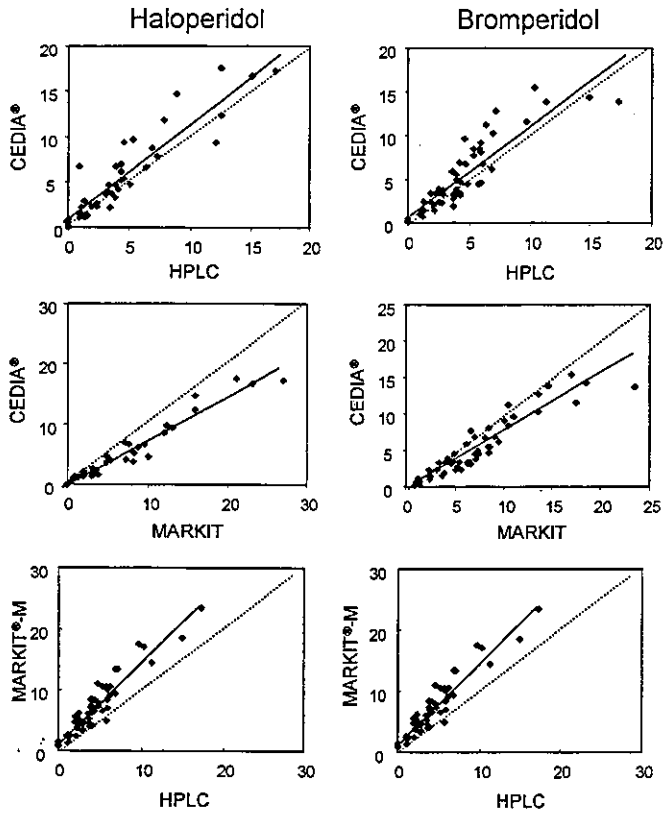

FIGURE 2. Correlations of plasma drug concentrations (ng/mL) among CEDIA®, MARKIT®-M, and HPLC methods in patients receiving haloperidol or bromperidol. The solid line was derived by linear regression of individuals. Dotted line indicates ideal linear regression (y = x).

has none of the several drawbacks associated with EIA (MARKIT®-M) assays.

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Different inhibitory effect of fluvoxamine on omeprazole metabolism between CYP2C19 genotypes

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Aims
Omeprazole is mainly metabolized by the polymorphic cytochrome P450 (CYP) 2C19. The inhibitory effect of fluvoxamine, an inhibitor of CYP2C19 as well as CYP1A2, on the metabolism of omeprazole was compared between different genotypes for CYP2C19.

Methods
Eighteen volunteers, of whom six were homozygous extensive metabolizers (EMs), six were heterozygous EMs and six were poor metabolizers (PMs) for CYP2C19, participated in the study. A randomized double-blind, placebo-controlled crossover study was performed. All subjects received two six-day courses of either daily 50 mg fluvoxamine or placebo in a randomized fashion with a single oral 40 mg dose of omeprazole on day six in both cases. Plasma concentrations of omeprazole and its metabolites, 5-hydroxyomeprazole, omeprazole sulphone, and fluvoxamine were monitored up to 8 h after the dosing.

Results
During placebo administration, geometric means of peak concentration (C_{max}), under the plasma concentration-time curve from 0 to 8 h (AUC(0,8 h)) and elimination half-life ($t_{1/2}$) of omeprazole were 900 ng ml^{-1}, 1481 ng ml^{-1} h, and 0.6 h in homozygous EMs, 1648 ng ml^{-1}, 4225 ng ml^{-1} h, and 1.1 h in heterozygous EMs, and 2991 ng ml^{-1}, 11537 ng ml^{-1} h, and 2.8 h in PMs, respectively. Fluvoxamine treatment increased C_{max} of omeprazole by 3.7-fold (95%CI, 2.4, 5.0-fold, $P < 0.01$) and 2.0-fold (1.4, 2.6-fold, $P < 0.01$), AUC(0,8 h) by 6.0-fold (3.3, 8.7-fold, $P < 0.001$) and 2.4-fold (1.7, 3.2-fold, $P < 0.01$), AUC(0,∞) by 6.2-fold (3.0, 9.3-fold, $P < 0.01$) and 2.5-fold (1.6, 3.4-fold, $P < 0.001$) and prolonged $t^{1}_{/2}$ by 2.6-fold (1.9, 3.4-fold, $P < 0.001$) and 1.4-fold (1.02, 1.7-fold, $P < 0.05$), respectively. However, no pharmacokinetic parameters were changed in PMs. The AUC(0,8 h) ratios of 5-hydroxyomeprazole to omeprazole were decreased with fluvoxamine in homozygous EMs ($P < 0.05$) and heterozygous EMs ($P < 0.01$).

Conclusions
Even a low dose of fluvoxamine increased omeprazole exposure in EMs, but did not increase omeprazole exposure in PMs after a single oral dose of omeprazole. These findings confirm a potent inhibitory effect of fluvoxamine on CYP2C19 activity. The bioavailability of omeprazole might, to some extent, be increased through inhibition of P-glycoprotein during fluvoxamine treatment.

Introduction

Omeprazole is one of the most widely used proton pump inhibitors for the treatment of gastric acid-related disorders [1]. Omeprazole is completely metabolized, mainly by hydroxylation catalyzed by CYP2C19 [2], which shows genetically determined polymorphism, yielding extensive metabolizers (EMs) and poor metabolizers (PMs) [3]. Since the rate of omeprazole hydroxylation correlates with the hydroxylation of S-mephenytoin, the metabolic ratio of 5-hydroxyomeprazole to omeprazole has been used to assess the activity of CYP2C19 [4]. Omeprazole is also metabolized by CYP3A4 to omeprazole sulphone. In PMs, this is the predominant metabolic pathway [5].

Fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), is metabolized in the liver by CYP2D6 and CYP1A2 [6]. Fluvoxamine is regarded as a potent CYP1A2 inhibitor based on many drug interactions of fluvoxamine with caffeine [7], clozapine [8, 9], olanzapine [10], imipramine [11, 12], amitriptyline [13], clomipramine [13, 14] and theophylline [15], each of which is a substrate for CYP1A2. In addition, it has been suggested that fluvoxamine inhibits the metabolism of CYP2C19 substrates such as citalopram [16], omeprazole [17, 18] and chloroguanide hydrochloride (INN, proguanil) [19]. Thus, fluvoxamine has been regarded as a potent inhibitor of not only CYP1A2 but also CYP2C19. Also, fluvoxamine increased plasma concentrations of alprazolam [20], a substrate of CYP3A4 [21], suggesting that fluvoxamine has an inhibitory effect on CYP3A4 to some degree.

Our previous study has shown that low doses of fluvoxamine in EMs for CYP2C19 decreased the area under the plasma concentration-time curve from time 0 to 8 h [AUC(0,8 h)] ratio of 5-hydroxyomeprazole : omeprazole by 3.4-fold [18], suggesting that fluvoxamine has a potent inhibitory effect on CYP2C19 activity. We presumed that no changes in omeprazole concentrations would be found in PMs if this interaction was only due to CYP2C19 inhibition by fluvoxamine. However, there is no published information about the difference in this interaction between EMs and PMs of CYP2C19. Therefore, in the present study, the inhibitory effects of fluvoxamine on the metabolism of omeprazole were compared between three different CYP2C19 genotypes.

Methods

Study design

Eighteen Japanese healthy volunteers (14 males and four females; age range 22–44 years; weight range 40–90 kg) participated in the study after written informed consent was obtained. The mutated alleles for CYP2C19, *CYP2C19*3(*3)* and *CYP2C19*2(*2)* had been identified using the PCR-RFLP methods of de Morais *et al.* [22], prior to this study. The CYP2C19 genotype analyses revealed five different patterns as follows: *1/*1 in 6, *1/*2 in 3, *1/*3 in 3, *2/*2 in 4 and *2/*3 in 2. These were divided into three groups, homozygous EMs (*1/*1, $n = 6$), heterozygous EMs (*1/*2 and *1/*3, $n = 6$) and PMs (*2/*2 and *2/*3, $n = 6$). The protocol was approved by the Ethics Committee of Hirosaki University School of Medicine.

A randomized double-blind placebo-controlled crossover study design in two phases was conducted at intervals of 2 weeks. Fluvoxamine (25 mg) as the capsule formulation containing a tablet formulation (Luvox®, Fujisawa Pharmaceutical Co., Ltd, Osaka, Japan) or matched placebo (as the capsule formulation with the same appearance and size of that of fluvoxamine) was given orally twice a day (09.00 h, 21.00 h) for 6 days. Nine volunteers each as a group were allocated to either of the different drug sequences: placebo-fluvoxamine or fluvoxamine-placebo. On day 6, they took a single oral 40 mg dose of omeprazole (Omepral®, AstraZeneca Co., Ltd, Osaka, Japan) and 25 mg dose of fluvoxamine or placebo after overnight fasting (09.00 h) with 240 ml of tap water. Compliance of test drugs was confirmed by pill-count. No other medications were taken during the study periods. No meal was allowed until 4 h after the dosing (13.00 h). The use of alcohol, tea, coffee and cola was forbidden during the test days.

Blood sampling

Blood samples (10 ml each) for determination of omeprazole and its metabolites, 5-hydroxyomeprazole and omeprazole sulphone, and fluvoxamine, were taken into heparinized tubes just before and 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after the administration of omeprazole. Plasma was separated immediately and kept at −30°C until analysis.

Assay

Plasma concentrations of omeprazole and its metabolites, 5-hydroxyomeprazole and omeprazole sulphone were determined by HPLC methods described by Kobayashi *et al.* [23] with minor modification. The method was validated for the concentration range 10–10000 ng ml^{-1}. Intra- and inter-day relative standard deviations were less than 8.9% at the concentration 10 ng ml^{-1}. The limit of quantification was 10 ng ml^{-1} for each compound.

Plasma concentrations of fluvoxamine were determined by HPLC methods developed in our laboratory.

In brief, all solvents used were of HPLC grade (Wako Pure Chemical Industries, Kyoto, Japan). All reagents were purchased from Wako Pure Chemical Industries (Kyoto, Japan). After sample alkalization with 0.5 ml of NaOH (2.5 M), the test compound and internal standard, alprazolam, were extracted from 1000 μl of plasma using 4000 μl of chloroform-n-heptane (30 : 70, v/v). The organic phase was evaporated to dryness and the residue was dissolved with 800 μl of mobile phase. An aliquot (500 μl) of the solution was injected into a C_{18} STR ODS-II analytical column (5 μl, 150 x 4.6 mm I.D.). The mobile phase consisted of phosphate buffer (0.02 M, pH 4.6), perchloric acid (6 M) and acetonitrile (58.93 : 0.07 : 41, v/v) for fluvoxamine and was delivered at a flow rate of 0.6 ml min^{-1}. The peak was detected using a UV detector set at 254 nm for fluvoxamine. The method was validated for the concentration range 1–100 ng ml^{-1}, and good linearity ($r > 0.999$) was confirmed. Intra- and inter-day coefficient variations were less than 7.6% at the concentration 0.8 ng ml^{-1} for the test compound. Relative errors ranged from −5–10% and mean recoveries were 87–95%. The limit of quantification was 0.8 ng ml^{-1} for fluvoxamine.

Data analyses of pharmacokinetics

The peak concentration (C_{max}) and concentration peak time (t_{max}) were obtained directly from the original data. The area under the plasma concentration-time curve (AUC(0,8 h)) was calculated with use of the trapezoidal rule. The terminal rate constant (k_e) used for the extrapolation was determined by regression analysis of the log-linear part of the concentration-time curve for each subject. The elimination half-life was determined by $0.693/k_e$. AUC from zero to infinity (0,∞) was calculated by AUC (0,last) + C_{last}/k_e, where C_{last} is last detectable plasma drug concentration.

Statistical analyses

The paired *t*-test for the comparison of placebo *vs* fluvoxamine treatment was conducted on pharmacokinetic parameters, while Wilcoxon signed-rank test was performed on the parameter t_{max}. Percentages of placebo in pharmacokinetic parameters between the three genotype groups were compared using one-way ANOVA followed by Scheffe test, whereas percentages of placebo in parameter t_{max} were compared using the Kruskal–Wallis test. The comparison between the AUC of omeprazole during fluvoxamine coadministration in homozygous and heterozygous EMs groups, and during placebo administration in PMs was performed with the use of one-way ANOVA followed by Scheffe test. Correlations between the percentage of placebo AUC of omeprazole during fluvoxamine and AUC ratio of 5-hydroxyomeprazole : omeprazole were tested using Spearman rank test. A *P* value of 0.05 or less was regarded as significant. SPSS 8.0.1 for Windows (SPSS Japan Inc., Tokyo) was used for these statistical analyses.

Results

Although none of the subjects needed to be withdrawn from this study, mild to moderate side-effects were observed during fluvoxamine administration: mild to moderate nausea in six subjects, mild appetite loss in three subjects, mild drowsiness in five subjects, dry mouth in two subjects. These side-effects continued until day 6 and ameliorated the day after discontinuation of fluvoxamine. No adverse events were reported during placebo administration or after omeprazole plus placebo administration.

No differences between the CYP2C19 genotypes, homozygous EMs, heterozygous EMs and PMs were found in subject profiles, including age (mean ± SD, 25 ± 3, 26 ± 4 and 30 ± 6 years, $P = 0.135$), body weight (66 ± 14, 61 ± 15 and 62 ± 12 kg, $P = 0.807$) and genders (M/F; 5/1, 5/1and 4/2). Geometric mean (95% confidence interval) of trough plasma concentrations of fluvoxamine on day 6 were 19.8 (4.9, 44.9) in homozygous EMs, 21.4 (11.8, 41.3) in heterozygous EMs, and 19.2 (14.4, 39.2) ng ml^{-1} in PMs, respectively, which did not differ between CYP2C19 genotypes ($P = 0.902$).

Plasma concentration-time curves of omeprazole during both phases in each genotype group for CYP2C19 are shown in Figure 1. Compared with control, fluvoxamine treatment increased C_{max} of omeprazole by 3.7-fold (95%CI, 2.4, 5.0-fold, $P < 0.01$) and 2.0-fold (1.4, 2.6-fold, $P < 0.01$), AUC(0,8 h) by 6.0-fold (3.3, 8.7-fold, $P < 0.001$) and 2.4-fold (1.7, 3.2-fold, $P < 0.01$), AUC(0,∞) by 6.2-fold (3.0, 9.3-fold, $P < 0.01$) and 2.5-fold (1.6, 3.4-fold, $P < 0.001$) and prolonged $t_{1/2}$ by 2.6-fold (1.9, 3.4-fold, $P < 0.001$) and 1.4-fold (1.02, 1.7-fold, $P < 0.05$), respectively. However, no pharmacokinetic parameters were changed in PMs (Table 1). There were no differences in t_{max} of omeprazole between the control and fluvoxamine phases in any genotype patterns (Table 1).

There was a significant difference between the control AUC(0,8 h) of omeprazole in PMs during placebo administration and the AUC(0,8 h) of omeprazole after fluvoxamine in homozygous EMs and heterozygous EMs (ANOVA; $P = 0.024$). *Post hoc* analyses revealed significant difference between the AUC(0,8 h) during

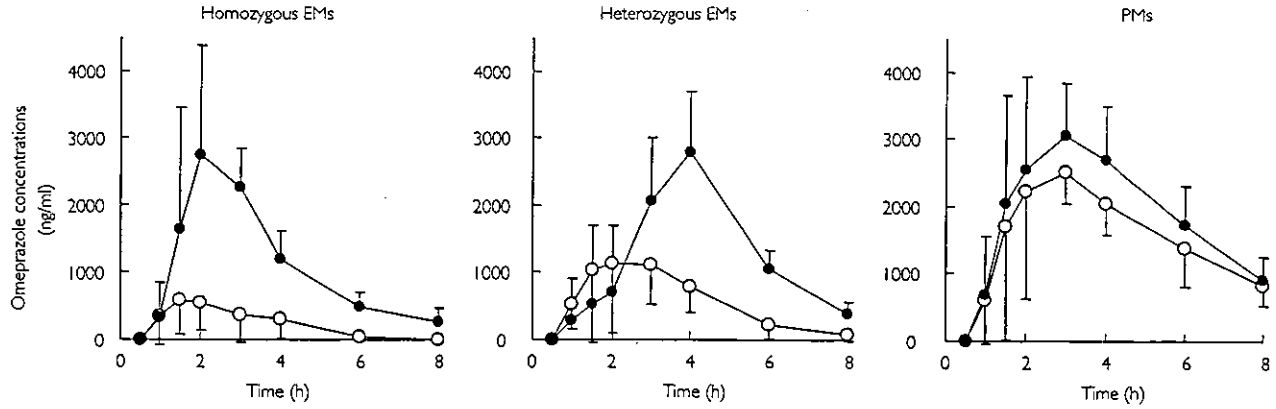

Figure 1

Mean plasma concentration-time curves of omeprazole during placebo and fluvoxamine treatment in homozygous extensive metabolizers (EMs) ($n = 6$), heterozygous EMs ($n = 6$) and poor metabolizers (PMs) ($n = 6$) for CYP2C19. Data are shown as mean and bars are SD. Data during control (○); data during fluvoxamine treatment (●)

Table 1

Pharmacokinetic parameters of omeprazole during placebo or fluvoxamine treatment in homozygous EMs, heterozygous EMs and PMs for CYP2C19

		Homozygous EMs ($n = 6$)	*P* value	Heterozygous EMs ($n = 6$)	*P* value	PMs ($n = 6$)	*P* value
C_{max} (ng ml^{-1})	With placebo	900 (617, 1287)	0.007	1648 (949, 2566)	0.009	2991 (2319, 3845)	0.101
	With fluvoxamine	3131 (1968, 4914)		3145 (2612, 3769)		3352 (2607, 4311)	
*t_{max} (h)	With placebo	1.75 (1.5, 4.0)	0.414	2.5 (1.0, 4.0)	0.059	2.0 (1.5, 4.0)	0.786
	With fluvoxamine	2.0 (1.5, 3.0)		4.0 (1.5, 4.0)		2.0 (1.5, 3.0)	
AUC(0,8 h) (ng ml^{-1} h)	With placebo	1481 (667, 2843)	0.001	4225 (2322, 6577)	0.002	11537 (8413, 15549)	0.112
	With fluvoxamine	7911 (5329, 11730)		9567 (8039, 11363)		13940 (10417, 18664)	
AUC(0,8) (ng ml^{-1} h)	With placebo	1483 (664, 2858)	0.002	4411 (2511, 6766)	0.001	15044 (10474, 21149)	0.209
	With fluvoxamine	8340 (5533, 12557)		10507 (9082, 12129)		17348 (12171, 24688)	
Elimination half-life (h)	With placebo	0.55 (0.40, 0.76)	<0.0001	1.08 (0.62, 1.67)	0.049	2.83 (2.12, 3.71)	0.277
	With fluvoxamine	1.39 (1.21, 1.60)		1.43 (1.04, 1.95)		2.59 (1.81, 3.64)	

*Data are shown as geometric mean (95% confidence interval); *t_{max} is given as median (range); P values were shown when compared with fluvoxamine.*

the placebo phase in PMs and the increased AUC(0,8 h) in homozygous EMs (Scheffe; $P = 0.032$), but not between AUC(0,8 h) during the placebo phase in PMs and increased AUC(0,8 h) in heterozygous EMs (Scheffe; $P = 0.108$).

Although there were almost no changes in pharmacokinetic parameters of the metabolites, 5-hydroxyomeprazole (Table 2) or omeprazole sulphone (Table 3) between control and fluvoxamine phases, the AUC(0,8 h) ratios of 5-hydroxyomeprazole to omeprazole were significantly decreased during fluvoxamine treatment to $17 \pm 5\%$ ($P < 0.05$) in homozygous EMs and to $49 \pm 15\%$ ($P < 0.01$) in heterozygous EMs (Table 2). In PMs, no pharmacokinetic parameters were changed.

Figure 2 shows the effect of CYP2C19 genotype on the mean fluvoxamine-mediated percent increase in pharmacokinetic parameters such as peak concentration (C_{max}), AUC(0,8 h), and elimination half-life. The fluvoxamine-mediated percent increase in C_{max} (ANOVA, $P = 0.0005$), AUC(0,8 h) ($P = 0.002$) and elimination half-life ($P < 0.0001$), but not t_{max} ($P = 0.300$) significantly differed between the three CYP2C19 genotypes. Figure 3 shows the effect of CYP2C19 genotype on the

Table 2
Pharmacokinetic parameters of 5-hydroxyomeprazole during placebo or fluvoxamine treatment in homozygous EMs, heterozygous EMs and PMs for CYP2C19

		Homozygous EMs ($n = 6$)	*P* value	Heterozygous EMs ($n = 6$)	*P* value	PMs ($n = 6$)	*P* value
C_{max} (ng ml^{-1})	With placebo	277 (165, 474)	0.011	340 (269, 486)	0.856	54 (33, 84)	0.098
	With fluvoxamine	154 (68, 325)		358 (292, 437)		74 (31, 158)	
*t_{max} (h)	With placebo	1.75 (1.0, 4.0)	0.854	2.5 (1.5, 6.0)	0.917	3.5 (2.0, 6.0)	0.129
	With fluvoxamine	2.0 (1.5, 3.0)		4.0 (1.5, 4.0)		3.5 (2.0, 4.0)	
AUC(0,8 h) (ng ml^{-1} h)	With placebo	586 (362, 978)	0.972	1083 (844, 1286)	0.560	262 (152, 422)	0.145
	With fluvoxamine	540 (262, 1072)		1201 (1025, 1405)		304 (139, 602)	
AUC ratio to omeprazole	With placebo	0.39 (0.20, 0.85)	0.019	0.25 (0.19, 0.32)	0.006	0.017 (0.009, 0.030)	0.736
	With fluvoxamine	0.07 (0.03, 0.13)		0.11 (0.10, 0.12)		0.018 (0.011, 0.030)	

*Data are shown as geometric mean (95% confidence interval); *t_{max} is given as median (range); P values were shown when compared with fluvoxamine.*

Table 3
Pharmacokinetic parameters of omeprazole sulphone during placebo or fluvoxamine treatment in homozygous EMs, heterozygous EMs and PMs for CYP2C19

		Homozygous EMs ($n = 6$)	*P* value	Heterozygous EMs ($n = 6$)	*P* value	PMs ($n = 6$)	*P* value
C_{max} (ng ml^{-1})	With placebo	82 (42, 149)	0.061	160 (89, 201)	0.025	274 (215, 347)	0.156
	With fluvoxamine	133 (80, 213)		316 (203, 483)		316 (198, 493)	
*t_{max} (h)	With placebo	2.5 (1.5, 4.0)	0.257	3.5 (3.0, 8.0)	0.083	7.0 (4.0, 8.0)	0.783
	With fluvoxamine	3.0 (3.0, 4.0)		6.0 (4.0, 8.0)		6.0 (4.0, 8.0)	
AUC(0,8 h) (ng ml^{-1} h)	With placebo	245 (107, 528)	0.012	758 (326, 970)	0.009	1442 (991, 2047)	0.062
	With fluvoxamine	717 (439, 1154)		1527 (962, 2376)		1710 (1114, 2590)	
AUC ratio to omeprazole	With placebo	0.16 (0.10, 0.26)	0.015	0.17 (0.12, 0.26)	0.340	0.10 (0.06, 0.15)	0.805
	With fluvoxamine	0.09 (0.07, 0.11)		0.15 (0.11, 0.20)		0.10 (0.06, 0.15)	

*Data are shown as geometric mean (95% confidence interval). *t_{max} is given as median (range). P values were shown when compred with fluvoxamine.*

mean fluvoxamine-mediated percent decrease in the AUC(0,8 h) ratio of 5-hydroxyomeprazole to omeprazole and the AUC(0,8 h) ratio of omeprazole sulphone to omeprazole. There was also a significant difference in the percent decrease in the AUC ratio of 5-hydroxyomeprazole to omeprazole between CYP2C19 genotypes ($P < 0.0001$), but not in the percent decrease in AUC(0,8 h) ratio of omeprazole sulphone to omeprazole ($P = 0.079$). The results of *post hoc* (Scheffe test) analyses are shown in Figures 2 and 3.

There was a significant correlation between the fluvoxamine-mediated percent increase in AUC(0,8 h) of omeprazole and the AUC ratio of 5-hydroxyomeprazole to omeprazole ($r_s = 0.886$, $P < 0.001$).

Discussion

Fluvoxamine has been regarded as a potent inhibitor of CYP1A2 [7–15], based on several drug–drug interaction studies. However, it is unlikely that the inhibitory effect of fluvoxamine on CYP1A2 had a significant effect upon the interaction in this study because the major enzyme catalyzing omeprazole metabolism is not CYP1A2, but CYP2C19 and CYP3A4.

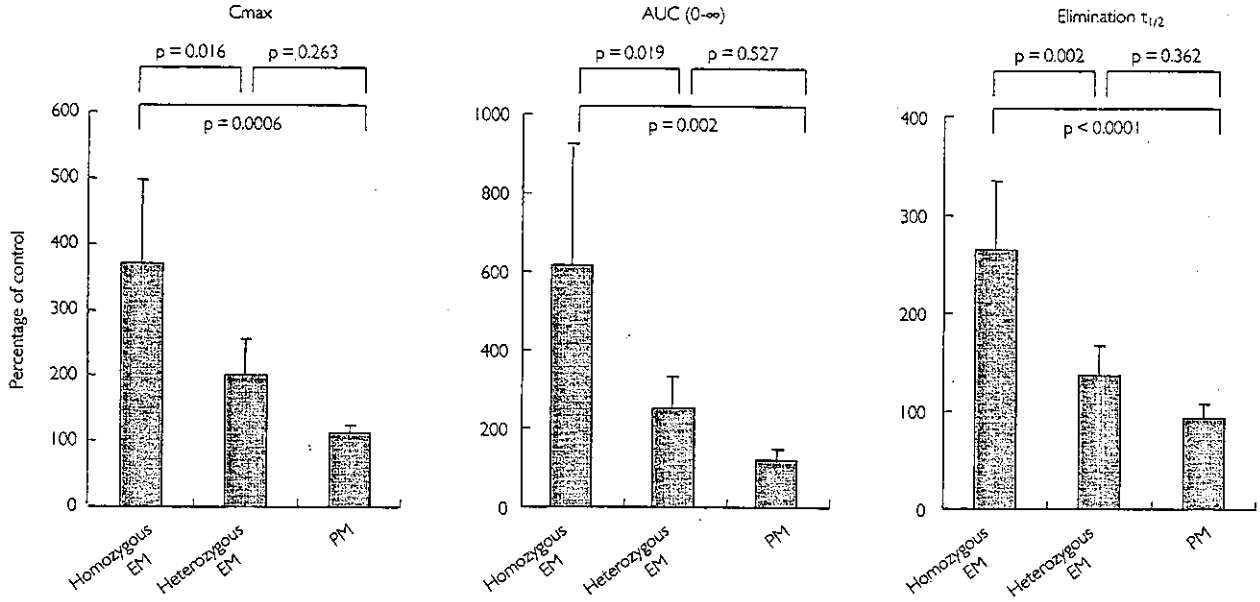

Figure 2
Effect of CYP2C19 genotype on the mean fluvoxamine-mediated percent increase in pharmacokinetic parameters such as peak concentration (C_{max}), area under concentration-time curve (AUC) and elimination half-life. Error bars indicate SD

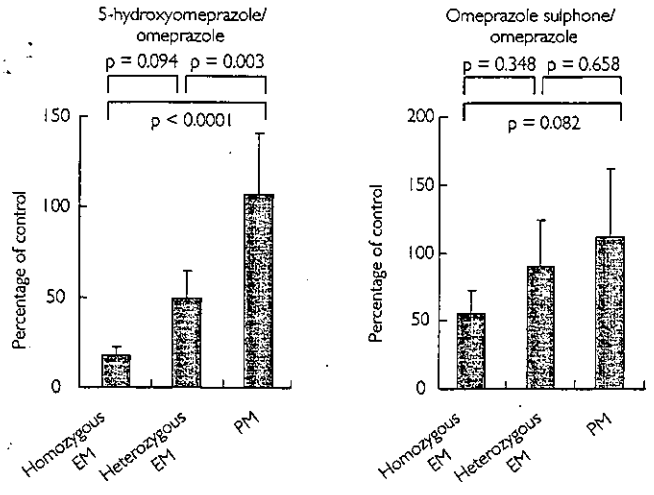

Figure 3
Effect of CYP2C19 genotype on the mean fluvoxamine-mediated percent decrease in the AUC ratio of 5-hydroxyomeprazole to omeprazole and the AUC ratio of omeprazole sulphone to omeprazole. Error bars indicate SD

The present study showed that a low dose of fluvoxamine (50 mg daily) significantly increased plasma omeprazole concentrations in both homozygous EMs and heterozygous EMs of CYP2C19, which is in line with our previous report [18]. Moreover, a pronounced reduction of the AUC ratio of 5-hydroxyomeprazole to omeprazole, which is regarded as an index of CYP2C19 activity, was found in homozygous EMs and heterozygous EMs. Therefore, this study confirms that fluvoxamine is a potent inhibitor of CYP2C19 in EMs of CYP2C19. On the other hand, in PMs, no difference in plasma concentration of omeprazole or the AUC ratio of 5-hydroxyomeprazole to omeprazole was found between control and fluvoxamine. This is a reasonable finding because CYP2C19, which fluvoxamine is expected to inhibit, has no activity in PMs. Based on these results in EMs and PMs therefore we concluded that the inhibitory effect of fluvoxamine on omeprazole metabolism was different between EMs and PMs. This phenomenon is in accordance with previous reports [24, 25].

There was a significant difference between the AUC(0,8 h) during placebo in PMs and the increased AUC(0,8 h) in homozygous EMs, but not between AUC(0,8 h) during placebo in PMs and the increased AUC(0,8 h) in heterozygous EMs. These findings were contrary to our expectations. Since the inhibitory effect of fluvoxamine occurs in a dose-dependent manner [18], pretreatment with 50 mg fluvoxamine daily for 5 days might not fully inhibit CYP2C19 activity in homozygous EMs, but it completely inhibited the CYP2C19 activity in heterozygous EMs, which is lower than that in in homozygous EMs.

The fluvoxamine-mediated percent increase in pharmacokinetic parameters of omeprazole except t_{max} and

the percent decrease in the AUC ratio of 5-hydroxyomeprazole to omeprazole significantly differed between the three CYP2C19 genotypes (Figure 2). Furthermore, a significant correlation between the percentage of control in AUC of omeprazole and the AUC ratio of 5-hydroxyomeprazole to omeprazole was found. These findings suggest that the inhibitory effect of fluvoxamine occurs in a gene-dose-dependent manner and is clearly influenced by CYP2C19 activity.

A recent *in vitro* study has shown some involvement of P-glycoprotein in omeprazole transport [26], while an *in vitro* study with cell lines has recently demonstrated that the inhibitory effect of fluvoxamine on P-glycoprotein is intermediate [27]. Therefore, these findings imply possible mechanisms other than CYP2C19 inhibition. The bioavailability of omeprazole might, to some extent, be increased through inhibition of omeprazole transporting back to the intestinal lumen after absorption by fluvoxamine treatment, though the contribution of P-glycoprotein to omeprazole disposition and the inhibitory effect of fluvoxamine on P-glycoprotein is under further *in vivo* investigation.

The increased AUC of omeprazole during fluvoxamine treatment in heterozygous EMs similar to that during placebo in PMs has significant clinical implications, although the increased AUC in homozygous EMs was still significantly lower than the AUC in PMs. Several studies have suggested that the CYP2C19 genotype influences the cure rate of gastric acid-related disorders including eradication rate of *H. pylori* [28–32]. PMs for CYP2C19 have significantly higher eradication rates of *H. pylori* following treatment with such proton pump inhibitors as omeprazole, lansoprazole and rabeprazole than do EMs [28–31]. Therefore, the combination therapy of omeprazole and low dose of fluvoxamine may be helpful in the treatment of acid-related disorders. In addition, lack of major changes in AUC of omeprazole during fluvoxamine treatment in PMs suggests that co-administration of low-dose fluvoxamine does not influence outcomes in the treatment of acid-related disorders in PMs. If CYP2C19 genotype is not available, co-administration of low-dose fluvoxamine may be safer than increasing the omeprazole dose because low-dose fluvoxamine increases omeprazole exposure selectively in EMs, but not in PMs. However, further study is necessary to determine whether or not co-administration of low dose of fluvoxamine is clinically relevant as adjunctive therapy for eradication of *H. pylori* because several subjects suffered from side-effects probably caused by low dose of fluvoxamine treatment in the present study.

Although no side-effects due to the increased omeprazole exposure during the fluvoxamine administration were observed under the conditions of this study, repeated administration of both omeprazole and fluvoxamine might cause some adverse reaction to omeprazole. Furthermore, adverse reactions to fluvoxamine itself (e.g. serotonin toxicity) should be carefully monitored when clinical doses of these drugs are concomitantly prescribed.

In conclusion, even a low dose of fluvoxamine increased omeprazole exposure in EMs, but did not increase omeprazole exposure in PMs. The increased AUC(0,8 h) in heterozygous EMs was similar to that in PMs. These findings confirm a potent inhibitory effect of fluvoxamine on CYP2C19 activity.

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Effects of various factors on steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone: lack of impact of MDR-1 genotypes

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Aims
An *in vitro* study has suggested that risperidone is a substrate of P-glycoprotein, which is coded by *MDR-1* gene. Thus, we studied the effects of major polymorphisms of the *MDR-1* gene on plasma drug concentrations.

Methods
Subjects were 85 schizophrenic patients receiving 3 mg twice daily of risperidone. Sample collections were conducted 12 h after the bedtime dosing. Plasma concentrations of risperidone and 9-hydroxyrisperidone were quantified using LC/MS/MS. *MDR-1* genotypes (*C3435T* and *G2677T/A*) and CYP2D6 genotypes were identified using PCR-RFLP methods.

Results
There was no difference in geometric mean (95% CI) of steady-state plasma concentration of risperidone between *C3435T* genotypes [*C/C, C/T, T/T*; 2.06 (1.63, 6.47), 2.96 (3.10, 7.91), 2.28 (1.81, 8.04) ng ml^{-1}, $P = 0.759$] or *G2677T/A* genotypes [*G/G, G/T* or *A, T* or *A/T* or *A*; 1.62 (0.08, 6.07), 2.64 (3.25, 7.10), 2.71 (2.77, 8.72) ng ml^{-1}, $P = 0.625$] or 9-hydroxyrisperidone between *C3435T* genotypes [38.3 (33.7, 50.1), 34.9 (32.9, 42.0), 35.7 (31.7, 42.3) ng ml^{-1}, $P = 0.715$] or *G2677T/A* genotypes [40.6 (33.0, 51.8), 35.0 (33.3, 42.4), 36.1 (32.8, 47.2) ng ml^{-1}, $P = 0.601$]. Multiple regression analyses including *CYP2D6* genotypes, sex, and age revealed that steady-state plasma concentration of risperidone correlated with the number of mutated alleles for *CYP2D6* (standardized partial correlation coefficients (beta) = 0.540, $P < 0.001$) and those of 9-hydroxyrisperidone (standardized beta = 0.244, $P = 0.038$) and active moiety (standardized beta = 0.257, $P = 0.027$) correlated with age.

Conclusions
These findings suggest that the MDR-1 variants are not associated with steady-state plasma concentration of risperidone or 9-hydroxyrisperidone, but *CYP2D6* genotypes and age are determinants of these concentrations.

Introduction

Risperidone is one of the representative atypical antipsychotic drugs and has potent antagonistic properties for serotonin 5-HT_2 and dopamine D_2 receptors [1, 2]. This drug is characterized by effectiveness against both positive and negative symptoms in the treatment of schizophrenia [3]. Furthermore it produces fewer side-effects, including extrapyramidal side-effects, than conventional antipsychotic drugs [4].

Risperidone mainly undergoes 9-hydroxylation, yielding an active metabolite 9-hydroxyrisperidone, while alicyclic dehydroxylation and oxidative N-dealkylation are the minor metabolic pathways [5]. 9-hydroxyrisperidone is mainly removed through renal excretion, while it undergoes little further hepatic metabolism [6]. Since 9-hydroxyrisperidone has similar pharmacological properties to risperidone, the sum of risperidone and 9-hydroxyrisperidone is regarded to contribute to the overall antipsychotic effects in the treatment of schizophrenia [7, 8]. At steady state, the majority of the active moiety is ascribable to 9-hydroxyrisperidone in schizophrenic patients who are extensive metabolizers for CYP2D6 [9, 10], suggesting the clinical importance of this metabolite in the ordinary population.

P-glycoprotein, which is encoded by MDR-1 gene, is involved in the acquisition of multidrug resistance phenotypes not only in cancer cells but also in normal tissues such as brain, kidney, liver and intestine [11, 12]. Its major physiological role is to serve as a barrier to entry and as an efflux mechanism for xenobiotics and cellular metabolites [13]. It has also suggested that P-glycoprotein may limit intestinal drug absorption to constrain oral drug bioavailability [14]. Hoffmeyer *et al.* [15] suggested that a single-nucleotide polymorphism in exon 26 of the MDR-1 gene (C3435T) was associated with a lower level of intestinal MDR-1 expression. Moreover it has been reported that another single-nucleotide polymorphism in exon 21 of the MDR-1 gene (G2677T/A) is also linked with a lower function of P-glycoprotein [16].

Recently, an *in vitro* study has shown that quetiapine and risperidone have stronger affinity to P-glycoprotein than other atypical antipsychotic drugs, suggesting that quetiapine and risperidone are substrates of P-glycoprotein [17]. However, there are no *in vivo* data indicating that quetiapine or risperidone as a substrate of P-glycoprotein is of clinically relevance. Therefore, we studied the effects of *MDR-1* genotypes on plasma concentrations of risperidone and 9-hydroxyrisperidone in schizophrenia. Furthermore, various other factors including CYP2D6 genotypes and age were examined using multiple regression analysis.

Methods

Subjects

The subjects were 85 schizophrenic Japanese inpatients (25 males and 60 females) who fulfilled the criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition. Some of the patients participated in our previous studies on the relationship between steady-state plasma drug concentrations and *CYP2D6* genotype [18, 19]. The mean ± SD (range) of age, body weight and duration of illness were 45.4 ± 14.8 (18–75) years, 59.1 ± 11.6 (37–105) kg and 175 ± 135 (4–448) months, respectively. The study was approved by the Ethics Committee of Hirosaki University Hospital, and written informed consent to participate in this study was obtained from the patients and their families.

Protocol

The subjects had received risperidone 3 mg twice a day (08.00 h and 20.00 h) for 4–68 weeks. The elimination half-lives of risperidone and 9-hydroxyrisperidone were reported to be 3–20 h and 20–29 h, respectively [5]. Therefore, plasma concentrations of these compounds had already reached steady state in all of the subjects before initiating the study. The drugs co-administered were flunitrazepam 1–6 mg day^{-1} in 56 cases, biperiden 4–6 mg day^{-1} in 37 cases, sennoside 12–60 mg day^{-1} in 28 cases, trihexyphenidyl 4–10 mg day^{-1} in 15 cases, diazepam 2–30 mg day^{-1} in 10 cases, lorazepam 1–3 mg day^{-1} in 8 cases and alprazolam 0.8–2.4 mg day^{-1} in 7 cases.

Assays for risperidone and 9-hydroxyrisperidone

Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured using a liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) method. Extraction procedure was as follows: 200 µl of 0.1 m phosphate buffer (pH 7), 50 µl of internal standard solution (R068808: Jansen Research Foundation) and 100 µl of methanol were added to 200 µl of plasma sample. Thereafter, 400 µl of 0.1 m borax was added. The mixture was vortexed and poured over an Extrelut NT 1 (Merck) column, which was eluted with 7 ml of ethyl acetate. The eluate was evaporated under a nitrogen stream at 65°C, and was redissolved in 100 µl of methanol which was again evaporated under a nitrogen stream at 65°C. The residues were redissolved in 200 µl of acetonitrile : 0.01 m ammonium acetate (50 : 50, pH 9.0), and 5 µl were injected onto the LC-MS-MS system. The system consisted of API 3000 (Sciex) and a column (Hypersil BDS C18 100 ′ 4.6, 3 µm). The

mobile phase was gradient ammonium acetate (0.01 M, pH 9.0)-acetonitrile. Among the fragment ions of the compounds, the mass-to-charge ratio (m/z) 207.0 for risperidone, m/z 191.0 for 9-hydroxyrisperidone, and m/z 201.0 for the internal standard, were selected for ion monitoring. The lower limit of detection was 0.1 ng ml^{-1} for risperidone and 9-hydroxyrisperidone, and the values of the intra-assay and inter-assay coefficients of variation were less than 5% at all the concentrations (0.1–100 ng ml^{-1}) of calibration curves for both compounds.

Analyses for MDR-1 and CYP2D6 genotypes

For the determination of *MDR-1* and *CYP2D6* genotype, DNA was isolated from peripheral leucocytes by a guanidium isothiocyanate method. The *C3435T* and *G2677T/A* alleles were detected by PCR-RFLP methods as described by Tanabe *et al.* [20]. The *CYP2D6*1 (*1)*, *CYP2D6*3* and *CYP2D6*4* alleles were identified by allele specific PCR analysis according to Heim & Meyer [21]. A long-PCR analysis was used to detect the *CYP2D6*5 (*5)* allele [22]. The *CYP2D6*10 (*10)* allele was identified as the C188T mutation using a two-step PCR analysis as described by Johansson *et al.* [23]. The *CYP2D6*14 (*14)* allele was identified as the G1846T/A mutation using a two-step PCR analysis as by Kubota *et al.* [24]. The *CYP2D6*2* does not result in decreased CYP2D6 activity. Therefore, the *CYP2D6*2* was regarded as the wild type (wt) allele, together with the *CYP2D6*1*.

Data analyses

Steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone were adjusted by drug concentration × body weight/median of body weight (58 kg) in sample population. Active moiety concentration was calculated from the sum of risperidone and 9-hydroxyrisperidone concentrations.

Haplotype analysis was restricted to *C3435T* and *G2677T/A* on the basis of linkage disequilibrium observed between both positions. To obtain a large difference in P-glycoprotein activity, specific genotypes were assigned to simple haplotype pairs, i.e. homozygous wild type allele at both positions, heterozygous at both positions, homozygous mutated alleles at both positions.

The comparison of the plasma drug concentrations and each *MDR-1* genotype was performed with use of one-way ANOVA. Multiple regression analyses were used to detect correlation between plasma drug concentrations and several factors including *MDR-1* genotypes (*C3435T* and *G2677T/A*), *CYP2D6* genotype, age and gender. The number of mutated allele was used as independent variables and gender difference was analyzed using dummy variables (male = 0, female = 1). A P value of 0.05 or less was regarded as significant. SPSS 7.5.1 for Windows SPSS Japan Inc., Tokyo, was used for these statistical analyses.

Results

The patients had the following *MDR-1* genotypes: *C/C* (23 cases), *C/T* (41) and *T/T* (21) for *C3435T* and *G/G* (10), *G/T* or *A* (44) and *T* or *A/T* or *A* (31) for *G2677T/A*, respectively. There was no difference in body weight, gender distribution or age between *C3435T* genotype or between *G2677T/A* genotype.

No difference was found in geometric means (95% confidence intervals) of steady-state plasma concentration of risperidone between *C3435T* genotypes [*C/C, C/T, T/T*; 2.06 (1.63, 6.47), 2.96 (3.10, 7.91), 2.28 (1.81, 8.04) ng ml^{-1}, $F = 0.276$, d.f. = 2,82, $P = 0.759$] or *G2677T/A* genotypes [*G/G, G/T* or *A*, *T* or *A/T* or *A*; 1.62 (0.08, 6.07), 2.64 (3.25, 7.10), 2.71 (2.77, 8.72) ng ml^{-1}, $F = 0.337$, d.f. = 2,82, $P = 0.715$]. The steady-state plasma concentration of 9-hydroxyrisperidone did not differ between *C3435T* genotypes [38.3 (33.7, 50.1), 34.9 (32.9, 42.0), 35.7 (31.7, 42.3) ng ml^{-1}, $F = 0.473$, d.f. = 2,82, $P = 0.625$] or *G2677T/A* genotypes [40.6 (33.0, 51.8), 35.0 (33.3, 42.4), 36.1 (32.8, 47.2) ng ml^{-1}, $F = 0.255$, d.f. = 2,82, $P = 0.775$]. There was no difference in active moiety concentration between *C3435T* genotypes or between *G2677T/A* genotypes (Table 1).

Haplotype pattern of *C3435T* and between *G2677T/A* genotypes was as follows: *C/C* and *G/G* in eight cases, *C/T* and *G/T* or *A* in 26 cases, and *T/T* and *T* or *A/T* or *A* in 14 cases. Other cases were excluded from analyses. No difference was found in plasma concentrations of risperidone ($F = 0.379$, d.f. = 2,45, $P = 0.686$), 9-hydroxyrisperidone ($F = 0.787$, d.f. = 2,45, $P = 0.461$) or active moiety between these three groups ($F = 0.372$, d.f. = 2,45, $P = 0.691$) (Table 1).

The patients had the following CYP2D6 genotypes: *wt/wt* (37 patients), **10/wt* (30), **5/wt* (9), **10/*10* (5), **5/*10* (3) and **4/*14* (1), respectively. A patients with **4/*14* was regarded as a poor metabolizer. These patients were divided into three groups according to the number of mutated alleles; no mutated allele in 37, one mutated allele in 39 and two mutated alleles in nine patients. Multiple regression analyses including *C3435T* and *G2677T/A* allele for *MDR-1*, *CYP2D6* genotypes, gender difference and age showed that the steady-state plasma concentration of risperidone correlated with the number of mutated alleles for *CYP2D6* (standardized partial correlation coefficients (beta) = 0.539,

Table 1
Steady-state plasma concentrations of risperidone, 9-hydroxyrisperidone and active moiety between *C3435T* and *G2677T/A* genotypes and their haplotypes

Genotype	n	Risperidone (ng ml^{-1})	9-hydroxyrisperidone (ng ml^{-1})	Active moiety (ng ml^{-1})
C3435T				
C/C	23	2.06 (1.63, 6.47)	38.3 (33.7, 50.1)	41.8 (36.8, 55.0)
C/T	44	2.96 (3.10, 7.91)	34.9 (32.9, 42.0)	39.8 (37.4, 48.5)
T/T	21	2.28 (1.81, 8.04)	35.7 (31.7, 42.3)	40.2 (35.6, 53.2)
Significance		0.759	0.625	0.842
G2677T/A				
G/G	10	1.62 (0.08, 6.07)	40.6 (33.0, 51.8)	43.1 (33.6, 57.7)
G/T, A	44	2.64 (3.25, 7.10)	35.0 (33.3, 42.4)	39.4 (37.4, 48.6)
T, A/T, A	31	2.71 (2.77, 8.72)	36.1 (32.8, 47.2)	41.0 (37.6, 52.8)
Significance		0.715	0.601	0.775
Haplotype				
C/C and G/G	8	1.68 (-0.36, 7.88)	43.3 (34.2, 57.8)	46.0 (34.3, 65.6)
C/T and G/T, A	26	2.88 (2.64, 7.90)	35.7 (32.5, 44.6)	40.0 (36.1, 51.6)
T/T and T, A/T, A	14	1.88 (1.23, 6.21)	36.5 (31.0, 49.1)	49.5 (33.5, 54.0)
Significance		0.686	0.461	0.691

Data are shown as geometric mean (95% confidence interval). Active moiety is sum of risperidone plus 9-hydroxyrisperidone.

Table 2
Standardized partial correlation coefficients and multiple correlation coefficients between plasma drug concentrations and various factors in 85 schizophrenic patients receiving risperidone

Variables	Standardized partial correlation coefficient: Risperidone	9-hydroxyrisperidone	Active moiety
MDR-1 genotypes			
C3435T	0.004	−0.017	−0.013
G2677T/A	0.023	−0.013	−0.003
CYP2D6 genotypes	0.540***	−0.083	0.122
Age	0.121	0.244*	0.257*
Gender	0.022	−0.102	−0.081
Multiple correlation coefficient	0.573***	0.253	0.302

*$P < 0.05$, ***$P < 0.001$. Active moiety is sum of risperidone plus 9-hydroxyrisperidone.*

$P < 0.001$) and that plasma concentration of 9-hydroxyrisperidone (standardized beta = 0.244, $P = 0.038$) and active moiety (standardized beta = 0.257, P = 0.027) correlated with age (Table 2).

Discussion

P-glycoprotein is found in the epithelial cells lining the luminal surface of many organs often associated with an excretory or barrier function, that is, the hepatic bile canalicular membrane, renal proximal tubule, villus-tip enterocyte in the small intestine, and the endothelial cells making up the blood-brain and blood–testes barriers [11, 12, 14, 25]. Recently, a polymorphism in exon 26 (C3435T) of this gene was shown to correlate with intestinal P-glycoprotein expression and function in humans. Carriers homozygous for this polymorphism (TT) showed more than two-fold lower P-glycoprotein expression and higher digoxin plasma concentrations

compared with the CC group [15]. However, the role of P-glycoprotein in pharmacokinetics or pharmacodynamics has not yet been fully proven in psychiatric field.

A recent *in vitro* study has examined the activity of P-glycoprotein towards four atypical and two conventional antipsychotics and a proven substrate, verapamil, by their P-glycoprotein ATPase activity, a putative measure of P-glycoprotein affinity [17]. The Michaelis-Menten equation was applied to the data. The rank order of the ratio V_{max}/K_m was: verapamil (2.6) > quetiapine (1.7) > risperidone (1.4) > olanzapine (0.8) > chlorpromzaine (0.7) > haloperidol (0.3) = clozapine (0.3). The atypical antipsychotics quetiapine and risperidone were relatively good P-glycoprotein substrates, although their affinities were not as high as verapamil. These results suggest that P-glycoprotein is likely to influence absorption in the small intestine and/or excretion in liver or kidney of all atypical antipsychotics to various degrees. Therefore, we assumed that MDR-1 genotypes would have some impact on the pharmacokinetics of risperidone even at steady state.

Contrary to our expectation, the steady-state plasma concentration of risperidone was not different between *MRD-1* genotypes. It appears that *MDR-1* variants were not associated with the steady-state plasma concentration of risperidone. We have some plausible explanations for these negative findings. First, when *MDR-1* variants alter the activity of P-glycoprotein in the small intestine, which limits oral bioavailability, peak concentrations of risperidone may differ between *MDR-1* genotypes. However, plasma concentrations of risperidone were monitored at 12 h after the dosing in the present study. Therefore, any difference in peak plasma concentration of risperidone caused by *MDR-1* variants was offset by large interindividual variability in risperidone metabolism during the elimination phase. Second, since a previous study suggesting low contribution of renal excretion (4%) to overall drug elimination [5], the contribution of biotransformation of risperidone to risperidone disposition might be much larger than that of the excretion via P-glycoprotein in liver or kidney.

9-hydroxyrisperidone is mainly removed through renal excretion [6]. P-glycoprotein exists in the renal proximal tubule [25]. Thus, it is more likely that the steady-state plasma concentration of 9-hydroxyrisperidone depends upon P-glycoprotein activity. However, no change was found between the *MDR-1* genotypes in this study. This finding suggests either that 9-hydroxyrisperidone is not such a specific substrate of P-glycoprotein or that renal excretion of the compound may be mediated by other transporters. Likewise, the *MRD-1* variants were not associated with the active moiety concentration. This is a reasonable finding in the light of lack of association between the MDR-1 variants and the steady-state plasma concentration of 9-hydroxyrisperidone. Active moiety concentration is predominantly influenced by the steady-state plasma concentration of 9-hydroxyrisperidone because the steady-state plasma concentration of 9-hydroxyrisperidone was much higher than that of risperidone in this study. Therefore, we concluded that it is unlikely that *MRD-1* variants have clinical implications based on changes in plasma drug concentrations.

In the present study, multiple regression analyses showed strong correlation between the number of mutated alleles for *CYP2D6* and steady-state plasma concentration of risperidone. This finding is in accordance to previous studies [10, 18, 19, 26]. Several *in vitro* studies demonstrating that 9-hydroxylation is mainly catalyzed by CYP2D6 also support the results of this study [27, 28]. Furthermore, the steady-state plasma concentration of 9-hydroxyrisperidone or active moiety concentration was not correlated with number of mutated alleles for *CYP2D6*, which is also in line with previous findings [10, 18, 19, 26]. Although it is still unknown whether elimination of 9-hydroxyrisperidone is mediated in liver, this finding may be explained some possibility that further metabolism of 9-hydroxyrisperidone is catalyzed by CYP2D6, to some extent. From a clinical point of view, active moiety concentration is more important when investigating the relationship between clinical outcomes and pharmacokinetics. From this perspective, it is unlikely that *CYP2D6* variants have clinical implications based on changes in the plasma concentration of risperidone.

A significant correlation was found between the steady-state plasma concentration of 9-hydroxyrisperidone and age. This is a new finding, but it is not surprising because renal function tends to decrease with advancing of age. The active moiety concentration also correlated with age. Although there have been no data indicating clear association between clinical effect of risperidone and plasma drug concentration, a recent study suggested that the higher plasma concentration of active moiety tends to have a higher rate of extrapyramidal symptoms [29, 30]. Therefore, careful dose setting and possibly therapeutic drug monitoring is needed in elderly patients.

In conclusion, the present study suggests that *MDR-1* genotypes are unlikely to affect steady-state plasma concentration of risperidone or 9-hydroxyrisperidone. However *CYP2D6* genotypes and age are determinants affecting risperidone and 9-hydroxyrisperidone, respectively. Particularly careful administration is necessary in

elderly patients because the active moiety concentration depends on age.

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The Effects of *Ginkgo biloba* Extracts on the Pharmacokinetics and Pharmacodynamics of Donepezil

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The effects of ginkgo supplementation on the steady-state plasma concentration of donepezil and the activity of cholinesterase in red blood cells and cognitive function were examined. Fourteen inpatients with Alzheimer's disease received donepezil 5 mg/day, supplemented with extracts of Ginkgo biloba *90 mg/day for 30 days. Blood samples were collected before and during ginkgo supplementation and 30 days after its discontinuation, together with an assessment of cognitive function. Plasma drug concentration was measured using high-performance liquid chromatography (HPLC), and cholinesterase in red blood cells was measured using Ellman methods. Cognitive function was evaluated using the Mini-Mental Scale Examination (MMSE). Plasma concentration of donepezil during ginkgo supplementation (mean ± SD [95% confidence interval]; 24.4 ± 12.6 ng/mL [17.1-31.7 ng/mL]) was not significantly different from that before ginkgo supplementation (22.7 ± 10.3 ng/mL [16.8-28.7 ng/mL]) or that 4 weeks after its discontinuation (25.0 ± 12.9 ng/mL [17.6-32.4 ng/mL]). There was no significant difference between cholinesterase in red blood cells before ginkgo supplementation (1.75 ± 0.21 U [1.63-1.87 U]), during ginkgo supplementation (1.91 ± 0.27 U [1.76-2.07 U]), and 4 weeks after its discontinuation (1.83 ± 0.29 U [1.66-2.00 U]). Ginkgo supplementation did not alter MMSE scores throughout the study. The present study shows that ginkgo supplementation does not have major impact on the pharmacokinetics and pharmacodynamics of donepezil.*

Keywords: *Donepezil; Ginkgo biloba; pharmacokinetics; pharmacodynamics; drug-herb interaction; Alzheimer's disease*

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Donepezil is a centrally and selectively acting acetylcholinesterase inhibitor. It has been reported that donepezil is effective in the treatment of cognitive impairment and memory loss in patients with Alzheimer's disease and is well tolerated when 5 mg daily of the drug is prescribed.[1-3] In a clinical trial survey, significant correlation has been demonstrated between the plasma concentration of donepezil and the percentage of acetylcholinesterase inhibition.[2,4] The 50% inhibition of acetylcholinesterase activity is obtained at the plasma drug concentration of 15.6 ng/mL, and a plateau of the inhibition is reached at a plasma concentration of donepezil higher than 50 ng/mL. Although a therapeutic range of donepezil concentration in plasma has not fully been proven yet, improvement of cognitive impairment was significantly correlated with the plasma concentration of donepezil.[2] Therefore, information about a potential factor affecting plasma drug concentration is clinically important.

Ginkgo is derived from the leaf of *Ginkgo biloba*. It has been used for cognitive disorders, peripheral vascular disease, age-related macular degeneration, vertigo, tinnitus, erectile dysfunction, and altitude sickness.[5,6] Several studies suggest that ginkgo may stabilize or improve cognitive performance in patients with Alzheimer's disease and multi-infarct dementia.[7-9]

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Although ginkgo is frequently administered with donepezil in patients with Alzheimer's disease or multi-infarct dementia based on these findings,[10] there is no information about a drug interaction between donepezil and ginkgo. Thus, we examined the effect of the extract of *Ginkgo biloba* on the pharmacokinetics and pharmacodynamics of donepezil in elderly patients with Alzheimer's disease.

METHODS

Subjects

Fourteen patients (6 males and 8 females; age range: 65-80 years; weight range: 40-75 kg) with Alzheimer's disease who were admitted to Kuroishi-Akebono Hospital (Kuroishi, Japan) and who were treated with donepezil participated in the study. Concomitant medications were flunitrazepam 2 mg/day in 4 patients, zolpidem 10 mg/day in 4 patients, fluvoxamine 75 mg/day in 2 patients, fluvastatin 20 mg/day in 4 patients, trapidil 300 mg/day in 2 patients, digitoxin 0.0025 mg/day in 2 patients, furosemide 20 to 40 mg/day in 3 patients, spironolactone 25 to 50 mg/day in 3 patients, nifedipine 20 to 40 mg/day in 2 patients, benidipine 4 mg/day in 2 patients, theophylline 400 mg/day in 1 patient, and sennoside 24 to 48 mg/day in 6 patients. The protocol was approved by the Ethics Committee of Hirosaki University School of Medicine. Written informed consent was obtained from all patients or their families.

Study Design

The patients received an oral 5-mg dose of donepezil once a day (8 p.m.) for at least 20 weeks. After no fluctuation of mental status for 28 days was confirmed, ginkgo 90 mg/day (Ichoha Sainoshin, Aihoupu Co., Kagoshima, Japan) was also administrated for 30 days. Concomitant medications were fixed without dosage adjustment during the study period. Blood samplings (16 mL each) for the plasma concentration of donepezil and cholinesterase in red blood cells were taken into heparinized tubes 12 hours after donepezil dosing (8 a.m.), which was performed before and during ginkgo supplementation and 30 days after its discontinuation. Then, 10 mL of blood was separated to plasma immediately, and the plasma was stored at –30°C until analysis. The remaining 6 mL of whole blood was transferred to the laboratory of Sumikin Biosciences Co. (Sagamihara, Kanagawa, Japan) to determine cholinesterase activity in red blood cells. On each day of blood sampling during the protocol, cognitive function was evaluated using the Mini-Mental Scale Examination (MMSE). A rater was blind to the protocol or regimen of each patient.

Assay

The plasma concentration of donepezil was quantified using high-performance liquid chromatography (HPLC) developed in our laboratory.[11] The limit of determination of donepezil was 1.5 ng/mL, and the coefficient of variation (CV) was less than 5%. Cholinesterase activity in red blood cells was determined using Ellman methods with spectroscopy.[12]

Statistical Analysis

Statistical analyses were performed by using Friedman rank tests with SPSS software. All tests were two-tailed and considered to be statistically significant when the *p*-value was less than 0.05.

RESULTS

The plasma concentration of donepezil, the cholinesterase activity in red blood cells, and the MMSE scores before and during ginkgo supplementation and 4 weeks after its discontinuation are summarized in Table I, and intraindividual changes in these data are shown in Figure 1. The plasma concentration of donepezil during ginkgo supplementation (mean ± SD [95% confidence interval]; 24.4 ± 12.6 ng/mL [17.1-31.7 ng/mL]) was not significantly different from that before ginkgo supplementation (22.7 ± 10.3 ng/mL [16.8-28.7 ng/mL]) or that 4 weeks after its discontinuation (25.0 ± 12.9 ng/mL [17.6-32.4 ng/mL]) (Table I). There was no significant difference between cholinesterase in red blood cells before ginkgo supplementation (1.75 ± 0.21 U [1.63-1.87 U]), during ginkgo supplementation (1.91 ± 0.27 U [1.76-2.07 U]), and 4 weeks after its discontinuation (1.83 ± 0.29 U [1.66-2.00 U]) (Table I). Ginkgo supplementation did not alter MMSE scores throughout the study.

DISCUSSION

In vitro studies have reported that donepezil is metabolized to N-dealkyldonepezil and O-demethyldonepezil, whose reactions were catalyzed by CYP3A4 and CYP2D6, respectively.[13] An in vivo study has also demonstrated that ketoconazole, an inhibitor of CYP3A4, significantly decreases donepezil clearance, suggesting that CYP3A4 is involved in the metabolism of donepezil.[14]

Table I Plasma Drug Concentration, Cholinesterase in Red Blood Cells, and Mini-Mental Scale Examination (MMSE) before and during Ginkgo Supplementation and after Its Discontinuation in 14 Patients with Alzheimer's Disease

	Before	During	After	Significance
Plasma drug concentration (ng/mL)	22.7 ± 10.3 (16.8, 28.7)	24.4 ± 12.6 (17.1, 31.7)	25.0 ± 12.9 (17.6, 32.4)	*ns*
Cholinesterase in red blood cells (U)	1.75 ± 0.21 (1.63, 1.87)	1.91 ± 0.27 (1.74, 2.07)	1.83 ± 0.29 (1.66, 2.00)	$p < 0.01$
MMSE (score)	9.0 ± 7.8 (4.5, 13.5)	8.7 ± 7.7 (4.3, 13.1)	8.7 ± 7.9 (4.1, 13.3)	*ns*

Data are shown as mean ± SD (95% confidence interval).

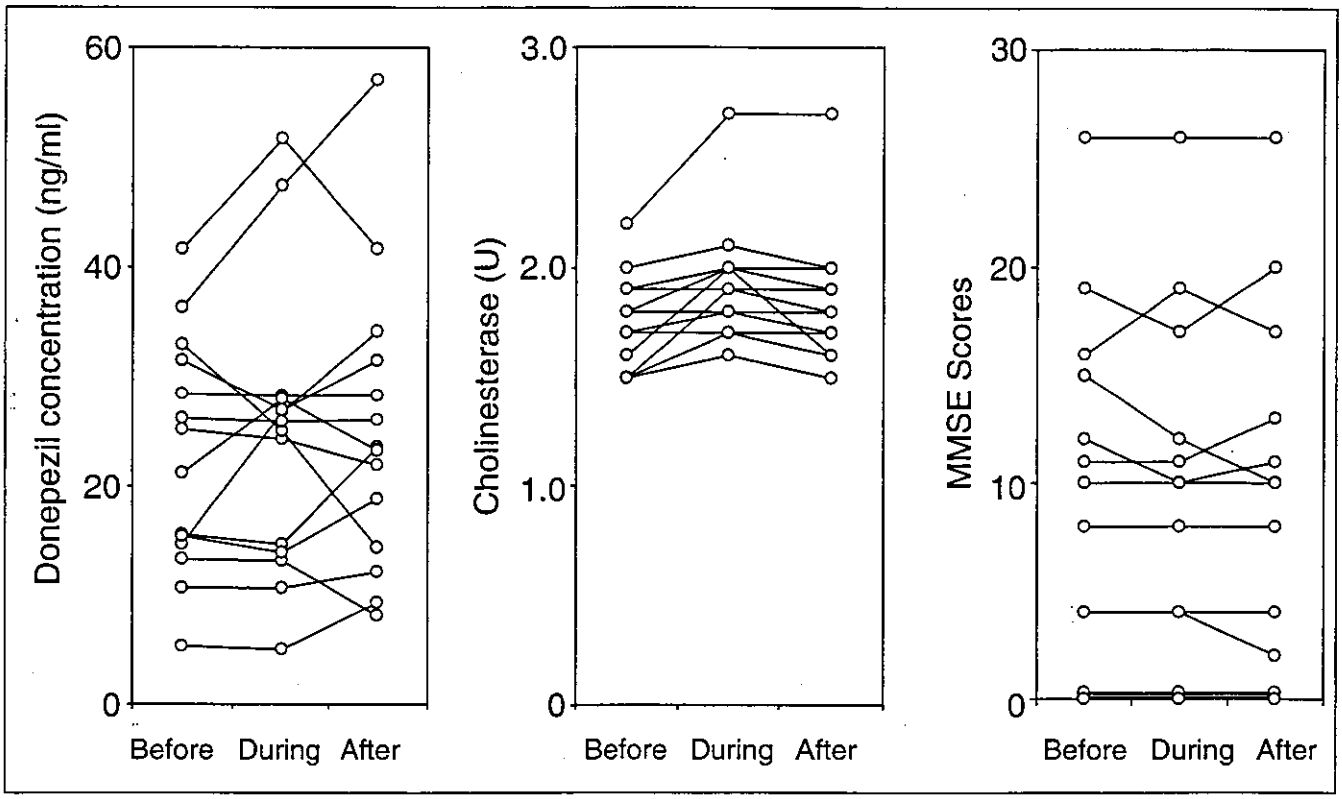

Figure 1. Intraindividual changes in steady-state plasma concentration of donepezil (left), cholinesterase in red blood cells (middle), and Mini-Mental Scale Examination (MMSE) (right) before and after ginkgo supplementation and 4 weeks after its discontinuation in 14 patients with Alzheimer's disease.

Galluzzi et al[15] have reported a case in which a patient with Alzheimer's disease fell into a coma after taking a combination of ginkgo and trazodone. Ginkgo flavonoids increase the production of 1-(m-chlorophenyl)piperazine (m-CPP), an active metabolite of trazodone, which, by acting on benzodiazepine binding sites, releases γ-aminobutyric acid (GABA) through an agonistic action on presynaptic serotonin 5-HT_2 and α_2-adrenergic receptors/located GABAergic activity. Our previous study reported that carbamazepine, an inducer of CYP3A4, decreased the steady-state plasma concentration of trazodone, suggesting the involvement of CYP3A4 in the metabolism of trazodone.[16] Thus, it is possible that ginkgo has an inducing effect on CYP3A4 as well as another flavonoid, St. John's wort.[17,18] In addition, a recent animal study

has shown that the feeding of ginkgo markedly increases the CYP content and CYP2B and CYP3A mRNA in liver.[19] Therefore, we presumed that ginkgo supplementation with donepezil would cause some drug interaction.

The results of this study, however, showed that ginkgo did not alter the steady-state plasma concentration of donepezil, which means that ginkgo does not have an inhibitory or inducing effect on the metabolism of donepezil. A recent pharmacokinetic study has shown that ginkgo supplementation did not affect the single point ratios of 1'-hydroxymidazolam/midazolam and debrisoquine urinary recovery ratios, which are regarded as a simple index of CYP3A4 and CYP2D6, respectively.[18] These findings support our conclusion that ginkgo does not have any effect on the metabolism of donepezil catalyzed by CYP2D6 and CYP3A4. In the clinical situation, therefore, little concern about the pharmacokinetic interaction between donepezil and ginkgo is needed in the treatment of Alzheimer's disease.

It should be noted that the current study did not measure acetylcholinesterase and pseudocholinesterase separately but just cholinesterase (sum of acetyl- and pseudocholinesterase). Pseudocholinesterase is mainly located in plasma, while acetylcholinesterase is located in red blood cells, to a considerable extent. Therefore, cholinesterase activity in red blood cells is regarded to be roughly reflected by acetylcholinesterase activity. In the present study, cholinesterase in red blood cells was significantly elevated during ginkgo supplementation, although it was a small difference. These findings are in discord with our observation that no change in the pharmacokinetics of donepezil was seen in light of the significant correlation between the plasma concentration of donepezil and the percentage of acetylcholinesterase inhibition.[4] Ginkgo itself may have some effects on cholinesterase activity.

Several studies demonstrated that ginkgo itself improves cognitive impairment or memory loss.[6-9] However, although ginkgo increased cholinesterase activity in this study, ginkgo supplementation did not alter MMSE scores. A plausible explanation for this discrepancy may be our shorter additional treatment (30 days) than previous reports or that patients with extremely severe Alzheimer's disease (0 points in MMSE scores in 4 patients throughout the study period) were included in this study.

In conclusion, ginkgo supplementation does not have major impact on the pharmacokinetics and pharmacodynamics of donepezil. There was no indication in this study of any adverse drug-herb interaction between donepezil and ginkgo during their combined use in the treatment of Alzheimer's disease.

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