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Comparative pharmacokinetics and bioequivalence of two tablet formulations of 2 mg risperidone in healthy Thai male volunteers

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Key words
risperidone – 9-hydroxyrisperidone – pharmacokinetics – bioequivalence – HPLC-UV

Abstract. Background: Risperidone is an atypical antipsychotic drug with potent serotonin and moderate dopamine antagonistic properties. It possesses good bioavailability following oral administration. Risperidone is primarily converted by the cytochrome P450 2D6 (CYP2D6) and 3A4 (CYP3A4) enzymes to 9-hydroxyrisperidone, its active metabolite with equivalent potency to the parent compound. Objective: This study aimed to compare the pharmacokinetics and determine bioequivalence of two risperidone immediate release oral tablets, a test formulation (Risperidone GPO® or "Test") and a reference formulation (Risperdal® or "Reference"). Method: A single-dose, randomized, fasting, 2-period, 2-sequence, crossover study design with a 2-week washout period was conducted in 23 healthy Thai male volunteers. Blood samples were collected predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72 and 96 h following an oral administration of 2 mg risperidone. The plasma concentrations of risperidone and 9-hydroxyrisperidone were determined by using a validated HPLC method. Pharmacokinetic parameters of Test and Reference were obtained by noncompartmental analysis. Results: The 90% confidence intervals for Test/Reference ratios of the pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) of both risperidone and its active metabolite (9-hydroxyrisperidone) fell within the acceptable bioequivalence range (80 – 125%) according to ASEAN guideline. Conclusion: The two risperidone formulations are bioequivalent. The test formulation may be used for generic substitution where applicable.

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Introduction

Risperidone, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7, 8,9-tetrahydro-4*H*-pyrido[1,2-α]pyrimidin-4-one, is an atypical antipsychotic drug used for treatment of psychotic disorders. It is more effective and produces less extrapyramidal side effect than typical antipsychotics [1]. The pharmacological action of risperidone results from its potent serotonin and moderate dopamine antagonistic properties [1, 2]. Moreover, it also, to a certain extent, affects α_1-adrenergic, α_2-adrenergic and histamine (H_1) receptors. The binding profile of risperidone for 5-HT_{2A}, 5-HT_{1D}, 5-HT_{1A}, D_2, H_1, α_1 and α_2 receptors was 0.15, 3.90, 190.00, 3.77, 5.20, 2.70 and 8.00 nM, respectively [3]. Clinical studies have shown that risperidone is effective against the positive, negative and affective symptoms of schizophrenia [1].

Risperidone is well absorbed with 70% bioavailability following oral administration. Its absorption is not affected by food. After entering systemic circulation, various metabolites of risperidone are formed extensively in the liver by the genetically polymorphic CYP2D6 and CYP3A4 enzymes [4]. 9-Hydroxyrisperidone, the principal active metabolite, showed similar antipsychotic activity to the parent compound with high affinity to 5-HT_{2A} (1.21 nM) and D_2 (2.80 nM) receptors [3]. The plasma concentrations of risperidone and 9-hydroxyrisperidone are dose-dependent within the therapeutic dose range. Risperidone is rapidly distributed with a volume of distribution 1 – 2 l/kg. The protein

binding of risperidone and its metabolite are 90% and 77%, respectively, and the binding is non-competitive [5]. The plasma protein binding of both compounds in the elderly is not significantly different from that of young subjects. The half life of risperidone is 3 h in extensive metabolizers, and can be increased up to 20 h in poor metabolizers [6]. For 9-hydroxyrisperidone, the half-life is about 24 h in extensive metabolizers and longer in poor metabolizers. The overall mean elimination half-life of the active moiety appeared to be 20 h after single dose or multiple doses administration, which is similar in extensive and poor metabolizers [6]. Both risperidone and its metabolite are excreted approximately 70% in urine and 14% in feces [7]. The usual recommended dose for chronic schizophrenia of risperidone is 2 – 8 mg/day. The objective of this study was to compare the pharmacokinetics and determine bioequivalency of two risperidone immediate-release oral tablet formulations in healthy Thai male volunteers.

Materials and methods

Study products

The reference formulation (Risperdal®) marketed by Janssen Pharmaceutica, Japan was used in this study (Lot no. 846AHJ, expiration date 09/2011). The test formulation (Risperidone GPO®) was manufactured and distributed by the Government Pharmaceutical Organization, Thailand (Lot no. S520154, manufacturing date 03/2009, expiration date 03/2011). Pharmaceutical equivalence between Test and Reference was previously reported including the comparison of dissolution profiles of both formulations (dissolution profile performed in pH 1.2, 4.5 and 6.8 by Government Pharmaceutical Organization, unpublished data).

Study subjects

A total of 24 healthy Thai male volunteers (age between 18 and 25 years; body mass index (BMI) in the range of 18 – 25 kg/m^3) was recruited. All subjects needed to meet the criteria for physical examination, predefined clinical laboratory evaluation, and medical history to be eligible to enter the study. An informed consent was obtained from all participants prior to enrollment. It was essential that subjects refrained from smoking, drinking and taking others medications within 2 weeks before entering the study and during the study period. The exclusion criteria included; allergic reactions to risperidone or other ingredients in the formulation, subjects had history of diseases that could affect bioavailability of risperidone (such as, gastrointestinal, liver, renal diseases), a history of regular alcohol consumption, smoking (more than 10 cigarettes per day) or having participated in other clinical experiments within 1 month prior to our study. Subjects could be withdrawn from the study under the discretion of the physician investigator in case the subjects had experienced any serious adverse events during the study procedures or the study protocol was violated. However, the subjects could always voluntarily withdraw themselves from the study. The study was conducted in the compliance of the current version of the Declaration of Helsinki.

Study design

The study protocol was approved by the Ethics Committee of Naresuan University and Thailand Food and Drug Administration. A single-dose, randomized, fasting, 2-period, 2-sequence, crossover study design with a 2-week washout period was conducted. During each period of study, the subjects were admitted to Naresuan University Hospital, Naresuan University (Phitsanulok, Thailand). All volunteers were allocated to treatment using block randomization. Each subject received either a single dose of 2 mg risperidone tablet of Test or Reference with 240 ml of water after an overnight fast. They were then in the upright seated position for at least 30 min. At 2 h post-dose, a standardized sugar drink was given to each subject. The first standardized meal was provided at 4 h after the drug administration and water was allowed *ad libitum*.

Each blood sample (6 ml) was collected into a lithium heparin-coated plastic tube (Becton Dickinson and Company, Franklin Lakes, NJ, USA) by catheterized venipunc-

ture at forearms before dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36. 48, 72 and 96 h after the drug administration. Plasma samples were obtained by centrifugation at 5,000 rpm for 10 min at 4 °C (Beckman J2-MC, Beckman Coulter, Inc., Fullerton, CA, USA) and then kept in the cryogenic vials (Nalge Nunc International, Rochester, NY, USA) at –79 ± 2 °C for further analysis. After 2 weeks of washout period, the subjects were returned to the hospital. Similar procedures were performed when the alternative formulation was administered to each subject.

Sample analysis

Standard Risperidone, 9-hydroxyrisperidone and clozapine (internal standard) were obtained from Sigma-Aldrich distributed in Canada, Israel and USA, respectively. The analytical method was modified from the method of Avenoso et al. [9] by using isocratic high-performance liquid chromatography with UV detection. The HPLC system consisted of a LC-10ATVP pump, SIL-10-AD VP autoinjector (at the temperature of 24 ± 1 °C), and a SPD-20A UV-VIS detector (Shimadzu Corporation, Japan). HPLC separation was carried out by reversed phase chromatography on an Alltima C-18® column (particle size 5 μm, 250 × 4.6 mm i.d.) (Alltech Associated, Inc., Deerfield, IL, USA). The mobile phase consisted of 50 mM phosphate buffer (adjust to pH 3.1 by phosphoric acid): acetonitrile (74 : 26, v/v), run at a flow rate of 1.2 ml/min and with UV detection at 278 nm. The method was validated for signal to noise, extraction recovery, linearity, lower limit of quantification (LLOQ), accuracy, precision, specificity and stability according to the guideline [8, 10] before starting sample analysis.

For sample preparation, an aliquot of 1.0 ml of thawed plasma sample was transferred to a test tube. Then 1.0 ml of NaOH (2M) and 35 μl of clozapine (1 μg/ml) were added and mixed by using vortex mixer. The analytes were extracted from plasma by a liquid-liquid extraction with 5 ml of methyl t-butyl ether: isoamyl alcohol (isopentanol) (99 : 1) and back-extracted with 400 μl of KH_2PO_4 (pH 2.2). The organic layer was discarded and the acidic layer was separated and washed with diethylether. Then a 350 μl of each ready-prepared sample was injected into the HPLC system for quantitative analysis of risperidone and 9-hydroxyrisperidone.

Pharmacokinetic and statistic analysis

Individual pharmacokinetic parameters of risperidone and 9-hydroxyrisperidone after a single oral administration of a 2 mg risperidone tablet were estimated by non-compartmental analysis using WinNonlin Professional version 4.0.1 (Pharsight Corporation, Mountain View, CA, USA). Basically, the maximum plasma concentration (C_{max}) and the time to maximum plasma concentration (t_{max}) were obtained directly from the observed plasma concentration-time data. The area under the plasma concentration-time curve from zero to time t (AUC_{0-t}), where t is the time of the last quantifiable concentration (C_t), was calculated using the linear trapezoidal rule. From terminal log-decay phase, elimination rate constant (K_e) was predicted using the linear regression and $t_{1/2}$ was calculated as $0.693/K_e$. The $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/K_e$.

Natural log-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were subjected to an analysis of variance (ANOVA) with the factors sequence, subject nested within sequence, period and formulation using a general linear model procedure by SPSS for Windows standard V. 11.5 (SPSS, Inc., Chicago, IL, USA). To meet the acceptable bioequivalence criteria, 90% confidence intervals (90% CI) for the ratios (Test/Reference) of the geometric mean values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of both risperidone and 9-hydroxyrisperidone must be within the range of 0.80 – 1.25. The nonparametric two-tailed Wilcoxon signed rank test ($\alpha = 0.05$) was used to evaluate t_{max} differences between Test and Reference.

Results

Risperidone, 9-hydroxyrisperidone and clozapine (internal standard) were well separated with the retention times of 10.2, 7.2, and 19.9 min, respectively. No interfering

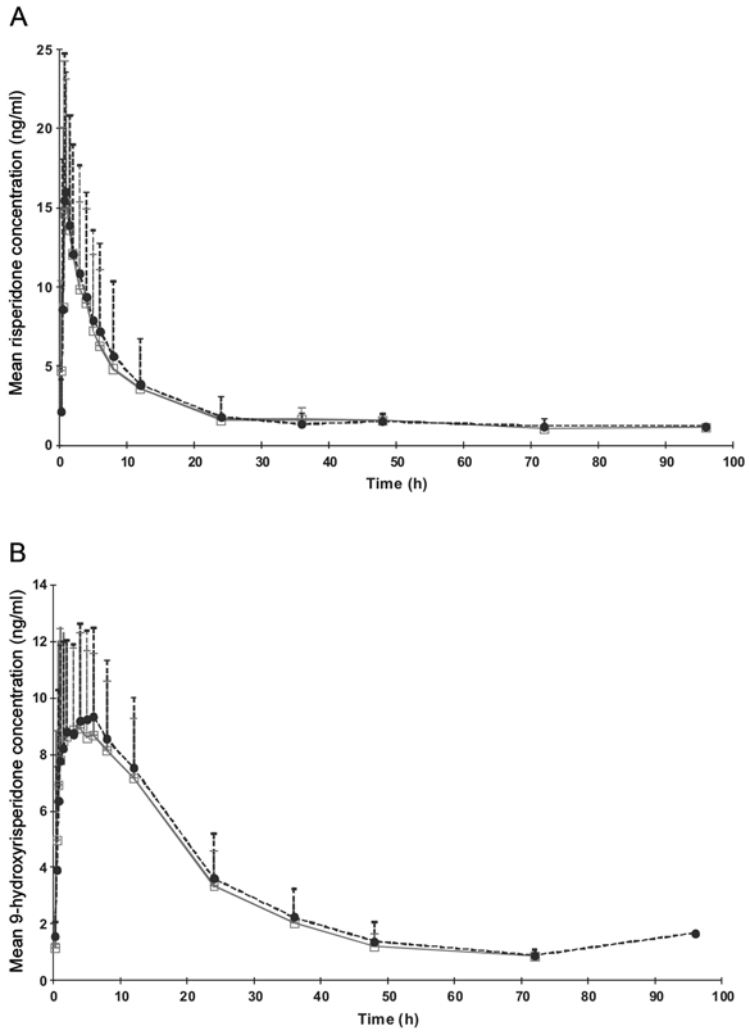

Figure 1. Mean Plasma concentration-time curve of risperidone (A) and 9-hydroxyrisperidone (B) after administration of a 2 mg risperidone tablet in healthy Thai male volunteers (n = 23). (□ = test formulation, ● = reference formulation)

peaks from endogenous substances were observed. The method was validated over the linearity range of 0.75 – 80 ng/ml (r^2 > 0.996) and the lower limit of quantitation (LLOQ) was 0.75 ng/ml for both risperidone and 9-hydroxyrisperidone.

Of the 24 subjects recuited, only 23 volunteers were enrolled. One subject was excluded due to low white blood count (WBC) observed on the day before the study start. The eligible subjects had an average age ± SD of 21.6 ± 0.78 years and an average body mass index (BMI) of 22.3 ± 1.80 kg/m^3. Pharmacokinetic parameters from all enrolled subjects were evaluated and used for bioequivalence evaluation.

The average plasma concentrations of risperidone and 9-hydroxyrisperidone at each sampling time for 23 subjects after a single dose of 2 mg risperidone tablet were shown in Figure 1. Individual pharmacokinetic parameters of risperidone and 9-hydroxyrisperidone were estimated and the average values of those for Test and Reference were summarized in Table 1. The statistical analysis of unbalanced data obtained from 23 volunteers was performed. ANOVA on log transformed data for both formulations did not show any statistically significant differences between C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ ($p > 0.05$) for the Test and Reference except for subject (sequence) effect. The 90% confidence intervals were confirmed by SPSS along with WinNonlin program and manual calculation by using the following equation [14]:

$$90\%CI = 100 \times e^{(\overline{Y}_T - \overline{Y}_R) \pm t_{(1-\alpha),N-2} \bullet \sqrt{\frac{EMS}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$$

$\overline{Y}_T$ and $\overline{Y}_R$ = Least square means of natural log-transformed pharmacokinetic parameters of test and reference formulations, respectively.
EMS = Error mean square
n_1 and n_2 = The number of volunteers in each sequence of treatment
N = The total number of volunteers in the study = $n_1 + n_2$

The point estimates (90% CIs) for the Test/Reference ratios of mean C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of risperidone were 1.02 (0.94 – 1.12), 0.96 (0.82 – 1.12) and 0.99 (0.85 – 1.14), respectively, and those of 9-hydroxyrisperidone were 0.95 (0.90 – 1.01), 0.93 (0.88 – 1.00) and 0.94 (0.88 – 1.00), respectively (Table 1). The Wilcoxon signed rank test for t_{max} did not show any significant difference between two formulations. In this study, powers of statistical tests for all pharmacokinetic parameters were greater than 80%.

The adverse effect was monitored throughout the study. The physical examination was performed at screening and at the end of the study. Body temperature and vital signs (including blood pressure and pulse rate) were evaluated before dosing and at time 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72

Table 1. Mean pharmacokinetic parameters, ratio of means and 90% confidence interval of test/reference ratios of risperidone and 9-hydroxyrisperidone after administration 2 mg risperidone tablets (n = 23).

Parameters	Geometric least square means		Ratio of means	90% CI (T/R)
	Risperidone GPO® (T)	Risperdal® (R)		
Risperidone				
C_{max} (ng/ml)	15.91	15.54	1.02	0.94 – 1.12
AUC_{0-t} (ng × h/ml)	76.24	79.20	0.96	0.82 – 1.12
$AUC_{0-\infty}$ (ng × h/ml)	86.89	87.97	0.99	0.85 – 1.14
t_{max} (h)	1.12 ± 0.74	0.99 ± 0.37		
$t_{1/2}$ (h)	4.91 ±3.60	4.67 ± 3.22		
9-Hydroxyrisperidone				
C_{max} (ng/ml)	9.23	9.67	0.95	0.90 – 1.01
AUC_{0-t} (ng × h/ml)	197.25	211.00	0.93	0.88 – 1.00
$AUC_{0-\infty}$ (ng × h/ml)	222.52	236.93	0.94	0.88 – 1.00
t_{max} (h)	5.62 ± 3.56	5.06 ± 3.10		
$t_{1/2}$ (h)	14.58 ± 7.96	14.07 ± 4.67		

Remarks: 1. t_{max} and $t_{1/2}$ demonstrated in arithmetic mean ± SD; 2. Bioequivalence acceptable range = 0.80 – 1.25.

and 96 h after the drug administration. There was no serious adverse effect occurring for both risperidone formulations in this study. All volunteers showed similar sedative action during Period I and II. Two volunteers reported about dizziness after intake of the Test formulation for 4 h and 1 volunteer had insomnia in the night in which the Reference formulation was administrated.

Discussion

In this study, the validated analytical method was modified from the previous reported methods [9, 11] by increasing the ratio of phosphate buffer, reducing pH of mobile phase and using a longer C18 column. As a result, the separation of analyses from endogenous interference peaks was improved. Even though the total run time lengthened up to 22 min, the sensitivity of the method was increased by reducing the LLOQ of both risperidone and 9-hydroxyrisperidone from 5 ng/ml to 0.75 ng/ml. The maximum plasma concentration of risperidone in Chinese and Korean subjects after a single dose of 2 mg risperidone was 8 – 17 ng/ml [12, 13] and, for 9-hydroxyrisperidone, was 10 – 11 ng/ml in Thai subjects (unpublished data). Therefore the sensitivity of the developed method was demonstrated to be adequate for determination of risperidone and 9-hydroxyrisperidone in human plasma and can be applied to a pharmacokinetic study.

The aim of this study was to evaluate the bioequivalence of two formulations of 2 mg risperidone tablets (Test formulation manufactured by the Government Pharmaceutical Organization, Thailand, and Reference formulation manufactured by Janssen Pharmaceutica). This study was designed to collect the blood samples up to 96 h after drug administration to ensure sufficient samples for evaluation of AUC_t in CYP2D6 poor metabolizers. Both formulations were readily absorbed from the gastrointestinal tract and the risperidone was detected in plasma from 15 to 30 min after dosing. The means of pharmacokinetic parameters between Test and Reference showed similar range for both risperidone and its active metabolite as previously reported [12]. The analysis of variance (ANOVA) did not detect any significant sequence, period and formulation effects. The subject (sequence) effect was found to be significant for natural log-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for both risperidone and 9-hydroxyrisperidone. This indicates the differences among the subjects enrolled in the study, must be related to the sample size and is not likely to have any clinical significance. There was no carryover effect observed in this study. The power of statistical tests for all pharmacokinetic parameters base on 23 volunteers was demonstrated to be sufficient

to detect the differences between the two formulations (> 80%), which indicated that the design in this study was acceptable.

The 90% confidence intervals for the Test/Reference ratios of mean C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for both risperidone and 9-hydroxyrisperidone were all within the acceptable bioequivalence range of 0.80 – 1.25, according to ASEAN guideline [15]. No significant difference in t_{max} was observed between two formulations. Therefore, it can be concluded that the bioavailability of risperidone was equivalent in terms of rate and extent of absorption between the test (Risperidone GPO®) and reference (Risperdal®) formulations and both formulations may be prescribed interchangeably.

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