

Bioequivalence study of Donepezil hydrochloride in healthy Korean volunteers

Yewon Choi¹, Su-jin Rhee¹, In-Jin Jang¹, Kyung-Sang Yu¹, Sung-Vin Yim² and Bo-Hyung Kim^{2*}

¹Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Hospital, Seoul 110-744, Korea, ²Department of Clinical Pharmacology and Therapeutics, Kyung Hee University College of Medicine and Hospital, Seoul 130-872, Korea

*Correspondence: B. H. Kim; Tel: +82-2-958-9326, Fax: +82-2-958-9559, E-mail: bhkim98@khu.ac.kr

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Donepezil is a centrally acting, reversible acetylcholinesterase inhibitor that is widely used for treating Alzheimer's disease. This study aimed to compare the pharmacokinetics of Bastia®, a test tablet formulation of donepezil hydrochloride 10 mg, with those of Aricept®, the reference tablet formulation of donepezil hydrochloride 10 mg, in healthy Korean male volunteers. A randomized, single-dose, two-way crossover study was conducted in 32 subjects. Subjects received a single dose of either test or reference compound and the alternate drug after a 4-week washout period. Serial blood samples for pharmacokinetic analysis were collected prior to dosing and periodically for 288 h after dosing for measurement of the plasma concentrations of donepezil. A non-compartmental method was used to estimate the pharmacokinetic parameters. The maximum concentration (C_{max}) and the area under the concentration-time curve from time zero to the time of the last quantifiable concentration (AUC_{288h}) for the two formulations were compared to evaluate bioequivalence. The C_{max} of the test and reference drugs were 27.58 ± 7.46 and 26.35 ± 6.51 $\mu\text{g/L}$ (mean \pm SD), respectively, while AUC_{288h} was 1080.14 ± 229.77 and 1043.07 ± 242.28 $\mu\text{g}\cdot\text{h/L}$ (mean \pm SD), respectively. The geometric mean ratios (90% confidence interval) of the C_{max} and AUC_{288h} of the two tablets were 1.043 (0.990-1.099) and 1.039 (1.013-1.065). In conclusion, the newly formulated tablet of donepezil hydrochloride 10 mg is bioequivalent to the currently marketed 10 mg tablet.

Introduction

Donepezil is a piperidine-based, reversible acetylcholinesterase inhibitor widely used for treating Alzheimer's disease. Its therapeutic effect is thought to result from increased levels of acetylcholine available for synaptic transmission in the central nervous system. When compared to placebo, daily doses of 5 or 10 mg donepezil improve both cognitive and global clinical functions in patients with mild to moderate Alzheimer's disease. A daily dose of 10 mg delays the deterioration in the activities of daily life compared to that observed with placebo treatment. [1] The daily-recommended dose of donepezil is 5 to 10 mg. [2] The dose can be increased depending on the patient's clinical condition.

In one study, the concentration of donepezil peaked (C_{max}) to

4.1 ± 1.5 h after dosing and the mean terminal half-life was found to be 81.5 ± 22.0 h. [3] The pharmacokinetics (PK) of donepezil were found to be linear and showed dose proportionality after the administration of single doses to healthy volunteers. [3] The elimination half-life of donepezil is about 70 h and the mean apparent plasma clearance (CL/F) is 0.13-0.19 L/h/kg. Donepezil is slowly absorbed from the gastrointestinal tract. Its absorption rate and extent of absorption are not influenced by food. Its relative oral bioavailability is 100%. The drug is excreted in the parent form by the kidney and extensively metabolized, mainly by CYP 3A4 and CYP 2D6, in the liver. The metabolites are mainly excreted into the feces through the bile. [4]

Bastia® is a generic preparation of donepezil hydrochloride, developed by Korea Pharma Co., Ltd. Although the innovator and the generic preparations are manufactured by different manufacturers, generic formulations can provide alternative therapeutic options if they are bioequivalent to the innovator preparation.

The aim of this study was to compare the PK of Bastia® (test

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drug) and Aricept® (reference drug) in healthy Korean male volunteers and to evaluate the bioequivalence of the two formulations.

Methods

Subjects

Thirty-two healthy Korean male subjects aged 19- to 55-years-old, with no history of gastrointestinal, cardiovascular, pulmonary, renal, neurologic, endocrinal, or hematologic disease, and with no hypersensitivity to medications were recruited in this study. They were not taking any other medications at the time of screening. All subjects submitted written informed consents before participating in the study. This study was approved by the Institutional Review Board of Kyung Hee University Hospital.

Drugs

The test formulation (donepezil hydrochloride, tablet 10 mg) was manufactured by Korea Pharma Co., Ltd., Seoul, Korea (lot no. 9005, Expiration 2012. 11. 25). The reference formulation (donepezil hydrochloride, tablet 10 mg) was manufactured by Daewoong Pharmaceutical Co., Ltd., Seoul, Korea (lot no. 055506, Manufactured 2009. 7. 8).

Study design

A randomized, single-dose, two-period, two-treatment, two-sequence, crossover study was conducted with 32 subjects randomized into 2 sequence groups. The number of subjects was calculated using published intra-individual coefficient of variation of C_{max} and AUC_{inf} (15% and 10%, respectively[5]), and considering dropouts. Subjects in one sequence group received the reference formulation in period 1 and the test formulation in period 2 with an interval of 4 weeks between periods, while subjects in the other group received the drugs in the opposite order. Subjects were admitted to the Clinical Trial Center, Kyung Hee University Hospital, from the day before dosing (Day -1) to the day after dosing (Day 2) of each period. They were instructed to abstain from drinking alcohol, smoking, intense physical activity, and intake of drinks containing xanthine. Subjects received one tablet of either the test or reference formulations with 240 mL of water in fasting state on the morning of the first day of each period. Water and food intake were restrained for 2 and 4 h, respectively, after drug administration. Serial blood samples for PK analysis were collected pre-dose (time 0), and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 96, 144, 240, and 288 h after dosing. Safety and tolerability were assessed based on adverse events, vital signs, 12-lead ECG, clinical laboratory values, and physical examination.

Plasma concentration

Blood samples (7 mL each) were collected and plasma was separated by centrifugation at 3,000 rpm for 10 min at 4°C and immediately stored at a temperature of -70°C or lower until analy-

sis. The plasma concentration of donepezil was determined by high-performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies, USA) tandem mass spectrometry (MS/MS, API 4000, Applied Biosystems/MDS SCIEX, Foster City, CA, USA). Donepezil (100 µg/mL in 50% methanol) was used as a reference standard.

Each plasma sample (300 µL) was mixed with 1,000 µL of cisapride (15 µg/mL in acetonitrile, internal standard), vortexed for 90 sec, and centrifuged at 13,000 rpm for 8 min. The supernatant (1,000 µL) was taken and evaporated under nitrogen gas. The mobile phase (200 µL) was added and injected into the HPLC system.

The calibration curve was obtained by fitting the peak area ratio of the internal standard and the nominal concentration (X) of analyte to the equation, $Y=aX + b$ (weighting $1/X^2$). The method was validated over the range of 0.1-50 ng/mL for donepezil, 5 times daily, for 5 days. The lower limit of quantitation (LLOQ) was 0.1 ng/mL. Assay precisions were 96.92-113.58% (interday) and 97.63-111.51% (intraday) and the coefficients of variation (CVs) were less than 2.96 % (0.25 ng/mL, interday) and 5.40 % (5 ng/mL, intraday).

Pharmacokinetic analysis

The C_{max} and the time to C_{max} (t_{max}) were directly obtained from the observed values. The area under the plasma concentration-time curve from time 0 to the last observed time point of 288 h (AUC_{288h}) was calculated using the linear trapezoidal approximation. The terminal elimination rate constant (k_e) was estimated from the terminal phase by log-linear regression and the terminal half-life was calculated as $\ln 2/k_e$. The AUC from time 0 to time infinity (AUC_{inf}) was calculated by adding AUC_{288h} to the extrapolated area beyond the last measurable plasma concentration (C_{last}), $AUC_{288h} + C_{last}/k_e$. The apparent clearance (CL/F) was calculated as $Dose/AUC_{inf}$. Pharmacokinetic parameters were calculated and estimated as non-compartmental using the BA Calc 2007 analysis program Ver. 1.0.0 (Ministry of Food and Drug Safety, MFDS, Cheongju-si, Chungcheongbuk-do, Korea).

Statistical analysis

A general linear mixed effect model was used to compare the C_{max} and AUC_{288h} between the test and reference formulations. The period, sequence, and formulation variables were used as fixed effects, and subjects nested within sequence were used as random effects. In the mixed effect model, the logarithmic forms of C_{max} and AUC were used, and the least square mean differences of C_{max} and AUC with 90% confidence interval (90% CI) between both formulations were exponentiated to obtain the geometric mean ratio and its 90% CI. Bioequivalence was assessed based on the criteria of conventional bioequivalence with a range of 0.8-1.25. These statistical analyses were performed using the K-BE Test 2007, Ver. 1.1.0 (Ministry of Food and Drug Safety, MFDS, Cheongju-si, Chungcheongbuk-do, Korea).

RESULTS

The mean values of age, weight, and height of the 32 subjects were 24.5 ± 3.8 (19-39) years, 68.9 ± 9.2 (56-90) kg, and 175.4 ± 4.7 (165-184.4) cm, respectively (mean \pm SD (range)). Among the 32 subjects, 5 subjects were excluded because of non-compliance and 2 subjects experienced adverse events (AEs). Vomiting was reported 4 h after administration of the reference tablet in one subject and headache occurred 4 h after administration of the test tablet in another subject. These two subjects were dropped from the study. The remaining 25 subjects completed the study, and donepezil was well-tolerated in these subjects.

The pharmacokinetic parameters for the reference and test compounds are shown in Table 1. The concentration-time

curves for both formulations were similar, including both the absorption and disposition phases (Fig. 1). The median t_{\max} of the test drug (2.0 h, range 1.5-3.0 h) was similar to that of the reference drug (2.0 h, range 1.0-4.0 h), and the mean half-life of donepezil was similar for the test (70.40 ± 10.08 h) and reference (72.81 ± 11.78 h) formulations. The ratios of the geometric means (90% CI) for C_{\max} , AUC_{288h} and AUC_{inf} were 1.043 (0.990-1.099), 1.039 (1.013-1.065), and 1.035 (1.009-1.061), respectively. The 90% CIs are within the bioequivalence range of 0.800-1.250. The mean value of CL/F of the test formulation was similar to that of the reference formulation (Table 1). In addition, no systematic within-subject difference was observed for the C_{\max} and AUC_{288h} of the reference and test formulations

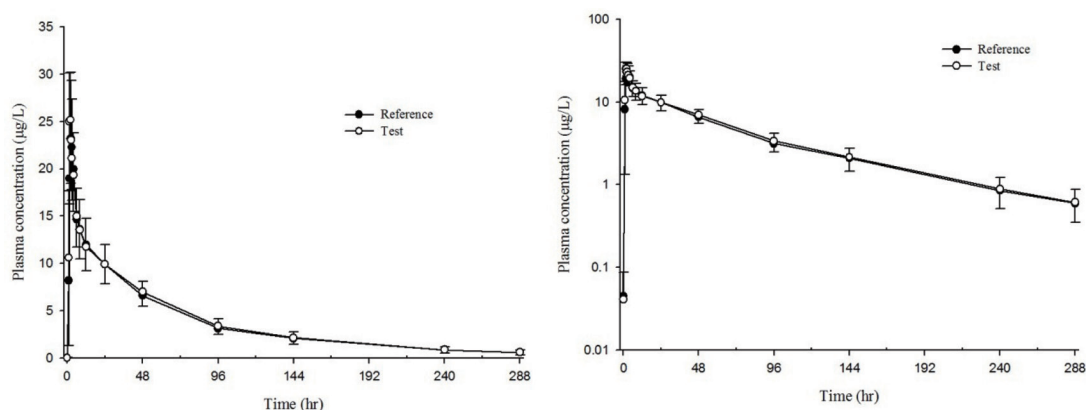


Figure 1. Mean plasma concentration-time profiles of donepezil after single oral administration of reference formulation (filled circles) and test formulation (open circles). Bars represent standard deviations. (Left, linear; right, log-linear)

Table 1. Pharmacokinetic parameters of donepezil administered as the reference drug and test formulation

Parameters	Reference (N=25)		Test (N=25)		Geometric mean ratio (90% CI) ^b
	Mean \pm SD	CV(%)	Mean \pm SD	CV(%)	
t_{\max} (h) ^a	2.0 (1.0-4.0)		2.0 (1.5-3.0)		
C_{\max} (µg/L)	26.35 \pm 6.51	24.71	27.58 \pm 7.46	27.04	1.043 (0.990-1.099)
AUC_{144h} (µg·h/L)	867.37 \pm 188.69	21.75	897.97 \pm 180.10	20.06	1.035 (1.010-1.062)
AUC_{240h} (µg·h/L)	1008.30 \pm 230.20	22.83	1044.24 \pm 218.32	20.91	1.039 (1.013-1.064)
AUC_{288h} (µg·h/L)	1043.07 \pm 242.28	23.23	1080.14 \pm 229.77	21.27	1.039 (1.013-1.065)
AUC_{inf} (µg·h/L)	1108.44 \pm 268.52	24.23	1143.71 \pm 254.17	22.22	1.035 (1.009-1.061)
$AUC_{extra,144h}$ ^a (%)	20.37 (13.97-29.33)		21.98 (13.46-30.86)		
$AUC_{extra,240h}$ ^a (%)	8.38 (4.80-15.25)		8.59 (4.51-13.53)		
$AUC_{extra,288h}$ ^a (%)	5.26 (2.73-10.88)		5.30 (2.55-8.59)		
Half-life (h)	72.81 \pm 11.78	16.18	70.4 \pm 10.08	14.31	
CL/F (L/h)	9.65 \pm 2.87	29.77	9.27 \pm 2.63	28.42	

All values, except for $AUC_{extra,144h}$, $AUC_{extra,240h}$ and $AUC_{extra,288h}$, are presented as the arithmetic mean \pm standard deviation and coefficient of variation (%). t_{\max} , time to peak concentration; C_{\max} , peak plasma concentration; AUC_t , area under the plasma concentration-time curve from 0 to t h; $AUC_{extra}(\%)$, % extrapolated AUC_t , which was calculated as $[(AUC_{inf} - AUC_t) / AUC_{inf}]$; CL/F, apparent clearance. ^aMedian value [min – max]. ^bGeometric mean ratio of test/reference, exponentiation of least square mean difference (90% CI) of logarithmic transformed C_{\max} and AUC values.

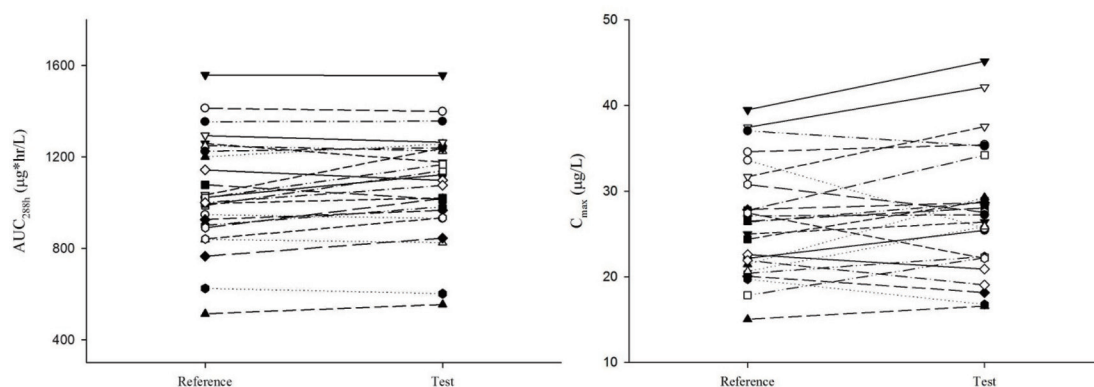


Figure 2. Individual subject's C_{max} and AUC_{288h} values for donepezil reference formulation and test formulation (Left: AUC_{288h} , Right: C_{max})

(Fig. 2).

Discussion

This study shows that the test formulation of donepezil, Bastia®, as 10 mg tablets is bioequivalent to the reference formulation, Aricept® (10 mg tablets), based on the 90% CI of the geometric mean ratios of C_{max} and AUC_{288h} . The mean values of AUC_{inf} , half-life, and CL/F were similar for both formulations. The observed pharmacokinetic values are consistent with previously reported donepezil PK data.[3] Orally administered donepezil (10 mg) was well tolerated. Of 32 participants who received the drug, AEs were reported in only two subjects, one after receiving the test compound and the other after receiving the reference compound. These AEs have been reported in a previous study[6] and occurred 4 h after administration of the reference or test tablets. Therefore, these AEs were considered to be drug-related.

This study was conducted according to a randomized two-way crossover design, which is generally used for studying bioequivalence. The blood-sampling period of 288 h was long enough to completely characterize the absorption and elimination phases of donepezil. Considering that the terminal half-life is 50-70 h, as shown here and in the previous study, the washout period of 4 weeks used in the present study is 10-fold higher than the half-life of donepezil and is sufficient to eliminate the first dose of the drug completely prior to the second administration.[3] Furthermore, for 13 subjects, the donepezil concentrations measured just prior to the second dose were below the LLOQ and for the other subjects, the values ranged from 0.11 to 0.24 ng/mL, which are less than 1% of the C_{max} concentration. Thus, a washout period of 4 weeks is sufficient to ensure that the drug is eliminated and there is no carry-over effect in the second phase of the study.

The softwares used to analyze the data, BA-Calc 2007 (Ver.1.0.0) and K-BE Test 2007 (Ver 1.1.0), were originally developed in 2007 in the College of Pharmacy at Kyung-hee University in Korea, with the support of the Ministry of Food and Drug

Safety.[7] However, there are other widely used software packages for analyzing pharmacokinetic parameters and assessing bioequivalence. These include Phoenix® WinNonlin® (Ver. 6.3, Pharsight, Mountain View, CA), which uses the same statistical methodology to assess bioequivalence as that used in K-BE Test 2007. When the pharmacokinetic data from the current study were analyzed using Phoenix® WinNonlin®, the results were similar to those obtained by K-BE Test 2007. There were minor differences in some parameter values, but the differences were less than 0.01% and did not affect the bioequivalence conclusions.

Because the mean half-life of donepezil is long (50-70 h), the sampling period had to be correspondingly long. This long sampling schedule could affect subject compliance in terms of admission periods and outpatient visits for pharmacokinetic sampling. To account for this, data at longer sampling times, 240 and 288 h, could be omitted and a truncated AUC can be used to evaluate bioequivalence. We additionally conducted a bioequivalence analysis using a truncated AUC for the data where AUC_{extra} (%) was around 20% or less (Table 1). The 90% CIs of the truncated AUCs (AUC_{14h} and AUC_{240h}) were similar to AUC_{288h} and AUC_{inf} and were within the range of 0.8-1.25. Therefore, the truncated AUCs, AUC_{14h} or AUC_{240h} , could be used as surrogates for AUC_{288h} to reduce sampling time for this bioequivalence study.

Alzheimer's disease occurs in the elderly. Previous pharmacokinetic studies have shown that the elimination half-life of donepezil in elderly patients is almost twice as long as that in young healthy volunteers.[8,9] However, the exposure of the drug including C_{max} , CL/F and AUC measured during therapeutic drug monitoring of elderly patients with Alzheimer's disease are comparable to those observed in young healthy volunteers. The difference of the half-life between the two age groups is probably due to a large distribution volume in the elderly. Still, further clinical studies are required to assess age-related differences in the disposition of the drug.

The pharmacokinetics of donepezil obtained after administra-

tion of test and reference drugs show that both formulations have similar pharmacokinetic characteristics and are bioequivalent with respect to the 90% CIs of the geometric mean ratios of C_{max} and AUC. Both formulations were well tolerated. All of these findings suggest that Bastia® could be a therapeutic alternative for Aricept®.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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