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Drug–drug interactions of Z-338, a novel gastroprokinetic agent, with terfenadine, comparison with cisapride, and involvement of UGT1A9 and 1A8 in the human metabolism of Z-338

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Abstract

In the present study, the inhibitory properties of *N*-[2-(diisopropylamino)ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxamide monohydrochloride trihydrate (Z-338), a novel gastroprokinetic agent, were investigated and compared with those of cisapride to establish its potential for drug–drug interactions. There was no notable inhibition of terfenadine metabolism or of any of the isoforms of cytochrome P450 (CYP1A1/2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4) by Z-338 in in vitro studies using human liver microsomes. Z-338 was mainly metabolized to its glucuronide by UGT1A9 (UDP glucoronosyltransferase 1 family, polypeptide A9) and UGT1A8, and did not show marked inhibition of P-glycoprotein activity. On the other hand, cisapride strongly inhibited CYP3A4 and markedly inhibited CYP2C9.

Furthermore, we used the whole-cell patch-clamp technique to investigate the effects of Z-338 and cisapride on potassium currents in human embryonic kidney (HEK) 293 cells transfected with the human ether-a-go-go-related gene (hERG). Z-338 had no significant effect on hERG-related current at the relatively high concentration of 10 μM. In contrast, the inhibition by Z-338 was very small compared with that of cisapride at 10 nM, which was a thousand-fold lower concentration.

In the prediction method for the drug interaction between terfenadine and cisapride based on the K_i and PK parameters, we suggest the possibility that terfenadine mainly affect the QT interval, since its plasma concentration would be markedly increased, but cisapride may not be changed. Thus, in contrast with cisapride, Z-338 did not inhibit CYP and the hERG channel, and is predominantly metabolized by glucuronide conjugation, Z-338 is considered unlikely to cause significant drug–drug interactions when coadministered with CYP substrates at clinically effective doses.

Keywords: Z-338; Terfenadine; Cisapride; hERG; CYP inhibition

1. Introduction

Serious adverse effects caused by drug–drug interactions are a great cause for concern, and much significant information can be obtained from in vitro studies (Ito et al., 1998; Furuta et al., 2001a, 2002). Z-338, *N*-[2-(diisopropylamino)ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxamide monohydrochloride trihydrate, is a novel gastroprokinetic agent synthesized by ZERIA Pharmaceutical (Nakajima et al., 2000; Ogishima et al., 2000; Furuta et al., 2001b). Z-338 is under development for the treatment of functional dyspepsia, and is now in clinical trials (Oliver et al., 2000). Recently, cisapride, another gastrointestinal proki-

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netic agent, has been associated with drug–drug interactions during concomitant therapy with antifungal agents, macrolides or antidepressants (Bedford and Rowbotham, 1996; Michalets and Williams, 2000; Simard et al., 2001). In particular, fatal cardiovascular toxicity, with arrhythmia and torsade de pointes is very serious regarding coadministration of cisapride and cytochrome P450 (CYP) 3A4 inhibitors, e.g. the nonsedating antihistamine terfenadine (Brown, 1997; Pelov et al., 1999; Dresser et al., 2000; Paakkari, 2002). Both cisapride and terfenadine can inhibit the human ether-a-go-go-related gene (hERG) K^+ channel (Mitcheson et al., 2000) and both drugs have now been withdrawn from the market. Furthermore, since CYP3A4 is involved in the metabolism of both cisapride and terfenadine, competitive inhibition is expected to cause elevation of their plasma concentrations (Dresser et al., 2000). However, investigations concerning the mechanism of interaction of cisapride and terfenadine have not been performed, so that it has not been possible to predict the plasma concentrations of both drugs. In this paper, we compared the in vitro inhibitory effects of cisapride and Z-338 on CYP inhibition using human liver microsomes and on K^+ currents in hERG-transfected (HEK) 293 cells in order to predict drug interactions in the clinical setting. Furthermore, the principal UDP glycosyltransferase (UGT) isoforms involved in the metabolism of Z-338 and the effect of Z-338 on P-glycoprotein, an efflux transporter, were investigated to assess its potential for drug–drug interactions.

2. Materials and methods

2.1. Chemicals

Z-338 and internal standard (*N*-[2-(diisopropylamino)ethyl]-2-[(2,4,5-trimethoxybenzoyl)amino]-1,3-thiazole-4-carboxamide maleate) were synthesized at ZERIA Pharmaceutical. Cisapride was extracted and purified from Risamol granules and its chemical purity was more than 99% by HPLC method (Yoshitomi Pharmaceutical, Osaka, Japan). Terfenadine alcohol form, terfenadine carboxyl form, resorufin, 7-ethoxyresorufin, furafylline, sodium diethyldithiocarbamate trihydrate, sulfaphenazole, 6β-hydroxytestosterone and ketoconazole were purchased from ULTRAFINE Chemicals (Manchester, UK). Terfenadine, clomipramine, dicumarol, 8-methoxypsoralen, 7-benzyloxyresorufin, orphenadrine hydrochloride, tolbutamide, quinidine hydrochloride monohydrate, chlorzoxazone and tranylcypromine were purchased from Sigma (St. Louis, MO, USA). α-Naphthoflavone and chlorpropamide were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Coumarin, 7-hydroxycoumarin, 5-hydroxy-1-tetralone, 2-benzoxazolinone and aniline were purchased from Wako (Osaka, Japan). Omeprazole was purchased from Union Quimico Farmaceutica (Barcelona, Spain). Hydroxytolbutamide, (*S*)-mephenytoin, (±)-4′-hydroxymephenytoin, bufuralol, 1′-hydroxybufuralol and 6-hydroxychlorzoxazone were purchased from Sumika Chemical Analysis Service (Osaka, Japan). All other reagents used were of at least analytical or high-performance liquid chromatography (HPLC) grade.

2.2. Inhibition by Z-338, cisapride and terfenadine of drug-metabolizing activity in human liver microsomes

Pooled microsomes from 10 male human livers were obtained from the International Institute for the Advancement of Medicine (Exton, PA, USA) with the approval of the local ethics committee and the coroner.

We conducted inhibition studies in vitro using human liver microsomes with Z-338, cisapride and terfenadine. The reaction mixture (total volume 0.5 ml) consisted of 100 mM Na, K-phosphate buffer, 0.1 mM EDTA, NADPH-generation system (2.5 mM $NADP^+$, 25 mM D-glucose-6-phosphate, 2 unit glucose-6-phosphate dehydrogenase, 10 mM $MgCl_2$), water, the substrate and the inhibitor. The mixture was incubated at 37 °C. The reaction was terminated by adding acetonitrile. The production of terfenadine alcohol metabolite and the disappearance of cisapride and terfenadine were determined by HPLC (Shimadzu, Kyoto, Japan). Terfenadine and its metabolites were separated by cyanopropyl-column (Develosil CN-UG-5, 4.6×250 mm, Nomura Chemical, Aichi, Japan) and monitored by fluorescence detector (Excitation 230 nm, Emission; 280 nm). Cisapride is monitored at 307 nm of UV detector connected to a ODS column (Capcell Pak C18, 4.6×250 mm, Shiseido, Tokyo, Japan).

The following marker substrate activities were used to evaluate the potential inhibitory effects of Z-338 and cisapride on CYP isoforms in human liver microsomes: 7-ethoxyresorufin deethylation activity (CYP1A1/2; Burke et al., 1985, 1994), coumarin hydroxylation activity (CYP2A6; Chang et al., 1994), 7-benzyloxyresorufin debenzylation activity (CYP2B6; Burke et al., 1985, 1994), tolbutamide hydroxylation activity (CYP2C9; Knodell et al., 1987, Miners and Birkett, 1996), (*S*)-mephenytoin hydroxylation activity (CYP2C19; Wrighton et al., 1993), bufuralol hydroxylation activity (CYP2D6; Yamazaki et al., 1994), chlorzoxazone hydroxylation activity (CYP2E1; Peter et al., 1990), testosterone 6β-hydroxylation activity (CYP3A4; Imaoka et al., 1989), terfenadine hydroxylation activity (CYP3A4; Yun et al., 1993).

Inhibition by the selective chemical inhibitors α-naphthoflavone (CYP1A1/2), furafylline (CYP1A1/2), 8-methoxypsoralen (CYP2A6), orphenadrine (CYP2B6), sulfaphenazole (CYP2C9), omeprazole (CYP2C19), tranylcypromine (CYP2C19), quinidine (CYP2D6), anilline (CYP2E1), diethyldithiocarbamate (CYP2E1) and ketoconazole (CYP3A4) was also investigated. The K_i values were calculated from Dixon plots based on a competitive inhibition model (Dixon, 1953).

2.3. Incubation with human UGT isoforms in a cDNA-expression system

For the metabolic study using UGT isoforms (SUPERSOMES, Gentest, MA, USA), the reaction mixture (total volume 0.25 ml) consisted of 125 mM Tris–HCl buffer (pH 7.4) containing 0.004% Triton X, 4 mM $MgCl_2$, 0.8 mM UDP-glucuronic acid (UDPGA) and 1 mg/ml microsomal protein from the enzyme cDNA-expression system. The mixtures were activated by preincubation at 37 °C for 20 min without Z-338. The reaction was then initiated by adding Z-338 and incubation was continued for 2 h. The reaction was terminated by adding methanol, followed by addition of internal standard. The mixture was centrifuged at 9000×*g* for 10 min. The supernatant was injected onto an HPLC column and the disappearance of Z-338 was determined according to previous method (Furuta et al., 2001b).

The maximum metabolic rate (V_{max}) and Michaelis constant (K_m) values were determined from a non-linear least-squares regression program (MULTI; Yamaoka et al., 1981) according to Eq. (1):

$$v = V_{max} \times C/(K_m + C) \quad (1)$$

where v is metabolic rate and C is Z-338 concentration.

To determine the kinetic parameters, incubation time employed were 1 h for UGT1A8 and 5 min for UGT1A9, respectively.

2.4. Inhibition assay of P-glycoprotein

Inhibition assays were conducted according to Hunter et al. (1993). TC7 cells were maintained in high glucose Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 1 mM non-essential amino acids and penicillin–streptomycin–fungizone at 100 U/ml, 100 μg/ml and 0.25 μg/ml, respectively. For the P-glycoprotein inhibition assay, the cells were seeded at 1×10^5 cells/cm^2 on porous polycarbonate membrane inserts in 24-well Transwell™ (Costar, Corning, NY, USA) plates and used at days 13–25 post-seeding.

The basolateral-to-apical permeability of ^{3}H-digoxin at 1 μM was determined in the absence and presence of Z-338 at 0.5, 5 and 50 μM, respectively, in 5 mM HBSS-HEPES (pH 7.4). Cyclosporin A (10 μM) and verapamil (50 μM) were used as reference inhibitors. Inhibitors, when present, were added to both the apical and the basolateral sides.

2.5. Effect of Z-338 and cisapride on potassium currents in hERG-transfected HEK293 cell

The hERG-transfected HEK293 cells were obtained from Wisconsin Alumni Research Foundation (WI, USA). The cells were thawed and subcultured for at least two generations before use in the study. Culture medium was prepared by adding 10% fetal bovine serum (GIBCO, CA, USA) and ×1 penicillin–streptomycin (Sigma) to Dulbecco's modified Eagle's medium (GIBCO). As a selective medium, geneticin (GIBCO) was added to the above medium to a final concentration of 400 μg/ml. The experiments were carried out using the whole-cell patch-clamp technique. Cells were cultured for 24–72 h and disseminated on collagen-coated cover glasses (collagen-coated type I; Iwaki Glass, Funabashi, Japan). The test preparations were applied after the current amplitude had been stabilized. The temperature of the extracellular solution in the chamber was maintained at 37 °C during the measurement. The chamber solution contained the following: NaCl (137 mM), KCl (4 mM), $CaCl_2$ (1.8 mM), $MgCl_2$ (1 mM), glucose (10 mM) and HEPES (10 mM, pH 7.4 with NaOH). Recordings began at 10 min after application of the test substance, at which time activating currents were evoked by a 0.75-s step of depolarizing potential of 0 mV following a holding potential of −70 mV, and tail currents were evoked by a 0.75-s step of −50 mV following the activating step. Membrane currents were recorded by use of an Axopatch 200B patch-clamp amplifier (Axon Instruments, CA, USA). Application concentrations were 1 and 10 μM for Z-338 in water, and 10 and 100 nM for cisapride in dimethyl sulfoxide (DMSO), respectively. Maximum concentration of DMSO is 0.1% in incubation mixture.

2.6. Prediction of drug–drug interactions with terfenadine in the clinical setting

Based on the K_i values obtained from in vitro inhibition studies with human liver microsomes, the plasma concentrations of cisapride and terfenadine were predicted by method by Ito et al. (1998) as follows.

The pharmacokinetic data used were obtained from the following sources. C_{max} was 118.8 ng/ml for cisapride (Shiina and Miwa, 1985) and 1.544 ng/ml for terfenadine (Garteiz et al., 1982) after clinical dosing of 40 mg of cisapride and 60 mg of terfenadine, respectively. The ratio of the concentration in the blood to that in the plasma (R_{BP}=1.0) and the ratio of the drug concentration in the liver to that in the plasma (R_{LP}=34.6) for cisapride were from the data in rats, therefore the ratio of the drug concentration in the liver to that in the blood (R_{LB}) was same as R_{LP} (Michiels et al., 1987). The unbound fraction in the plasma (f_{plasma}=0.12) for cisapride, and f_{plasma} (=0.03) and R_{LB} (=5.2) for terfenadine, were from the literature (Miyake, 2002; McTavish et al., 1990; Leeson et al., 1982). We used R_{BP} of 1 for terfenadine, and the hepatic blood flow (Q_h=1610 ml/min), the first-order rate constant for gastrointestinal absorption (K_a=0.1 min^{-1}) and the fraction absorbed from the gastrointestinal tract into the portal vein (F_a=1) were used (Ito et al., 2002).

The inflow concentration (I_{in}) of the inhibitor into the liver after oral administration can be predicted, the maximum concentration of the inhibitor in the blood (I_{max}),

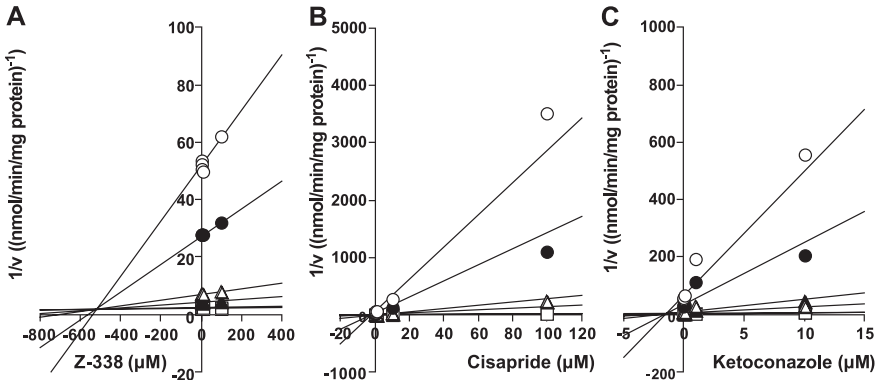

Fig. 1. Dixon plots of inhibition by Z-338 (A), cisapride (B) and ketoconazole (C) on terfenadine metabolic activity in human liver microsomes (○) 0.5 μM, (●) 1 μM, (△) 5 μM, (▲) 10 μM, (□) 50 μM, (■) 100 μM.

and the free concentration of drug in the liver ($I_{u,liver}$) and as follows:

$$I_{in} = I_{max} + (k_a \times dose \times F_a)/Q_h \quad (2)$$

$$I_{max} = C_{max} \times R_{BP} \quad (3)$$

$$I_{u,liver} = I_{in} \times f_{plasma} \times R_{LB}/R_{BP} \quad (4)$$

The degree of changed in plasma concentration caused by the drug–drug interaction is determined as $1+I_{u,liver}/K_i$.

3. Results

3.1. Inhibition by Z-338, cisapride and terfenadine of drug-metabolizing activity in human liver

We first investigated the effects of reaction time on terfenadine elimination rate and alcohol metabolite production rate. Both rates were individually maintained with reaction time up to 15 min. The alcohol metabolite production rate accounted for approximately one-third of terfenadine elimination rate. In the following experiments, a reaction time of 5 min was employed, for which the initial rate was maintained almost unchanged.

Eadie-Hofstee plots for elimination of terfenadine and terfenadine hydroxylation activity were constructed to examine if the inhibitions were competitive or not, and these plots showed that both Z-338 and cisapride competitively inhibited enzyme activity in this study (data not shown). The K_i values of Z-338 for elimination of terfenadine and for terfenadine hydroxylation activity were estimated to be 504.7 and 362.5 μM, respectively. Cisapride inhibited elimination of terfenadine and terfenadine hydroxylation activity with K_i values of 1.5 and 2.3 μM, respectively. Figs. 1 and 2 show Dixon plots for the effect of Z-338 or cisapride on elimination of terfenadine and terfenadine hydroxylation activity. The K_i values of ketoconazole, as positive control, for elimination of terfenadine and for terfenadine hydroxylation activity were estimated to be 1.5 and 0.5 μM, respectively. Cisapride inhibited elimination of terfenadine and terfenadine hydroxylation activity with K_i values of 1.7 and 2.3 μM, respectively. By contrast, Z-338 had no appreciable inhibitory effect on elimination of terfenadine (K_i=520.0 μM) and terfenadine hydroxylation activity (K_i=347.8 μM), with K_i values higher than the maximum concentration of Z-338 added (100 μM). These K_i values were similar to those

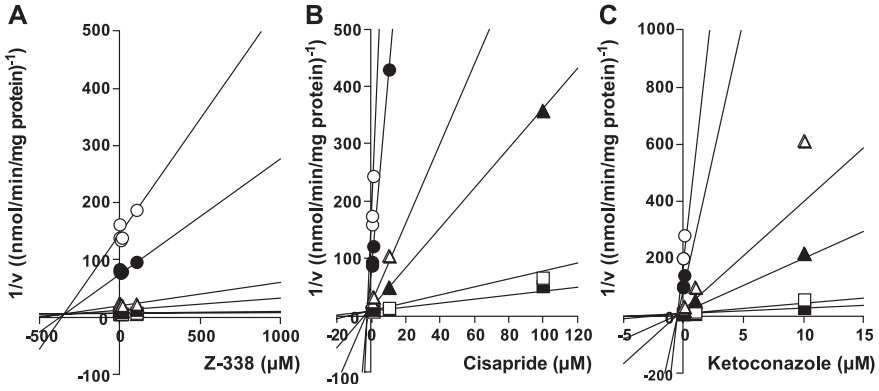

Fig. 2. Dixon plots of inhibition by Z-338 (A), cisapride (B) and ketoconazole (C) on terfenadine hydroxylation activity in human liver microsomes (○) 0.5 μM, (●) 1 μM, (△) 5 μM, (▲) 10 μM, (□) 50 μM, (■) 100 μM.

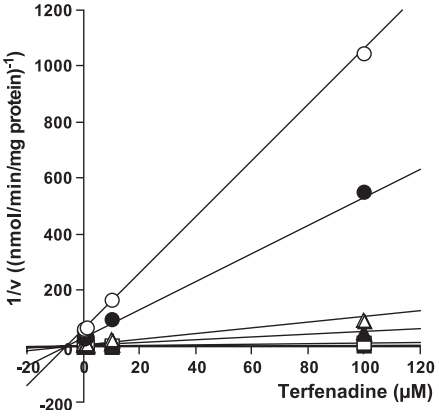

Fig. 3. Dixon plots of inhibition by terfenadine on cisapride metabolic activity in human liver microsomes (○) 0.5 μM, (●) 1 μM, (△) 5 μM, (▲) 10 μM, (□) 50 μM, (■) 100 μM.

obtained from Eadie-Hofstee plots. The K_i value of terfenadine for elimination of cisapride was estimated to be 6.2 μM (Fig. 3). Table 1 shows K_i values of Z-338 and cisapride for the CYP isoforms in human liver microsomes. Z-338 had no inhibitory effects on CYP isoforms, and K_i values of Z-338 for these CYP isoforms were higher than those of cisapride added.

Table 1
Inhibition constants (K_i values, μM) on CYP isoforms of human liver microsomes by Z-338, cisapride and specific CYP inhibitors on several enzyme metabolic activities

	Z-338	Cisapride	Inhibitor
CYP1A1 and 1A2			
7-ethoxyresorufin deethylation activity	225.5	148.1	0.021 (α-naphthoflavone) 3.14 (furafylline)
CYP2A6			
Coumarin hydroxylation activity	140.5	76.3	1.31 (8-methoxypsoralen)
CYP2B6			
7-benzyloxyresorufin debenzylation activity	Not inhibited	Not inhibited	2315.8 (orphenadrine)
CYP2C9			
Tolbutamide hydroxylation activity	1030.8	15.7	1.16 (sulfaphenazole)
CYP2C19			
S-mephenytoin hydroxylation activity	$\geqslant 10^4$	164.0	8.72 (omeprazole) 53.1 (tranylcypromine)
CYP2D6			
Bufuralol hydroxylation activity	135.1	101.9	0.042 (quinidine)
CYP2E1			
Chlorzoxazone hydroxylation activity	2624.7	2254.3	33.8 (aniline) 9.33 (diethyldithiocarbamate)
CYP3A4			
Testosterone 6β-hydroxylation activity	$\geqslant 10^4$	0.95	0.30 (ketoconazole)
Terfenadine hydroxylation activity	347.8	2.3	0.5 (ketoconazole)

The K_i values were calculated from Dixon plots based on a competitive inhibition model.

3.2. Incubation with human UGT isoforms in a cDNA-expression system

The results of in vitro studies on glucuronide conjugation are shown in Fig. 4. The metabolic rate of Z-338 with UGT control microsomes was 3.21% of the parent drug added at the start of the incubation during 2 h of incubation time. When Z-338 was incubated with UGT1A9, 100% of the added Z-338 was metabolized during 30 min of incubation time, and 64.80% of the added Z-338 was metabolized with UGT1A8 during 2 h of incubation time. The metabolic rates of Z-338 with UGT1A1, 1A3, 1A4, 1A6, 1A10, 2B7 and 2B15 were below 10% of the parent drug added at the start of the incubation. The kinetic parameters of V_{max} and K_m for UGT1A8 were 11.11 nmol/min/mg protein of expressed microsomes and 1.43 mM, respectively, and those for UGT1A9 were 15.24 nmol/min/mg protein of expressed microsomes and 43.13 μM, respectively (Fig. 5). The values of V_{max}/K_m for UGT1A8 and 1A9 were 0.008 and 0.353 ml/min/mg protein of expressed microsomes, respectively.

3.3. Inhibition assay of P-glycoprotein

The aim of this study was to assess the P-glycoprotein inhibition potential of Z-338. We measured inhibition of the basolateral-to-apical permeability of ^{3}H-digoxin in the Caco-2 TC7 cell model (Table 2). Cyclosporin A (10 μM) and verapamil (50 μM) showed 66.0% and 64.4%, respectively, inhibition of the basolateral-to-apical permeability of ^{3}H-digoxin. In contrast, Z-338 at 0.5, 5 and 50 μM showed 20.1%, 11.1% and 21.1% inhibition, respectively. These values were 25% or less and it appeared that the weak inhibition was not concentration-dependent.

3.4. Effect of Z-338 and cisapride on potassium currents in hERG-transfected HEK293 cell

Compared with the peak of the preapplication tail current, which was assigned a value of 1, the relative tail

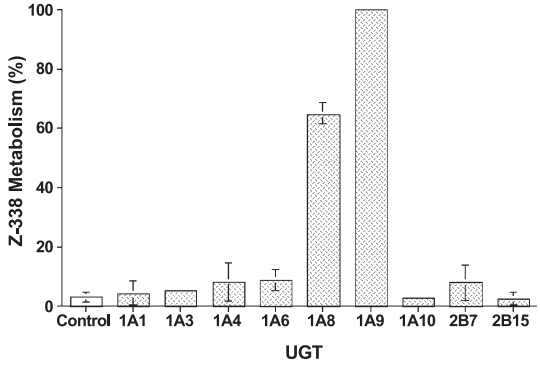

Fig. 4. Metabolism of Z-338 by human UGT isoforms in a cDNA-expression system. The values show the percent reduction to initial values (mean±SD, n=3). Incubation time employed was 2 hours.

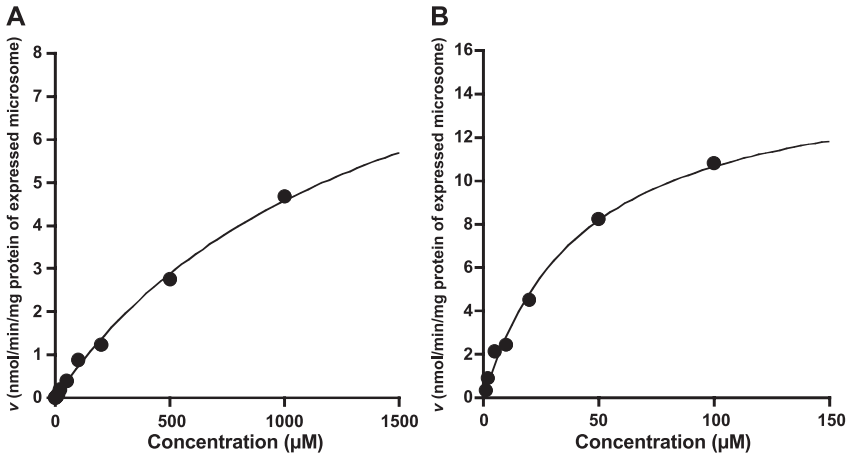

Fig. 5. Simulation curve for metabolic rate of Z-338 by human UGT 1A8 (A) and 1A9 (B) in a cDNA-expression system. Incubation time employed were 1 hour for UGT1A8 and 5 min for UGT1A9, respectively. (A); Vmax=11.11 nmol/min/mg protein of expressed microsome, Km=1426.51 μM, Vmax/Km=0.008 mL/min/mg protein of expressed microsome. (B); Vmax=15.24 nmol/min/mg protein of expressed microsome, Km=43.13 μM, Vmax/Km=0.353 mL/min/mg protein of expressed microsome.

currents at 10 min with water and DMSO, the vehicle control, were 95±2% and 94±3%, respectively. With 100 nM of cisapride, however, the relative current was 22%, indicating a strongly decreased potassium current. In contrast, with 10 μM of Z-338, the relative current was 83±5%, which was not statistically significantly different from the vehicle control value (Fig. 6).

3.5. Prediction of drug–drug interactions between cisapride and terfenadine in the clinical setting

Based on the K_i values obtained from in vitro inhibition studies with human liver microsomes, the plasma concentrations of cisapride and terfenadine were calculated. The maximum inflow concentration into liver (I_{in}) were calculated to be 2603.3 ng/ml (5.6 μM) of cisapride and 3728.3 ng/ml (2.6 μM) of terfenadine, respectively (Eq. (2)), when 40 mg of cisapride and 60 mg of terfenadine would be coadministered. The unbound intracellular concentration in the liver ($I_{u,liver}$) was calculated to be 10.8 μg/ml (23 μM) for cisapride and 582 ng/ml (1.2 μM) for terfenadine, respectively. The values of $1+I_{u,liver}/K_i$ for cisapride and terfenadine were 15.6 and 1.2, respectively.

Table 2
Inhibition of Z-338 on ^{3}H-digoxin permeation in the Caco-2 TC7 cell model

	Concentration (μM)	% inhibition			
		1st	2nd	3rd	Mean
Control		−8.5	5.6	2.9	0.0
Z-338	0.5	4.4	35.6	20.3	20.1
Z-338	5	24.8	15.9	−7.4	11.1
Z-338	50	20.8	25.3	17.2	21.1
Cyclosporin A	10	52.6	73.4	72.0	66.0
Verapamil	50	74.0	60.2	59.1	64.4

4. Discussion

There have been many reports on CYP isoforms in human liver microsomes, and specific substrates for these isoforms have been identified (Burke et al., 1985, 1994; Chang et al., 1994; Knodell et al., 1987; Wrighton et al., 1993; Yamazaki et al., 1994; Peter et al., 1990; Imaoka et al., 1989; Yun et al., 1993; Furuta et al., 2001b, 2002). Notably, the prediction of potential drug–drug interactions by using in vitro inhibition constants provides significant information to prevent such clinical incidents. It has been shown that the gastrointestinal motility-improving agent cisapride strongly inhibits CYP3A4, the main metabolic enzyme in the human liver. Cisapride caused serious drug–drug interactions with the antihistamine terfenadine, azole antifungal drugs, macrolides and antidepressants (Bedford and Rowbotham, 1996; Brown, 1997; Michalets and Williams, 2000; Simard et al., 2001). Therefore, in this study, we examined the inhibition potential of Z-338 on several CYP isoforms. In addition, we compared the inhibitory effect of Z-338 with those of cisapride on the CYP isoforms.

Terfenadine, a pro-drug, is metabolized to its active metabolite, terfenadine carboxyl form, via an alcohol form (Garteiz et al., 1982; Yun et al., 1993). However, the formation of terfenadine carboxyl form is negligible in in vitro studies using human liver microsomes. Because terfenadine alcohol form, also referred to hydroxyl terfenadine, is considered to be instructive for evaluation of terfenadine metabolism, both the elimination rate of terfenadine and the production rate of hydroxy terfenadine were monitored in this study. Other reports have shown the production of a piperidine carbinol derivative in the dealkylation process of terfenadine (Jurima-Romet et al., 1994), but we could not obtain a standard for determination of this metabolite. Both the elimination rate of terfenadine and the production rate of hydroxy terfenadine were linear during

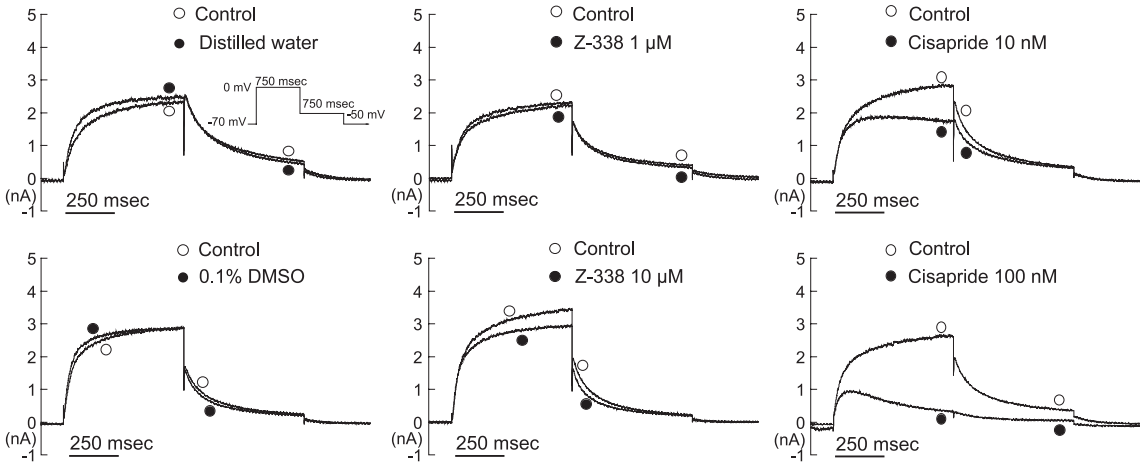

Fig. 6. Typical hERG current traces in the absence and presence of Z-338 or cisapride. The panels show activating and tail currents in a hERG-transfected HEK293 cell under control conditions and after 10 min treatment with drugs. Currents were elicited by a depolarizing pulse to 0 mV (750 ms) and tail currents were recorded during a step to −50 mV (750 ms).

the first 15 min of incubation. One-third of the total elimination of terfenadine was due to production of hydroxy terfenadine, implying that the remaining two-thirds of total metabolism was likely due to N-dealkylation.

Cisapride strongly inhibited the elimination of terfenadine and the formation of hydroxy terfenadine with a potency slightly less than that of ketoconazole, a well-established CYP3A4 inhibitor. The K_i values were the same for the elimination of terfenadine and the production of hydroxy terfenadine, suggesting that CYP3A4 is the main enzyme involved in both processes. The K_i value of terfenadine for cisapride elimination was 6.2 μM. In contrast, Z-338 had negligible inhibitory activity on terfenadine metabolism, with K_i values of over 100 μM. The inhibition of various CYP isoforms by cisapride and Z-338 is shown in Table 1. Z-338 did not significantly inhibit any of the CYP isoforms tested, with all calculated K_i values being greater than those of cisapride.

Since Z-338 is metabolized mainly by glucuronide conjugation rather than by oxidation, we investigated the involvement of UGT isoforms using cDNA expression systems. In this in vitro metabolic study, the main metabolite of Z-338, Z-338 glucuronide, was produced by UGT1A8 and UGT1A9, with negligible involvement of other isoforms. The metabolic parameter K_m for UGT1A9 was much smaller than that for UGT1A8, and the intrinsic clearance by UGT1A9 was 45 times that by UGT1A8; therefore, the principal isoform involved in the formation of Z-338 glucuronide was UGT1A9. UGT1A9 is expressed in human intestine and transfers glucuronide to the hydroxyl groups of acetaminophen or quercetin; UGT1A8 is expressed in human liver and metabolizes scopoletin or propafol (Radominska-Pandya et al., 1999).

Recently, drug–drug interactions mediated by the multidrug resistance efflux transporter P-glycoprotein have attracted much attention (Hunter et al., 1993). In particular, there have been reports of interactions with digoxin or cyclosporin A, known substrates of P-glycoprotein. Therefore, the P-glycoprotein inhibition potential of Z-338 was assessed by measuring ^{3}H-digoxin permeation in the Caco-2 TC7 cell model. The inhibition of P-glycoprotein activity was less than 25% at 0.5–50 μM of Z-338 and was not concentration-dependent; however, both cyclosporin A and verapamil inhibited P-glycoprotein by more than 60% in this system. These findings suggest that Z-338 is unlikely to be a P-glycoprotein inhibitor.

The fatal drug–drug interaction between terfenadine and cisapride is believed to be due to their inhibitory effects on the K^+ channel. The effects of Z-338 and cisapride on potassium currents were investigated by the whole-cell patch-clamp technique in HEK293 cells transfected with hERG. Cisapride drastically inhibited the potassium current at 100 nM, but Z-338 at concentrations up to 10 μM had no significant effect on the relative tail current compared with the solvent control.

Prediction of the maximum plasma concentration of Z-338 from the therapeutic dose suggests concentrations of 35.2 ng/ml (78 nM) at 100 mg and 139 ng/ml (309 nM) at 300 mg; therefore, the plasma concentration would not achieve levels that would prolong the QT interval. On the other hand, the IC_{50} of cisapride on the hERG channel and the plasma concentration of cisapride at clinically used doses were reported to be 44.5 nM (Rampe et al., 1997) and 118.8 ng/ml (300 nM, Shiina and Miwa, 1985), respectively, which have the potential to cause cardiovascular events even if cisapride is administered alone. In vitro assay method using hERG-transfected cells is convenient and sensitive for screening the potential of induction of cardiac arrhythmia. Recently, co-expression system of hERG channel assembled MinK-related peptide 1 (MiRP1), a small integral membrane subunit, has been developed (Anantharam et al., 2003). Since the reliability of the co-expression system to evaluate effects of drugs on the hERG current has not yet been elucidated well, we have conducted the hERG assay without

MiRP1 expression in this study. We intended to clarify the actual inhibitory potential of Z-338 by further investigations including the action potential duration in isolated guinea pig papillary muscle and in in vivo anesthetized dogs.

The occurrence of fatal drug–drug interactions between terfenadine and cisapride can be seen as a significant incident that has prompted reconsideration of the selection criteria for concomitant medications. Rampe reported that IC_{50} value on hERG channel currents was 56.0 nM for terfenadine, it causes QT interval prolongation as well as cisapride (Rampe et al., 1997). However, the relationship between elevation of plasma concentration and K^+ channel inhibition after coadministration of terfenadine and cisapride was not sufficiently clear. In the our prediction, the maximum unbound intracellular concentrations in the liver after simultaneous administration of 40 mg of cisapride and 60 mg of terfenadine were calculated to be 10.8 μg/ml (23 μM) of cisapride and 582 ng/ml (1.2 μM) of terfenadine. Therefore, $1+I_{u,liver}/K_i$ was calculated to be 15.6 for terfenadine and 1.2 for cisapride. The plasma concentration of terfenadine is extremely low after administration without inhibitors; however, our results predict that after simultaneous administration with cisapride the plasma concentration of terfenadine may increase markedly to be 16-fold higher than the plasma concentration without cisapride. Furthermore, the absorption rate of terfenadine might be increased since cisapride promote the gastrointestinal motility. These observations have some assumptions but suggest that the potentially lethal drug–drug interaction between cisapride and terfenadine, mediated by prolongation of the QT interval, could be caused by the pronounced increase in the plasma concentration of terfenadine after coadministration of cisapride, rather than caused by increment of cisapride concentration by terfenadine.

On the other hand, Z-338 does not appear to inhibit CYP3A4, therefore, significant drug–drug interactions with substrates for CYP3A4, e.g. terfenadine, are unlikely to occur in vivo after coadministration with Z-338.

In conclusion, in contrast to cisapride, Z-338 does not inhibit CYP or the hERG channel and is predominantly metabolized by UGT1A9 and 1A8. Therefore, Z-338 is considered unlikely to cause significant drug–drug interactions when coadministered with CYP substrates at clinically effective doses.

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