digoxin concentration, whereas heterozygous (CT) subjects presented with intermediate levels.[10] Our additional analysis of 20 patients tested at all 4 doses also suggested that TT subjects have higher FLV concentration-to-dose ratio compared with CC or CT subjects, although the number of subjects was small and dose-dependent effects of the 3435 C>T genotype could not be detected clearly.

Two studies[2,3] using a single oral dose of 50 mg FLV reported that the area under the plasma or serum concentration-time curve (area under the curve, or AUC) of FLV was significantly associated with CYP2D6 activity. A significant correlation between AUC of FLV and CYP1A2 activity was found in 1 study using a single oral dose of 50 mg FLV.[1] These studies suggested that pharmacokinetics of FLV depended on CYP2D6 and CYP1A2 activity at lower doses such as 50 mg FLV. However, a 2003 study[4] using 200 mg/d FLV did not find effects of CYP1A2 and CYP2D6 on the pharmacokinetics of FLV, and the possible explanation for this result was thought to be saturation of CYP1A2 and CYP2D6 at high doses of FLV. Saturation kinetics of CYP2D6 have been demonstrated for other drugs such as paroxetine.[19] CYP1A2 has also been characterized by saturation kinetics for other drugs such as theophylline[20] and caffeine.[21] Several other studies[22–24] have shown nonlinear kinetics for FLV, which is considered to be caused by the saturation of CYP1A2 and CYP2D6. In our study, FLV concentration-to-dose ratio also increased with increasing treatment dose. The 3435 C>T genotype had a significant effect on plasma FLV concentration only at the highest dose of FLV, 200 mg/day, likely because of an increasing number of subjects with saturated CYP1A2 and CYP2D6 at this higher dose.

An in vitro study[25] suggested that FLV had an ABCB1 inhibitory effect. Therefore, ABCB1 may be partly involved in nonlinear kinetics of FLV, and ABCB1 inhibitory effect of FLV may be different among *ABCB1* genotype groups. However, our study did not investigate these points.

As mentioned earlier, several reports have suggested that CYP2D6 is involved in the metabolism of FLV. Several mutated alleles of the *CYP2D6* gene causing absent activity such as *3, *4, and *5, and decreased activity such as *10, have been reported. Accordingly, in subjects having mutated alleles of CYP2D6, the CYP2D6 pathway may be easily saturated at relatively lower doses, and ABCB1 has a greater impact on FLV metabolism. Further studies analyzing genotype of *ABCB1* with that of *CYP2D6* are needed to investigate FLV pharmacokinetics in details.

The 3435 C>T polymorphism genotyped in our study causes no amino acid change. There may be other functional polymorphisms in significant linkage disequilibrium with 3435 C>T polymorphism that affect the pharmacokinetics of FLV. For instance, the G2677A/T polymorphism in exon 21 causes an amino acid change and is associated with expression of P-glycoprotein.[26,27] This polymorphism is found to be in significant linkage disequilibrium with 3435 C>T polymorphism.[26] Further studies investigating other polymorphisms including G2677A/T are needed.

In addition, it would be interesting to investigate effects of *ABCB1* gene polymorphisms on FLV pharmacokinetics at doses higher than 200 mg/d because high doses of SSRIs are occasionally needed to obtain a therapeutic effect in the treatment of psychiatric disorders such as obsessive-compulsive disorder.

CONCLUSION

This study suggests that pharmacokinetics of FLV depend on 3435 C>T genotype of *ABCB1* at high doses such as 200 mg/d. However, sample size of the present was quite small, and only 4 subjects carried the TT genotype of 3435 C>T in the 200 mg/d treatment group. Further studies with a larger sample size are needed to clarify the extent of involvement of *ABCB1* gene polymorphism on FLV pharmacokinetics.

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REFERENCES

1. Spigset O, Carleborg L, Hedenmalm K, et al. Effect of cigarette smoking on fluvoxamine pharmacokinetics in humans. *Clin Pharmacol Ther.* 1995;58:399–403.
2. Carrillo JA, Dahl ML, Svensson JO, et al. Disposition of fluvoxamine in humans is determined by the polymorphic CYP2D6 and also by the CYP1A2 activity. *Clin Pharmacol Ther.* 1996;60:183–190.
3. Spigset O, Granberg K, Hägg S, et al. Relationship between fluvoxamine pharmacokinetics and CYP2D6/CYP2C19 phenotype polymorphisms. *Eur J Clin Pharmacol.* 1997;52:129–133.
4. Gerstenberg G, Aoshima T, Fukasawa T, et al. Effects of the CYP2D6 genotype and cigarette smoking on the steady-state plasma concentration of fluvoxamine and its major metabolite fluvoxamino acid in Japanese depressed patients. *Ther Drug Monit.* 2003;25:463–468.
5. Ohara K, Tanabe S, Ishibashi K, et al. CYP2D6*10 alleles do not determine plasma fluvoxamine concentration/dose ratio in Japanese subjects. *Eur J Clin Pharmacol.* 2003;58:659–661.
6. Gottesman MM, Pastan I, Ambudkar SV. P-glycoprotein and multidrug resistance. *Curr Opin Genet Dev.* 1996;6:610–617.
7. Ambudkar SV, Dey S, Hrycyna CA, et al. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol.* 1999;39:361–398.
8. Tanigawara Y. Role of P-glycoprotein in drug disposition. *Ther Drug Monit.* 2000;22:137–140.
9. Terao T, Hisanaga E, Sai Y, et al. Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier. *J Pharm Pharmacol.* 1996;48:1083–1089.
10. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA.* 2000;97:3473–3478.
11. Kurata Y, Ieiri I, Kimura M, et al. Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin Pharmacol Ther.* 2002;72:209–219.
12. Verstuyft C, Schwab M, Schaeffeler E, et al. Digoxin pharmacokinetics and MDR1 genetic polymorphisms. *Eur J Clin Pharmacol.* 2003;58:809–812.
13. Chowbay B, Cumaraswamy S, Cheung YB, et al. Genetic polymorphisms in MDR1 and CYP3A4 genes in Asians and the influence of MDR1 haplotypes on cyclosporin disposition in heart transplant recipients. *Pharmacogenetics.* 2003;13:89–95.

14. Bonhomme-Faivre L, Devocelle A, Saliba F, et al. MDR1 C3435T polymorphism influences cyclosporine A dose requirement in liver-transplant recipients. *Transplantation*. 2004;78:21–25.
15. Zheng H, Webber S, Zeevi A, et al. Tacrolimus dosing in pediatric heart transplant patients is related to CYP3A5 and NDR1 gene polymorphisms. *Am J Transplant*. 2003;3:477–483.
16. Uhr M, Steckler T, Yassouridis A, et al. Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood-brain barrier deficiency due to mdr1a P-glycoprotein gene disruption. *Neuropsychopharmacology*. 2000;22:380–387.
17. Roberts RL, Joyce PR, Mulder RT, et al. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenomics J*. 2002; 2:191–196.
18. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. Text Revision. APA: Washington, DC; 2000.
19. Sawamura K, Suzuki Y, Someya T. Effects of dosage and CYP2D6-mutated allele on plasma concentration of paroxetine. *Eur J Clin Pharmacol*. 2004;60:553–557.
20. Dahlqvist R, Billing B, Miners JO, et al. Nonlinear metabolic disposition of theophylline. *Drug Monit*. 1984;6:290–297.
21. Kamimori GH, Lugo SI, Penetar DM, et al. Dose-dependent caffeine pharmacokinetics during severe sleep deprivation in humans. *Int J Clin Pharmacol Ther*. 1995;32:182–186.
22. Härtter S, Wetzel H, Hiemke C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography. *Clin Chem*. 1992;38:2082–2086.
23. Spigset O, Öhman R. A case of fluvoxamine intoxication demonstrating nonlinear elimination pharmacokinetics. *J Clin Psychopharmacol*. 1996; 16:254–255.
24. Spigset O, Granberg K, Hägg S, et al. Non-linear fluvoxamine disposition. *Br J Clin Pharmacol*. 1998;45:257–263.
25. Weiss J, Dormann SK, Martin-Facklam M, et al. Inhibition of P-glycoprotein by newer antidepressants. *J Pharmacol Exp Ther*. 2003; 305:197–204.
26. Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: Relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther*. 2001;297:1137–1143.
27. Siegmund W, Ludwig K, Giessmann T, et al. The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. *Clin Pharmacol Ther*. 2002;72: 572–583.

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PHARMACOKINETICS AND DISPOSITION

Terbinafine increases the plasma concentration of paroxetine after a single oral administration of paroxetine in healthy subjects

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Abstract
Objective Paroxetine is believed to be a substrate of CYP2D6. However, no information was available indicating drug interaction between paroxetine and inhibitors of CYP2D6. The aim of this study was to examine the effects of terbinafine, a potent inhibitor of CYP2D6, on pharmacokinetics of paroxetine.
Methods Two 6-day courses of either a daily 150-mg of terbinafine or a placebo, with at least a 4-week washout period, were conducted. Twelve volunteers took a single oral 20-mg dose of paroxetine on day 6 of both courses. Plasma concentrations of paroxetine were monitored up to 48 h after dosing.
Results Compared with the placebo, terbinafine treatment significantly increased the peak plasma concentration (C_{max}) of paroxetine, by 1.9-fold (6.4±2.4 versus 12.1±2.9 ng/ml, $p<0.001$), and the area under the plasma concentration-time curve from zero to 48 h [AUC (0–48)] of paroxetine by 2.5-fold (127±67 vs 318±102 ng/ml, $p<0.001$). Elimination half-life differed significantly (15.3±2.4 vs 22.7±8.8 h, $p<0.05$), although the magnitude of alteration (1.4-fold) was smaller than C_{max} or AUC.
Conclusion The present study demonstrated that the metabolism of paroxetine after a single oral dose was inhibited by terbinafine, suggesting that inhibition of CYP2D6 activity may lead to a change in the pharmacokinetics of paroxetine. However, further study is required to confirm this phenomenon at steady state.

Keywords Paroxetine · Terbinafine · CYP2D6 · Interaction

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Introduction

Paroxetine is one of the selective serotonin transporter inhibitors (SSRI) and is widely used in the treatment of mental disorders, including depression, panic disorders, and obsessive compulsive disorder [1, 2]. Paroxetine is almost completely absorbed following oral administration. However, the drug undergoes extensive first pass metabolism [3, 4]. As a result, less than 50% of a single dose of paroxetine reaches the general circulation. Paroxetine is eliminated by metabolism involving oxidation, demethylation, and conjugation [5].

An early in vivo study using healthy volunteers demonstrated a cosegregation between paroxetine and sparteine metabolism, and that steady-state plasma concentration of paroxetine in poor metabolizers (PM) was higher than extensive metabolizers (EM) [6, 7]. Moreover, an in vitro study demonstrated that quinidine and quinine, both of which are potent inhibitors, inhibit paroxetine demethylation in human liver microsomes from EM, but did not inhibit that from PM [8]. Based on these findings, it is evident that paroxetine is primarily metabolized by the cytochrome CYP2D6. However, there are few in vivo data,

indicating that paroxetine concentration is influenced by CYP2D6 modulators despite the fact that numerous in vivo and in vitro studies used paroxetine as a potent inhibitor of CYP2D6 [9–12].

Terbinafine is an orally active allylamine antifungal agent with a primarily fungicidal action in vitro [13, 14]. Clinical trials have demonstrated that orally administered terbinafine is effective in the treatment of dermatophyte infection of the skin [15]. Several in vitro studies demonstrated that terbinafine has a potent inhibitory effect on CYP2D6 activity [16–18]. Several in vivo studies indicated that terbinafine inhibited dextromethorphan [19] and desipramine [20], both of which are substrates of CYP2D6, suggesting an inhibitory effect of terbinafine on CYP2D6 in vivo. Moreover, several case reports demonstrated two cases who suffered from desipramine toxicity [21] and nortriptyline toxicity [22] induced by terbinafine. Terbinafine increased CYP2D6-mediated amitriptyline and nortriptyline concentration for at least 6 months [23], suggesting terbinafine inhibited CYP2D6-mediated metabolism of these antidepressants. Therefore, it is more likely that terbinafine affects the disposition of paroxetine. To our knowledge, however, there is no information about drug–drug interaction between terbinafine and paroxetine. The aim of this study was to confirm the effects of terbinafine on the pharmacokinetics of paroxetine.

Methods

Subjects

Twelve healthy Japanese volunteers (nine males, three females) were enrolled in this study. Their mean±SD of age (range) was 24.8±2.5 (20–35) years and mean body weight was 58.3±8.5 (46–75) kg. The Ethics Committee of Hirosaki University School of Medicine approved this study protocol, and written informed consent had been obtained from each participant before any examinations.

Study design

A randomized crossover study design was conducted at intervals of 4 weeks. One capsule containing either 125 mg of terbinafine or a matched placebo with 240 ml of tap water was given once daily at 0800 hours for 6 days. Compliance of thetest drug was confirmed by pill-count. No other medications were taken during the study periods. No meal was allowed until 4 h after dosing (1300 hours). The use of alcohol, tea, coffee and cola was forbidden during the test days.

Sample collections

Blood samplings (10 ml each) for determination of paroxetine were taken into heparinized tubes just before and 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h after the administration of paroxetine. Plasma was separated immediately and kept at −30°C until analysis. At the same time as the blood samplings, blood pressure and heart rate were measured. Any adverse events were reported by each subject.

Assay

Plasma concentrations of paroxetine were measured using a high-performance liquid chromatography method developed in our laboratory. In brief, extraction procedure was as follows: to 2,000 μl of plasma sample was added 500 μl of 0.5 M NaOH, 100 μl of internal standard solution (trifluperidol 200 μg/ml) and 100 μl of methanol. Thereafter, the tubes were vortex-mixed for 10 s and 5μml of n-heptane–chloroform (70:30, v/v) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 2,500 *g* for 10 min at 4°C, and the organic phase was evaporated in vacuo at 40°C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved in 500 μl of mobile phase, then 400 μl were injected onto the HPLC system. The HPLC system consisted of Shimadzu LC-10AT high-pressure pumps, a Shimadzu CTO-10AVP column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan) and a Shimadzu SIL-10ADVP (500-μl injection volume) (Tokyo, Japan) and a column (STR-ODS II C18 150×4.6, 3 μm) (Tokyo, Japan). The mobile phase was phosphate buffer (0.02 M, pH=4.6), acetonitrile and perchloric acid (60%) (57.25: 42.5: 0.25, v/v/v). The lowest limit of detection and quantification were 0.5 and 1.0 ng/ml, respectively, and the values of the intra-assay and inter-assay coefficient of variation were less than 10 % at all the concentrations (1.0–150 ng/ml) of calibration curves for paroxetine.

CYP2D6 genotypes

For the determination of *CYP2D6* genotype, DNA was isolated from peripheral leukocytes by a guanidium isothiocyanate method. Genotypings of CYP2D6 were performed using AmpliChip CYP450 Test DNA chip (Roche Diagnostics). The AmpliChip CYP450 Test provides materials for genotyping two cytochrome genes, encompassing 31 known mutations in the CYP2D6 gene, including duplication and gene deletion (CYP2D6*2, *3, *4, *5, *6, *7, *8, *9, *10AB, *11, *14A, *15, *17, *19, *20, *25, *26, *29, *30, *31, *35, *36, *40, *41, *1XN,

*2XN, *4XN, *10XN, *!7XN, *5XN, *41XN). The alleles without activity were *3, *4, *5, *6, *7, *8, *11, *14A, *15, *19, *20, *40. The alleles which lead to the decreased enzyme activity were CYP2D6*9, *10, *17, *29, *36, *41.

Date 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 (0–48)] was calculated with use of the lin-lin trapezoidal rule. AUC from zero to infinity [AUC(0–∞)] and elimination half-life were determined by non-compartment model with WinNonlin Professional software (Pharsight, Cary, N.C., USA). The terminal elimination rate constant (k_e) was determined by log-linear regression of the final data points (4). The apparent elimination half-life of the log-linear phase ($t_{1/2}$) was calculated as follows: $0.693/k_e$.

Statistical analysis

Data are shown as means±SD in tables and figures. Paired *t*-test was used for the comparison of the plasma drug concentrations between two phases, i.e., placebo and terbinafine. The comparison of $t_{max}$was performed using the Wilcoxon signed-sample test. A *p*value of 0.05 or less was regarded as significant. Geometric mean ratios to corresponding values in placebo phase with 95% confidence intervals were used for detection of significant differences. When the 95% confidence interval did not cross 1.0, the result was also regarded as significant. When the calculated 90% confidence intervals with logarithmic transformation of pharmacokinetic data (C_{max}, AUC(0–48) and total AUC) fell within, on average, 80–125% for the ratio of the paroxetine plus terbinafine to paroxetine only, we regarded the paroxetine plus terbinafine as bioequivalent to paroxetine only. SPSS 13.0J for Windows (SPSS Japan, Tokyo) was used for these statistical analyses.

Results

The subjects had the following CYP2D6 genotypes: *wt/wt* (4 subjects), **10/wt* (6), **10/*10* (1) and **5/*10* (1), respectively. No subjects regarded as poor metabolizers were included. These patients were divided into three groups according to the number of mutated alleles: no mutated allele in 4, one mutated allele in 6 and two mutated alleles in 2 subjects.

Appetite loss (n=4, n=6), abdominal disturbance (n=4, n=7), diarrhea (n=1, n=1), asthenia (n=5, n=7) and sleepiness (n=3, n=4) were observed in control and terbinafine phases, respectively. All of these side effects were mild to moderate and recovered at longest within 2 days after paroxetine doses.

Plasma drug concentration-time curves during both placebo and terbinafine treatments are shown in Fig. 1. Their pharmacokinetic parameters are summarized in Table 1, and individual data are shown in Table 2. There were three subjects whose plasma concentrations of paroxetine 48 h after paroxetine dosing in control phase were under the detectable quantification (1.0 ng/ml). Although we were not able to calculate accurate averages in 12 subjects, we calculated the average at 48 h in Fig. 1, using the half of limit of quantification (0.5 ng/ml) in the three subjects. Extrapolated AUC of paroxetine was 9.3% for control and 24.9% for terbinafine, respectively.

The C_{max} of paroxetine during terbinafine treatment was higher than the corresponding value during placebo by 1.87-fold [95% confidence interval (95% CI), 1.49, 2.57-fold]. The AUC (0–48) of paroxetine during trebinafine treatment was higher than placebo by 2.53-fold (1.85, 4.58-fold). The total AUC of paroxetine during trebinafine treatment was higher than placebo by 2.88-fold (1.99, 5.41-fold). Elimination $t_{1/2}$ of paroxetine during trebinafine was significantly longer than that during placebo [1.35-fold (1.14, 1.70-fold)]. No change was found in t_{max} [1.14-fold (0.99, 1.36-fold)].

Bioequivalence analyses showed that 90% confidence interval of log-transformed C_{max}, AUC (0–48) and total AUC between two treatments were 130–148%, 116–127 and 118–128%, respectively.

There was significant correlation between number of mutated alleles for CYP2D6 and Cmax (r_s=0.772, p<0.01), AUC(0–48) (r_s=0.724, p<0.05) and total AUC (r_s=0.724, p<0.05). Significant correlations were found between

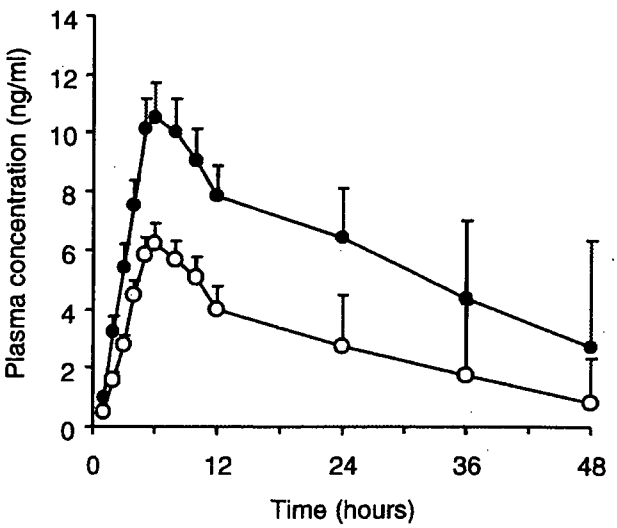

Fig. 1 Mean plasma concentration-time curves of paroxetine after a single oral 20 mg dose of paroxetine. *Open circles* indicate control and *solid circles* indicate terbinafine. *Error bars* indicate standard error

Table 1 Effects of terbinafinee treatment on paroxetine pharmacokinetic parameters after a single oral 20-mg dose of paroxetine in 12 healthy volunteers

Parameters	Control	Terbinafine	Ratio to control
C_{max}(ng/ml)	6.5±2.4	12.1±2.9***	1.92 (1.71, 2.22)
t_{max}(h)	5.0 (4.0–10.0)	6.0 (5.0–8.0)	-
AUC (0–48) (ng.h/ml)	127±67	318±102***	2.64 (2.07, 3.64)
AUC (0–∞) (ng.h/ml)	150±78	443±172***	3.03 (2.46, 4.08)
Vd/F (l)	3,599±1,515	1,577±528***	0.47 (0.36, 0.76)
CL/F (l/h)	167±76	58±44***	0.35 (0.25, 064)
Elimination half-life (h)	15.3±2.3	22.7±8.8*	1.39 (1.13, 1.86)

Data are shown as mean±SD for pharmacokinetic parameters and geometric mean
(95% confidence interval) for ratio to control
Data for tmax are shown as median and range
C_{max}, peak concentration; t_{max}, time to peak concentration in plasma; AUC (0–48), are under plasma concentration-time curve from 0 to 48 h; AUC (0–∞), AUC from 0 to infinity; Vd/F, apparent volume of distribution; CL/F, apparent total clearance
*p<0.05, ***p<0.001

number of mutated alleles for CYP2D6 and ratio of terbinafine phase to placebo for Cmax (r_s=−0.628, p<0.05), AUC(0–48) and (r_s=−0.774, p<0.01), but not total AUC (r_s=−0.477, ns). Elimination half-life was not correlated with number of mutated alleles for CYP2D6 (r_s=0.211, ns).

There was no relationship between total AUC and the observed side effects after single dose of paroxetine. No difference was found in different sequence (placeco–terbinafine versus terbinafine–placebo).

Discussion

The results of this study showed a significant increase in plasma concentration of paroxetine (C_{max} and AUC) during terbinafine treatment. Additionally, the paroxetine pharmacokinetics were not regarded as bioequivalent according to EMEA and FDA guidance. These findings imply that terbinafine increases the bioavailability of paroxetine or inhibits the metabolism of paroxetine. Our result was in accordance with the previous studies of dextrometrophan [19] and desipramine [20]. Paroxetine is almost completely absorbed following oral administration. However, because paroxetine undergoes extensive first pass metabolism, less than 50% of a single dose of paroxetine reaches the general circulation, suggesting that the first pass effect of paroxetine is more than 50% [4]. Therefore, terbinafine might inhibit the first pass effect of paroxetine in this study.

Furthermore, prolongation of elimination of half-life was observed during terbinafine coadministration. This suggests that terbinafine inhibits metabolism of paroxetine. Previous studies confirmed the decrease in their metabolites, 2-hydroxydesipramine [20] and dextrophan [19], during terbinafine as well as the increase in the substrates of CYP2D6, suggesting that terbinafine inhibits biotransformation from despramine to 2-hydroxydesipramine and from dextrometrophan to dextrophan. Unfortunately, we were unable to measure the metabolites of paroxetine because of undetectable levels in both phases.

Numbers of mutated alleles for CYP2D6 correlated well with Cmax and AUC, but not with elimination half-life in this study. This finding suggests that CYP2D6 activity is associated with the first pass effect of paroxetine. Furthermore, we found a significant relationship between CYP2D6 genotype and the magnitude of this interaction. In subjects having lower CYP2D6 activity, the inhibitory effect of

Table 2 Characteristics of healthy subjects and their individual data

No.	Age	Gender	Weight	CYP2D6	C_{max}(ng/ml)		AUC (0–∞) (ng.h/ml)		Elimination half-life (h)	
					Control	Terbinafine	Control	Terbinafine	Control	Terbinafine
1	29	Male	75	*1/*10	4.7	10.9	88	448	16.2	38.4
2	25	Male	63	*2/*10	6.2	14.2	123	547	12.4	29.6
3	21	Male	64	*1/*1	3.2	6.8	66	99	11.7	8.8
4	21	Female	47	*1/*2	4.3	11.6	72	419	17.0	15.8
5	26	Female	49	*2/*5	6.4	10.2	129	350	13.8	16.6
6	25	Male	57	*1/*1	4.8	10.2	78	350	13.3	16.6
7	20	Male	52	*5/*10	12.3	16.2	304	830	16.1	30.5
8	24	Female	46	*10/*10	9.3	16.9	286	574	16.2	22.9
9	22	Male	54	*1/*10	6.4	10.9	166	406	15.6	18.5
10	24	Male	62	*2/*10	6.9	9.9	141	409	14.1	33.0
11	25	Male	64	*1/*1	6.3	14.9	161	385	18.9	15.7
12	35	Male	66	*1/*10	6.9	12.4	185	510	19.0	25.9

C_{max}, peak concentration; AUC (0–∞), AUC from 0 to infinity;

terbinafine is expected to have been smaller. This confirms that these interactions resulted from CYP2D6 inhibition.

An earlier in vivo study demonstrated that the interphenotype difference in metabolism was less prominent at steady state than after a single dose [7]. There is ongoing controversy as to whether or not the steady-state plasma concentration of paroxetine is different between CYP2D6 genotypes. A recent study indicated no relationship between CYP2D6 genotypes and steady-state plasma concentration of paroxetine [24], although there were significant differences between the CYP2D6 genotypes [25, 26]. Therefore, the magnitude of our interaction might be reduced after repeated doses. Thus, further studies are required to confirm our finding even at steady state.

Severely depressed patients tend to suffer from dermatophytosis in skin, hair and nails due to the difficulty in self-care. On the other hand, systemic fungal infections remain a major clinical problem in immunocompromised patients and such patients tend to have negative thinking [27]. From a clinical point of view, it is more likely that terbinafine is added to depressed patients treated with paroxetine. Thus, interaction between paroxetine and terbinafine should be kept in mind by physicians. Although there is no information about drug interaction between repeated doses of these drugs, dosage of paroxetine should be adjusted based on our result showing the increased exposure of paroxetine during terbinafine.

Limitations of this study included sampling time and washout period. We had a 4-week washout period in this study. Extrapolated AUC of paroxetine was 9.3% for control and 24.9% for terbinafine. If we took blood samples for a longer period (e.g., 72 h), more accurate parameters of paroxetine might have been calculated. In addition, a case report suggested that the effect of terbinafine on CYP2D6-mediated amitriptyline and nortriptyline concentration continues for at least 6 months [23]. Madani et al. [20] suggest that the inhibitory effect of terbinafine on CYP2D6 measured by desipramine continues for 4 weeks, while Abdel-Rahman et al. [19] suggest that CYP2D6 inhibition of terbinafine measured by dextrometrophan metabolic ratios in most subjects return to baseline at 4 weeks after discontinuation of terbinafine. Although half dose (125 mg) and shorter duration (7 days) than previous studies by Madani et al. (250 mg for 21 days) and Abdel-Rahman et al. (250 mg for 14 days) were used in our study and no difference was found in different sequences (placeco–terbinafine versus terbinafine–placebo), the inhibitory effect of terbinafine on CYP2D6 might remain until the control phase.

In conclusion, the present study showed that terbinafine increased paroxetine exposure, probably because of an increase in bioavailability through CYP2D6 inhibition. A change in regulation of CYP2D6 may lead to significant alteration of paroxetine pharmacokinetics.

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References

1. Burrows GD, McIntyre IM, Judd FK, Norman TR (1988) Clinical effects of serotonin reuptake inhibitors in the treatment of depressive illness. J Clin Psychiatry 49(Suppl):18–22
2. Gunasekara NS, Noble S, Benfield P (1998) Paroxetine. An update of its pharmacology and therapeutic use in depression and a review of its use in other disorders. Drugs 55:85–120
3. Kaye CM, Haddock RE, Langley PF, Mellows G, Tasker TC, Zussman BD, Greb WH (1989) A review of the metabolism and pharmacokinetics of paroxetine in man. Acta Psychiatr Scand Suppl 350:60–75
4. van Harten J (1993) Clinical pharmacokinetics of selective serotonin reuptake inhibitors. Clin Pharmacokinet 24:203–220
5. Haddock RE, Johnson AM, Langley PF, Nelson DR, Pope JA, Thomas DR, Woods FR (1989) Metabolic pathway of paroxetine in animals and man and the comparative pharmacological properties of its metabolites. Acta Psychiatr Scand (Suppl) 350:24–26
6. Sindrup SH, Brosen K, Gram LF, Hallas J, Skjelbo E, Allen A, Allen GD, Cooper SM, Mellows G, Tasker TC et al (1992) The relationship between paroxetine and the sparteine oxidation polymorphism. Clin Pharmacol Ther 51:278–287
7. Sindrup SH, Brosen K, Gram LF (1992) Pharmacokinetics of the selective serotonin reuptake inhibitor paroxetine: nonlinearity and relation to the sparteine oxidation polymorphism. Clin Pharmacol Ther 51:288–295
8. Bloomer JC, Woods FR, Haddock RE, Lennard MS, Tucker GT (1992) The role of cytochrome P4502D6 in the metabolism of paroxetine by human liver microsomes. Br J Clin Pharmacol 33:521–523
9. Skjelbo E, Brosen K (1992) Inhibitors of imipramine metabolism by human liver microsomes. Br J Clin Pharmacol 34:256–261
10. Ramamoorthy Y, Tyndale RF, Sellers EM (2001) Cytochrome P450 2D6.1 and cytochrome P450 2D6.10 differ in catalytic activity for multiple substrates. Pharmacogenetics 11:477–487
11. Laugesen S, Enggaard TP, Pedersen RS, Sindrup SH, Brosen K (2005) Paroxetine, a cytochrome P450 2D6 inhibitor, diminishes the stereoselective O-demethylation and reduces the hypoalgesic effect of tramadol. Clin Pharmacol Ther 77:312–323
12. Saito M, Yasui-Furukori N, Nakagami T, Furukori H, Kaneko S (2005) Dose-dependent interaction of paroxetine with risperidone in schizophrenic patients. J Clin Psychopharmacol 25:527–532
13. Clayton YM (1989) In vitro activity of terbinafine. Clin Exp Dermatol 14:101–103
14. Balfour JA, Faulds D (1992) Terbinafine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. Drugs 43:259–284
15. Abdel-Rahman SM, Nahata MC (1997) Oral terbinafine: a new antifungal agent. Ann Pharmacother 31:445–456
16. Abdel-Rahman SM, Marcucci K, Boge T, Gotschall RR, Kearns GL, Leeder JS (1999) Potent inhibition of cytochrome P-450 2D6-mediated dextromethorphan O-demethylation by terbinafine. Drug Metab Dispos 27:770–775
17. Vickers AE, Sinclair JR, Zollinger M, Heitz F, Glanzel U, Johanson L, Fischer V (1999) Multiple cytochrome P-450s involved in the

metabolism of terbinafine suggest a limited potential for drug-drug interactions. Drug Metab Dispos 27:1029–1038
18. Venkatakrishnan K, von Moltke LL, Greenblatt DJ (2000) Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. Clin Pharmacokinet 38:111–180
19. Abdel-Rahman SM, Gotschall RR, Kauffman RE, Leeder JS, Kearns GL (1999) Investigation of terbinafine as a CYP2D6 inhibitor in vivo. Clin Pharmacol Ther 65:465–472
20. Madani S, Barilla D, Cramer J, Wang Y, Paul C (2002) Effect of terbinafine on the pharmacokinetics and pharmacodynamics of desipramine in healthy volunteers identified as cytochrome P450 2D6 (CYP2D6) extensive metabolizers. J Clin Pharmacol 42:1211–1218
21. van der Kuy PH, Hooymans PM (1998) Nortriptyline intoxication induced by terbinafine. Br Med J 316:441
22. O'Reardon JP, Hetznecker JM, Rynn MA, Baldassano CF, Szuba MP (2002) Desipramine toxicity with terbinafine. Am J Psychiatry 159:492
23. Castberg I, Helle J, Aamo TO (2005) Prolonged pharmacokinetic drug interaction between terbinafine and amitriptyline. Ther Drug Monit 27:680–682
24. Ueda M, Hirokane G, Morita S, Okawa M, Watanabe T, Akiyama K, Shimoda K (2006) The impact of CYP2D6 genotypes on the plasma concentration of paroxetine in Japanese psychiatric patients. Prog Neuropsychopharmacol Biol Psychiatry 30:486–491
25. Sawamura K, Suzuki Y, Someya T (2004) Effects of dosage and CYP2D6-mutated allele on plasma concentration of paroxetine. Eur J Clin Pharmacol 60:553–557
26. Lam YW, Gaedigk A, Ereshefsky L, Alfaro CL, Simpson J (2002) CYP2D6 inhibition by selective serotonin reuptake inhibitors: analysis of achievable steady-state plasma concentrations and the effect of ultrarapid metabolism at CYP2D6. Pharmacotherapy 22:1001–1006
27. Penzak SR, Reddy YS, Grimsley SR (2000) Depression in patients with HIV infection. Am J Health Syst Pharm 57:376–386

Effect of Itraconazole on Pharmacokinetics of Paroxetine: The Role of Gut Transporters

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Abstract: A recent in vitro study has shown that paroxetine is a substrate of P-glycoprotein. However, there was no in vivo information indicating the involvement of P-glycoprotein on the pharmacokinetics of paroxetine. The aim of this study was to examine the effects of itraconazole, a P-glycoprotein inhibitor, on the pharmacokinetics of paroxetine. Two 6 day courses of either 200 mg itraconazole daily or placebo with at least a 4 week washout period were conducted. Thirteen volunteers took a single oral 20 mg dose of paroxetine on day 6 of both courses. Plasma concentrations of paroxetine were monitored up to 48 hours after the dosing. Compared with placebo, itraconazole treatment significantly increased the peak plasma concentration (C_{max}) of paroxetine by 1.3 fold (6.7 ± 2.5 versus 9.0 ± 3.3 ng/mL, $P < 0.05$) and the area under the plasma concentration-time curve from zero to 48 hours [AUC (0–48)] of paroxetine by 1.5 fold (137 ± 73 versus 199 ± 91 ng*h/mL, $P < 0.01$). Although elimination half-life differed significantly (16.1 ± 3.4 versus 18.8 ± 5.9 hours, $P < 0.05$), the alteration was small (1.1 fold). The present study demonstrated that the bioavailability of paroxetine was increased by itraconazole, suggesting a possible involvement of P-glycoprotein in the pharmacokinetics of paroxetine.

Key Words: paroxetine, itraconazole, P-glycoprotein, interaction

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INTRODUCTION

Recently, it has become increasingly evident that drug transporters have a pivotal role in the pharmacokinetics of numerous drugs, with therapeutic implications.[1–6] Numerous studies have revealed that targeted expression of drug uptake and efflux transport to specific cell membrane domains allows for the efficient directional movement of many drugs in clinical use.[1–6] Transport by ATP-dependent efflux pumps such as P-glycoprotein influences the intestinal absorption[7,8] and renal[9,10] or hepatic elimination[11] and central nervous system concentrations[8] of many drugs.

Paroxetine is one of the selective serotonin transporter inhibitors and is widely used in the treatment of mental disorders, including depression, panic disorders, and obsessive-compulsive disorder.[12] Because paroxetine is metabolized by cytochrome isoenzyme P450 (CYP) 2D6[13] and inhibits CYP2D6 activity,[14,15] drug–drug interaction with paroxetine through CYP2D6 inhibition has been a significant concern. On the other hand, we showed a case in which digitalis intoxication was induced by coadministration of paroxetine through P-glycoprotein inhibition.[16] Also, a recent in vitro study demonstrated that P-glycoprotein inhibitory activity with paroxetine was more potent than the known P-glycoprotein inhibitor quinidine.[17] Another in vitro study reported that the cerebrum concentrations of paroxetine were higher in knockout mice.[18] Therefore, paroxetine is not only an inhibitor, but also a substrate of P-glycoprotein. To date, however, there are no in vivo data indicating that paroxetine as a substrate of P-glycoprotein is of clinical relevance.

The triazole antifungal agent, itraconazole, has a wide spectrum of antifungal activity in vitro.[19] Several studies have demonstrated a drug interaction between itraconazole and neuropsychiatric agents, including midazolam,[20] triazolam,[21] alprazolam,[22] haloperidol,[23] and bromperidol,[24] presumably as a result of inhibition of CYP3A4 by itraconazole. Meanwhile, P-glycoprotein reversal agents, including itraconazole, have been demonstrated to alter the pharmacokinetic properties of coadministered agents in therapeutic areas.[25,26] The effect of itraconazole was concentration-dependent with cimetidine's apparent permeability value for basolateral-to-apical transport decreasing from 3.96 to 1.92 × 10^{-6} cm/second ($P < 0.05$), resulting in a 50% decrease in efflux ratio. The MDR1-mediated transport of [3H]digoxin was inhibited by ketoconazole and itraconazole, and slightly by miconazole, suggesting that itraconazole has an inhibitory effect on P-glycoprotein.

Based on these findings, it is possible that itraconazole affects the pharmacokinetics of paroxetine. To our knowledge, there is no information about a drug interaction between itraconazole and paroxetine. The aim of this study was to confirm the effects of itraconazole, a transporting inhibitor, on the disposition of paroxetine.

METHODS

Subjects

Thirteen healthy Japanese volunteers (10 male, 3 female) were enrolled in this study. Their mean ± standard deviation

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age (range) was 24.2 ± 3.5 years (range, 21–35 years) and mean body weight was 57.3 ± 7.2 kg (range, 45–67 kg). The Ethics Committee of Hirosaki University School of Medicine approved the study protocol, and written informed consent was obtained from each participant before any examinations.

Study Design

A randomized crossover study design was conducted at intervals of 4 weeks. Two 50 mg capsules of itraconazole twice daily (8 AM, 8 PM) or matched placebo with 240 mL of tap water were given for 6 days. The volunteers took a single oral 20 mg dose of paroxetine at 9 AM on day 6 with 240 mL of tap water. Compliance of test drug was confirmed by pill count. No other medications were taken during the study periods. No meal was allowed until 4 hours after dosing (1 PM). The use of alcohol, tea, coffee, and cola was forbidden during the test days.

Sample Collections

Blood samplings (10 mL each) for determination of paroxetine were taken into heparinized tubes just before and 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 hours after the administration of paroxetine. Plasma was separated immediately and kept at –30°C until analysis. At the time of blood samplings, blood pressure and heart rate were measured. Any adverse events were reported by subjects.

Assay

Plasma concentrations of paroxetine were measured using a high-performance liquid chromatographic method developed in our laboratory. In brief, the extraction procedure was as follows: to 2 mL of plasma sample was added 500 μL of 0.5 M NaOH, 100 μL of internal standard solution (200 μg/mL trifluperidol), and 100 μL of methanol. Thereafter, the tubes were vortex-mixed for 10 seconds and 5 mL of n-heptane–chloroform (70:30, v/v) was added as extraction solvent. After 10 minutes of shaking, the mixture was centrifuged at 2500*g* for 10 minutes at 4°C, and the organic phase was evaporated to dryness in vacuo at 40°C (TAITEC VC-960; Shimadzu, Kyoto, Japan). The residue was dissolved in 500 μL of mobile phase. A total of 400 μL was injected onto the HPLC system. The HPLC system consisted of Shimadzu LC-10AT high-pressure pumps, a Shimadzu CTO-10AVP column oven, a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan), a Shimadzu SIL-10ADVP (500 μL injection volume) (Tokyo, Japan), and a column (STR-ODS II C18 150 × 4.6, 3 μm) (Shimadzu, Tokyo, Japan). The mobile phase was phosphate buffer (0.02 M, pH = 4.6), acetonitrile, and perchloric acid (60%) (57.25: 42.5: 0.25, v/v/v). The lower limit of detection was 0.5 ng/mL for paroxetine, and the values of the intraassay and interassay coefficients of variation were less than 5% at all the concentrations (2.5–150 ng/mL) of the calibration curve for paroxetine.

Date Analyses of Pharmacokinetics

The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were obtained directly from the original data. The area under the plasma concentration-time curve [AUC (0–48)] was calculated using the lin-lin trapezoidal rule. AUC from zero to infinity [AUC(0–∞)] and elimination half-life were determined by noncompartment model with WinNonlin Professional software (Pharsight Co., Cary, NC).

Statistical Analysis

Data are shown as mean ± standard deviation in tables and mean ± standard error in figures. Paired *t* test was used for the comparison of the plasma drug concentrations between 2 phases, ie, placebo and itraconazole. The comparison of t_{max} was performed using the Wilcoxon signed-sample test. A *P* value of 0.05 or less was regarded as significant. Geometric mean ratios to corresponding values in the placebo phase with 95% confidence intervals were used for detection of significant difference. When the 95% confidence interval did not cross 1.0, the result was also regarded as significant. SPSS 13.0J for Windows SPSS Japan Inc., Tokyo, was used for these statistical analyses.

RESULTS

Appetite loss (n = 4, n = 7), abdominal disturbance (n = 4, n = 6), diarrhea (n = 1, n = 1), asthenia (n = 5, n = 6), and sleepiness (n = 3, n = 4) were observed in control and itraconazole phases, respectively. These side effects were mild to moderate and occurred 2 hours after doses of paroxetine and all recovered, at most, within 2 days after the doses.

Plasma drug concentration-time curves during both placebo and itraconazole treatments are shown in Figure 1, and their pharmacokinetic parameters are summarized in Table 1. There were 3 subjects whose plasma concentrations of paroxetine 48 hours after paroxetine dosing in the control phase were under the detection limit (0.5 ng/mL). We were not able to calculate the accurate average in all 13 subjects because of the 3 unavailable data. Therefore, we do not show

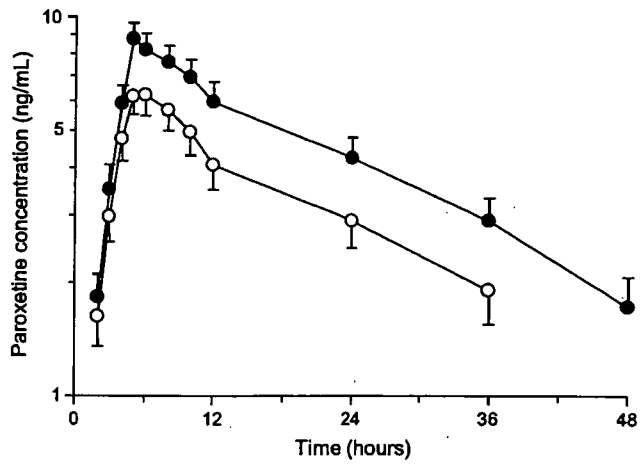

FIGURE 1. Mean plasma concentration-time curves of paroxetine after a single oral 20 mg dose of paroxetine. Open circles are control and solid circles are itraconazole treatment (100 mg twice daily for 6 days). Error bars indicates standard error. Control at 48 hours is not shown because of undetectable concentrations in 3 subjects.

TABLE 1. Effects of Itraconazole Treatment (200 mg for 6 days) on Paroxetine Pharmacokinetic Parameters After a Single Oral 20 mg Dose of Paroxetine in 13 Healthy Volunteers

Parameters	Control	Itraconazole	Ratio to Control
C_{max} (ng/mL)	6.7 ± 2.5	9.0 ± 3.3*	1.30 (1.01, 1.77)
t_{max} (h)	5.0 (4.0–8.0)	5.0 (5.0–8.0)	1.06 (0.92, 1.19)
AUC (0–48) (ng*h/mL)	137 ± 73	199 ± 91**	1.51 (1.08, 2.36)
AUC (0–∞) (ng*h/mL)	165 ± 93	256 ± 141**	1.56 (1.14, 2.39)
Cl/F (L/hr)	159 ± 82	101 ± 48*	0.64 (0.53, 0.87)
Vd/F (L)	3479 ± 1576	2499 ± 1015*	0.73 (0.61, 0.98)
Elimination half-life (h)	16.1 ± 3.4	18.8 ± 5.9*	1.14 (1.01, 1.34)

*$P < 0.05$, **$P < 0.01$, comparted with control.
Data are shown as mean ± SD for pharmacokinetic parameters except for t_{max}.
Data for t_{max} are shown as median (range).
Ratio to control are shown as geometric mean (95% confidance interval).
C_{max}, peak concentration; t_{max}, time to peak concentration in plasma;
AUC (0–48), are under plasma concentration-time curve from 0 to 48 hours;
AUC (0–∞), AUC from 0 to infinity; Cl/F, apparent total clearance; Vd/F, apparent volume of distribution.

the data at 48 hours in Figure 1. The percentage extrapolated AUC were 15 ± 7% for control and 21 ± 8% for itraconazole.

The paroxetine C_{max} during itraconazole treatment was higher than the corresponding value during placebo by 1.30-fold [95% confidence interval (95% CI), 1.01–1.77-fold]. The AUC (0–48) of paroxetine during itraconazole treatment was higher than placebo by 1.51-fold (1.08–2.36-fold). The total AUC of paroxetine during itraconazole treatment was higher than placebo by 1.56-fold (1.14–2.39-fold). Elimination $t_{1/2}$ of paroxetine during itraconazole was significantly longer than that during placebo [1.14-fold (1.01–1.34-fold)]. No change was found in t_{max} [1.06-fold (0.92–1.19-fold)].

There was no relationship between total AUC and the observed side effects after a single dose of paroxetine.

DISCUSSION

The results of this study showed a significant increase in plasma concentration of paroxetine (C_{max} and AUC) during itraconazole treatment. These findings imply that itraconazole increases the bioavailability of paroxetine or decreases the total clearance of paroxetine. Although itraconazole prolonged the elimination $t_{1/2}$ of paroxetine in this study, the alteration was small (14%). Therefore, it appears that the bioavailability of paroxetine was increased by itraconazole, which might be attributed to increased absorption of paroxetine in the small intestine or inhibition of extraction into bile in the liver.

Severely depressed patients tend to have dermatophytosis in skin, hair, and nails as a result of difficulties with self-care. On the other hand, systemic fungal infections remain a major clinical problem in immunocompromised patients and such patients tend to have negative thinking.[27] From a clinical point of view, it is more likely that itraconazole would be added to depressed patients treated with paroxetine. Thus, a pharmacokinetic interaction between paroxetine and itraconazole should be kept in mind by physicians, although the magnitude may not be large.

Several in vitro and in vivo studies have consistently suggested that paroxetine is both a substrate and an inhibitor of cytochrome isoenzyme P450 (CYP) 2D6.[13,14] Therefore, drug–drug interaction with paroxetine through only CYP2D6 inhibition has been a concern. On the other hand, the stimulation of the inhibition of the metabolic activities mediated by CYP1A2, CYP2D6, or CYP2E1 by 15 minute preincubation was not observed for any of the antifungal drugs, suggesting that these antifungal drugs, including itraconazole, are not mechanism-based inhibitors.[28] Furthermore, the ratio of risperidone/9-hydroxyrisperidone, an index of CYP2D6 activity, did not differ before itraconazole treatment (0.14 ± 0.13), after itraconazole treatment (0.15 ± 0.13), and 1 week after discontinuation (0.14 ± 0.13) ($P > 0.05$).[29] Therefore, it is unlikely that itraconazole inhibits paroxetine metabolism catalyzed by CYP2D6.

Because itraconazole is regarded as an inhibitor of CYP3A[20,21] as well as P-glycoprotein[25,26] based on several in vitro and in vivo investigations, it is possible that significant interaction between these drugs occurs as a result of inhibition of CYP3A. Consistently, drug interaction with itraconazole showed a large prolongation of elimination half-life of test drugs.[20–22] However, although statistically significant, alteration of elimination of half-life of paroxetine was small (14%). In addition, there were no data indicating the involvement of CYP3A4 in the metabolism of paroxetine but only CYP2D6. Thus, it seems that the drug interaction did not lead to an inhibitory effect of itraconazole on hepatic CYP3A.

An in vitro study reported that the cerebrum concentrations of paroxetine were higher in knockout mice, suggesting that paroxetine is a substrate of P-glycoprotein.[17] Meanwhile another study showed that neither verapamil nor P-glycoprotein-selective antagonist PGP-4008 affected the intracellular accumulation of [3H]paroxetine, [14C]phenytoin, [3H]clozapine, or [14C]carbamazepine in bovine retinal endothelial cells, indicating that these drugs are not substrates for P-glycoprotein.[30] We do not have a clear explanation for this discrepancy. Furthermore, in vitro studies are required to confirm the affinity of paroxetine as a substrate of P-glycoprotein.

There was no relationship between total AUC and the observed side effects after a single dose of paroxetine in this study. However, it is possible that itraconazole inhibits the activity of transporter(s) in the blood–brain barrier, resulting in higher paroxetine concentration in the brain. When both paroxetine and itraconazole are administered repeatedly for a long time, or if a more potent inhibitor of transporters than itraconazole is administered concomitantly with paroxetine, it would appear that careful monitoring of patients is required.

In conclusion, the present study showed that itraconazole increased paroxetine exposure, probably because of an increase in bioavailability through P-glycoprotein inhibition. Changes in the regulation of transporters such as P-glycoprotein may lead to a significant alteration of paroxetine pharmacokinetics.

REFERENCES

1. Ayrton A, Morgan P. Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica*. 2001;31:469–497.

2. Kim RB. Transporters and xenobiotic disposition. *Toxicology.* 2002;27: 181–182, 291–297.
3. Hochman JH, Yamazaki M, Ohe T, et al. Evaluation of drug interactions with P-glycoprotein in drug discovery: in vitro assessment of the potential for drug–drug interactions with P-glycoprotein. *Curr Drug Metab.* 2002; 3:257–273.
4. Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet.* 2003;42:59–98.
5. Fromm MF. Importance of P-glycoprotein for drug disposition in humans. *Eur J Clin Invest.* 2003;33(Suppl 2):6–9.
6. Sun J, He ZG, Cheng G, et al. Multidrug resistance P-glycoprotein: crucial significance in drug disposition and interaction. *Med Sci Monit.* 2004;10: RA5–14.
7. Suzuki H, Sugiyama Y. Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. *Eur J Pharm Sci.* 2000; 12:3–12.
8. Fromm MF. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *Int J Clin Pharmacol Ther.* 2000;38:69–74.
9. Inui KI, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. *Kidney Int.* 2000;58:944–958.
10. Lee W, Kim RB. Transporters and renal drug elimination. *Annu Rev Pharmacol Toxicol.* 2004;44:137–166.
11. Hooiveld GJ, van Montfoort JE, Meijer DK, et al. Function and regulation of ATP-binding cassette transport proteins involved in hepatobiliary transport. *Eur J Pharmacol Sci.* 2001;12:525–543.
12. Gunasekara NS, Noble S, Benfield P. Paroxetine. An update of its pharmacology and therapeutic use in depression and a review of its use in other disorders. *Drugs.* 1998;55:85–120.
13. Yoon YR, Cha IJ, Shon JH, et al. Relationship of paroxetine disposition to metoprolol metabolic ratio and CYP2D6*10 genotype of Korean subjects. *Clin Pharmacol Ther.* 2000;67:567–576.
14. Solai LK, Pollock BG, Mulsant BH, et al. Effect of nortriptyline and paroxetine on CYP2D6 activity in depressed elderly patients. *J Clin Psychopharmacol.* 2002;22:481–486.
15. Bertelsen KM, Venkatakrishnan K, Von Moltke LL, et al. Apparent mechanism-based inhibition of human CYP2D6 in vitro by paroxetine: comparison with fluoxetine and quinidine. *Drug Metab Dispos.* 2003;31:289–293.
16. Yasui-Furukori N, Kaneko S. Digitalis intoxication induced by paroxetine co-administration. *Lancet.* 2006;367:788.
17. Weiss J, Dormann SM, Martin-Facklam M, et al. Inhibition of P-glycoprotein by newer antidepressants. *J Pharmacol Exp Ther.* 2003; 305:197–204.
18. Uhr M, Grauer MT, Holsboer F. Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. *Biol Psychiatry.* 2003;54:840–846.
19. Borgers M, Degreef H, Cauwenbergh G. Fungal infections of the skin: infection process and antimycotic therapy. *Curr Drug Targets.* 2005;6: 849–862.
20. Olkkola KT, Backman JT, Neuvonen PJ. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther.* 1994;55:481–485.
21. Varhe A, Olkkola KT, Neuvonen PJ. Oral triazolam is potentially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther.* 1994;56:601–607.
22. Yasui N, Kondo T, Otani K, et al. Effect of itraconazole on the single oral dose pharmacokinetics and pharmacodynamics of alprazolam. *Psychopharmacology.* 1998;139:269–273.
23. Yasui N, Kondo T, Otani K, et al. Effects of itraconazole on the steady-state plasma concentrations of haloperidol and its reduced metabolite in schizophrenic patients: in vivo evidence of the involvement of CYP3A4 for haloperidol metabolism. *J Clin Psychopharmacol.* 1999;19:149–154.
24. Furukori H, Kondo T, Yasui N, et al. Effects of itraconazole on the steady-state plasma concentrations of bromperidol and reduced bromperidol in schizophrenic patients. *Psychopharmacology.* 1999;145:189–192.
25. Takara K, Tanigawara Y, Komada F, et al. Cellular pharmacokinetic aspects of reversal effect of itraconazole on P-glycoprotein-mediated resistance of anticancer drugs. *Biol Pharm Bull.* 1999;22:1355–1359.
26. Sakaeda T, Iwaki K, Kakumoto M, et al. Effect of micafungin on cytochrome P450 3A4 and multidrug resistance protein 1 activities, and its comparison with azole antifungal drugs. *J Pharm Pharmacol.* 2005;57: 759–764.
27. Penzak SR, Reddy YS, Grimsley SR. Depression in patients with HIV infection. *Am J Health Syst Pharm.* 2000;57:376–386.
28. Niwa T, Inoue-Yamamoto S, Shiraga T, et al. Effect of antifungal drugs on cytochrome P450 (CYP) 1A2, CYP2D6, and CYP2E1 activities in human liver microsomes. *Biol Pharm Bull.* 2005;28:1813–1816.
29. Jung SM, Kim KA, Cho HK, et al. Cytochrome P450 3A inhibitor itraconazole affects plasma concentrations of risperidone and 9-hydroxyrisperidone in schizophrenic patients. *Clin Pharmacol Ther.* 2005;78:520–528.
30. Maines LW, Antonetti DA, Wolpert EB, et al. Evaluation of the role of P-glycoprotein in the uptake of paroxetine, clozapine, phenytoin and carbamazepine by bovine retinal endothelial cells. *Neuropharmacology.* 2005;49:610–617.

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No association between CYP2D6 polymorphisms and personality trait in Japanese

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What is already known about this subject
- CYP2D6 mediates, to some extent, the synthesis of the neurotransmitters, serotonin and dopamine in the brain. A positive relationship between CYP2D6 activity and personality has been suggested, while other studies have failed to find a relationship.
- Therefore, because of these inconsistent findings, the association between CYP2D6 polymorphism and interindividual variability in personality has not yet been resolved.

What this study adds
- The detailed genotypes for *CYP2D6* were identified using DNA tip in a large number of Japanese subjects. However there were no correlations between scores of personality and *CYP2D6* genotypes.
- This study demonstrated that there was no significant association between CYP2D6 activity and personality trait in the Japanese population.

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Aims
The polymorphic enzyme CYP2D6 is expressed not only in liver but also in brain at low concentrations. CYP2D6 mediates, to some extent, the synthesis of the neurotransmitters, serotonin and dopamine. We investigated a possible association between the genetic polymorphism of CYP2D6 and individual personality trait.

Methods
Mentally and physically healthy volunteers were recruited ($n = 342$). Temperament and Character Inventory (TCI) and *CYP2D6* genotyping were performed in all subjects. We detected mutated alleles which were identified using the Amplichip CYP450 DNA chip.

Results
The number of phenotypes, assumed by genotype for ultrarapid metabolizers (UM), extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM) were 4 (1.1%), 262 (76.6%), 75 (21.9%) and 1 (0.3%), respectively. There were no differences in scores for novelty seeking, harm avoidance, reward dependence or persistence among the *CYP2D6* phenotypes. The number of mutated alleles for CYP2D6 did not differ for scores of novelty seeking, harm avoidance, reward dependence or persistence. In subitem analyses, only RD3 (attachment) had a significant difference both in the *CYP2D6* phenotype ($P < 0.05$) and genotype ($P < 0.05$).

Conclusions
This study did not demonstrate a significant association between CYP2D6 activity and personality trait because of the small interindividual variability in CYP2D6 activity within the Japanese population.

Introduction

Cloninger *et al.* [1] demonstrated that human personality consists of seven dimensions including three temperament dimensions and four character dimensions, and on the basis of this model they developed the temperament and character inventory (TCI), a questionnaire for assessing personality traits. Of the temperament dimensions, which include novelty seeking, harm avoidance, reward dependence, and persistence, three have been assumed to be related to monoamine neurotransmitters, novelty seeking with dopaminergic activities, harm avoidance with serotonergic activities and reward dependence with noradrenergic activity.

It has been reported that CYP2D6 is expressed not only in the liver but also in the brain at low concentrations [2]. The CYP2D6 in the brain has been shown to interact with the dopamine transporter and it has been suggested that it plays a role in the catabolism and processing of neurotransmitters [3–5]. Genetic polymorphism in CYP2D6 has been associated with smoking behaviour and this modification may occur through the involvement of CYP2D6 in the dopaminergic pathway. In addition, CYP2D6 mediates the synthesis of the neurotransmitters, serotonin and dopamine, from tyramine and 5-methoxytryptamine [2]. Since these neurotransmitters are linked with personality trait according to Cloninger's model, it is likely that genetic polymorphism of CYP2D6 is associated with individual personality. However, the association is inconsistent. Several studies have suggested a positive relationship between CYP2D6 activity and personality [6–8], while another study failed to find the relationship [9].

Therefore, the aim of the present study was to clarify the possible relationship between genotypes of *CYP2D6* polymorphism and behavioural traits in a large sample of Japanese, as measured by the TCI.

Methods

This study was approved by the Ethics Committee of Hirosaki University School of Medicine. The subjects enrolled consisted of Japanese students and medical staff within the medical school ($n = 342$, male = 166, female = 176). Their mean age was 29.9 ± 11.4 years (range 18–69 years). After giving the subjects a full description of the study, written informed consent to participate was obtained from each of them. The subjects were asked to complete the 240 items of the Japanese version of the TCI, whose reliability and validity had been established by Kijima *et al.* [10]. The 240 items were allocated into four factors of temperaments (novelty seeking, harm avoidance, reward dependence and persistence) and three factors of characters (self-directedness, co-operativeness and self-transcendence), where self-transcendence consisted of self-forgetfulness, transpersonal identification and spiritual acceptance. Genotyping of CYP2D6 was performed using the AmpliChip CYP450 test® DNA chip (Roche Co., Tokyo, Japan). The AmpliChip CYP450 Test provides materials for genotyping the *CYP2D6* gene, encompassing 31 known mutations (CYP2D6*2, *3, *4, *5, *6, *7, *8, *9, *10AB, *11, *14, *15, *17, *18, *19, *20, *25, *26, *29, *30, *31, *35, *36, *40, *41, *1XN, *2XN, *4XN). The alleles which led to no enzyme activity are CYP2D6*3, *4, *5, *6, *7, *8, *11, *14, *15, *19, *20 and *40. The alleles which led to decreased enzyme activity are CYP2D6*9, *10, *17, *29, *36 and *41. CYP2D6*2 and *35 are regarded as normal enzyme activity. Based on the *CYP2D6* genotypes, subjects were grouped into four main phenotypes: poor metabolizer (PM) (two deficit alleles, e.g. *5/*5), intermediate metabolizer (IM) (either two reduced activity alleles or one reduced activity allele and one no activity allele, e.g. *4/*10, *5/*10, *10/*10), extensive metabolizer (EM) (one or more wild type alleles, e.g. *1/*1, *1/*2, *1/*5, *1/*10), and ultrarapid metabolizer (UM) (more than two copies of wild type, e.g. *1/*1XN, *1/*2XN). The mean scores for the seven factors of the TCI were compared among UM, EM and IM and between the number of mutated alleles using ANOVA. Because only one of our subjects was a PM, PM was excluded from the statistical analysis. The result was defined as being significant at $P < 0.05$.

Results

The genotypes of our subjects were *1/*1 ($n = 65$, 19.0%), 1/*2 ($n = 33$, 9.6%), *1/*4 ($n = 1$, 0.3%), *1/*5 ($n = 95$, 4.4%), *1/*10 ($n = 95$, 27.8%), *1/*14 ($n = 5$, 0.3%), *1/*36 ($n = 1$, 0.3%), *1/*41 ($n = 5$, 1.5%), *1/*1xN ($n = 2$, 0.6%), *1/*2xN ($n = 1$, 0.3%), *2/*2 ($n = 7$, 2.0%), *2/*4 ($n = 1$, 0.3%), *2/*5 ($n = 6$, 1.8%), *2/*10 ($n = 30$, 8.8%), *2/*14 ($n = 1$, 0.3%), *2/*41 ($n = 1$, 0.3%), *2/*2xN ($n = 1$, 0.3%), *4/*10 ($n = 1$, 0.3%), *5/*5 ($n = 1$, 0.3%)), *5/*10 ($n = 19$, 5.6%), *5/*36 ($n = 1$, 0.3%), *10/*10 ($n = 50$, 14.6%), *10/*14 ($n = 1$, 0.3%), *10/*41 ($n = 31$, 0.9%). The number of phenotypes, assumed by genotype, for UM, EM, IM and PM were 4 (1.1%), 262 (76.6%), 75 (21.9%) and 1 (0.3%), respectively. There were no differences in the mean scores for the seven TCI factors between the phenotypes, assumed by genotype (Table 1). In subitem analyses, only RD3 (Attachment) had a significant difference in *CYP2D6* phenotype ($P = 0.017$).

The number of *CYP2D6* genotypes based on the number of mutated alleles was 109 (31.9%) for zero

Table 1
Comparison of the temperament and character inventory (TCI) subitem scores among phenotypes assumed by *CYP2D6* genotypes

	UM ($n=4$)	EM ($n=262$)	IM ($n=75$)	PM ($n=1$)	Significance
Novelty seeking	18.3 (16.7, 19.8)	21.4 (20.9, 22.0)	21.5 (20.4, 22.5)	27	NS
Harm avoidance	19.3 (13.2, 25.3)	19.8 (19.0, 20.6)	20.0 (18.6, 21.2)	17	NS
Reward dependence	17.5 (15.9, 19.1)	14.7 (14.3, 15.1)	15.5 (14.7, 16.2)	15	NS
Persistence	4.8 (1.2, 8.3)	4.4 (4.1, 4.6)	4.2 (3.8, 4.5)	7	NS
Self-directedness	32.0 (24.1, 40.0)	26.1 (25.3, 26.9)	26.8 (25.2, 28.4)	27	NS
Co-operativeness	31.5 (27.3, 35.7)	28.0 (27.4, 28.6)	28.4 (27.2, 29.6)	32	NS
Self-transcendence	7.8 (4.5, 11.0)	10.7 (10.1, 11.3)	9.6 (8.4, 10.7)	13	NS

Data are mean (95% confidence interval); UM; ultra rapid metabolizer, EM, extensive metabolizer, IM; intermediate metabolizer, PM; poor metabolizer.

Table 2
Comparison of the temperament and character inventory (TCI) subitem scores among the number of mutated alleles for CYP2D6

	Number of mutated alleles			
	0 ($n=109$)	1 ($n=157$)	2 ($n=76$)	Significance
Novelty seeking	21.2 (20.3, 22.1)	21.6 (20.8, 22.3)	21.5 (20.5, 22.6)	NS
Harm avoidance	20.3 (19.1, 21.4)	19.4 (18.5, 20.4)	20.0 (18.7, 21.3)	NS
Reward dependence	14.6 (13.9, 15.2)	14.9 (14.4, 15.4)	15.4 (14.7, 16.2)	NS
Persistence	4.3 (3.9, 4.6)	4.5 (4.2, 4.8)	4.2 (3.8, 4.6)	NS
Self-directedness	26.4 (25.2, 27.7)	26.0 (25.0, 27.1)	26.8 (25.2, 28.4)	NS
Co-operativeness	27.8 (26.7, 28.8)	28.2 (27.4, 29.0)	28.5 (27.3, 29.7)	NS
Self-transcendence	9.7 (8.9, 10.6)	11.3 (10.5, 12.1)	9.7 (8.6, 10.8)	$P<0.05$

Data are mean (95% confidence interval).

alleles, 157 (45.9%) for one allele, and 76 (22.2%) for two alleles, respectively. The number of mutated alleles for CYP2D6 did not differ with the scores of TCI for six factors except self-transcendence which differed significantly. (Table 2). *Post hoc* analysis showed a significant difference between 0 mutated allele *vs.* 1 mutated allele ($P = 0.034$). In subitem analyses, only RD3 (attachment) had significant difference in *CYP2D6* genotype ($P = 0.034$).

Discussion

The results of this study showed that scores of TCI (novelty seeking, harm avoidance, reward dependence and persistence) were not different between *CYP2D6* phenotypes, as assumed by *CYP2D6* genotype in Japanese. There are three possibilities to explain these results. First, since we had only one PM in the present study, PM was not included in statistical analyses. In addition, four UMs were not enough to detect the difference. This is ascribable to a narrower variation of CYP2D6 activity than that in previous studies using Caucasians. Therefore, the relatively small difference in CYP2D6 activity in Japanese was not enough to detect differences in personality. Second, most of our subjects were medical students and medical staff. Although statistical analyses were not performed, it is possible that the characteristics of our subjects deviated and had small interindividual variability in personality trait. Last, although cross validation between the original TCI and its Japanese version was performed, there is a possibility

that differences in religion or race between Caucasians and Asians may result in less sensitivity of detection, such that a cultural difference cannot be excluded.

Similar to our finding, there was no difference between EM and IM in Japanese subjects [18]. Only three genotypes of *10/*10 and *1/*1 and *1/*10 were identified in this study, although the frequency of mutations other than CYP2D6*10 which predominantly affects CYP2D6 activity is relatively high in Japanese, e.g. CYP2D6*5. Therefore, this negative association between CYP2D6 activity and personality traits in Japanese was first demonstrated by our detailed study.

In conclusion, the present study demonstrated no significant association between CYP2D6 activity and personality trait in Japanese subjects. A significant factor affecting this outcome is the extremely rare frequency of subjects with outlying activity, leading to low interindividual variability in CYP2D6 activity in Japanese subjects.

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References

1 Cloninger CR, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. Arch Gen Psychiatry 1993; 50: 975–90.

2 Hiroi T, Imaoka S, Funae Y. Dopamine formation from tyramine by CYP2D6. Biochem Biophys Res Commun 1998; 249: 838–43.

3 Seeman P, Niznik HB. Dopamine receptors and transporters in Parkinson's disease and schizophrenia. FASEB J 1990; 4: 2737–44.

4 Gonzalez FJ, Meyer UA. Molecular genetics of the debrisoquin-sparteine polymorphism. Clin Pharmacol Ther 1991; 50: 233–8.

5 Tyndale R, Aoyama T, Broly F, Matsunaga T, Inaba T, Kalow W, Gelboin HV, Meyer UA, Gonzalez FJ. Identification of a new variant CYP2D6 allele lacking the codon encoding Lys–281: possible association with the poor metabolizer phenotype. Pharmacogenetics 1991; 1: 26–32.

6 Bertilsson L, Alm C, De Las Carreras C, Widen J, Edman G, Schalling D. Debrisoquine hydroxylation polymorphism and personality. Lancet 1989; 1: 555.

7 Llerena A, Edman G, Cobaleda J, Benitez J, Schalling D, Bertilsson L. Relationship between personality and debrisoquine hydroxylation capacity. Suggestion of an endogenous neuroactive substrate or product of the cytochrome P450 2D6. Acta Psychiatr Scand 1993; 87: 23–8.

8 Roberts RL, Luty SE, Mulder RT, Joyce PR, Kennedy MA. Association between cytochrome P450 2D6 genotype and harm avoidance. Am J Med Genet B Neuropsychiatr Genet 2004; 127: 90–3.

9 Suzuki E, Kitao Y, Ono Y, Iijima Y, Inada T. Cytochrome P450 2D6 polymorphism and character traits. Psychiatr Genet 2003; 13: 111–3.

10 Kijima N, Saito R, Takeuchi M, Yoshino A, Ono Y, Kato M, Kitamura T. Cloninger's seven-factor model of temperament and character and Japanese version of Temperament and Character Inventory (TCI). Arch Psychiatr Diagn Clin Eval 1996; 7: 379–99 (in Japanese).

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Regular Article

Different serum concentrations of steady-state valproic acid in two sustained-release formulations

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Abstract Recently a new sustained-release formulation of valproic acid has been developed in Japan. The sustained-release mechanism of the new formulation was different from the conventional formulation. The aim of the present study was to compare the pharmacokinetic characteristics of valproic acid in two sustained-release formulations. Different sustained-release formulations of valproic acid (Depakene R and Selenica R) were administered in a randomized cross-over fashion in repeated doses in 24 psychiatric patients. After ≥4 weeks administration of valproic acid once daily, blood samples were taken just before (0 h) and 8, 12, 24 h after the morning dose. Blood sampling was performed in the same manner in the same patients 4 weeks after switching from one to the other formulation of valproic acid. Serum concentrations of valproic acid at 0 h (50.7 ± 19.4 vs 44.9 ± 21.8 μg/mL, $P < 0.05$) and 24 h (52.3 ± 19.54 vs 6.2 ± 22.2 μg/mL, $P < 0.05$) were significantly higher during Selenica R than during Depakene R treatment, whereas the serum concentration of valproic acid at 8 h (49.7 ± 19.2 vs 62.4 ± 25.6 μg/mL, $P < 0.01$) was significantly lower during Selenica R treatment than during Depakene R treatment. Serum concentrations of valproic acid at 12 h were not different. The present study demonstrated that steady-state serum concentrations were different because of the different dissolution profiles. When a prescription for valproic acid is switched from one drug to the other, prescribers should be aware that the therapeutic drug monitoring data are not consistent.

Key words dissolution, steady-state serum concentration, sustained-release formulation, valproic acid.

INTRODUCTION

Successful long-term treatment of patients with epilepsy requires selection of an appropriate anti-epileptic regimen, optimal dosing and patient compliance.[1] Recent advances in the choice of treatment options are transforming the global management of these patients.[1] Although the achievement of seizure freedom remains the primary goal of any anti-epileptic treatment, issues associated with drug acceptability and tolerability, and with quality of life have gained increasing attention as major determinants of ultimate therapeutic success.[1,2] Sustained-release formulations of anti-epileptic drugs can be very helpful in achieving treatment objectives. Stable serum levels without marked peak-to-trough fluctuations, reduced frequency of dosing and the possibility of dosing flexibility may all improve compliance, patient satisfaction and ultimately quality of life.[3–5]

Valproic acid has been widely used in the last decade and is now considered a relatively safe and effective anticonvulsant agent.[6] Recently, several investigators have proposed its use in the treatment of anxiety, alcoholism and mood disorders.[7,8] Valproic acid is characterized by dose-limited absorption, non-linear plasma protein binding, and multiple metabolic pathways of elimination.[6,9,10] Once absorbed, valproic acid is largely bound to plasma proteins and has a relatively small volume of distribution. Its concentration in

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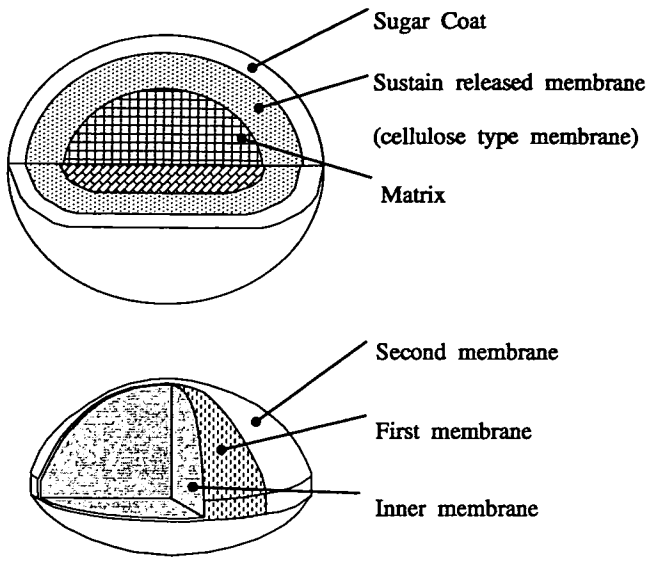

Figure 1. Cross-section of the slow-release Depakene R and Selenica R tablets.

cerebrospinal fluid is approximately one-tenth that in plasma and is directly correlated with the concentration found in tears.[10] At therapeutic doses, valproic acid half-life varies from 10 to 20 h in adults, while it is significantly shorter (6–9 h) in children.[6,10] Valproic acid undergoes extensive liver metabolism.[10,11] Numerous metabolites have been positively identified and there is reasonable evidence that several of them contribute to its pharmacological and toxic actions.[10,11]

Valproic acid is available in different dosage forms for parenteral and oral use. All available oral formulations are almost completely bioavailable, but they differ in dissolution characteristics and absorption rates. The sustained-release formulation (Depakene R, Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan) can therefore be given once or twice daily.[12,13] The tablet core consists of a matrix structure that is covered with the sustained-released membrane. The elution of valproic acid is controlled as the substance passes through the core of the matrix structure and further through the sustained-released membrane (Fig. 1).

Recently another sustained-release formulation containing 200 mg valproic acid (Selenica R, Nikken Chemicals Co. Ltd, Tokyo, Japan) has been developed and available in Japan.[14] Selenica R has a double-coating system that provides a mechanism that is different from the conventional sustained-release formulation (Fig. 1). We therefore compared the pharmacokinetic parameters in two formulations of valproic acid in psychiatric patients.

METHODS

The Ethics Committee of Hirosaki University School of Medicine approved this study protocol, and written informed consent was been obtained from each participant before any examinations.

The subjects were 24 patients (15 male, nine female) receiving valproic acid, who were diagnosed as having bipolar disorders and schizophrenia. The mean (and range) of age and bodyweight were 51 years (21–68 years) and 58 kg (38–98 kg), respectively. Twelve patients received valproic acid at 08.00 hours in the conventional sustained-release formulation, Depakene R, for at least 4 weeks, and the other 12 patients received the valproic acid at 08.00 hours in the new sustained-release formulation, Selenica R for at least 4 weeks. Co-administered medications were as follows: risperidone ($n = 10$), olanzapine ($n = 8$), haloperidol ($n = 4$), zotepine ($n = 3$), levomepromadine ($n = 3$), lithium ($n = 2$), flunitrazepam ($n = 12$), brotizolam ($n = 8$), diazepam ($n = 5$), biperiden ($n = 7$), and sennoside ($n = 13$). These medications were fixed throughout the study period. After ≥4 weeks of administration, blood sampling (5 mL each) was performed just before and 8, 12 and 24 h after administration. The sustained-release formulation of valproic acid administered to the patients was switched to the other type. Four weeks after the switching, blood sampling (5 mL each) was performed in the same way. Clinical global impression (CGI) score for patient psychiatric condition was monitored at blood sampling.[15] There was no difference between administration days of Depakene R and Selenica R.

The serum samples were frozen and kept at –20°C until analysis. Serum concentrations of valproic acid were quantified with enzyme immunoassay (EIA). The detection limit was 1.0 μg/mL. The inter- and intra-assay coefficient of variation (CV) for plasma concentrations of valproic acid were <5.5% for all quality control concentrations.

Pharmacokinetic parameters were determined on a non-compartment model with WinNonLin software (Pharsight, Cary, NC, USA). Apparent volume of distribution (Vd/F), absorption constant (Ka), elimination constant (Ke), lag time (Tlag) were calculated as first parameters. Area under the drug concentration–time curve (AUC), apparent clearance (CL/F), time to peak concentration (T_{max}), peak concentration (C_{max}), peak concentration at steady state (Css_{max}) and minimum concentration at steady state (Css_{min}) were estimated as second parameters.

Statistical analysis was performed by the use of repeated measures of ANOVA in SPSS (SPSS, Chicago, IL, USA). Post-hoc analysis was done using Bonferroni

correction with paired *t*-test. All tests were two-tailed and were considered to be statistically significant for $P < 0.05$.

RESULTS

There were no patients who had an adverse event after switching valproic acid formulation. Daily change in serum concentration of valproic acid during treatments with Depakene R and Selenica R are shown in Fig. 2. Serum concentrations of valproic acid at 0 h (44.9 ± 21.8 vs 50.7 ± 19.4 μg/mL, $P < 0.05$) and 24 h (46.2 ± 22.2 vs 52.3 ± 19.5 μg/mL, $P < 0.05$) during Depakene R treatment were significantly lower than those during Selenica R treatment (Fig. 2). Serum concentration of valproic acid at 8 h (62.4 ± 25.6 vs 49.7 ± 19.2 μg/mL, $P < 0.01$) during Depakene R treatment was significantly higher than the concentration during Selenica R treatment (Fig. 2). However, no difference was found in steady-state serum concentration of valproic acid at 12 h (62.4 ± 25.6 vs 49.7 ± 19.2 μg/mL, n.s.; Fig. 2).

The results of pharmacokinetics parameters simulated by the Pharmacokinetic Analysis program (WinNonLin) are shown in Table 1. The Tlag (1.3 ± 1.4 vs 6.8 ± 1.6 h, $P < 0.001$) and Tmax (9.4 ± 2.2 vs 15.8 ± 2.1 h, $P < 0.001$) during Selenica R treatment were significantly later than during Depakene R treatment. No difference in the estimated C_{ss}max and C_{ss}min were found between the two formulations (Table 1).

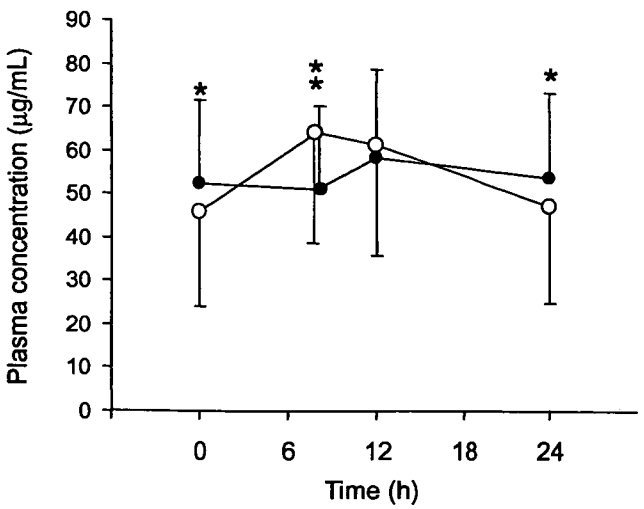

Figure 2. Steady-state serum concentration–time curves after repeated oral doses of Depakene R and Selenica R. Error bars indicate SD. (○), Depakene R; (●), Selenica R. $*P < 0.05$, $**P < 0.01$.

Table 1. Estimated pharmacokinetic parameters in patients receiving repeated doses of valproate in two sustained-release formulations ($n = 24$)

Parameters	Depakene R (mean ± SD)	Selenica R (mean ± SD)	*P*
Vd/F (L)	5.45 ± 0.59	7.22 ± 2.14	<0.01
Ka (1/h)	0.092 ± 0.063	0.068 ± 0.026	<0.05
Ke (1/h)	0.075 ± 0.022	0.073 ± 0.026	NS
T lag (h)	1.3 ± 1.4	6.8 ± 1.6	<0.001
AUC (h·μg/mL)	1334 ± 531	1305 ± 467	NS
CL/F (L/h)	0.50 ± 0.32	0.49 ± 0.26	NS
Tmax (h)	9.4 ± 2.2	15.8 ± 2.1	<0.001
Cmax (μg/mL)	43 ± 23	46 ± 19	NS
Cssmax (μg/mL)	65 ± 20	60 ± 20	NS
Cssmin (μg/mL)	35 ± 12	31 ± 11	NS

AUC (0–∞), area under the curve from 0 to infinity; Cl/F, apparent total clearance; C_{max}, peak concentration; Css_{max}, peak concentration in the steady state; Css_{min}, trough concentration in the steady state; Ka, absorption constant; Ke, elimination constant; Tlag, lag time; t_{max}, time to peak concentration in serum; Vd/F, apparent volume of distribution.

There was no difference in CGI scores between Depakene R and Selenica R (3.9 ± 1.2 vs 4.0 ± 1.3, n.s.).

DISCUSSION

Bioequivalence is an important component of the development of anti-epileptic drugs.[16] Development of new formulations after the original testing of any drug requires demonstration that the compounds are therapeutically equivalent and additional efficacy studies may not be required. Sustained-release formulations may reduce toxicity with a lower maximum blood concentration (C_{max}) and improve efficacy with a higher minimum blood concentration (C_{min}). Obtaining an equivalent AUC while slowing gastrointestinal transit and avoiding food effects and dose dumping among a population with epilepsy with individual variability requires extensive engineering of the formulation.[16]

The results of the preliminary study using a single oral dose showed no differences in C_{max} or AUC of valproic acid between the new and conventional formulations,[17] suggesting that these two formulations are bioequivalent. However, absorption speed, that is, Tlag, and hence T_{max} were very different between the two formulations in the single oral dose study. These findings suggest that valproic acid dissolved from the Depakene R tablet appears earlier in the blood and brain than that from the Selenica R tablet. These

findings resulted from the different mechanisms of the sustained release between Depakene R and Selenica R, namely, the elution of valproic acid in Depakene R is controlled in such a way that the substance passes through the core of a matrix structure and further through the sustained-released membrane, while Selenica R has a mixed membrane composed of ethylcellulose and methacrylate copolymer-L (Fig. 1).[14]

The result of a repeated-dose study in psychiatric patients showed significant differences in steady-state serum concentration of valproic acid at 0, 8 or 24 h after administration. Steady-state serum concentrations at 0 and 24 h during Selenica R were higher than those during Depakene R, while the steady-state serum concentration at 8 h during Selenica R was lower than during Depakene R. The difference may be explained by the difference in Tlag, and hence T_{max}, observed in the single-dose study and the difference in dissolution. As a result, the fluctuation of serum valproic acid concentration in the steady state during Selenica R is shifted to 5–6 h later compared with Depakene R. Because blood sampling for therapeutic drug monitoring (TDM) of valproic acid is generally recommended just before administration, previous therapeutic drug monitoring data are not available when a sustained-release preparation is switched to another formulation. Simulation curve of serum concentration of valproic acid in the steady state including C_{ss}max and C_{ss}min from 1-point sampling data should be available for TDM.

In contrast, no changes in the mental status of psychiatric patients were observed between Selenica R treatment and Depakene R treatment in the present study. This is explained by similar exposure (AUC) in the two formulations. Thus, when a sustained-release preparation is switched to another formulation, the inconsistency of TDM data does not necessarily mean alternation in clinical response.

Many medications were concomitantly administered with valproic acid in Selenica R and Depakene R formulations. However, it is unlikely that the difference in the two formulations led to the significant difference in the results of the present study because co-administered medications were fixed throughout the study period.

Steady-state serum concentrations of valproic acid in the present study were slightly lower than the therapeutic concentration for epilepsy. If doses are escalated up to therapeutic levels, we concluded that the significant difference of TDM data does not necessarily mean alternation in clinical response, because the drug C_{ss}max or exposure (AUC) in the two formulations probably associated with pharmacodynamic action are not different.

In order to improve compliance, a small tablet is preferable for patients, particularly for children. The size of the current commercial once-a-day controlled-release tablet is as follows: Depakote Tablet (Abbott Laboratories, Abbott Park, IL, USA; containing 500 mg divalproex sodium), 0.9 × 1.9 cm, 1.0 g; Depakene R 200 mg Tablet, 0.66 × 1.06 cm, 0.52 g; and Selenica R 200 mg Tablet, 0.50 × 0.92 cm, 0.25 g. From the point of view of easy swallowing, Selenica R Tablet may have the advantage.

In conclusion, the present study demonstrated that steady-state serum concentrations of valproic acid were different because of the different dissolution profiles. When drug prescription for valproic acid is switched from one drug to the other, prescribers should know that TDM data are not consistent.

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REFERENCES

1. Pugh CB, Garnett WR. Current issues in the treatment of epilepsy. *Clin. Pharm.* 1991; **10**: 335–358.
2. Buck D, Jacoby A, Baker GA, Chadwick DW. Factors influencing compliance with antiepileptic drug regimes. *Seizure* 1997; **6**: 87–93.
3. Bialer M. Pharmacokinetic evaluation of sustained release formulations of antiepileptic drugs. Clinical implications. *Clin. Pharmacokinet.* 1992; **22**: 11–21.
4. Cramer J, Vachon L, Desforges C, Sussman NM. Dose frequency and dose interval compliance with multiple antiepileptic medications during a controlled clinical trial. *Epilepsia* 1995; **36**: 1111–1117.
5. Pellock JM, Smith MC, Cloyd JC, Uthman B, Wilder BJ. Extended-release formulations: Simplifying strategies in the management of antiepileptic drug therapy. *Epilepsy Behav.* 2004; **5**: 301–307.
6. Perucca E. Pharmacological and therapeutic properties of valproate: A summary after 35 years of clinical experience. *CNS Drugs* 2002; **16**: 695–714.
7. Bowden CL, Singh V. Valproate in bipolar disorder: 2000 onwards. *Acta Psychiatr. Scand. Suppl.* 2005; **426**: 13–20.
8. Nasrallah HA, Ketter TA, Kalali AH. Carbamazepine and valproate for the treatment of bipolar disorder: A review of the literature. *J. Affect. Disord.* 2006; **95**: 69–78.