Japanese and Koreans), the prevalence of PM phenotype is only 1% but the distribution of enzyme activity is significantly shifted toward lower values in EMs compared to Caucasian EMs [Kalow, 1991]. In PMs, aripiprazole exposure is increased by 80% and accompanied by a 30% decrease in exposure to the dehydrogenated putative active metabolite, leading to a net increase in the total active moieties from a given dose of aripiprazole, compared to EMs [Abilify®, 2002]. Similarly, co-administration of aripiprazole with quinidine, a potent inhibitor of CYP2D6 enzyme, results in a more than two-fold (112%) increase in aripiprazole exposure in EMs. Hence, it is conceivable that an increase in aripiprazole concentration can be anticipated with other potent CYP2D6 inhibitors (e.g. paroxetine) that may be co-prescribed with aripiprazole.

Pharmacokinetic bridging-studies are usually conducted when regulatory drug approval is sought in various countries. For aripiprazole, pharmacogenetic-guided pharmacokinetic bridging-studies focusing on CYP2D6 appear to be warranted among Asian, Caucasian and other populations who display genetically determined inter-ethnic differences in CYP2D6 activity. These data may provide guidance for rational use of aripiprazole and facilitate its registration in different populations or countries as well.

The CYP3A4 enzyme also contributes to metabolism of aripiprazole *via* dehydrogenation and is subject to genetic regulation. It is estimated that 60% to 90% of interindividual variation in catalytic function is determined by hereditary factors [Ozdemir *et al.* 2000]. However, the identity of the precise genetic loci regulating CYP3A4 function remains elusive. More than 30 SNPs have been discovered within *CYP3A4*, but the majority either occur at low frequency (<5%) in human populations or have a minimal impact on enzyme function [Lambda *et al.* 2002a; Lambda *et al.* 2002b]. An unequivocal prediction of CYP3A4 catalytic function solely with a genotypic test is not yet feasible. A further complicating factor is the extensive overlap in substrate selectivity between CYP3A4 and CYP3A5, suggesting that a genetic deficiency in CYP3A4 activity can be partially compensated by the CYP3A5 enzyme. Taken together, these data suggest that variability in CYP2D6 function due to genetic factors, or drug-drug interactions, influences the pharmacokinetics, clinical efficacy and, presumably, concentration-dependent side effects of aripiprazole [Kubo *et al.* 2005].

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Aripiprazole is thought to stabilize dopamine and serotonin neurotransmitter systems in various brain regions in a graded and selective manner depending on the existing endogenous dopaminergic or serotonergic tone. The underlying mechanism of action of aripiprazole in psychotic disorders is likely more complex than what would have been anticipated solely by simple partial agonist effects at the dopamine D2 receptor. In particular, differences in local cellular environment and variability in the type or concentration of the signaling partners for neurotransmitter receptors may also influence clinical response to aripiprazole [Lawler *et al.* 1999; Roth, 2000; Shapiro *et al.* 2003]. Available data from clinical trials in carefully selected patient populations suggest that aripiprazole is largely devoid of metabolic side effects frequently observed with atypical antipsychotics. Still, patients do not uniformly respond to aripiprazole while other patients fail to benefit from drug therapy. Insomnia, anxiety, akathisia, and worsening of psychosis have been noted during aripiprazole treatment in a minority of patients [Swainston-Harrison and Perry, 2004; Ramaswamy *et al.*, 2004; Reeves and Mack, 2004]. Pharmacogenomic biomarkers of therapeutic response to aripiprazole would be desirable to identify the patient subpopulations in whom aripiprazole is more likely to display its antipsychotic effects while minimizing the risk for adverse effects. This would further support its position as a first line agent in the treatment of psychosis and provide unequivocal therapeutic differentiation from other frequently prescribed antipsychotics such as olanzapine.

From the point of therapeutic sciences and psychotropic drug development, the implications of genetic testing for antipsychotic drug targets can be dramatic; it may mean that the choice of drugs – not only the dosage - will be guided by the genetic make up of individuals. Hence, pharmacogenomic tests may decrease the segment of the population for which and to whom drugs can be marketed [Williams-Jones and Corrigan, 2003]. Conversely, prior knowledge of genetic determinants of efficacy and safety may allow targeting of discrete subpopulations in clinical trials and demonstration of "proof of concept" in a smaller number of patients. In theory, this should significantly reduce research and development costs and expedite the regulatory approval of newer atypical antipsychotic candidates. This would also be an aid for rational prescription of aripiprazole as well as its therapeutic differentiation from other atypical antipsychoti c compounds. It is plausible that pharmaco-genomic biomarkers for aripiprazole may inform and guide the development of other partial dopamine-serotonin agonists such as bifeprunox that share similar drug targets [Newman-Tancredi *et al.* 2005].

It should be emphasized that some of the atypical antipsychotics will soon be eligible for regulatory approval as a generic formulation (e.g. risperidone and olanzapine within the next 5 years) [Grady *et al.* 2003]. It is conceivable that a combination of factors ranging from unmet patient needs to market forces, amendments in regulatory policies and competition by generic formulations may provide further motivation on the part of the pharmaceutical industry to develop genetic biomarkers of response to aripiprazole [Melzer *et al.* 2003; Williams-Jones and Burgess, 2004; Williams-Jones, 2005]. The discipline of science and technology studies (STS) and the attendant research literature focus on precisely such complex issues dealing with implementation of pharmacogenomics and other emerging biotechnologies in the clinic [Williams-Jones and Graham, 2003; Hedgecoe and Martin, 2003; Hedgecoe, 2003]. Unfortunately, the expertise already available in the STS research community does not always find its way to the mainstream medical research literature [Hedgecoe, 2004; Webster *et al.* 2004]. Therefore, there is an acute need for more extensive collaborations and consultations among pharmacogeneticists, bioethicists and experts dealing with

STS for an expeditious and equitable development of genetic biomarkers of treatment outcomes with newer atypical antipsychotic agents [Corrigan and Williams-Jones, 2005; Melzer *et al.* 2005; Ozdemir *et al.* 2005; Williams-Jones and Ozdemir, 2005; Smart *et al.* 2004; Webster *et al.* 2004].

As clinical utility of aripiprazole is extended to more diverse groups of patients, well-beyond those who meet the stringent and narrowly-defined inclusion and exclusion criteria used in clinical trials, psychiatrists should continue to use their clinical judgment and pharmacovigilance against previously unrecognized safety or efficacy issues. When pharmacogenomic testing is made available for use as part of routine patient care in psychiatry, it will be necessary to take into account that the human genome displays a high level of plasticity in regulation of gene expression, not to mention our incomplete understanding of the mechanisms responsible for posttranscriptional and posttranslational modifications on products of gene expression [Nebert *et al.* 2003; Collier, 2003]. On the path from the discovery of a genetic biomarker of psychotropic drug response in the laboratory, to a commercially available test applied at the point of patient care, the attendant ethical and therapeutic policy implications of pharmacogenomic tests and their integration with other types of (e.g. proteomics-based) biomarkers will also need to be considered.

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ABBREVIATIONS

EPS = Extrapyramidal symptoms
HGP = Human genome project
HTR1A = Serotonin-1A receptor gene
HTR2A = Serotonin-2A receptor gene
NUDR = Nuclear deformed epidermal autoregulatory factor
OPC-14597 = Aripiprazole
SNP = Single nucleotide polymorphism
STS = Science and technology studies

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Effect of verapamil on pharmacokinetics and pharmacodynamics of risperidone: In vivo evidence of involvement of P-glycoprotein in risperidone disposition

Objective: **A recent in vitro study has shown that risperidone is a substrate of P-glycoprotein. The aim of this study was to confirm the effects of verapamil, a P-glycoprotein inhibitor, on the pharmacokinetics of risperidone.**
Methods: **Two 6-day courses of either 240 mg verapamil daily, an inhibitor of P-glycoprotein, or placebo were administered in a randomized crossover fashion with at least a 4-week washout period. Twelve male volunteers took a single oral 1-mg dose of risperidone on day 6 of both courses. Plasma concentrations of risperidone, 9-hydroxyrisperidone, and prolactin were monitored up to 24 hours after dosing.**
Results: **Compared with placebo, verapamil treatment significantly increased the peak plasma concentration of risperidone by 1.8-fold and the area under the plasma concentration–time curve (AUC) from 0 to 24 hours of risperidone by 2.0-fold but did not alter the elimination half-life. The AUC from 0 to 24 hours of 9-hydroxyrisperidone, but not other pharmacokinetic parameters, was significantly increased during verapamil treatment. However, the AUC from 0 to 4 hours and the AUC from 0 to 8 hours of prolactin concentrations were not increased by verapamil treatment despite the pharmacokinetic alterations.**
Conclusion: **This study demonstrated that the bioavailability of risperidone was increased by verapamil, suggesting in vivo involvement of P-glycoprotein in the pharmacokinetics of risperidone. (Clin Pharmacol Ther 2005;78:43-51.)**

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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 adenosine triphosphate–dependent efflux pumps, such as P-glycoprotein, influences the intestinal absorption,[7,8] renal[9,10] or hepatic elimination,[11] and central nervous system concentrations[8] of many drugs.

Risperidone is one of the representative atypical antipsychotic drugs and has potent antagonistic properties for serotonin 5-HT_2 and dopamine D2 receptors.[12,13] This drug is characterized by its effectiveness against both positive and negative symptoms in the treatment of schizophrenia.[14] Furthermore, it produces fewer side effects, including extrapyramidal side effects, than conventional antipsychotic drugs.[15] A recent in vitro study has examined the activity of P-glycoprotein toward 4 atypical and 2 conventional antipsychotics and a proven substrate, verapamil, by their P-glycoprotein adenosine triphosphatase activity, a putative measure of P-glycoprotein affinity.[16] The rank order of the ratio of maximum velocity to Michaelis-Menten constant was as follows: verapamil (2.6) > quetiapine fumarate (INN, quetiapine) (1.7) > risperidone (1.4) > olanza-

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pine (0.8) > chlorpromazine (0.7) > haloperidol (0.3). The atypical antipsychotics quetiapine fumarate and risperidone were relatively good P-glycoprotein substrates, although their affinities were not as high as that of verapamil. These results suggest that P-glycoprotein is likely to influence absorption in the small intestine or excretion in the liver or kidney of all atypical antipsychotics to various degrees. However, there are no in vivo data indicating that quetiapine fumarate or risperidone as a substrate of P-glycoprotein is of clinical relevance, although some in vivo data have shown a lack of impact of the multidrug resistance 1 (MDR1) genotype on steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone.[17]

Verapamil, a short-term inhibitor of mainly P-glycoprotein, has been used to increase the therapeutic effectiveness of cytotoxic anticancer drugs in cancer chemotherapy.[18] More recently, P-glycoprotein reversal agents including verapamil have been demonstrated to alter the pharmacokinetic properties of coadministered agents in therapeutic areas other than oncology.[19]

The aim of this study was to confirm the effects of verapamil, a transporting inhibitor, on the disposition of risperidone and its active metabolite, 9-hydroxyrisperidone. Prolactin response to risperidone was also examined to clarify the effect of P-glycoprotein activity modulated by verapamil on the pharmacodynamics of risperidone.

METHODS

Subjects

Twelve healthy Japanese male volunteers were enrolled in this study. Their mean age (±SD) was 24.0 ± 2.0 years (range, 20-28 years), and their mean body weight was 64.8 ± 6.2 kg (range, 53-86 kg). The Ethics Committee of Hirosaki University School of Medicine, Hirosaki, Japan, 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 40-mg tablets of verapamil (Vasolan; Eisai Pharmaceutical, Tokyo, Japan) 3 times daily (at 8 AM, 1 PM, and 6 PM) or matched placebo with 240 mL of tap water was given for 6 days. The volunteers took a single oral 1-mg dose of risperidone at 9 AM on day 6 with 240 mL of tap water. Compliance with taking the 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 (at 1 AM). The use of alcohol, tea, coffee, and cola was forbidden during the test days.

Sample collections

Blood samples (5 mL each) for determination of risperidone and 9-hydroxyrisperidone and prolactin concentrations were taken into heparinized tubes just before and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after the administration of risperidone. Plasma was separated immediately and kept at −30°C until analysis. At the same time of blood sampling, blood pressure, heart rate, and Udvalg for Klinicke Undersøgelser (UKU) side effects rating scales[20] were monitored. The UKU consisted of 18 items, ie, psychic (concentration difficulties, asthenia, sleepiness, failed memory, and depression), extrapyramidal (dystonia, rigidity, hypokinesia, hyperkinesia, tremor, akinesia, and increased salivation), and autonomic (accommodation disturbances, reduced salivation, constipation, micturition disturbance, orthostatic dizziness, and palpitation) side effects, and was classified from 0 to 3 for each item.

Assay

Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured via the liquid chromatography–tandem mass spectrometry method described by Yasui-Furukori et al.[17] In brief, the extraction procedure was as follows: to 200 μL of plasma sample was added 200 μL of 0.1-mol/L phosphate buffer (pH 7), 50 μL of internal standard solution (R068808; Jansen Research Foundation, Beerse, Belgium), and 100 μL of methanol. Thereafter, 400 μL of 0.1-mol/L Borax (Sigma Coatings, Hasselt, Belgium) was added. The mixture was whirled in a vortex blender and poured over an Extrelut NT 1 (Merck, Boston, Mass) 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-mol/L ammonium acetate (50/50, pH 9.0), and 5 μL was injected onto the liquid chromatography–mass spectrometry–mass spectrometry system. The system consisted of API 3000 (Applied Biosystems, Foster City, Calif) and a column (Hypersil BDS C18, 100 × 4.6, 3 μm [Chemco Scientific, Brussels, Belgium]). The mobile phase was gradient ammonium acetate (0.01 mol/L, 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 for risperidone and 9-hydroxyrisperidone, and the values of the intra-assay and interassay coefficient of variation were less than 5% at all of the concentrations (0.1-100 ng/mL) of calibration curves for both compounds.

Plasma concentrations of prolactin were quantitated via enzyme immunoassay (IMX Prolactin Dainapack; Dainabot, Tokyo, Japan). The lower limit of detection was 0.6 ng/mL, and the values of the interassay coefficient of variation were 3.7%, 3.5%, and 3.5% at the concentrations of 8, 20, and 40 ng/mL for prolactin, respectively.

Cytochrome P450 2D6 genotypes

For the determination of cytochrome P450 (CYP) 2D6 genotype, deoxyribonucleic acid was isolated from peripheral leukocytes by a guanidium isothiocyanate method. The *CYP2D6*1*, *CYP2D6*3*, and *CYP2D6*4* alleles were identified by allele-specific polymerase chain reaction (PCR) analysis according to Heim and Meyer.[21] A long-PCR analysis was used to detect the *CYP2D6*5* allele.[22] The *CYP2D6*10* allele was identified as the C188T mutation by use of 2-step PCR analysis as described by Johansson et al.[23] The *CYP2D6*14* allele was identified as the G1846T/A mutation by use of 2-step PCR analysis as described by Kubota et al.[24] *CYP2D6*2* does not result in decreased CYP2D6 activity. Therefore *CYP2D6*2* was regarded as the wild-type (wt) allele, together with *CYP2D6*1*.

Date analyses of pharmacokinetics

The peak concentration (C_{max}) and concentration peak time (t_{max}) were obtained directly from the original data. 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 ($t_{½}$) was calculated as follows: 0.693/k_e. The area under the plasma concentration–time curve (AUC) from 0 to 24 hours [AUC(0-24)] was calculated with use of the linear-linear trapezoidal rule. AUC from time 0 to infinity [AUC(0-∞)] or total AUC was calculated by AUC(0-24) + C_{last}/k_e, where C_{last} was the plasma drug concentration at the last detectable time point.

Statistical analysis

Data are shown as mean ± SD in tables and mean ± SE in figures. Paired *t* test was used for the comparison of the plasma drug concentrations and scores of clinical assessments between 2 phases (ie, placebo and verapamil). The comparison of t_{max} was performed by use of the Wilcoxon signed-sample test. One-way ANOVA was used to compare CYP2D6 genotype effect on this interaction. A *P* value of .05 or less was regarded as significant. Geometric mean ratios to corresponding values in the placebo phase with 95% confidence intervals (CIs) were used for detection of significant differences. When the 95% CI did not cross 1.0, the result was also regarded as significant. SPSS 12.0J for Windows (SPSS Japan, Tokyo, Japan) was used for these statistical analyses.

RESULTS

The subjects had the following CYP2D6 genotypes: wt/wt in 3 subjects, **10*/wt in 5, **10/*10* in 1, and **5/*10* in 3. No subjects regarded as poor metabolizers were included. The subjects were divided into 3 groups according to the number of mutated alleles: there were no mutated alleles in 2 subjects, 1 mutated allele in 6, and 2 mutated alleles in 4.

Pharmacokinetics

Plasma drug concentration–time curves during both placebo and verapamil treatments are shown in Fig 1, and their pharmacokinetic parameters are summarized in Table I. The C_{max} of risperidone during verapamil treatment was higher than the corresponding value during the placebo phase by 1.76-fold (95% CI, 1.36- to 2.49-fold). The AUC(0-24) of risperidone during verapamil treatment was higher than during placebo by 1.97-fold (95% CI, 1.37- to 3.01-fold). The total AUC of risperidone during verapamil treatment was higher than during placebo by 1.82-fold (95% CI, 1.24- to 2.82-fold). No change was found in t_{max} (0.88-fold [95% CI, 0.60- to 1.52-fold]) or elimination $t_{1/2}$ (0.92-fold [95% CI, 0.78- to 1.19-fold]) of risperidone.

The geometric mean ratio of C_{max} of 9-hydroxyrisperidone was 1.14 (95% CI, 0.99- to 1.36-fold). The geometric mean ratios of AUC(0-24) and total AUC of 9-hydroxyrisperidone were 1.46-fold (95% CI, 1.00- to 2.24-fold) and 1.50-fold (95% CI, 0.76- to 2.89-fold), respectively. No differences in t_{max} (1.07-fold [95% CI, 0.78- to 1.68-fold]) or elimination $t_{1/2}$ (1.15-fold [95% CI, 0.83- to 1.74-fold]) were found.

The C_{max} values of active moiety (risperidone plus 9-hydroxyrisperidone) (1.34-fold [95% CI, 1.19- to 1.55-fold]) were increased during verapamil treatment. The AUC(0-24) (1.39-fold [95% CI, 1.28- to 1.52-fold]) and total AUC (1.38-fold [95% CI, 1.24- to 1.57-fold]) of active moiety during verapamil treatment were significantly higher than the corresponding value before the coadministration.

The AUC ratio of risperidone to 9-hydroxyrisperidone was not altered (1.21-fold [95% CI, 0.78- to 2.31-fold]). The mean magnitude (±SD) of the risperidone-verapamil interaction was 3.2 ± 2.5–fold in subjects with no mutated allele, 1.7 ± 0.3–fold in subjects with 1 mutated allele,

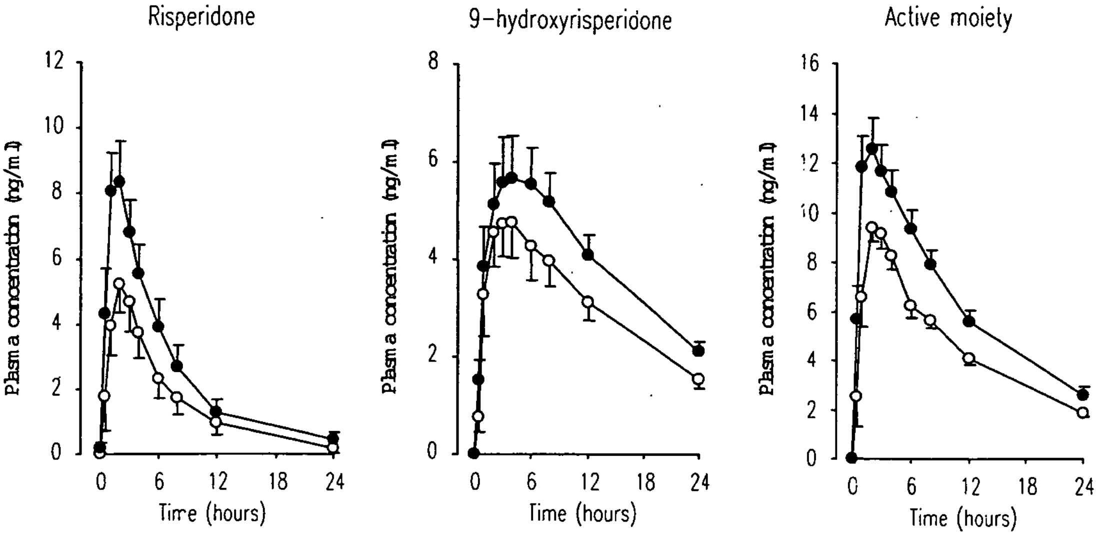

Fig 1. Mean plasma concentration–time curves of risperidone, 9-hydroxyrisperidone, and active moiety after single oral 1-mg dose of risperidone. *Open circles* indicate placebo and *solid circles* indicate verapamil. *Error bars* indicate SE.

Table I. Pharmacokinetic parameters of drug concentrations after single oral 1-mg dose of risperidone during placebo and verapamil treatments

	Placebo	*Verapamil*	*Significance*
Risperidone			
C_{max} (ng/mL)	5.9 ± 3.1	10.0 ± 4.2	$P < .001$
t_{max} (h)	2.0 (0.75-3.0)	1.0 (1.0-6.0)	NS
AUC(0-24) (ng · h/mL)	32.6 ± 29	56.2 ± 37.6	$P < .001$
Total AUC (ng · h/mL)	39.4 ± 34.9	62.8 ± 43.1	$P < .001$
$t_{1/2}$ (h)	3.9 ± 1.9	3.6 ± 2.2	NS
9-Hydroxyrisperidone			
C_{max} (ng/mL)	5.4 ± 2.4	6.2 ± 2.9	$P < .01$
t_{max} (h)	4.0 (1.0-12.0)	4.0 (1.0-12.0)	NS
AUC(0-24) (ng · h/mL)	73.9 ± 31.7	94.8 ± 37.3	$P < .01$
Total AUC (ng · h/mL)	105.9 ± 40.9	136.8 ± 46.3	$P < .05$
$t_{1/2}$ (h)	13.4 ± 10.1	13.4 ± 9.3	NS
Active moiety			
C_{max} (ng/mL)	10.2 ± 2.3	13.9 ± 4.1	$P < .01$
AUC(0-24) (ng · h/mL)	110.0 ± 22.0	154.22 ± 39.3	$P < .001$
Total AUC (ng · h/mL)	137.2 ± 28.6	194.29 ± 60.0	$P < .001$

Data are shown as mean ± SD, except for t_{max}, which is shown as median and range.

C_{max}, Peak concentration; t_{max}, time to peak concentration in plasma; NS, not significant; AUC(0-24), area under plasma concentration–time curve from 0 to 24 hours; Total AUC, area under plasma concentration–time curve from time 0 to infinity; $t_{1/2}$, elimination half-life.

and 2.0 ± 1.3–fold in subjects with 2 mutated alleles (*df* = 2,9; F = 1.72; *P* = not significant).

Pharmacodynamics

UKU score–time curves and plasma concentration–time curves of prolactin and during both treatments are shown in Figs 2 and 3, and the pharmacokinetic parameters of prolactin are summarized in Table II. Although there were mild side effects (chest pain) in 1 subject, no clinically significant adverse events were reported during days 1 to 5. Mild to moderate psychic side effects (eg, concentration difficulty, latency, and sleepiness) were observed from 2 to 12 hours after the risperidone administration in almost all subjects. There was no change in the peak UKU score (4.7 ± 2.3 versus 3.2 ± 1.6, P = not significant; 0.67-fold [95% CI, 0.52- to 1.15-fold]). The area under the UKU score–time curve during placebo was significantly higher than that during verapamil (29.7 ± 21.3 versus 14.4 ± 10.8, $P < .05$). However, the geometric mean ratio of the area under the UKU score–time curve was 0.53 (95% CI, −0.29- to 3.05-fold).

The geometric mean ratio of C_{max} of prolactin between placebo and verapamil treatments was 1.08 (95% CI, 0.96- to 1.27-fold). Those of AUC(0-4), AUC(0-8), AUC(0-24), and incremental AUC of prolactin were 1.15-fold (95% CI, 1.01- to 1.26-fold), 1.12-fold (95% CI, 1.02- to 1.25-fold), 1.24-fold (95% CI, 1.12- to 1.39-fold), and 1.20-fold (95% CI, 0.73- to 2.23-fold), respectively.

DISCUSSION

The results of this study showed a significant increase in the plasma concentration of risperidone (C_{max} and AUC) during verapamil treatment. These findings imply that verapamil increases the bioavailability of risperidone or decreases the total clearance of risperidone. However, because verapamil did not alter the elimination $t_{1/2}$ of risperidone in this study, it appears that only the bioavailability of risperidone was increased by verapamil, which might be attributed to increased absorption of risperidone in the small intestine or inhibition of extraction to bile in the liver. Our recent study showed that verapamil increased the bioavailability of fexofenadine,[25] probably through P-glycoprotein inhibition. Because a recent jejunal single-pass perfusion study suggested that verapamil treatment did not alter the permeability of fexofenadine,[26] it is more likely that the interaction between verapamil and risperidone occurs in the first-pass liver extraction process.

Our previous report showed a lack of effects of major polymorphisms of the *MDR1* gene on steady-state plasma drug concentrations in 85 schizophrenic patients receiving 3 mg risperidone twice daily.[17] When *MDR1* variants alter the activity of P-glycoprotein in the small intestine, which limits oral bioavailability, the peak concentration of risperidone may differ between *MDR1* genotypes. However, plasma concentrations of risperidone were monitored at 12 hours after dosing in the present study. Therefore any difference in the peak plasma concentration of risperidone caused by *MDR1* variants was offset by large interindividual variability in risperidone metabolism during the elimination phase.

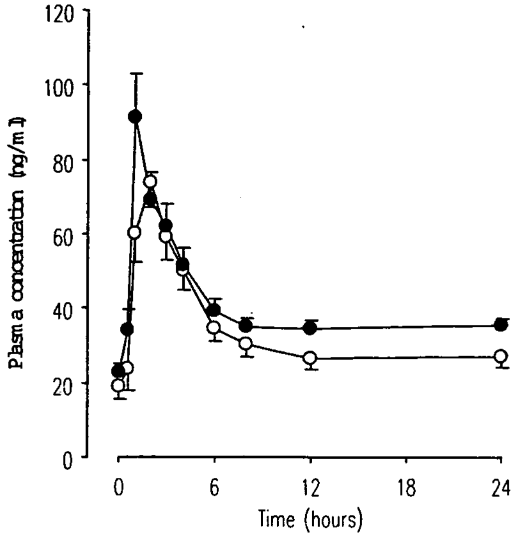

Fig 2. Mean Udvalg for Klinicke Undersøgelser (UKU) side effect rating scale score–time curves after single oral 1-mg dose of risperidone. *Open circles* indicate placebo and *solid circles* indicate verapamil. *Error bars* indicate SE.

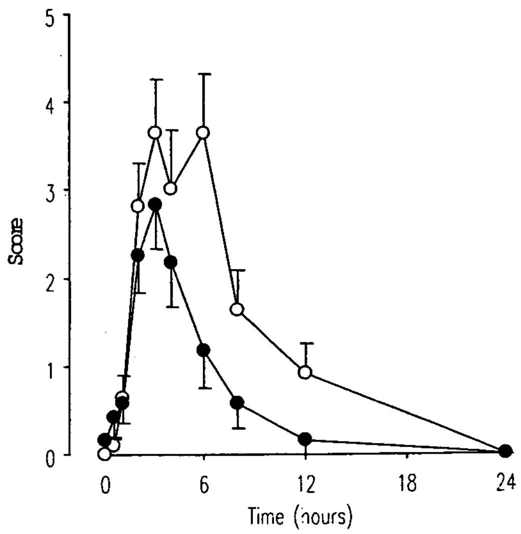

Fig 3. Mean plasma concentration–time curves of prolactin after single oral 1-mg dose of risperidone. *Open circles* indicate placebo and *solid circles* indicate verapamil. *Error bars* indicate SE.

Verapamil is regarded as an inhibitor of CYP3A, as well as P-glycoprotein, on the basis of several in vitro and in vivo investigations.[27-29] Fang et al[30] demonstrated that CYP3A is a major enzyme catalyzing 9-hydroxylation of risperidone. On the basis of these facts, therefore, it is possible that significant interaction between these drugs

Table II. Pharmacokinetic parameters of prolactin concentration and AUC ratios of prolactin to drug concentration after single oral 1-mg dose of risperidone during placebo and verapamil treatments

	Placebo	*Verapamil*	*Significance*
C_{max} (ng/mL)	84.4 ± 21.8	95.6 ± 39.1	NS
AUC(0-4) (ng · h/mL)	15.5 ± 10.4	26.0 ± 13.3	NS
AUC(0-8) (ng · h/mL)	25.6 ± 18.8	42.3 ± 24.4	NS

Data are shown as mean ± SD.
AUC(0-4), Area under plasma concentration–time curve from 0 to 4 hours; AUC(0-8), area under plasma concentration–time curve from 0 to 8 hours.

occurs as a result of inhibition of CYP3A. Our previous in vitro study showed that intrinsic clearance for (+)-9-hydroxylation of risperidone catalyzed by CYP2D6 was 25.2 ± 12.2 $\mu L \cdot min^{-1} \cdot mg^{-1}$, whereas that for (+)- and (−)-9-hydroxylation of risperidone catalyzed by CYP3A was 6.65 ± 2.15 $\mu L \cdot min^{-1} \cdot mg^{-1}$ and 3.89 ± 1.74 $\mu L \cdot min^{-1} \cdot mg^{-1}$, respectively, suggesting that CYP2D6 was more predominant than CYP3A in clinically relevant concentrations of risperidone.[31] In fact, there was no difference in the elimination half-life of risperidone, which is well influenced by drug metabolism. In addition, although alternative contribution of CYP3A to risperidone metabolism in subjects with low CYP2D6 activity is assumed, the magnitude of this interaction in subjects with 2 mutated alleles for CYP2D6 was not different from that in other genotype groups. Thus it seems that the drug interaction did not lead to an inhibitory effect of verapamil on hepatic CYP3A. However, the possibility that inhibition of CYP3A in the small intestine during verapamil treatment caused the increased absorption of risperidone cannot be excluded.

Also, verapamil is a nonspecific inhibitor of several membrane transport proteins, including P-glycoprotein and organic anion transporting polypeptide (OATP) A.[32] In contrast, there are no data indicating that OATPs have any role in risperidone penetrating membrane. In the current study, therefore, the possibility that verapamil alters risperidone pharmacokinetics through an inhibition of OATPs cannot be excluded.

The metabolite 9-hydroxyrisperidone is mainly removed through renal excretion,[33] whereas P-glycoprotein exists in the renal proximal tubule.[34] Consequently, it is possible that the plasma concentration of 9-hydroxyrisperidone depends on P-glycoprotein activity, and we assumed that the difference in 9-hydroxyrisperidone concentration would be larger. However, the pharmacokinetic parameters of 9-hydroxyrisperidone were not influenced by verapamil treatment as much as risperidone. 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. In addition, the formation of 9-hydroxyrisperidone is mainly catalyzed by CYP2D6,[31] but plasma concentrations of 9-hydroxyrisperidone are not dependent CYP2D6 activity.[35-38] Further study is required to clarify the disposition of 9-hydroxyrisperidone.

Likewise, verapamil was associated with the active moiety concentration. This is a reasonable finding in light of the alteration of plasma concentrations of risperidone. Apart from steady state, the active moiety concentration is influenced by both plasma concentrations of risperidone and 9-hydroxyrisperidone because the plasma concentration of 9-hydroxyrisperidone was similar to that of risperidone after a single oral dose of risperidone.[39] From this pharmacokinetic point of view, we concluded that P-glycoprotein modulation has potential clinical implications based on changes in peak plasma concentrations.

The major physiologic role of P-glycoprotein is to serve as a barrier to entry and as an efflux mechanism for xenobiotics and cellular metabolites.[40] Not only may P-glycoprotein limit intestinal drug absorption to constrain oral drug bioavailability, but the rate of P-glycoprotein efflux transport can also mediate brain penetration of lipophilic drugs.[41,42] This is based on several kinetic studies showing large differences in brain concentration between the knockout animal [*mrd1a* (−/ −) and *mrd1a/1b* (−/ −) mice] and normal animal [*mrd1a* (+/+) and *mrd1a/1b* (+/+) mice].[43] Therefore interindividual variability of P-glycoprotein function in the brain contributes to this variability of clinical response to neuropsychiatric agents.

A few studies have suggested that inhibition of P-glycoprotein in the central nervous system affects pharmacodynamic alteration. Loperamide produced no respiratory depression when administered alone, but respiratory depression occurred when loperamide (16 mg) was given with quinidine at a dose of 600 mg.[44] These changes were not explained by increased plasma loperamide concentrations. This study demonstrates the potentially important drug interactions that occur by inhibition of P-glycoprotein.

Several positron emission tomography studies suggested that risperidone disposition in plasma is not associated with that in the brain.[45,46] Because the inhibition of P-glycoprotein activity during verapamil treatment probably leads to elevation of risperidone concentrations in the brain, we assumed that the prolactin concentration or prolactin concentration normalized by plasma concentration should be increased during verapamil treatment. In addition, several animal studies showing that the brain concentration of P-glycoprotein substrates increased after verapamil treatment support our hypothesis, although these studies used experimental doses of verapamil.[47,48]

Contrary to our expectation, the prolactin concentration was not affected during verapamil treatment. We do not have a clear explanation for this finding. A previous study demonstrated that verapamil infusion decreased the prolactin concentration in patients with hyperprolactinemia induced by sulpiride, which is a dopamine D_2 antagonist, but increased the prolactin concentration in patients with hyperprolactinemia induced by prolactinoma.[49] Further studies with respect to the pharmacodynamic effect of verapamil are required.

The area under the UKU score–time curve during placebo was significantly higher than that during verapamil treatment, although the active moiety concentration was increased during verapamil treatment in this study. The possibility that this finding is ascribable to more sequence effects in the placebo-verapamil group than that in the verapamil-placebo group cannot be excluded entirely.

In conclusion, this study showed that verapamil increased risperidone exposure, probably because of an increase in bioavailability through P-glycoprotein inhibition. Change in the regulation of transporters such as P-glycoprotein, though not simple, may lead to significant alteration of risperidone pharmacokinetics.

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Dose-Dependent Interaction of Paroxetine With Risperidone in Schizophrenic Patients

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Abstract: Augmentation with paroxetine (10–40 mg/d) for antipsychotic treatment may improve the negative symptoms in schizophrenic patients but involves a risk of drug-drug interaction. We studied the effects of paroxetine on plasma concentrations of risperidone and 9-hydroxyrisperidone and their clinical symptoms in risperidone-treated patients. Twelve schizophrenic inpatients with prevailingly negative symptoms receiving risperidone 4 mg/d were, in addition, treated with incremental doses of paroxetine for 12 weeks (10, 20, and 40 mg/d for 4 weeks each). Plasma concentrations of risperidone and 9-hydroxyrisperidone were quantified with liquid chromatography–mass spectrometry mass-mass spectrometry together with clinical assessments before and after each phase of the 3 paroxetine doses. Risperidone concentrations during coadministration of paroxetine 10, 20, and 40 mg/d were 3.8-fold (95% confidence interval, 3.2–5.8, $P < 0.01$), 7.1-fold (95% confidence interval, 5.3–16.5, $P < 0.01$), and 9.7-fold (95% confidence interval, 7.8–22.5, $P < 0.01$) higher than that before paroxetine coadministration, respectively. Active moiety (risperidone plus 9-hydroxyrisperidone) concentration was not increased during the paroxetine 10 mg/d (1.3-fold, not significant) or 20 mg/d (1.6-fold, not significant), but were significantly increased by 1.8-fold (95% confidence interval, 1.4–2.7, $P < 0.05$) during the paroxetine 40 mg/d. Significant improvement in negative symptoms was observed from 10 to 40 mg/d of paroxetine, whereas scores in extrapyramidal side effects during 20 and 40 mg/d of paroxetine were significantly higher than baseline score. This study indicates that paroxetine increases plasma risperidone concentration and active moiety concentration in a dose-dependent manner. Low-dose coadministration of paroxetine with risperidone may be safe and effective in the treatment of schizophrenic patients with negative symptoms.

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Negative symptoms play an important role in schizophrenia and are related to deficits in global functioning and global outcome. The atypical neuroleptics have proven to be effective in treating negative symptoms. However, although clinical experience appears to show that they have advantages over the traditional neuroleptics in treating negative symptoms, superiority has not always been statistically confirmed, and the treatment results in individual patients in everyday clinical practice are often not satisfying.[1] Therefore, other drug treatment options also have to be carefully considered.

Although the role and mechanism of antidepressant drugs as an adjunct in the treatment of schizophrenic patient with negative symptoms remain unclear, there is some evidence that adding an antidepressant that is a selective serotonin reuptake inhibitor to antipsychotic agents improves negative symptoms of schizophrenia.[2] However, a problem is the risk of possible pharmacodynamic and/or pharmacokinetic interactions when using antidepressants as an adjunctive therapy. Particularly, the pharmacokinetic consequences of combination of selective serotonin reuptake inhibitors and neuroleptics should be carefully considered. The selective serotonin reuptake inhibitors differ substantially in their potential for pharmacokinetic drug interactions, which reflects a difference in their effect on cytochrome P450 isoenzymes (CYP) in the liver.[3]

Risperidone is an atypical antipsychotic with a potent serotonin 5-HT_2 and a moderate dopamine D_2 antagonistic activity.[4] It is effective in the treatment of both positive and negative symptoms of schizophrenia and has a lower potential to cause extrapyramidal symptoms, compared with classic antipsychotic agents.[5] Risperidone is metabolized in the liver by CYP, mainly by 9-hydroxylation and, to a lesser extent, by *N*-dealkylation and 7-hydoxylation.[6] Several in vitro studies with human liver microsomes and recombinantly expressed microsomes have demonstrated that the formation of 9-hydroxyrisperidone is predominantly catalyzed by CYP2D6 and, to a lesser extent, CYP3A4.[7,8] Furthermore, several in vivo studies have shown that the steady-state plasma concentration of risperidone is increased with the number of mutated alleles for CYP2D6 in schizophrenic patients.[9–12] Thus, CYP2D6 is predominantly involved in the risperidone metabolism.

Augmentation of paroxetine to risperidone is relatively common and proposed for the treatment of negative symptoms of schizophrenia and other psychiatric disease, for example, major depression or obsessive-compulsive disease.[13,14] Spina et al[15] suggest that a significant pharmacokinetic interaction of this combination occurs through CYP2D6 inhibition. However, there is no information about therapeutic and toxic dose of paroxetine when added to risperidone.

Therefore, this study was designed to investigate the dose effects of paroxetine on the steady-state plasma

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concentrations of risperidone, 9-hydroxyrisperidone, and active moiety during incremental doses of paroxetine. Moreover, changes in psychiatric symptoms and neuroleptic side effects occurring contemporaneously with concomitant administration of paroxetine were evaluated.

MATERIAL AND METHODS

Subjects

Twelve Japanese schizophrenic inpatients (7 women and 5 men), who fulfilled the criteria for schizophrenia, according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* and had negative symptoms in spite of undergoing risperidone therapy and required selective serotonin reuptake inhibitor therapy, were selected for the study. The mean ± SD of age was 52.3 ± 10.3 years (range, 33–67), body weight was 67.6 ± 8.2 kg (range, 57–83), and duration of illness was 298.8 ± 110.9 months (range, 84–482). 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

Before paroxetine coadministration, subjects had been treated for at least 6 weeks (range, 6–156) with fixed dose of risperidone. Dose of risperidone was 4 mg/d, and frequency was twice daily (8 am and 8 pm). The elimination half-lives of risperidone and 9-hydroxyrisperidone were reported to be 3 to 20 hours and 20 to 29 hours, respectively. Therefore, plasma concentrations of these compounds already reached steady state in all subjects before initiating the study. Dosage and frequency of administration of risperidone were fixed throughout the duration of the study. Paroxetine 10 mg once a day (8 pm) was coadministered to all the subjects for the first 4 weeks, followed by 20 mg/d for the next 4 weeks, and finally, 40 mg/d for the last 4 weeks. Concomitant treatment with other medications, flunitrazepam (1–3 mg/d) in 6 patients, nitrazepam (10 mg/d) in 1 patient, brotizolam (0.5 mg/d) in 1 patient, etizolam (2–6 mg/d) in 2 patients, zolpidem (7.5 mg/d) in 1 patient, sennoside (12–24 mg/d) in 3 patients, etilefrine hydrochloride (15–20 mg/d) in 2 patients, amezinium metilsulfate (20 mg/d) in 3 patients, atorvastatin calcium hydrate (20 mg/d), and benidipine hydrochloride (8 mg/d) in 1 patient, was also fixed throughout the study period. None of the subjects received drugs known as inhibitors or inducer of CYP2D6 or 3A4 in risperidone metabolism.[16]

Plasma sample collections were performed before and 4 weeks after each dose sequence of 10, 20, and 40 mg/d of paroxetine coadministration. Sampling for pharmacokinetics determination was drawn at 8 am (approximately 12 hours after the last dose of risperidone and the paroxetine dose and before the morning dose of risperidone).

On the same days as the blood samplings, psychiatric symptoms and side effects were evaluated by Positive and Negative Syndrome Scale (PANSS),[17] the Calgary Depression Scale for Schizophrenics (CDSS),[18] and the Udvalg for Kliniske Undersøgelser (UKU) side effects rating scale,[19] respectively. Patients were evaluated by the same investigator. This investigator from other hospital was not involved in the study patient care and was blind to drug regimens and result of drug concentrations. However, he had access to the nursing charts.

Each of the 30 items of the PANSS was assessed by 7-scale steps from 1 (not present) to 7 (extremely severe). The 25 items were divided into 5 clusters: negative (emotional withdrawal, passive/apathetic withdrawal, lack of spontaneity, poor rapport, blunted affect, and active social avoidance), excitement (excitement, poor impulse control, hostility, and tension), cognitive (conceptual disorganization, disorientation, difficulty in abstract thinking, mannerisms and posturing, and poor attention), positive (delusions, unusual thought content, grandiosity, suspiciousness/persecution, and hallucinations), and depression (anxiety, guilt feelings, depression, somatic concern, and preoccupation) symptoms as classified by Lindenmayer et al.[20] Nine items of the Japanese version of the CDSS, which was a depression scale for schizophrenia patients, were assessed by total scores. Nineteen items were selected from the UKU side effects rating scale to assess the side effects of risperidone, which were divided into 3 subgroups: psychic (concentration difficulties, asthenia, sleepiness, failing memory, depression, and tension), extrapyramidal (dystonia, rigidity, hypokinesia, hyperkinesias, tremor, akathisia, and increased salvation), and autonomic (accommodation disturbances, reduced salvation, constipation, micturition disturbances, orthostatic dizziness, and palpitation) side effects.

Assay

Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured using the liquid chromatography–mass spectrometry mass-mass spectrometry method. The extraction procedure was as follows: 200 μL of 0.1 mol/L phosphate buffer (pH 7), 50 μL internal standard (R068808, Janssen Research Foundation, Beerse, Belgium), and 100 μL methanol were added, and after this, 400 μL of 0.1 mol/L borax was added. The mixture was vortexed and poured over an Extrelut NT 1 (Merck, Boston, Massachusetts) column, which was eluted with 7 mL ethyl acetate. The eluate was evaporated under a nitrogen stream at 65°C. The residues were redissolved in 200 μL acetonitrile/0.01 mol/L ammonium acetate (50/50, pH 9.0), and 5 μL was injected onto the liquid chromatography–mass spectrometry mass-mass spectrometry system. The system consisted of API 3000 (Sceix) and a column (Hypersil BDS C18, 100 × 4.6 mm, 3-μm particle size, Chemco Scientific, Brussels, Belgium). The mobile phase was an ammonium acetate (0.01 mol/L, pH 9.0)–acetonitrile gradient. Among the fragment ions of the compounds, the mass-to-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, and the values of the interassay coefficient of variation were less than 5% at all the concentrations of calibration curves for risperidone and 9-hydroxyrisperidone.

The *CYP2D6*1* (**1*), *CYP2D6*3*, and *CYP2D6*4* alleles were identified by allele-specific polymerase chain reaction analysis according to Heim and Meyer.[21] A long-polymerase chain reaction analysis was used to detect the *CYP2D6*5* (**5*) allele.[22] The *CYP2D6*10* (**10*) allele was identified as the *C188T* mutation using a 2-step polymerase chain reaction analysis as described by Johansson et al.[23] The *CYP2D6*14* (**14*) allele was identified as the *G1846T/A* mutation using a 2-step polymerase chain reaction analysis as described 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*.

Statistical Analyses

The repeated measurement of analysis of variance followed by Bonferroni correction and Friedman test followed by Bonferroni correction were used for the comparison of the plasma drug concentrations and scores of clinical assessments, respectively, among 4 phases, that is, before and during paroxetine coadministration at 10, 20, and 40 mg/d. Correlations between the changes in plasma concentrations of risperidone, 9-hydroxyrisperidone, and active moiety were tested using linear regression analysis. A P value of 0.05 or less was regarded as significant. SPSS 12.0 for Windows (SPSS Japan Inc, Tokyo, Japan) was used for these statistical analyses.

RESULTS

Plasma concentrations of risperidone and 9-hydroxyrisperidone, active moiety, and metabolic ratio (risperidone/9-hydroxyrisperidone) before and during paroxetine coadministration are summarized in Table 1. Mean plasma concentrations of risperidone during coadministration of paroxetine 10, 20, and 40 mg/d were 3.8-fold (95% confidence interval [CI], 3.2–5.8, $P < 0.01$), 7.1-fold (95% CI, 5.3–16.5, $P < 0.01$), and 9.7-fold (95% CI, 7.8–22.5, $P \leq 0.01$) significantly higher than those before paroxetine coadministration. No significant change was found in 9-hydroxyrisperidone concentrations between baseline and coadministration of paroxetine 10 mg/d (0.9-fold), 20 mg/d (0.9-fold), and 40 mg/d (0.8-fold). Active moiety was not increased during the paroxetine 10 mg/d (1.3-fold, not significant) or 20 mg/d (1.6-fold, not significant), but was significantly increased by 1.8-fold (95% CI, 1.4–2.7, $P < 0.05$) during the paroxetine 40 mg/d. Metabolic ratio increased significantly during coadministration of paroxetine 10 mg/d by 4.2-fold (95% CI, 3.4–6.2, $P < 0.001$), 20 mg/d by 8.2-fold (95% CI, 6.0–16.0, $P < 0.001$), and 40 mg/d by 12.6-fold (95% CI, 9.6–26.8, $P < 0.001$).

Five *CYP2D6* genotypes were identified in the patients: 2 heterozygotes of the **1* and **2* alleles, 6 heterozygotes of the **1* and **10*, 1 heterozygote of the **2* and **10*, 2 homozygotes of the **10*, and 1 heterozygote of **5* and **10*. These patients were divided into 3 groups according to the number of mutated alleles: 0 mutated allele in 2 patients, 1 mutated allele in 7 patients, and 2 mutated alleles in 3 patients. Large differences in the percentage of control in risperidone concentration and baseline ratios with paroxetine coadministration were observed between *CYP2D6* genotype groups. They had smaller change in risperidone concentration during paroxetine coadministration, although it was not statistically significant because of small number of subjects. There were significant correlations in paroxetine-mediated increase in plasma concentration of risperidone during 10 mg/d ($r_s = -0.671$, $P < 0.05$), 20 mg/d ($r_s = -0.804$, $P < 0.01$), and 40 mg/d ($r_s = -0.832$, $P < 0.001$) (Fig. 1).

Scores of negative symptoms were significantly reduced after coadministration of paroxetine 10 mg ($P < 0.05$), 20 mg ($P < 0.01$), and 40 mg ($P < 0.01$), respectively, whereas those in total PANSS, excitement, cognitive, positive, and depression symptoms were unchanged (Table 2). No changes were found in scores of total CDSS throughout the study. Scores in UKU side effects rating scale were increased significantly in total by paroxetine 40 mg

TABLE 1. Changes in Drug Concentrations, Before and After Coadministration With Incremental Doses of Paroxetine in 12 Schizophrenic Patients

	Paroxetine Dose (mg/d)			
	0	**10**	**20**	**40**
Risperidone (ng/mL)	10.4 ± 18.5	25.9 ± 24.7*	41.9 ± 29.4*	58.5 ± 41.7*
Ratio to baseline		3.8 (3.2, 5.8)	7.1 (5.3, 16.5)	9.7 (7.8, 22.5)
9-OH risperidone (ng/mL)	38.0 ± 18.2	35.1 ± 15.5	33.6 ± 15.5	32.6 ± 23.7
Ratio to baseline		0.9 (0.8, 1.0)	0.9 (0.6, 2.1)	0.8 (0.5, 2.0)
Active moiety (ng/mL)	48.4 ± 35.2	61.0 ± 37.2	75.5 ± 43.8	91.1 ± 64.3†
Ratio to baseline		1.3 (1.2, 1.5)	1.6 (1.2, 2.3)	1.8 (1.4, 2.7)
Metabolic Ratio	0.2 ± 0.2	0.7 ± 0.5‡	1.2 ± 0.4‡	1.8 ± 0.4‡
Ratio to baseline		4.2 (3.4, 6.2)	8.2 (6.0, 16.0)	12.6 (9.6, 26.8)
Paroxetine (ng/mL)	—	11.0 ± 12.8	45.0 ± 42.4	189.3 ± 138.5

Drug concentrations are shown as mean ± SD (range). Ratio to baseline are shown as geometric mean (95% CI). 9-OH risperidone indicates 9-hydroxyrisperidone; active moiety, risperidone plus 9-OH risperidone; metabolic ratio, risperidone/9-OH risperidone.

*$P < 0.01$, compared with baseline and obtained by Bonferroni multiple comparison.

†$P < 0.05$, compared with baseline and obtained by Bonferroni multiple comparison.

‡$P < 0.001$, compared with baseline and obtained by Bonferroni multiple comparison.

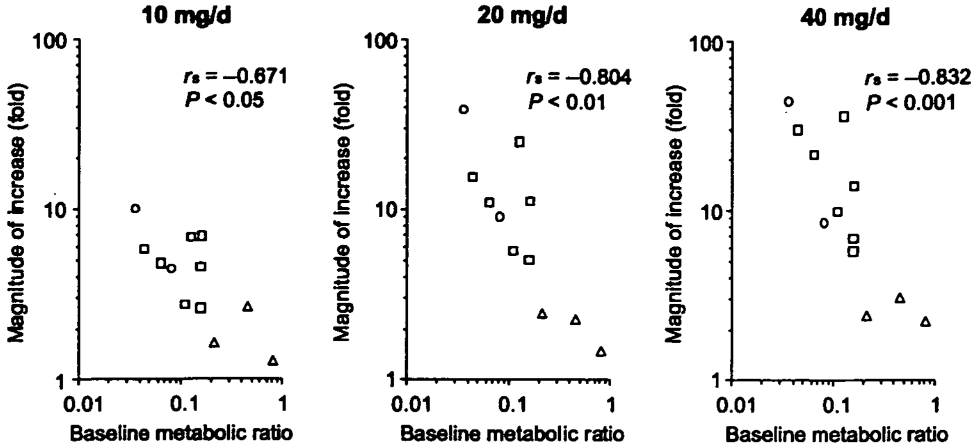

FIGURE 1. Correlations between baseline concentration ratio of 9-hydroxyrisperidone to risperidone and the magnitude of paroxetine-mediated increase in risperidone concentration in schizophrenic patients receiving 4 mg/d of risperidone. Open circles indicate data of patients with no mutated allele for CYP2D6. Open squares and triangles indicate data of patients with 1 and 2 mutated alleles for CYP2D6, respectively.

coadministration ($P < 0.05$) and in extrapyramidal symptoms by 20 mg ($P < 0.05$) and 40 mg ($P < 0.05$), whereas there were no significant differences in psychic and autonomic symptoms.

DISCUSSION

The results of this study showed that coadministration of paroxetine resulted in significant increase in the plasma concentration of risperidone, which is in line with previous in vivo interaction studies with paroxetine,[8] fluoxetine,[25] and thioridazine.[26] Plasma concentrations of risperidone during coadministration of paroxetine 10, 20, and 40 mg/d were increased by 3.8-fold, 7.1-fold, and 9.7-fold, respectively, suggesting that the interaction between paroxetine and risperidone occurs in a dose-dependent manner. In addition, metabolic ratio increased significantly during coadministration of paroxetine 10 mg/d by 4.2-fold, 20 mg/d by 8.2-fold, and 40 mg/d by 12.6-fold. Thus, this finding implies that CYP2D6 catalyzing 9-hydroxylation plays important role in this interaction. Surprisingly, only even 10 mg/d of paroxetine has a potent inhibitory effect on risperidone metabolism. Therefore, careful monitoring is required even when low dose of paroxetine is added to neuroleptics catalyzed by CYP2D6, such as perphenazine, thioridazine, and zuclopenthixol.

TABLE 2. Changes in PANSS, CDSS, and UKU Scores Before and After Coadministration With Incremental Doses of Paroxetine in 12 Schizophrenic Patients

	Paroxetine Dose (mg/d)			
	0	10	20	40
PANSS scores				
Total	72.2 ± 14.6	67.4 ± 16.6	60.0 ± 10.0	64.9 ± 13.3
Negative	24.9 ± 3.7	18.9 ± 6.0*	17.2 ± 5.9†	18.8 ± 4.1†
Excitement	8.0 ± 1.5	8.3 ± 2.3	8.1 ± 1.6	8.7 ± 2.5
Cognitive	13.6 ± 4.2	13.3 ± 5.2	11.5 ± 2.2	13.6 ± 4.1
Positive	13.0 ± 4.4	13.6 ± 4.6	12.5 ± 3.1	12.5 ± 2.7
Depression	12.7 ± 5.0	13.3 ± 4.6	10.8 ± 2.9	11.3 ± 4.4
CDSS scores				
Total	3.4 ± 2.1	2.9 ± 2.6	2.3 ± 2.1	1.9 ± 2.1
UKU scores				
Total	3.2 ± 2.5	3.4 ± 3.1	4.7 ± 3.3	6.3 ± 4.6*
Psychic	1.5 ± 1.6	1.3 ± 1.7	1.6 ± 1.6	2.5 ± 2.2
Extrapyramidal	0.5 ± 0.8	1.0 ± 1.2	1.8 ± 1.9*	2.3 ± 2.7*
Autonomic	1.2 ± 1.2	1.1 ± 1.4	1.3 ± 1.5	1.4 ± 1.9

Data are shown as mean ± SD.

*$P < 0.05$, compared with baseline and obtained by Bonferroni multiple comparison.

†$P < 0.01$, compared with baseline and obtained by Bonferroni multiple comparison.

As with risperidone, paroxetine metabolism is primarily involved in mediation by CYP2D6 in the liver,[27] whereas several in vitro studies have shown that paroxetine is an inhibitor of CYP2D6.[27–30] An in vivo study has shown that 83% of subjects phenocopied to PM status after receiving the paroxetine 20 mg/d, as measured by dextromethorphan/dextrorphan urinary ratio.[31] Jeppesen et .al[32] have demonstrated that CYP2D6 activity was proportionately decreased with incremental dose of paroxetine, and 3 of the 6 volunteers changed phenotype from extensive metabolizers to poor metabolizers after only a single oral dose of 40 or 80 mg paroxetine, as measured by sparteine. Moreover, paroxetine 20 mg daily resulted in a change in all individuals from ultrarapid metabolizers to normal extensive metabolizers, as measured by debrisoquine metabolic ratio, and the high paroxetine concentrations in 2 subjects treated with 40 mg daily caused them to be poor metabolizers.[33] In addition, a recent in vitro study with human liver microsome demonstrated that paroxetine exhibited a preincubation-dependent increase in inhibitory potency, suggesting mechanism-based inhibition of CYP2D6 by paroxetine.[34] Based on these findings, therefore, the inhibitory effect of paroxetine on CYP2D6 activity is potent and occurs in a dose-dependent manner, which supports our findings.

No change was found in 9-hydroxyrisperidone throughout the study period. This finding was also obtained in previous other studies with paroxetine[8] and fluoxetine.[25] Although we do not have clear explanation for it, paroxetine might inhibit the further elimination of 9-hydroxyrisperidone because paroxetine affects other metabolic pathways of risperidone such as 7-hydroxylation and *N*-dealkylation or both.

There were significant correlations between paroxetine-mediated increase in risperidone concentration during each phase of paroxetine coadministration and baseline metabolic ratio of risperidone/9-hydroxyrisperidone that is regarded as index of CYP2D6 activity. Moreover, *CYP2D6* genotype was linked with the baseline metabolic ratio of risperidone/9-hydroxyrisperidone. This is not a surprising finding because patients with mutated alleles have low activity of CYP2D6, which paroxetine is expected to inhibit. Thus, the magnitude of this drug interaction can be predicted by CYP2D6 activity.

However, another mechanism should also be considered. 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,[35] while an in vitro study with cell lines has recently demonstrated that the inhibitory effect of paroxetine on P-glycoprotein is potent.[36] In addition, a population-based, nested, case-control study suggested an increased risk of toxicity of digoxin, which is a substrate of P-glycoprotein,[37] after initiation of paroxetine (odds ratio, 2.8; 95% CI, 1.6–4.7).[38] Therefore, the bioavailability of risperidone might, to some extent, be increased through inhibition of risperidone transporting back to the intestinal lumen after absorption by paroxetine treatment, although the contribution of P-glycoprotein to risperidone disposition and inhibitory effect of paroxetine on P-glycoprotein are under further in vivo investigation.

Although interpretation of pharmacodynamic results must be limited because double-blind methodology was not conducted in the present study, it is noteworthy that significant changes in clinical symptoms or side effects were observed during paroxetine coadministration (Table 2). Improvement of negative symptoms observed during the administration of paroxetine from 10 to 40 mg/d in this study suggests that an activating effect of augmentation of risperidone on chronic patients occurs even with a low dose of paroxetine. Coadministration of 20 and 40 mg/d of paroxetine led to worsening of extrapyramidal side effects probably because of elevation in active moiety concentration. This interaction may have clinical implication leading to potentially toxic total plasma risperidone levels. Careful clinical observation and monitoring of plasma risperidone levels may be useful whenever paroxetine is coadministered with risperidone, and low doses of paroxetine should be recommended for augmentation therapy. If no improvement of negative symptoms is observed, carefully monitored titration of paroxetine is required.

In trials using antidepressants for the treatments of depressive symptoms or negative symptoms in schizophrenia, it has been described that the improvement of the positive symptoms might be delayed and/or that a relapse of positive symptoms can occur in patients who already had a partial full remission of psychotic symptoms.[39,40] In fact, our previous study reported that 3 of 12 patients had a worsening of excitement symptoms after adding fluvoxamine to haloperidol.[41] However, no relapse in any patients was observed throughout the study period. The increased plasma concentration of active moiety might be attributed to avoidance of this kind of risk, although extrapyramidal side effects increased.

In conclusion, paroxetine increases plasma concentrations of risperidone and active moiety in a dose-dependent manner. This drug interaction may be explained by an inhibitory effect of CYP2D6 activity proportionately with paroxetine doses. Low-dose coadministration of paroxetine with risperidone may be safe and effective in the treatment of schizophrenic patients with negative symptoms.

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