Arch Pharm Res Vol 29, No 6, 525-533, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Pharmacokinetics and Bioequivalence Evaluation of Risperidone in Healthy Male Subjects with Different *CYP2D6* Genotypes

Hea-Young Cho[1] and Yong-Bok Lee[1,2]

[1]Clinical Trial Center, Chonnam National University Hospital, Gwangju 501-757, Korea and [2]College of Pharmacy and Institute of Bioequivalence and Bridging Study, Chonnam National University, Gwangju 500-757, Korea

(Received May 10, 2006)

The aim of this study was to evaluate the bioequivalence of risperidone in healthy male subjects representing different *CYP2D6* genotypes with respect to risperidone, 9-hydroxyrisperidone (9-OH-risperidone), and active moiety. A total of 506 Korean subjects were genotyped for *CYP2D6*10* by means of allele-specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Based on the genotype analysis, 24 subjects, 7 homozygous for *CYP2D6*1*, 10 for **10*, and 7 heterozygous for **10*, were recruited and received a single oral dose of 2 mg risperidone tablet in this study. Serum concentrations of risperidone and 9-OH-risperidone up to 48 h were simultaneously determined. There were no significant differences of the active moiety, risperidone, and 9-OH-risperidone between the two preparations in $AUC_{0-\infty}$ and C_{max}. The 90% confidence intervals (CIs) for the ratio of means of the log-transformed $AUC_{0-\infty}$ and C_{max} for the active moiety, risperidone, and 9-OH-risperidone were all within the bioequivalence acceptance criteria of 0.80-1.25. The *CYP2D6*10* allele particularly was associated with higher serum concentrations of risperidone and the risperidone/9-OH-risperidone ratio compared with the *CYP2D6*1* allele. The results demonstrate that the two preparations of risperidone are bioequivalent and it can be assumed that they are therapeutically equivalent and exchangeable in clinical practice. Furthermore, the pharmacokinetic parameters of risperidone and the risperidone/9-OH-risperidone ratio are highly dependent on the *CYP2D6* genotypes.

Key words: Risperidone, 9-Hydroxyrisperidone, Pharmacokinetics, Bioequivalence, *CYP2D6* genotype, Healthy subjects

INTRODUCTION

Many factors, such as dietary intake, age, and concurrent drug therapies, affect a person's response to medications. Importantly, genetic makeup determines inherent pharmacokinetics, giving rise to inter-individual differences in drug absorption, distribution, metabolism, and excretion (Evans and Relling, 1999; Rogers *et al.*, 2002).

Cytochromes are the major Phase I biotransformation enzymes, responsible for maintaining homeostasis in metabolizing both endogenous substances and exogenous xenobiotics (Ramamoorthy *et al.*, 2001; Daniel and David, 2002). *CYP2D6* metabolizes clinically important drugs including neuroleptics, tricyclic antidepressants, selective serotonin reuptake inhibitors, beta adrenergic drugs and opioids (Brosen and Rasmussen, 1996). The *CYP2D6* gene has turned out to be extremely polymorphic with more than 70 allelic variants described so far (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). The active allele is *CYP2D6*1* and the most common inactive alleles are *CYP2D6*4*, *CYP2D6*3* and *CYP2D6*5*, where the entire gene locus is deleted. In Caucasians, approximately 8% are poor metabolizers for *CYP2D6*. In Asian peoples, there is a low incidence (1%) of poor metabolism and there is no published data on the incidence of ultrarapid metabolism (Bathum *et al.*, 1999; Wilson *et al.*, 2001; Bertilsson *et al.*, 2002). Despite of the low frequency of poor metabolizers of *CYP2D6* substrates in the Asian populations, they have been found to carry a high frequency (51%) of the C_{188} to T_{188} mutation in exon 1, which causes a Pro_{34}→Ser amino acid substitution leading to a form of an unstable enzyme with lower metabolic activity (Johansson *et al.*, 1994). This mutation, which is a

Correspondence to: Yong-Bok Lee, College of Pharmacy and Institute of Bioequivalence and Bridging Study, Chonnam National University, Gwangju 500-757, Korea
Tel: 82-62-530-2931, Fax: 82-62-530-0106
E-mail: leeyb@chonnam.chonnam.ac.kr

defining mutation for *CYP2D6*10* allele, is relatively rare in Caucasian populations (Armstrong *et al.*, 1994). Therefore, in the Asian populations, *CYP2D6*10* allele seems to be more clinically important than other *CYP2D6* mutant alleles (Gan *et al.*, 2002; Ono *et al.*, 2002).

Risperidone, 3-[2-[4-(6-fluoro-1,2-benzisoxazole-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido [1,2-a]pyrimidin-4-one, is pharmacologically characterized by both a selective serotonin-5HT$_2$ and a dopamine D$_2$ receptor antagonistic properties (Janssen *et al.*, 1988; Leysen *et al.*, 1988). The drug is claimed to be effective in the treatment of both positive (hallucinations, delusions, and thought disorders) and negative (emotional withdrawal, blunted effect, and loss of speech) symptoms of schizophrenia and to have a low incidence of extrapyramidal side effects compared to classic antipsychotics (Claus *et al.*, 1992; Chouinard and Arnott, 1993). Therapeutic dose range is 3-8 mg, twice daily, which may need to be reduced in special patient groups (elderly and renal diseased) (Mannens *et al.*, 1993).

Risperidone is extensively converted in the liver. The main metabolite of risperidone, 9-OH-risperidone, has a similar pharmacological activity as the parent compound, and the serum concentration of the active moiety is thus the sum of the serum concentrations of risperidone and 9-OH-risperidone (Huang *et al.*, 1993). The first and major metabolic step in risperidone metabolism is alicyclic hydroxylation of the tetrahydropyridopyrimidinone ring at the 9-position which is catalized by *CYP2D6* (He and Richardson, 1995; Fang *et al.*, 1999). Thus, inter-individual variability in serum levels of the parent drug or metabolite can occur. Therefore, serum level monitoring may help to determine the required dose of the drug.

We have previously investigated the frequency of the single nucleotide polymorphisms (SNPs) on *CYP2D6* in Korean population, and then subjects have been recruited on the basis of their *CYP2D6* genotypes. The purposes of this study were to determine the pharmacokinetic parameters of two preparations of risperidone 2 mg tablet and to compare these parameters statistically to evaluate the bioequivalence between the two preparations, Riperidone tablet (test preparation, manufactured by Whan In Pharm. Co., Seoul, Korea) and Risperdal® tablet (reference preparation, manufactured by Janssen Pharmaceutica, Beerse, Belgium). Furthermore, typical pharmacokinetic parameters such as $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{1/2}$, and the relative total clearance (CL/F, only risperidone) of active moiety, risperidone, and 9-OH-risperidone in healthy subjects representing different *CYP2D6* genotypes were obtained and compared. In addition, *in vitro* dissolution of two preparations was tested according to United States Pharmacopoeia (USP) 26 (2003) and KFDA guideline for dissolution testing of solid oral products.

MATERIALS AND METHODS

Genotyping method

A 3 mL blood sample was obtained from each subject into EDTA tubes (BD Vacutainer™, UK). Genomic DNA was isolated from peripheral leukocytes by Wizard® Genomic DNA Purification Kit (Promega, WIS, U.S.A.), according to the guidelines of the manufacturer. For the determination of the *CYP2D6* genotype, PCR was carried out with minor modifications to amplify the portions of exon 1 containing C188T mutation as described by Johansson *et al.* (1994). For *CYP2D6*10* allele genotype, PCR was carried out in 20 μL reaction mixture containing PCR PreMix, 200-300 ng genomic DNA and 10 pmol each primer. Sense primer (5'-ACCAGGCCCCTCCAC-CGG-3') annealed intron, 196 bp upstream of intron/exon 1 junction and antisense primer (5'-TCTGGT AGGGGA-GCCTCAGC-3' and 5'-GTGGTGGGGCATCCTCAGG-3') annealed exon 1 starting from position 302 to 321 and L302 to 320. PCR was initiated by an initial denaturation at 94°C for 5 min followed by 30 cycles, each consisting of denaturation at 94 for 1 min, annealing at 68.5°C for 1 min, and extention at 72°C for 1 min. Final extention was performed at 72°C for 5 min. The amplified 534 bp fragment consisted of 196 bp of intron and 338 bp of exon 1. PCR products were electrophoresed on 2% agarose gel and visualized by ultraviolet transillumination after staining with ethidium bromide.

Subjects

A total of 506 subjects were genotyped for the *CYP2D6*10* allele. Of the 506 individuals analyzed, 136 were homozygous for the *CYP2D6*1* wild type allele (**1/*1*, 26.88%), 137 for the **10* allele (**10/*10*, 27.08%) and 233 were heterozygous for these alleles (**1/*10*, 46.05%). The allele frequencies of *CYP2D6*1* and **10* were thus 49.90% and 50.10%, respectively.

And then twenty-four subjects have been recruited on the basis of their *CYP2D6* genotypes, **1/*1* for 7, **1/*10* for 7 and **10/*10* for 10 subjects. They range in age from 22 to 30 years (24.63 ± 1.74 years), in weight from 51.1 to 73.1 kg (63.17 ± 7.33 kg), and in height from 165.1 to 182.5 cm (173.04 ± 4.64 cm). This sample size, which was calculated from the results of the preliminary study by posterior power analysis, was shown to have sufficient statistical power greater than 80% to detect 20% differences in the pharmacokinetic parameters between the two preparations (α=0.05).

Each subject was selected after passing a clinical screening procedure including a physical examination and laboratory tests (blood analysis; hemoglobin, hematocrit, RBC, WBC, platelet, differential counting of WBC, total proteins, albumin, sGOT, sGPT, alkaline phosphatase,

total bilirubin, cholesterol, creatinine, blood urea nitrogen, and glucose fasting and urine analysis; specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC and cast). These measures were necessary to ensure that the existing degree of variation would not be due to an influence of disease or other medications. Subjects were excluded if they were possibly sensitive to this type of preparations, had a history of any illness of hepatic, renal or cardiovascular systems, or had taken alcohol or other preparations within 4 weeks prior to the study.

Test and reference preparations

The test preparation, Riperidone (containing 2 mg of risperidone, lot No. 3501, Whan In Pharmaceutical Company, Seoul, Korea) and the reference preparation, Risperdal (containing 2 mg of risperidone, lot No. 7536, Janssen Pharmaceutica, Beerse, Belgium), were supplied as tablets.

Study design

This study was a single-dose, randomized, two-treatment, two-period crossover design consisting of a one-week washout period between the doses. All subjects were hospitalized at 8:00 PM one day before this study and fasted 12 h before each drug administration and 4 h after dose administration. Each subject received a single dose of either preparation (reference or test) of risperidone 2 mg with 240 mL of spring water at 8:30 AM. At 4, 10, 22, 28, 34 and 46 h after oral administration, all subjects were given standardized meals and were not allowed any drugs and alcohol or xanthine-containing foods and beverages during each study period. The subjects were continuously monitored by Chonnam National University Hospital staff throughout the confined period of study. Blood pressure and pulse rate were checked before and after the study.

This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practice. The protocol was approved by an institutional review board at Institute of Bioequivalence and Bridging Study, Chonnam National University, Gwangju, Korea, and informed consent was obtained from the subjects after explaining the nature and purpose details of the study in accordance with Korean Guideline for Bioequivalence Test.

Blood sampling

Blood samples were drawn into Vacutainer® (5 mL, Becton Dickinson and Company, U.S.A.) through indwelling cannula at the following times; predose (to serve as a control), at 20, and 40 min and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, and 48 h after the administration. The heparinized normal saline injectable solution, 0.3 mL, was flushed into the cannula to prevent blood clotting after each blood sampling. The blood samples were centrifuged for 15 min at 1500 *g*, and the serum was transferred to polyethylene tubes and stored at -80°C until the HPLC assay.

Assay validation

Concentrations of risperidone and its therapeutically active main metabolite, 9-OH-risperidone in serum were analyzed using a validated HPLC method with ultraviolet (UV) detection at 280 nm. Briefly, serum (1 mL) was mixed with 100 μL of internal standard (azelastine, 1 μg/mL in methanol), 100 μL of methanol and 500 μL of 1 mol/L sodium hydroxide by vortex-mixing for 3 sec. The sample was extracted with 4 mL of ethyl acetate by vortex-mixing for 2 min and centrifuged at 3000 *g* for 10 min. The organic layer (upper phase) was transferred to a clean test tube and back-extracted with 150 μL of 0.025 mol/L sulfuric acid by vortex-mixing for 1 min and centrifuged at 3000 *g* for 5 min. The aqueous layer was alkalinized with 30 μL of 1 mol/L sodium hydroxide and evaporated in a gentle stream of nitrogen. Sample aliquots of 50 μL volume were injected directly onto the HPLC system. The HPLC system consisted of a model LC-10A*i* isocratic pump (Shimadzu, Kyoto, Japan), equipped with a Rheodyne 7725 injection valve (Rheodyne, Cotati, CA, U.S.A.) and a model SPD 10A*vp* UV-VIS detector (Shimadzu, Kyoto, Japan). The separation was performed on Phenomenex (Torrance, CA, U.S.A.) LUNA C_{18}(2) column (3 μm particle size, 150 mm×3.0 mm i.d.), using a mobile phase consisting of 40 mmol/L ammonium acetate:methanol:acetonitrile (35:35:30, v/v/v) at the flow rate of 0.3 mL/min. The effluent was monitored at UV wavelength of 280 nm. Detector output was quantitated on a model Class LC-10 integrator (Shimadzu, Kyoto, Japan).

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by standard noncomparmental methods (Gibaldi and Perrier, 1982) using WinNonlin® software (Pharsight Corporation, CA, U.S.A.), a pharmacokinetic data analysis program. Active moiety was defined as the sum of risperidone and 9-OH-risperidone. The C_{max} and T_{max} were determined by the inspection from individual serum concentration-time profiles. The $AUC_{0-\infty}$ was calculated as $AUC_{0-t}+C_t/\lambda_Z$, where AUC_{0-t} is calculated by the linear trapezoidal rule from 0 to 48 h and C_t is the last measurable concentration. The $t_{1/2}$ was calculated as $0.693/\lambda_Z$, where λ_Z is terminal rate constant. The CL/F was calculated using formula $CL/F=dose/AUC_{0-\infty}$, where F is oral bioavailability. Metabolic ratio (MR) was defined as the ratio of the C_{max} for risperidone and 9-OH-risperidone.

Statistical analysis

Bioequivalence (BE) was assessed by mean of an ANOVA for crossover design and calculating 90% CIs of the ratio test/reference (T/R) (Westlake, 1972; Mandallaz and Mau, 1981; Locke, 1984). Statistical evaluations for $AUC_{0-\infty}$ and C_{max} of the active moiety, risperidone, and 9-OH-risperidone, after ln-transformation, were performed by ANOVA for two-way crossover design using the general linear model (GLM) procedure with sequence, subject within sequence, period, and formulation effect. For estimation of BE, the 90% CIs of the geometric mean ratio test/reference for $AUC_{0-\infty}$ and C_{max} were calculated assuming a multiplicative model and a parametric (Shuirmann's 2 one-sided *t*-test) approach (Chow and Liu, 1992). Comparisons of T_{max} values were made with the non-parametric Hodges-Lehmann's confidence interval approach assuming an additive model. The range of BE for parametric analysis was set to the commonly accepted 0.8-1.25 of the pharmacokinetic parameters for the $AUC_{0-\infty}$ and C_{max} of the active moiety, risperidone, and 9-OH-risperidone, and the range of equivalence for non-parametric analysis was set to the ±20% of the reference mean. All statistical comparisons were performed with a type 1 error (a) of 0.05 using Equiv Test™ software (Equiv Test 2.0™, Statistical Solution Ltd., 2001).

Data were only used reference serum data and analyzed by Kruskal-Wallis non-parametric rank analysis of variance to compare an overall difference among genotypes. A *P* value of less than 0.05 was considered to be statistically significant.

Dissolution test

The *in vitro* dissolution tests, as second equivalence criterion of two risperidone preparations, were carried out using the dissolution apparatus II (paddle method) of USP at 37±0.5°C, 50 rpm and 900 mL of the four dissolution media, pH 1.2, 4.0, 6.8 buffer solutions, and water. Drug release tests were conducted on 12 individual dosage units of the test and the reference preparation used in the BE studies. Samples were removed at 5, 10, 15, 30, and 45 min, filtered and assayed by HPLC coupled with UV detector at 260 nm. Finally, the dissolved risperidone content was expressed as percent of stated amounts.

The acceptance criteria for assessment of equivalence of dissolution profiles between two preparations were as follows. When the average dissolution from reference preparation reached 85% within 15 min, the average dissolution from test preparation should also reach 85% within 15 min. When the average dissolution from reference preparation reached 85% after more than 15 min, the average dissolution from test preparation should not be deviated by more than 15% from that of the reference preparation at two time points (Guideline for Bioequivalence Test, 2002). In addition, to compare dissolution profiles, the f_2 tests were used. An f_2 value ≥ 50 indicated a sufficiently similar dissolution profile (Guidance for Industry, 1997).

RESULTS AND DISCUSSION

Assay validation

The method was validated according to FDA guidance and international guidelines (Shah *et al.*, 2000; Guidance for Industry, 2001). The retention times for 9-OH-risperidone, risperidone and internal standard (azelastine) were approximately 5.5, 7.5 and 10.5 min, respectively. No endogenous substances interfered with the detection of 9-OH-risperidone, risperidone or I.S. at their retention times (figure not shown). The intra- and inter-day coefficients of variation for risperidone and 9-OH-risperidone were less than 13.53 and 14.32%, respectively, over the 0.2 to 50 ng/mL range. The lower limit of quantitation (LLOQ), based on a background approximately 10 times, was 0.2 ng/mL for both of risperidone and 9-OH-risperidone; at this concentration the accuracies were 120.00 and 95.00% while precisions remained below 12.60 and 12.16%, respectively. Correlation coefficients for calibration curves were 0.999 for both of risperidone and 9-OH-risperidone, respectively. Accuracy and precision were calculated using interpolated concentration of quality control (QC) samples at 3 concentrations. The QC standards for risperidone and 9-OH-risperidone were shown to have acceptable accuracies and precisions, with bias ≤10.00 and ≤14.00, and coefficients of variance ≤13.70 and ≤14.23, respectively. The mean absolute recovery of risperidone and 9-OH-risperidone determined from 5 aliquots of QC samples was found to be 89.17 ± 4.74 and 97.11 ± 4.50%, respectively.

Clinical observations

Both risperidone preparations administered orally were well tolerated. The most commonly reported adverse events after intake of either preparation were somnolence, lethargy, and headache, which occurred in all subjects. All adverse events were mild and resolved without treatment. There were no protocol violations and all 24 subjects completed the study.

Pharmacokinetic characteristics

The mean serum concentration-time profiles of the active moiety, risperidone, and 9-OH-risperidone for the test and the reference preparations are shown in Fig. 1, and descriptive statistics of the derived pharmacokinetic parameters such as $AUC_{0-\infty}$, C_{max}, T_{max} and $t_{1/2}$ for two preparations are summarized in Table I. The average $t_{1/2}$ of risperidone and 9-OH-risperidone in healthy male Korean subjects was consistent with values reported in

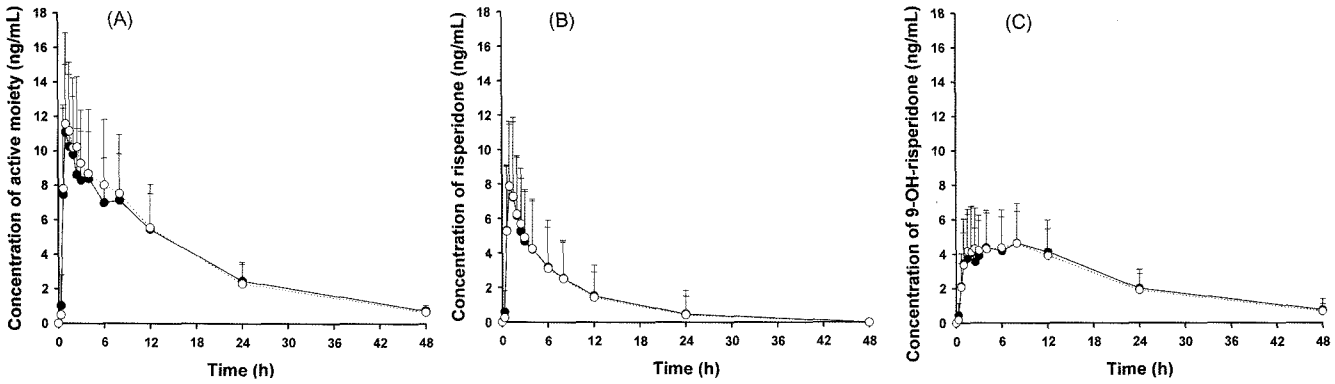

Fig. 1. Mean (± S.D.) serum concentration-time curves for (A) the active moiety, (B) risperidone, and (C) 9-OH-risperidone following oral administration of Risperdal tablet (●, reference) and Riperidone tablet (○, test) at the risperidone dose of 2 mg to 24 subjects. Vertical bar represents the standard deviation of the mean.

Table I. Pharmacokinetic parameters for the active moiety, risperidone, and 9-OH-rieperidone with the two risperidone preparations after oral administration of 2 mg risperidone to 24 subjects

	Geometric mean		Arithmetic mean		S.D.		Min/Max		C.V. (%)	
	Ref.	Test	Ref.	Test	Ref.	Test	Ref.	Test	Ref.	Test
Active moiety										
$AUC_{0-\infty}$ (ng·h/mL)	182.91	186.38	187.13	192.72	40.46	52.64	113.35/267.73	125.40/324.15	21.62	27.31
C_{max} (ng/mL)	11.78	13.17	12.48	13.95	4.07	4.80	4.68/ 19.87	6.19/ 23.86	32.61	34.41
T_{max} (h)			1.44	1.67	0.77	1.13	0.67/ 4.00	0.67/ 6.00	53.47	67.66
$t_{1/2}$ (h)			13.03	12.34	2.87	2.51	10.09/ 20.72	8.52/ 17.88	22.03	20.34
Risperidone										
$AUC_{0-\infty}$ (ng·h/mL)	40.90	41.06	58.93	57.24	46.88	44.04	6.25/189.51	8.04/154.39	79.55	76.94
C_{max} (ng/mL)	7.73	8.37	8.96	9.40	4.31	4.43	1.25/ 19.17	2.20/ 21.93	48.10	47.13
T_{max} (h)			1.17	1.17	0.39	0.47	0.67/ 2.00	0.67/ 2.50	33.33	40.17
$t_{1/2}$ (h)			4.99	4.74	2.74	2.29	1.81/ 12.74	1.57/ 9.09	54.91	48.31
9-OH-risperidone										
$AUC_{0-\infty}$ (ng·h/mL)	134.54	134.92	137.88	137.36	32.30	27.00	93.55/233.09	101.68/209.10	23.43	19.66
C_{max} (ng/mL)	5.11	5.33	5.61	5.83	2.47	2.62	1.81/ 10.92	1.91/ 12.60	44.03	44.94
T_{max} (h)			7.46	6.27	5.34	5.08	1.00/ 24.00	1.00/ 24.00	71.58	81.02
$t_{1/2}$ (h)			17.86	18.20	4.96	11.33	12.19/ 32.23	10.22/ 69.64	27.77	62.25

Test = test preparation, Ref. = reference preparation.
$AUC_{0-\infty}$: area under the serum concentration-time curve from 0 h to infinity, C_{max}: maximum concentration, T_{max}: time to reach C_{max}, $t_{1/2}$: terminal half-life.

the literature (range ~20 h) (Huang *et al.*, 1993). The average CL/F (L/h) of the test and the reference preparations were 69.70 ± 65.33 *vs* 90.18 ± 126.97, respectively.

Bioequivalence analysis with *CYP2D6* genotypes

No significant sequence effect was found for all of the pharmacokinetic parameters indicating that the crossover design was properly performed. Significant F test values were found between the subjects and subject nested sequence (SEQ) for $AUC_{0-\infty}$, C_{max} and T_{max} indicating a substantial inter-subject variation in the pharmacokinetics of risperidone from the two preparations. However, a SEQ effect in the two-way crossover design did not impair the BE conclusion.

BE preparations are usually considered as therapeutically equivalent. BE is generally accepted if the 90% CIs for the main parameters $AUC_{0-\infty}$ and C_{max} lie within an acceptance range of 0.8 to 1.25. Table II shows the results of the BE analysis of the main pharmacokinetic parameters such as $AUC_{0-\infty}$, C_{max} and T_{max} for the active moiety, risperidone, and 9-OH-risperidone. The parametric point estimates for mean of test preparation/mean of reference preparation (μ_T/μ_R) of $AUC_{0-\infty}$ and C_{max} were 1.004 and 1.083, respectively, and the parametric 90%

Table II. Statistical comparisons of pharmacokinetic parameters for the active moiety, risperidone, and 9-OH-risperidon of risperidone test (T) and reference (R) preparations (n=24)

	Statistical method	Active moiety		Risperidone		9-OH-risperidone	
Parameters	Multiplicative Model	T/R Point estimate	90% CI	T/R Point estimate	90% CI	T/R Point estimate	90% CI
$AUC_{0-\infty}$ (ng·h/mL)	Schuirmann's 2 one-sided *t*-test (parametric)	1.019	0.956-1.087	1.004	0.924-1.090	1.003	0.953-1.055
C_{max} (ng/mL)		1.118	1.005-1.243	1.083	0.990-1.183	1.043	0.914-1.190
	Additive model	T-R Point estimate	90% CI Boundary[1]	T-R Point estimate	90% CI Boundary[1]	T-R Point estimate	90% CI Boundary[1]
T_{max} (h)	Hodges-Lehmann's confidence interval (non-parametric)	0.043	-0.250-0.750 (±0.288)	-0.000	-0.250-0.250 (±0.235)	-1.125	-3.250-1.250 (±1.492)

[1]± 20% of mean of reference preparation.

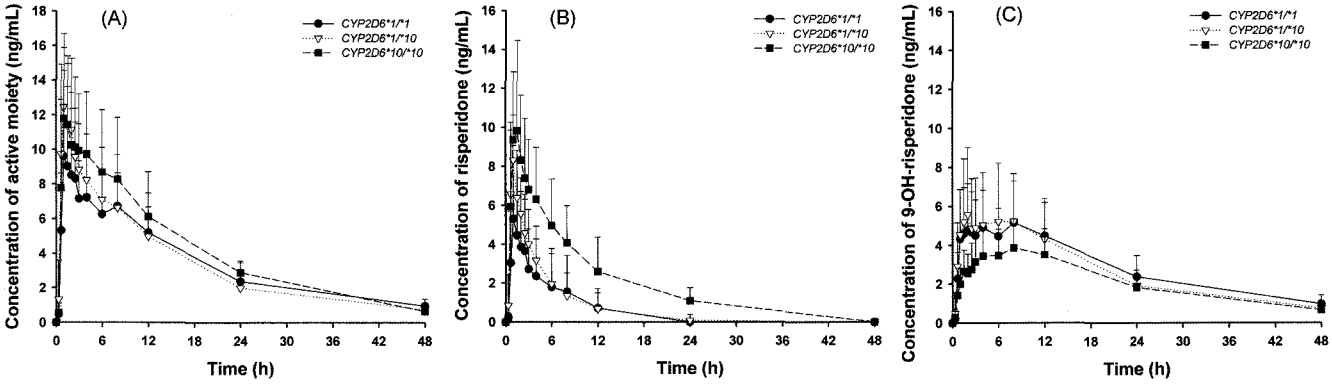

Fig. 2. Mean (± S.D.) serum concentration-time curves of (A) the active moiety, (B) risperidone, and (C) 9-OH-risperidone with different *CYP2D6* genotypes after a single oral dose of risperidone (2 mg). Vertical bar represents the standard deviation of the mean. (●) *CYP2D6*1/*1*, (▽) *CYP2D6*1/*10*, (■) *CYP2D6*10/*10*.

CIs for $AUC_{0-\infty}$ and C_{max} were entirely within the BE acceptance limits of 0.8-1.25. The detailed BE analysis for the T_{max} under the assumption of a non-parametric model was also given in Table II. Based on the results from the non-parametric Hodges-Lehmann's estimator and the paired *t*-test, there were no significant differences ($P>0.05$) in T_{max} and $t_{1/2}$ values for two risperidone preparations.

The *CYP2D6*10* allele was particularly associated with higher serum levels of risperidone compared with the *CYP2D6*1* allele. However, the serum concentrations of the active moiety did not differ between the genotype groups (Fig. 2). Thus, as the parent compound and metabolite are considered pharmacologically equipotent, the polymorphic metabolism is in this case not expected to be of clinical significance. It is to be pointed out that in all the published studies, there was a large overlap in the steady state serum concentrations of neuroleptics between the different genotype groups, indicating that other factors in addition to the *CYP2D6* genotype are of major importance for the interindividual variability in pharmacokinetics.

In addition, the pharmacokinetic parameters in relation to the different *CYP2D6* genotypes were summarized in Table III. The $AUC_{0-\infty}$, C_{max} and CL/F of risperidone and $AUC_{0-\infty}$ of 9-OH-risperidone differed significantly ($P<0.01$) among the three groups. The mean of MR and $t_{1/2}$ of risperidone were significantly different among the three groups ($P<0.01$) (Fig. 3).

Dissolution test

The risperidone is practically soluble in water. Both preparations released more than 85% risperidone within 15 min in a pH 1.2 buffer solution, 30 min in pH 4.0 and 6.8 buffer solutions and 45 min in water (Table IV). Analytical results for the release profiles of two risperidone preparations were very similar in at all dissolution media and an f_2 value in water was 71.7. The dissolution profiles of two risperidone preparations in water are shown in Fig. 4.

CONCLUSIONS

The results of this study confirmed that the two preparations of risperidone (Riperidone and Risperdal tablets) are bioequivalent with respect to the rate and extent of absorption in healthy subjects and with respect to the *in vitro* dissolution profiles. The calculated 90% CIs for

Table III. Pharmacokinetic parameter values for the active moiety, risperidone, and 9-OH-rieperidone in healthy Korean subjects with *CYP2D6*1/*1, CYP2D6*1/*10,* and *CYP2D6*10/*10* genotypes after intake of a single dose of risperidone (2 mg) orally

Variable	*CYP2D6*1/*1* (n=7)	*CYP2D6*1/*10* (n=7)	*CYP2D6*10/*10* (n=10)	Kruskal-Wallis test
Metabolic ratio	1.03±0.79	1.95±1.58	2.77±2.00	P=0.000**
Active moiety				
$AUC_{0-\infty}$ (ng·h/mL)	182.56± 37.75	172.89±32.71	207.00±55.57	P=0.152
C_{max} (ng/mL)	11.44± 4.31	14.17± 4.07	13.78± 4.69	P=0.259
T_{max} (h)	1.68± 0.70	1.31± 0.58	1.63± 1.29	P=0.300
$t_{1/2}$ (h)	15.12± 3.10	11.91± 1.55	11.53± 1.86	P=0.002**
Risperidone				
$AUC_{0-\infty}$ (ng·h/mL)	30.22± 31.20	36.05±20.21	93.01±43.02	P=0.000**
C_{max} (ng/mL)	5.97± 3.73	9.12± 2.32	11.47± 4.48	P=0.003**
T_{max} (h)	1.24± 0.51	0.99± 0.37	1.25± 0.37	P=0.093
$t_{1/2}$ (h)	3.45± 1.51	3.35± 0.74	6.91± 2.44	P=0.000**
CL/F (l/h)	167.60±151.32	65.97±21.74	28.37±18.74	P=0.000**
9-OH-risperidone				
$AUC_{0-\infty}$ (ng·h/mL)	159.26± 24.40	146.50±29.38	116.26±16.27	P=0.000**
C_{max} (ng/mL)	6.38± 2.26	6.67± 3.44	4.60± 1.35	P=0.055
T_{max} (h)	5.36± 3.86	6.04± 6.09	8.50± 5.11	P=0.075
$t_{1/2}$ (h)	17.98± 3.25	19.06±15.01	17.35± 4.73	P=0.194

Mean±S.D., *P<0.05, **P<0.01, significant difference compared with genotypes.
$AUC_{0-\infty}$: area under the serum concentration-time curve from 0 h to infinity, C_{max}: maximum concentration, T_{max}: time to reach C_{max}, $t_{1/2}$: terminal half-life, CL/F: apparent total clearance.

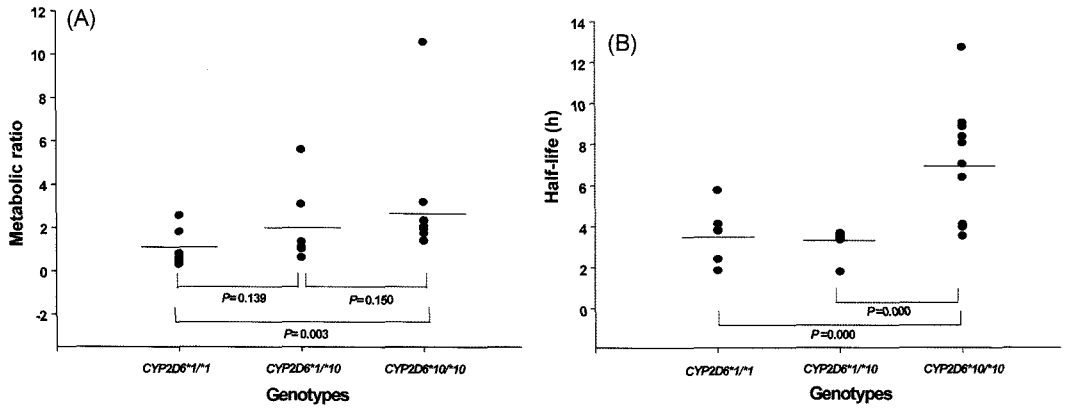

Fig. 3. Association of *CYP2D6* genotypes with (A) the metabolic ratio expressed as serum concentration ratio of risperidone to 9-OH-risperidone and (B) the half-life of risperidone in healthy male Korean subjects. The *CYP2D6* genotypes were **1/*1* (n=7), **1/*10* (n=7), and **10/*10* (n=10). The mean value in each group is indicated by a horizontal line.

Table IV. Dissolution data of two risperidone preparations in four dissolution media (n=12)

Dissolution media	pH 1.2[a]		pH 4.0[b]		pH 6.8[b]		Water[c]	
Preparations	Ref.	Test	Ref.	Test	Ref.	Test	Ref.	Test
Mean (min/max, %)	87.0 (85.0/88.8)	88.7 (86.3/91.5)	93.1 (88.4/101.2)	92.4 (89.4/97.1)	88.1 (86.1/91.6)	89.0 (84.7/92.4)	89.5 (81.6/95.8)	94.5 (92.5/96.8)
S.D.	1.36	1.86	5.04	2.83	1.86	3.20	5.88	1.49
C.V., %	1.56	2.10	5.41	3.06	2.11	3.60	6.57	1.58

[a] Percent of dissolved risperidone content within 15 min.
[b] Percent of dissolved risperidone content within 30 min.
[c] Percent of dissolved risperidone content within 45 min.
Test = test preparation, Ref. = reference preparation.
S.D.=standard deviation, C.V.=(S.D./mean) × 100%.

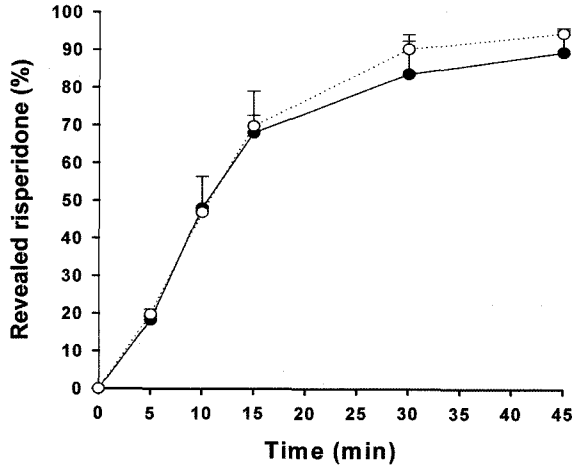

Fig. 4. Dissolution profiles of risperidone from Risperdal tablet (●, reference) and Riperidone tablets (○, test) in water. Data are presented as mean (n=12) ± S.D..

$AUC_{0-\infty}$ and C_{max} of Riperidone/Risperdal ratios (μ_T/μ_R) were well within the acceptable range defined by the US FDA. Therefore, it is concluded that the two preparations of risperidone can be used interchangeably in clinical practice.

Furthermore, the serum concentrations of risperidone and the MR (risperidone/9-OH-risperidone ratio) are highly dependent on the *CYP2D6* genotypes, and significant differences for pharmacokinetic parameters of risperidone and 9-OH-risperidone were observed among *CYP2D6*1/*1*, **1/*10* and **10/*10* groups ($P<0.01$). However, the lack of relationship between the genotype and the active moiety indicates that the *CYP2D6* polymorphism may be of limited importance for the clinical outcome during risperidone treatment.

ACKNOWLEDGEMENTS

This study was supported by the contract from Whan In Pharmaceutical Company, Seoul, Korea.

REFERENCES

Armstrong, M., Fairbrother, K., Idle, J. R., and Daly, A. K., The cytochrome P450 CYP2D6 allelic variant CYP2D6J and related polymorphisms in a European population. *Pharmacogenetic,* 4, 73-81 (1994).

Bathum, L., Skjelbo, E., Mutabingwa, T. K., Madsen, H., Horder, M., and Brosen, K., Phenotypes and genotypes for CYP2D6 and CYP2C19 in a black Tanzanian population. *Br. J. Clin. Pharmacol.,* 48, 395-401 (1999).

Bertilsson, L., Dahl, M. L., Dalén, P., and Al-Shurbaji, A., Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. *Br. J. Clin. Pharmacol.,* 53, 111-122 (2002).

Brosen, K. and Rasmussen, B. B., Selective serotonin re-uptake inhibitors: advances in basic research and clinical practice. *John Wiley & Sons Ltd.,* pp. 87-108, (1996).

Chouinard, G. and Arnott, W., Clinical review of risperidone. *Can. J. Psychiatry.*, 38, 89-95 (1993).

Chow, S. C. and Liu, J. P., Design and Analysis of Bioavailability and Bioequivalence Studies. Marcel Dekker Inc., New York, (1992).

Claus, A., Bollen, J., De Cuyper, H., Eneman, M., Malfroid, M., Peuskens, J., and Heylen, S., Risperidone versus haloperidol in the treatment of chronic schizophrenic inpatients: a multicentre double-blind comparative study. *Acta Psychiatr. Scand.,* 85, 295-305 (1992).

Daniel, W. N. and David, W. R., Clinical importance of the cytochromes P450. *Lance,* 360, 1155-1162 (2002).

Evans, W. E. and Relling, M. V., Pharmacogenomics: translating functional genomics into rational therapeutics. *Science,* 286, 487 (1999).

Fang. J., Bourin, M., and Baker. G. B., Metabolism of risperidone to 9-hydroxy-risperidone by human cytochromes P450 2D6 and 3A4. *Naunyn-Schmiedebergs Arch. Pharmacol.,* 359, 147-151 (1999).

Gan, S. H., Ismail, R., Wan Adnan, W. A., and Wan, Z., Correlation of tramadol pharmacokinetics and CYP2D6*10 genotype in Malaysian subjects. *J. Pharm. Biomed. Anal.,* 30, 189-195 (2002).

Gibaldi, M. and Perrier, D., Pharmacokinetics (2nd ed). Marcel Dekker Inc., New York, (1982).

Guidance for Industry: Bioanalytical Method Validation. US Food and Drug Administration, Center for Drug Evaluation and Research, (2001).

Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms. US Food and Drug Administration, Center for Drug Evaluation and Research, (1997).

Guideline for Bioequivalence Test. Korea Food and Drug Administration, (2002).

He, H. and Richardson, J. S., A pharmacological, pharmacokinetic and clinical overview of risperidone, a new antipsychotic that blocks serotonin 5-HT2 and dopamine D2 receptors. *Int. Clin. Psychopharmacol.,* 10, 19-30 (1995).

http://www.imm.ki.se/CYPalleles/ cyp2d6.htm

Huang, M. L., Van Peer, A., Woestenborghs, R., De Coster, R., Heykants, J., Jansen, A. A., Zylicz, Z., Visscher, H. W., and Jonkman, J. H., Pharmacokinetics of the novel antipsychotic agent risperidone and the prolactin response in healthy subjects. *Clin. Pharmacol. Ther.,* 54, 257-268 (1993).

Janssen, P. A., Niemegeers, C. J., Awouters, F., Schellekens, K. H., Megens, A. A., and Meert, T. F., Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S2 and dopamine-D2 antagonistic properties. *J. Pharmacol. Exp. Ther.,* 244, 685-693 (1988).

Johansson, I., Oscarson, M., Yue, Q. Y., Bertilsson, L., Sjoqvist, F., and Ingelman-Sundberg, M., Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. *Mol. Pharmacol.,* 46, 452-459 (1994).

Leysen, J. E., Gommeren, W., Eens, A., de Chaffoy de Courcelles, D., Stoof, J. C., and Janssen, P. A., Biochemical profile of risperidone, a new antipsychotic. *J. Pharmacol. Exp. Ther.,* 247, 661-670 (1988).

Locke, C. S., An exact confidence interval from untransformed data for the ratio of two formulation means. *J. Pharmacokinet. Biopharm.,* 12, 649-655 (1984).

Mandallaz, D. and Mau, J., Comparison of different methods of decision making in bioequivalence assessment. *Biometrics,* 37, 213-222 (1981).

Mannens, G., Huang, M. L., Meuldermans, W., Hendrickx, J., Woestenborghs, R., and Heykants, J., Absorption, metabolism, and excretion of risperidone in humans. *Drug Metab. Dispos.,* 21, 1134-1141 (1993).

Ono, S., Mihara, K., Suzuki, A., Kondo, T., Yasui-Furukori, N., Furukori, H., de Vries, R., and Kaneko, S., Significant pharmacokinetic interaction between risperidone and carbamazepine: its relationship with CYP2D6 genotypes. *Psychopharmacology,* 162, 50-54 (2002).

Ramamoorthy, Y., Tyndale, R. F., and Sellers, E. M., Cytochrome P450 2D6*1 and cytochrome P450 2D6*10 differ in catalytic activity for multiple substrates. *Pharmacogenetic,* 11, 477-487 (2001).

Rogers, J. F., Nafziger, A. N., and Bertino, J. S., Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. *Am. J. Med.* 113, 746-750 (2002).

Shah, V. P., Midha, K. K., Findlay, J. W., Hill, H. M., Hulse, J. D., McGilveray, U., McKay, G., Miller, K. J., Patnaik, R. N., Powell, M. L., Tonelli, A., Viswanathan, C. T., and Yacobi, A., Bioanalytical method validation-A revisit with a decade of progress. *Pharm. Res.,* 17, 1551-1557 (2000).

The United States Pharmacopoeia 26. Maryland: US Pharmacopeial Convention, Inc., pp. 2155, (2003).

Westlake, W. J., Use of confidence intervals in analysis of comparative bioavailability trials. *J. Pharm. Sci.,* 61, 1340-1341 (1972).

Wilson, J. F., Weale, M. E., Smith, A. C., Gratrix, F., Fletcher, B., Thomas, M. G., Bradman, N., and Goldstein, D. B., Population genetic structure of variable drug response. *Nat. Genet.,* 29, 265-269 (2001).