Arch Pharm Res Vol 28, No 4, 488-492, 2005

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

Pharmacokinetics and Bioequivalence of Haloperidol Tablet by Liquid Chromatographic Mass Spectrometry with Electrospray Ionization

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(Received November 11, 2004)

The purpose of this study is to investigate the bioequivalence of two haloperidol 5 mg tablets, Myung In haloperidol (Myung In Pharm. Co., Ltd., test drug) and Peridol® (Whanin Pharm. Co., Ltd., reference drug), and also to estimate the pharmacokinetic parameters of haloperidol in Korean volunteers. The bioavailability and pharmacokinetics of haloperidol tablets were examined on 24 healthy volunteers who received a single oral dose of each preparation in the fasting state in a randomized balanced 2 way crossover design. After an oral dosing, blood samples were collected for a period of 60 h. Plasma concentrations of haloperidol were determined using a liquid chromatographic electrospray mass spectrometric (LC-MS) method. The pharmacokinetic parameters were calculated with noncompartmental pharmacokinetic analysis. The geometric means of $AUC_{0\text{-}60h}$ and C_{max} between test and reference formulations were 17.21 ± 8.26 ng·h/mL vs 17.31 ± 13.24 ng·h/mL and 0.87 ± 0.74 ng/mL vs 0.85 ± 0.62 ng/mL, respectively. The 90% confidence intervals of mean difference of logarithmic transformed $AUC_{0\text{-}60h}$ and C_{max} were log0.9677~log1.1201 and log0.8208~log1.1981, respectively. It shows that the bioavailability of test drug is equivalent with that of reference drug. The geometric means of other pharmacokinetic parameters (AUC_{inf}, $t_{1/2}$, V_d/F, and CL/F) between test drug and reference drug were 21.75 ± 8.50 ng·h/mL vs 21.77 ± 15.63 ng·h/mL, 29.87 ± 8.25 h vs 29.60 ± 7.56 h, 11.51 ± 5.45 L vs 12.90 ± 6.12 L and 0.26 ± 0.09 L/h vs 0.31 ± 0.17 L/h, respectively. These observations indicate that the two formulation for haloperidol was bioequivalent and, thus, may be clinically interchangeable.

Key words: Haloperidol, LC-MS, Pharmacokinetic, Bioequivalence, Bioavailability

INTRODUCTION

The dopamine D_2 receptor antagonist haloperidol is prescribed as a high potency antipsychotic drug for the treatment of acute and chronic schizophrenia and other psychiatric disorders worldwide. The high efficacy is compromised by serious extrapyramidal adverse reactions (e.g., acute dystonia, pseudoparkinsonism, akathisia, and tardive dyskinesia), which occur with a high incidence (Casey *et al.,* 1998; Grohmann *et al.,* 1990). Despite that the therapeutic serum concentration of haloperidol ranges from 2 to 12 ng/mL, however, the range was reported from a number of clinical trials with a limited patient number (McEvoy 2002; Walter *et al.,* 1998; Altamura 1996).

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Haloperidol is extensively metabolized in the liver, and only 1% of the administered dose is excreted unchanged form to the urine (Forsman *et al.,* 1977). Approximately 50-60% of the dose is reported to be bio-transformed by the liver *via* glucuronidation *in vivo* (Someya *et al.,* 1992).

Haloperidol is used for the symptomatic management of psychotic disorders and Tourette's disorder (i.e., Gilles de la Tourette's syndrome), and also has been used in prevention and management of severe nausea and vomiting. For the symptomatic management of psychotic disorder or Tourette's disorder, the usual initial dosage of haloperidol is 3-5 mg two or three times daily (Šer *et al.,* 1997; Lacy 2001).

The purpose of this study was to determine the pharmacokinetic parameters of two brands of haloperidol 5 mg tablets and then to compare these parameters statistically to evaluate the bioequivalence of the two preparations. Myung In Haloperidol (Myung In Pharm. Co., Ltd., halo-

peridol 5 mg/tablet) was used as a test formulation while Peridol® (Whanin Pharm. Co., Ltd., haloperidol 5 mg/tablet) was used as the reference. Typical bioavailability parameters, such as AUC_t (the area under the plasma concentration–time curve from 0 to last sampling time) and C_{max} (the maximum plasma concentration) of haloperidol were compared for the bioequivalence evaluation.

MATERIALS AND METHODS

Drug and reagents

The test formulation of the study was Myung In haloperidol tablet (Haloperidol 5 mg, Lot No. 034302) from Myung In Pharm. Co. Ltd., and the reference formulation was Peridol® (Haloperidol 5 mg, Lot No. 3402) from Whanin Pharm. Co., Ltd. The drug, internal standard (i.e., chlorohaloperidol) and other reagents were purchased from Sigma Aldrich.

Subjects

In this bioequivalence study, 24 health volunteers were participated (age: 23.3 ± 1.65 years, weight: 63.6 ± 8.65 kg, height: 170.8 ± 7.26 cm). All subjects were selected after completing a thorough history and physical examination, and after a normal laboratory examination consisting of hematology, serum chemistry and urinalysis. All subjects were presented with full details of the investigation prior to consent. Each subject gave written informed consent to study procedures that were approved by the institutional review board of the Institute of Drug Development, Chungnam National University (Daejeon, Korea).

Study design

The administration of two haloperidol preparations to the subjects followed a balanced two-way crossover design with a week drug free interval between the two administrations. All subject were fasted for at least 10 h prior to the timing of the dose. At zero hour, an intravenous cannula was inserted into a forearm vein for sampling of blood. After baseline blood sampling, subjects were assigned randomly to receive the test or the reference formulation for the first dose of haloperidol 5 mg with 240 mL water and then respective alternative formulation for the second dose at a week later. All subjects abstained from food until the 4 h blood specimen was obtained. Blood samples were collected to assay the plasma haloperidol concentrations for 60 h at the following times: 1, 2, 3, 4, 5, 6, 9, 12, 24, 36, 48, and 60 h. Immediately following blood withdrawal, the sample were kept in ice bath and centrifuged within 30 min after sampling. The separated plasma samples were stored in -70°C until the assay (see below).

Processing of plasma sample for haloperidol HPLC assay

The initial steps included the addition of 50 μL of internal standard (i.e., chlorohaloperidol 500 ng/mL) and 500 μL of NaOH (2M) to 0.9 mL of plasma. A mixed organic solvent (8 mL), consisting of *n*-hexane and isoamylalcohol (99:1 V/V) was added to the mixture for liquid-liquid extraction for approximately 20 min. The mixture was centrifuged and the organic layer was evaporated to dryness under nitrogen stream. The residue was reconstituted with 50 μL of methanol, and an aliquot (10 μL) was injected onto the LC-MS system (see below; Hoja *et al.,* 1997; Arinobu *et al.,* 2002).

Liquid chromatography and mass spectrometry

The analysis of haloperidol sample was carried out using an Agilent 1100 HPLC system (Agilent Technology, Urdorf, Switzerland), equipped with an electrospray mass spectrometric system (Agilent Technology) and an autosampler (Agilent 1100 Series, Agilent Technology). The chromatographic separations were performed on a Bondapak C_{18} column (150×3.9 mm), with a flow rate of 0.4 mL/min. The mobile phase was a series steps of gradient consisting of 0.04M ammonium acetate buffer (solvent A) and methanol (solvent B) that follows: 60% B at 0 minutes, 80% B at 15 minutes and 100% B at 25 minutes until 32 minutes. The column was further equilibrated for 8 minutes before the introduction of the next sample. The operating conditions for the mass spectrometry were 140V for the fragmentor voltage, 10 L/min for the drying gas flow, 350°C for the drying gas temperature, and 30 psig for the nebuliser pressure. Quantifiers ions used for a haloperidol and a chlorohaloperidol were 376.3 and 392.3, respectively (Hoja *et al.,* 1997*).* Linearity was assessed in the ranges 0.1-5 ng/mL, respectively. Detection limits were 0.1 ng/mL. Correlation coefficients for calibration were better than 0.999, and intraday and interday variability were less than 10%.

Pharmacokinetic analysis

The pharmacokinetic parameters of haloperidol was preformed using noncompartmental pharmacokinetic methods with BA-Calc program (KFDA, Korea). The maximum haloperidol concentration (C_{max}) and the corresponding peak time (T_{max}) were determined by the inspection of the individual drug plasma concentration-time profiles. The elimination rate constant (k_e) was first estimated by log-linear least squares regression analysis of the last 4 concentration-time data pairs (i.e., 24 - 60 h). The elimination half-life ($t_{1/2}$) was calculated as $0.693/k_e$. The area under the curve to the last measurable concentration (AUC_t) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity

(AUC_{inf}) was calculated as $AUC_t + C_t/k_e$, where C_t is the last measurable concentration.

Statistical analysis

For the purpose of bioequivalence analysis AUC_t and C_{max} were considered as primary variables. Bioequivalence was assessed by means of an analysis of variance (ANOVA) for crossover design and calculating standard 90% confidence intervals of the ratio test/reference (T/R). AUC_t and C_{max} ANOVA were performed using logarithmically transformed AUC_t and C_{max}. The formulations were considered bioequivalent if the difference between two compared parameters was found not statistically significant (*i.e.*, $P \geq 0.05$) and 90% confidence intervals for these parameters fell within $80 \pm 125\%$ (log0.8-1.25). All statistical comparisons were carried out using K-BE test program (KFDA, Korea).

RESULTS AND DISCUSSION

HPLC-MS analysis

In this LC-MS method, no significant interferences were found for all samples and a representative chromatogram is depicted in Fig. 1. The retention time for haloperidol and the internal standard (chlorohaloperidol) were approximately 14.9 and 18.0 min, respectively. The quantification limit for haloperidol in human plasma was 0.1 ng/mL as evidence by the fact that the sample had a signal to noise ratio of 10. The coefficients variation of intraday and interday validation for human plasma were less then 7.90% and 7.34%, respectively, from haloperidol plasma concentration ranges from 0.1 to 5 ng/mL (Table I), indicating that the assay is valid for the bioequivalence study.

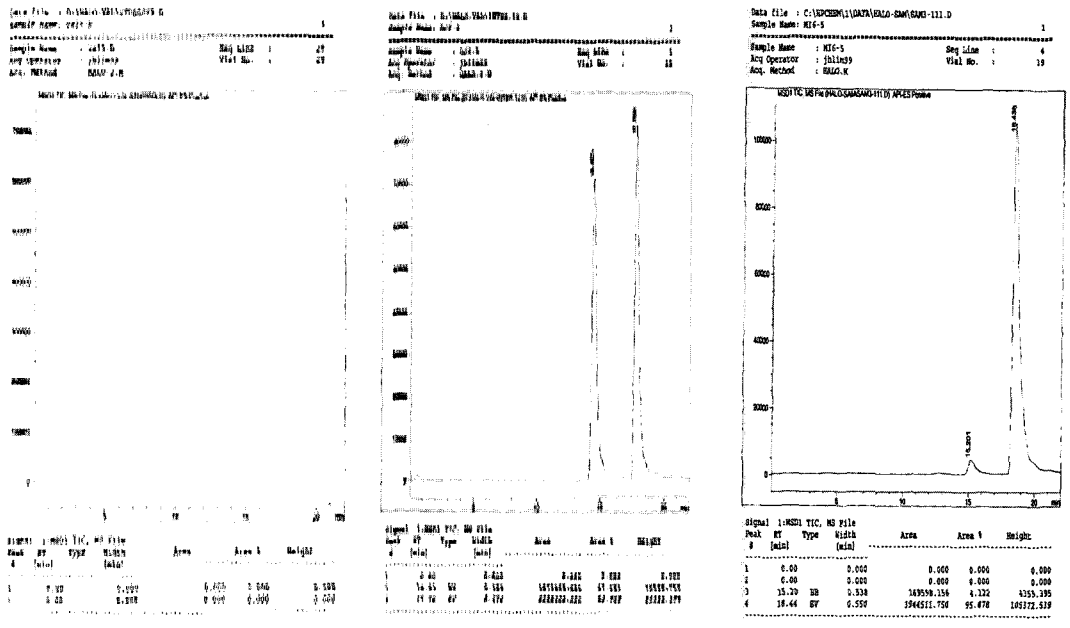
Table I. Precision and accuracy of the method for the determination of haloperidol in human plasma (n=5)

Concentration (ng/mL)	Accuracy (CV%)		Precision%
	Intraday	Interday	
0.1 (LOQ)	7.9	5.44	109.19
0.5	4.62	7.34	91.18
2	6.62	3.85	96.00
5	4.01	5.78	100.13

Pharmacokinetic analysis

All subjects completed study procedures without any remarkable adverse effect. However, some volunteers expressed a mild extrapyramidal effects of haloperidol. The mean plasma haloperidol concentration–time profiles are shown in Fig. 2. Almost identical plasma haloperidol concentration profiles were obtained from both formulations (Fig. 2). Table II summarized the pharmacokinetic parameters of $AUC_{0\text{-}60h}$, C_{max}, T_{max}, AUC_{inf}, $t_{1/2}$, V_d/F and CL/F, which were calculated with noncompartmental pharmacokinetic analysis by BA-Calc program (KFDA, Korea) (Table II).

The geometric means of $AUC_{0\text{-}60h}$ and C_{max} between



(A) (B) (C)

Fig. 1. Representative chromatograms of plasma extracts. (A) Drug free human plasma (Blank) (B) Blank plasma spiked with 5 ng/mL of haloperidol and 500 ng/mL chlorohaloperidol (internal standard) (C) Plasma sample of a subject at 5 h after a 5 mg oral dose of haloperidol. The area of haloperidol was calculated to be 0.48 ng/mL.

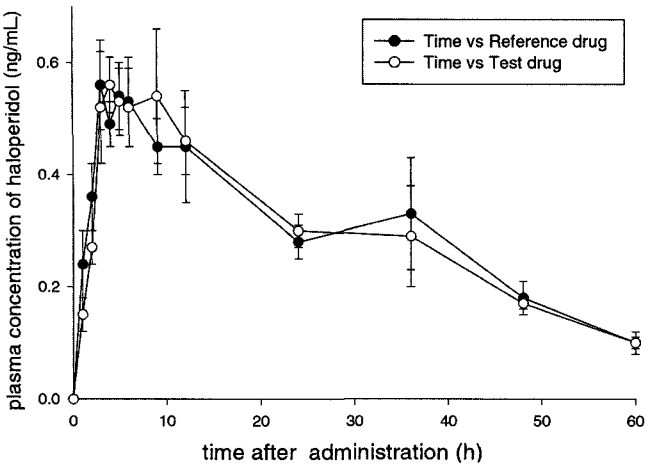


Fig. 2. Mean plasma haloperidol concentration-time profiles of healthy subjects following oral administration of haloperidol 5 mg. Vertical bars represent a standard deviation.

Table II. The pharmacokinetic parameters of haloperidol 5 mg tablets in volunteers after a single oral administration (mean ± standard deviation, n=24)

Parameters	Myung In haloperidol tablet (test drug)	Peridol® tablet (reference drug)	*t*-test
$AUC_{0\text{-}60h}$ (ng/mL/h)	17.21±8.26	17.31±13.24	N.S
AUC_{inf} (ng/mL/h)	21.75±8.50	21.77±15.63	N.S
C_{max} (ng/mL)	0.87±0.74	0.85± 0.62	N.S
T_{max} (h)	6.13±6.80	4.92± 2.60	
Clearance/F (L/h)	0.26±0.09	0.31± 0.17	N.S
Vd/F (L)	11.51±5.45	12.90± 6.12	N.S
Half life (h)	29.87±8.25	29.60± 7.56	N.S

test drug and reference drug were 17.21±8.26 ng·h/mL vs 17.31±13.24 ng·h/mL and 0.87±0.74 ng/mL vs 0.85±0.62 ng/mL, respectively. The mean terminal half-life for test and reference medications were 29.87 ± 8.25 and 29.60 ± 7.56 h, respectively. The terminal half-life for the drug in study was slightly longer than the those reported in other study (i.e., approximately 21 h) (McEvoy 2002).

Standard bioequivalence analysis

No significant sequence effect was found for all of the bioavailability parameters indicating that the crossover design was properly performed. Significant *F* test values were found between the subjects and subjects nested sequence (SEQ) for AUC_{60h} and C_{max}, indicating a substantial inter-subject variation in the pharmacokinetics of haloperidol from the two formulations (Table III). No significant period effect in AUC_{60h} and C_{max} was detected in this study. The detailed statistical and bioequivalence analyses for AUC_{60h} and C_{max} are listed in Table IV. The 90% confidence intervals for the mean ratio (T/R) of AUC_{60h} and

Table III. Analysis of variance test ($\alpha = 0.05$) for AUC_{60h} (log-transformed) and C_{max} (log-transformed) between test and reference drug of haloperidol

Source of variation	Log transform AUC_{60h} (F-test)	Log transform C_{max} (F-test)
Group or sequence	2.570(4.301)	4.295(4.301)
Period	2.518(4.301)	2.697(4.301)
Drug	1.472(4.301)	0.006(4.301)

Table IV. Statistics of bioequivalence analysis for log-transformed AUC_{60h} and log transformed C_{max} between test and reference drug of haloperidol

Range of criteria / Parameter	test/reference ratio (T/R)	F-value F<4.301	confidence limit log0.8 - 1.25
Log (AUC_{60h})	1.082	1.472	log0.9677 - 1.1201
Log (C_{max})	0.991	0.006	log0.8208- 1.1981

* F-value between test and reference formulation by ANOVA

C_{max} were log0.9677-1.1201 and log0.8208-1.1981 (Table IV), respectively, which were within the commonly accepted bioequivalence range of log0.80-1.25.

Summary and conclusion

The statistical comparison of AUC_{60h} and C_{max} clearly indicated no significant difference in the two brands of haloperidol 5 mg tablets. The 90% confidence intervals for the mean ratio (T/R) of AUC_{60h} and C_{max} were entirely within the acceptance range by the Korean guideline. Based on the pharmacokinetic and statistical results of this study, we can conclude that the Myung In haloperidol tablet is bioequivalent to Peridol® tablet, and that the two products can be considered interchangeable in medical practice.

ACKNOWLEDGEMENT

This study was supported by the contract, "Bioequivalence assessment of Myung In haloperidol tablet to Peridol® tablet after a single oral administration to healthy volunteers" from Myung In Pharm Co., Ltd., Seoul, Korea.

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