Songklanakarin J. Sci. Technol.
30 (3), 307-312, May - Jun. 2008

Songklanakarin Journal of Science and Technology
http://www.sjst.psu.ac.th

Original Article

Bioequivalence study of a generic Risperidone (Iperdal®) in healthy Thai male volunteers

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Received 3 April 2007; Accepted 28 May 2008

Abstract

The objective of this study was to compare the rate and extent of absorption of a generic risperidone (Iperdal®) with a reference formulation (Risperdal®) when given orally. The study was an open label, randomized, two-period, two-sequence, single dose cross-over design with a 2 weeks washout period in 16 healthy Thai male volunteers. Single oral dose of two 2-mg tablets of risperidone were administered and serial blood samples were collected from the antecubital vein before and at 0.17, 0.33, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 24 and 48 hours post dose. Risperidone plasma concentrations were assayed using a validated High Performance Liquid Chromatographic (HPLC)-UV method modified from Avenoso *et al*. (2000). Pharamcokinetic parameters i.e. C_{max}, $AUC_{0à48}$ and T_{max} were analyzed by noncompartment analysis. Variations of the data were analyzed by "Two Way Analysis of Variance" (ANOVA). Statistics were tested as stated in USP 28 guideline for bioequivalence study. The maximum concentration (C_{max}, ng/ml) of risperidone for the innovator and the generic product were 31.11±17.24 (range 5.64-56.78) and 32.58±19.77 (range 5.29-84.56) ng/ml, respectively. The area under the plasma concentration-time curve ($AUC_{0\rightarrow48}$) of the innovator and the generic product were 160.64±152.89 (range 18.57-550.32) and 144.03±127.37 (range 16.27-456.0) ng.hr/ml, respectively. The time to maximum concentration (T_{max}) of the innovator and the generic product were 0.97±0.41(range 0.5-2) and 1.02±0.32 (range 0.5-1.5) hr, respectively. The 90% confidence interval of the ratio of the ln-transformed of C_{max} and $AUC_{0à48}$ of both preparations were 89.39-112.99% and 80.02-107.28% respectively which were within the acceptance range of 80.00-125.00%. Therefore, it can be concluded that both preparations used in this study are bioequivalent in terms of both the rate and extent of absorption.

Keywords : bioequivalence, risperidone, Iperdal®, Risperdal®

1. Introduction

Risperidone, chemically classified as a benzisoxazole derivative, is an atypical antipsychotic agent and it is a selective monoaminergic antagonist with high affinity for serotonin type II ($5\text{-}HT_2$) and dopamine-D_2 antagonists (Nyberg *et al.*, 1993). Clinical trials in psychotic patients have shown that risperidone is effective in the treatment of both positive and negative symptoms of Schizophrenia (Marder *et al.*, 1997). Furthermore, risperidone therapy is less associated with extrapyramidal side effects as compared to the classical antipsychotic drugs. The U.S. Food and Drug Administration (FDA) approved the use of risperidone for

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the treatment of residual Schizophrenia, and a large prospective clinical study comparing risperidone with haloperidol has demonstrated that patients treated with risperidone have a lower risk of relapse than those treated with haloperidol. Due to the favorable clinical effects of risperidone, a substantial increase in its use has occurred during the last few years. Risperidone is rapidly and very well absorbed after administration orally. It is extensively metabolized by cytochrome P450 (CYP) 2D6 by hydroxylation. The principal metabolite is 9-hydroxyrisperidone. Hydroxylation of risperidone is subject to the same genetic CYP 2D6–related polymorphism as for debrisoquine and dextromethorphan. In poor metabolizers the half-life of risperidone was about 20 hours compared with about 3 hours in extensive metabolizers (Huang *et al.*, 1993). Plasma concentrations of risperidone, 9-hydroxyrisperidone, and risperidone plus 9-hydroxyrisperidone are dose proportional over the dosing range of 1 to 16 mg daily (0.5 to 8 mg twice a day). Risperidone exhibits linear elimination kinetics. Steady state is reached within 1 day for risperidone and within 5 days for the active fraction. Less than 1% of the drug is excreted unchanged in the feces (Heykants *et al.*, 1994).

Risperidone has been widely prescribed instead of the classical antipsychotic drugs due to its lower side effects. However, the cost of treatment with the innovator product is still too high for most patients. Therefore, many generic products have been developed. Interchangeability of the generic and the innovator products is determined by bioequivalence studies comparing the plasma concentration versus time curves of both products. They are considered bioequivalent when the rate and extent of bioavailability of the active ingredient in the two products are not significant different under suitable test conditions. In order to prevent the distribution of substandard products, bioequivalence studies are prerequisite for registration. Therefore, Iperdal®, a new generic formulation, is needed for bioequivalent testing.

2. Objectives

To investigate the bioequivalence of a generic risperidone (Iperdal®) and the reference formulation (Risperdal®) when given as a single 4 mg oral dose.

3. Materials and Methods

3.1 Drug formulations

Test product: **Iperdal®** 2 mg tablet by Atlantic Lab. Co. Ltd. (Thailand), 2038 Sukumvit Road, Bangkok, Thailand 10260; Lot No.: BN PD050008; MFG 10-06-2005, EXP 10-06-2008.

Reference product: **Risperdal®:** 2 mg tablet by Janssen Pharmaceutical N.V., Belgium; Lot No.: BN 03DB 922; MFG 04-2003, EXP 04-2006.

3.2 Instrumentation and Chemicals

A high performance liquid chromatography (HPLC) system, consisting of a Waters 2695 pump, autosampler (Waters Associates, Milford, MA, USA), and a Waters 2487 UV detector was used. The detector was a variable- wavelength UV detector set at 278 nm. The column was reverse-phase Symmetry C_{18} (4.6 mm x 250 mm HPLC column, particle size 5 mm, Waters Associates, Milford, MA, USA). A guard-pak precolumn module was used to obviate the effect of rapid column degeneration. All chemicals used were HPLC and/or analytical grade.

3.3 Subjects

The volunteers were given a detailed explanation of the purpose, protocol, and risk of the study, and each volunteer was given a written inform consent that was approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand (No. EC 47/400-002). 16 healthy Thai male volunteers, age 20-45 years, with a body mass index 18-24 kg/m^2 were enrolled in this study. All were in good health on the basis of medical history and physical examination. Routine blood tests, including complete blood count (CBC) with differential white blood cell count, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), direct bilirubin and albumin/ globulin were screened to exclude subject with abnormal hematological, liver, or kidney functions. None of the volunteers was a smoker or administered any medications continuously. Subjects with known contraindication or hypersensitivity to risperidone were excluded as well as those with known history of alcoholism or drug abuse. Drinking of alcoholic beverages, coffee and tea was not allowed at least one month prior to and during the entire period of this study.

3.4 Experimental design

The study was an open-labeled, randomized, single-dose, two-phases cross over designed with a 2 weeks washout period. The protocol was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University.

Phase 1: A single oral dose of risperidone (4 mg)

In the morning after an overnight fasting, 8 subjects received a single oral dose of 4 mg risperidone (two 2-mg tablets of Risperdal®). Another 8 subjects received a single oral dose of 4 mg risperidone (two 2-mg tablets of Iperdal®). The drug was administered with a glass of water (240 ml) under supervision. No food was taken at least 2 hours after ingestion of the drug. Volunteers remained upright during the first hour after drug administration and activities are limited as necessary.

A catheter was inserted into a forearm vein for the collection of blood sample, and was maintained patent by using 1 ml of a dilute heparin solution (100 unit/ml) after each sample. Venous blood samples (5 ml) were collected in heparinized tubes before drug administration and at 10, 20, 30, 45 min, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 hours post dose. Blood samples were centrifuged at 2,500 g for 10 minutes not later than 30 minutes after collection, and the plasma was separated and immediately stored at -70°C until assay.

Phase 2: A cross over of a single oral dose of risperidone (4 mg)

After 2 weeks of being free from the drug, the subjects received a single oral dose of risperidone alternately to the first phase after an overnight fasting. Venous blood samples were collected at the time interval before and after risperidone administration as previously done in Phase 1.

3.5 Sample analysis

The plasma risperidone concentrations were measured by using the HPLC method (applied from Avenoso *et al.*, 2000).

3.6 Mobile phase

The mobile phase consisted of 0.05 M potassium dihydrogenphosphate : acetonitrile (68 : 32 vol/vol) and adjusted to pH 3.80 with 25% phosphoric acid. The mobile phase was freshly prepared daily and filtered through 0.45 micropore filter (Nylon 66), then degassed by sonification for 10 minutes before using. The flow rate was 1 ml/min. All analyses were performed at room temperature (25±1°C).

3.7 Stock standard solution

A stock standard solution at a concentration of 1 mg/ml was prepared by dissolving 1 mg of standard risperidone in 0.1 N HCl. The solution was adjusted to 10 ml in a 10 ml volumetric flask. The stock solution was stable for at least 4 months at -20°C (Avenoso *et al.*, 2000). Working standard solution used to prepare a calibration curve was prepared by appropriate dilution of the stock standard solution with blank plasma.

3.8 Calibration curves

Calibration curves were prepared by adding a standard risperidone solution to blank human plasma so that the final concentrations in plasma were 2, 5, 10, 20, 50 and 100 ng/ml. The calibration curves for risperidone were linear in the range of 2-100 ng/ml. The lower limit of quantitation (LLOQ), expressed as the lowest concentrations at which percent deviation from the accuracy and precision are less than 15%.

3.9 Method validation

The method was validated according to the Thai FDA Guideline (Thai FDA, 2006) and the U.S. FDA Guidance (U.S. FDA, 2001).

3.10 Sample preparation

20 µl of clozapine (1mg/ml) as internal standard (I.S.) and 1 ml NaOH (2 M) were added to 1 ml of plasma. The tubes were vortex-mixed for 10 seconds and 4 ml of diisopropyl ether-isoamylalcohol (99 : 1, vol/vol) was added as extraction solvent. After 10 min shaking, the mixture was centrifuged at 3,000 g for another 10 min at 4°C and the organic phase (upper phase) was transferred to tubes containing 150 µl of KH_2PO_4 (0.1 M, pH 2.2 with 25% H_3PO_4), mixed for 1 min, and centrifuged at 3000 g for 5 min. The upper organic layer was carefully aspirated and 1 ml of diethyl ether was added. After gentle mixing, the ether phase was eliminated and a 60 µl aliquot of the remaining acid solution was injected into the HPLC system. The chromatographic conditions used in this study were good to separate risperidone from other endogenous substances in plasma.

3.11 Pharmacokinetic calculations

The area under the plasma concentration-time curve, $AUC_{0\text{-}48,}$ and $t_{½}$ (half-life time) were analyzed by non-compartment method, using WinNonlin program version 4.1 (Pharsight, Mountain View, CA). The maximum concentration, $C_{max,}$ and the time to maximum concentration, T_{max} were obtained by direct visualization of each subject's plasma concentration-time curve.

3.12 Bioequivalence evaluation Method Validation

The two products will be considered bioequivalent if the 90% confidence interval (CI) of the ratios of ln-transformed of C_{max} and $AUC_{0\rightarrow48}$ of both products were within the acceptance range for bioequivalence, i.e. 80.00-125.00%.

4. Results and Discussion

4.1 Method Validation

Chromatograms (not shown) have demonstrated that risperidone and the internal standard (clozapine) were clearly separated from endogenous substances with retention time of 4.6 and 6.4 minutes, respectively. This result indicated specificity of the analytical method.

Calibration curve of standard risperidone was linear over the range of 2-100 ng/ml. The correlation coefficient of calibration curve was 0.999. LLOQ: defined as the lowest concentration on the standard curve that can be analyzed with acceptable precision of 13.39%, was 2 ng/ml.

Accuracy of the test method for the three concentrations (5, 20 and 100 ng/ml) in five replicates were 100.14%, 100.04% and 100.05%, respectively.

Precision for this method were carried out by calculating the intra-day and inter-day precision at the three concentrations (5, 20 and 100 ng/ml) in five replicates. The intra-day precision (%CV) were 8.19%, 10.54% and 4.36%, respectively. The inter-day precision (%CV) were 13.94%, 8.43% and 4.12%, respectively.

Recovery: Mean extraction recoveries of risperidone at concentrations 5, 20 and 100 ng/ml were 109.52%, 106.10% and 98.72 %, respectively.

Stability: Risperidone was stable in freeze and thaw stability test. The percent change for risperidone concentrations were 4.76% and 0.45%, at the concentrations of 5 and 50 ng/ml, respectively. At room temperature, risperidone was found to be stable for 5 h (short term). The %-change at the concentrations of 5 and 50 ng/ml were 3.70 % and 3.33%, respectively. Risperidone was stable at -70°C for 30 days (long term) with 8.69% and 9.28% change at 5 and 50 ng/ml, respectively. For the post-preparative stability test, the samples were kept in the autosampler for 24 h before injection. The quantitative results indicated that risperidone was stable in the autosampler up to at least 24 h with 0% and 4.51% change at 5 and 50 ng/ml, respectively. Stock solution (1,000 ng/ml) stability of risperidone was found to be stable at -70°C for 14 days with 0.96% change .
Therefore, the analytical method used in this study was accurate, precise and rugged.

4.2 Pharmacokinetics study

Figure 1 demonstrates that the mean plasma concentrations of both products are almost identical throughout the whole period of the study (48 hours).

The maximum concentrations (C_{max}) of risperidone were 31.11±17.24 (range 5.64-56.78) and 32.58±19.77 (range 5.29-84.56) ng/ml for the innovator and the generic product, respectively. The area under the plasma concentration-time curves ($AUC_{0\rightarrow48}$) were 160.64±152.89 (range 18.57-550.32) and 144.03±127.37(range 16.27-456.09) ng.hr/ml for innovator and generic product, respectively. The time to maximum concentration (T_{max}, hr) was 0.97±0.41 (range 0.5-2) and 1.02±0.32 (range 0.5-1.5) hr for innovator and generic product, respectively. The T_{max} of both products were not significantly different and T_{max} of the test product was less than ±20% of the reference value.

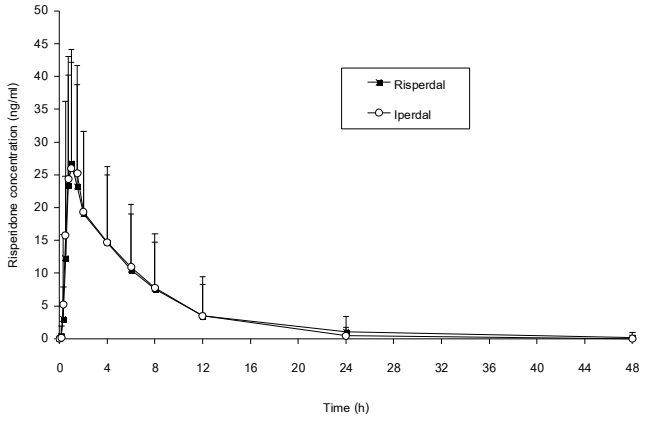

Figure 1. Mean plasma concentration-time curve of risperidone after a single oral dose of 4 mg of Risperdal®(--■--) and Iperdal®(--□--) in the 16 healthy male volunteers

4.3 Bioequivalence evaluation

The 90% confidence interval (CI) of the ratios of ln-transformed of C_{max} and $AUC_{0\rightarrow48}$ of both preparations were 89.39-112.99% and 80.02-107.28%, respectively (Table 4) which were within the acceptance range for bioequivalence, i.e. 80.00-125.00%.

Inter-individual variation found in this study (Table 1 to 3) could be due to many factors that can affect absorption, metabolism and excretion of the drugs such as age (Hunt *et al.*, 1992), (range 22-41 years, in this study). Genetic background of the volunteers is another important factor, as genetic polymorphism has been reported (Nyberg *et al.*, 1993) (about 6-8% in Caucasians but a very low percent of Asian are poor metabolizers). Although, the incidence of genetic polymorphism is very low in Asians, we could not exclude this effect from our study. It is to be pointed out that

Table 1. Pharmacokinetic parameters of risperidone after a single oral dose of Risperdal® in each subject.

Subject No.	T_{max} (hr)	C_{max} (ng/ml)	AUC (ng.hr/ml)	$T_{1/2}$ (hr)
1	1.00	43.96	187.80	2.95
2	1.00	5.64	29.91	7.08
3	1.00	49.62	131.00	2.22
4	1.00	28.51	144.66	4.84
5	0.50	16.78	18.57	1.55
6	0.50	16.83	41.64	2.63
7	2.00	9.50	67.22	3.67
8	1.00	56.78	212.94	2.74
9	1.00	44.77	550.32	16.00
10	0.50	51.99	181.90	2.18
11	0.75	19.96	35.61	2.29
12	0.75	48.41	403.95	5.91
13	0.75	14.96	38.15	2.38
14	0.75	19.66	45.95	2.97
15	1.50	47.25	334.86	7.35
16	1.50	23.13	145.73	4.62
Mean	**0.97**	**31.11**	**160.64**	**4.46**
S.D.	**0.41**	**17.24**	**152.89**	**3.56**
%CV	**42.27**	**55.42**	**95.18**	**79.82**
Maximum	**2.0**	**56.78**	**550.32**	**16.00**
Minimum	**0.5**	**5.64**	**18.57**	**1.55**
Max - Min	**1.5**	**51.14**	**531.75**	**14.45**

Table 2. Pharmacokinetic parameters of risperidone after a single oral dose of Iperdal® in each subject.

Subject No.	T_{max} (hr)	C_{max} (ng/ml)	AUC (ng.hr/ml)	$T_{1/2}$ (hr)
1	1.00	29.96	90.32	5.67
2	1.00	5.29	33.53	8.93
3	0.75	33.89	149.27	6.67
4	1.00	38.53	230.11	5.80
5	0.75	15.75	41.19	3.07
6	0.75	16.13	49.86	7.82
7	1.50	25.58	86.00	3.39
8	1.00	46.93	136.26	1.62
9	1.50	44.68	456.09	5.26
10	0.50	84.56	272.79	2.83
11	1.00	16.21	37.95	1.95
12	1.00	39.85	302.08	8.39
13	0.75	13.77	16.27	1.31
14	0.75	12.83	25.56	1.64
15	1.50	51.58	270.45	7.70
16	1.50	29.81	106.73	5.47
Mean	**1.02**	**32.58**	**144.03**	**4.85**
S.D.	**0.32**	**19.77**	**127.37**	**2.62**
%CV	**31.37**	**60.68**	**88.43**	**54.02**
Maximum	**1.50**	**84.56**	**456.09**	**8.93**
Minimum	**0.50**	**5.29**	**16.27**	**1.31**
Max - Min	**1.00**	**79.27**	**439.82**	**7.65**

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 (Cho HY and Lee YB, 2006). Food was unlikely to affect the absorption in this drug as it was previously reported that food does not affect either the rate or extent of absorption of risperidone (RxList, 2008). For the hepatic and renal function, there were some degree of difference in the functions. However, these functions were still within normal range. Therefore, they should not affect the elimination of the drugs.

4.4 Clinical observations

Both risperidone preparations were well tolerated and all subjects completed the study without serious side effects, except for marked sedation in all volunteers and orthostatic hypotension in one subject after taking Risperdal® during the Phase 1 study. Orthostatic hypotension has been reported previously (Marder *et al.,* 1997) and may be aggravated, in this case, may be by sleeplessness over the night before the study as this side effect was not found in this volunteer during the second phase of the study. All adverse effects were resolved without treatment.

5. Conclusions

Since the 90% CI of the ratios of test/reference of the two pharmacokinetic parameters i.e. C_{max} and $AUC_{0\rightarrow48}$ were 89.39–112.99% and 80.02–107.28% respectively, which were within the bioequivalence acceptance range i.e. 80.00-125.00%. Therefore, we can conclude that the Iperdal® and Risperdal® used in this study are bioequivalent in terms of both the rate and extent of absorption.

Table 3. Comparison of ratio of the Test (T) and the Reference (R) of C_{max} and AUC in each subject.

Subject No.	C_{max} (T)	C_{max} (R)	Frel (T/R)	AUC (T)	AUC (R)	Frel (T/R)
1	29.96	43.96	**0.68**	90.32	187.80	**0.48**
2	5.29	5.64	**0.94**	33.53	29.91	**1.12**
3	33.89	49.62	**0.68**	149.27	131.00	**1.14**
4	38.53	28.51	**1.35**	230.11	144.66	**1.59**
5	15.75	16.78	**0.94**	41.19	18.57	**2.22**
6	16.13	16.83	**0.96**	49.86	41.64	**1.20**
7	25.58	9.50	**2.69**	86.00	67.22	**1.28**
8	46.93	56.78	**0.83**	136.26	212.94	**0.64**
9	44.68	44.77	**1.00**	456.09	550.32	**0.83**
10	84.56	51.99	**1.63**	272.79	181.90	**1.50**
11	16.21	19.96	**0.86**	37.95	35.61	**1.07**
12	39.85	48.41	**0.82**	302.08	403.95	**0.75**
13	13.77	14.96	**0.92**	16.27	38.15	**0.43**
14	12.83	19.66	**0.65**	25.56	45.95	**0.56**
15	51.58	47.25	**1.09**	270.45	334.86	**0.81**
16	29.81	23.13	**1.29**	106.73	145.73	**0.73**

Table 4. Mean pharmacokinetic parameters (Mean ± S.D.) and 90% Confidence Interval of the ratios of Test/Reference of the $AUC_{0\rightarrow48}$ and C_{max} of both preparations (* Ln-transformed data)

Pharmacokinetic Parameters	Risperdal®	Iperdal®	90% Confidence Interval*
$AUC_{0\rightarrow48}$ (ng.hr/ml)	160.64 ± 152.89	144.03 ± 127.37	80.02 – 107.28
C_{max} (ng/ml)	31.11 ± 17.24	32.58 ± 19.77	89.39 – 112.99
T_{max} (hr)	0.97 ± 0.41	1.02 ± 0.32	-

Acknowledgements

The financial support of the Atlantic Laboratories Corporation Ltd., Bangkok, Thailand is highly acknowledged and appreciated.

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