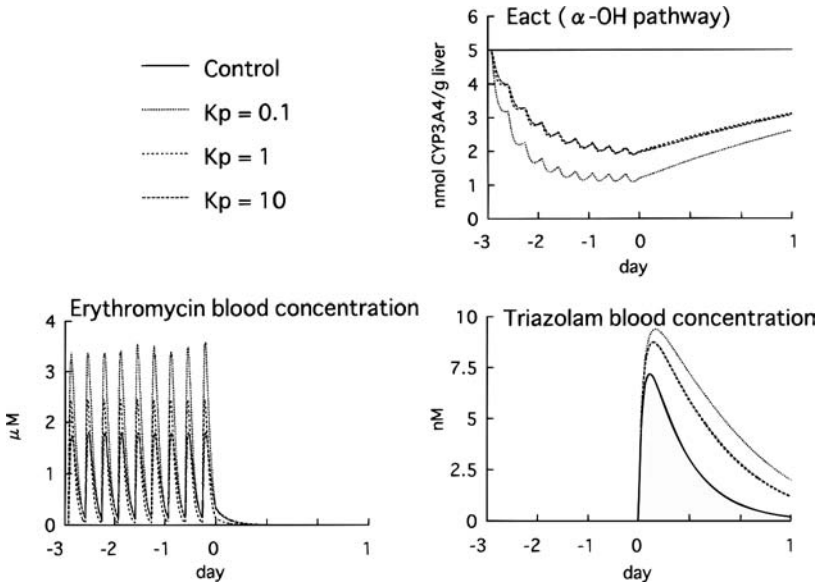

Fig. 7. Effect of Kp of erythromycin on the simulated hepatic CYP3A4 content and blood concentration of triazolam.

Because the inhibitory effect of erythromycin on triazolam metabolism as shown in this study could not be observed in the liver microsomes of male rats (data not shown), it is important to use human liver microsomes for the prediction of drug-drug interactions in humans. However, in future, it is important to confirm the validity of the present prediction methodology for mechanism-based inhibition in animal studies, selecting appropriate cases where the interaction can be clearly observed. In other words, the validity of the present methodology can be confirmed in appropriate animal models based on both in vitro (using e.g. liver microsomes for P450) and in vivo inhibition studies. Since invasive experiments are possible in this case, including measurements of the Kp of the inhibitor in the liver and enzyme activity in the liver, this may allow more accurate predictions to be made.

If a drug-drug interaction involving mechanism-based inhibition is investigated assuming competitive inhibition, the in vivo interaction would be underestimated and administration of the inhibitor could be hazardous. We propose that such in vitro studies considering mechanism of metabolic inhibition should be carried out during the drug developing phase so that in vivo drug-drug interactions can be quantitatively predicted.

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