Comparative Fasting Bioavailability of 2 Different Betahistine Dihydrochloride 24-mg Tablets: A Single-Dose, Randomized-Sequence, Open-Label, 2-Period Crossover Study in Healthy Thai Volunteers

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ABSTRACT

Objective: To evaluate the bioequivalence of 24 mg betahistine dihydrochloride tablets between the test product (Stei®) and the reference product (Serc®) in healthy Thai volunteers.

Methods: This was an open-label, randomized sequence, single-dose, two-period crossover study in 24 healthy volunteers. Half of the volunteers received a single dose of test product 24 mg and then reference product 24 mg after a minimum 7-day washout period. The remaining half of volunteers received the reference product first and then the test product with the same washout period. Blood samples were obtained at pre-dose and over 14 hours after dosing. Plasma concentrations of 2-pyridylacetic acid (2-PAA), a major metabolite of betahistine were quantified by using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Tolerability in volunteers were assessed during the study.

Results: Statistical comparison of the main pharmacokinetic parameters showed no significant difference between test and reference. The geometric mean ratios of 2-PAA between the test and reference products were 96.44%, 96.99%, and 94.56% for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, respectively. These pharmacokinetic parameter values lie within the FDA and European Medicines Agency specified bioequivalence limit (80-125%). No serious adverse events related to the studied drugs were found.

Conclusion: It can be concluded that these two betahistine dihydrochloride products were considered bioequivalent.

Keywords: Betahistine dihydrochloride, bioequivalence

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INTRODUCTION

Betahistine dihydrochloride (betahistine) is an analog of histamine with weak histamine H1 receptor agonist and more potent histamine H3 receptor antagonist properties. It is indicated for reducing the severity and frequency of vertigo attacks associated with Ménière's disease, and also demonstrates benefit in different types of peripheral vertigo.[1-7] Growing evidence suggests that its therapeutic effects result from histamine H3 receptor antagonist properties. Betahistine promotes blood flow of the inner ear by acting on the precapillary sphincter of the striavascularis, so it can normalize the imbalance between the

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production and resorption of endolymph. In addition, it may decrease the vestibular sensory input in the peripheral vestibular system and decrease activity of vestibular nuclei through neurotransmitter release.[7-9]

Betahistine is rapidly and completely absorbed after oral administration. The drug undergoes rapid and almost complete metabolism to 2-pyridyl acetic acid (2-PAA), a major metabolite with no pharmacological activity. Since there is no sensitive method for determining betahistine concentrations in plasma and urine after oral administration, pharmacokinetic analyses are therefore based on 2-PAA measurements in plasma and urine. The plasma concentration of 2-PAA reaches its maximum 1 hour after drug administration and declines with a half-life of approximately 3.5 hours.[10-12]

The original formulation of betahistine dihydrochloride is currently marketed in Thailand under the brand name Serc®. An introduction of generic formulation would provide cost savings for patients and society. However, the bioequivalence study is required for registration of pharmaceutical products, in order to prove efficacy and safety of the generic formulation.[13] Therefore, the present study aimed to determine the bioequivalence of the generic and original formulations of 24 mg betahistine dihydrochloride tablets.

MATERIALS AND METHODS

Study drugs

Stei® manufactured by Sriprasit Pharma Co., Ltd., Thailand (lot no. TR12048) and Serc® manufactured by Abbott Healthcare S.A.S., France (lot no. 627349), were used as test and reference products, respectively. Both products were prepared as tablets containing 24 mg betahistine dihydrochloride.

Volunteers

Healthy Thai volunteers both male and female whose age ranged from 18 to 45 year old with a body mass index between 18-25 kg/m^2, were informed of the details and purposes of the present study and provided written informed consent before participation. Volunteers were assessed to be in good physical condition by clinical screening which included history taking, physical examination and the following laboratory tests: complete blood count, blood urea nitrogen, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, fasting blood sugar, urinalysis and hepatitis B surface antigen. Pregnant or lactating women or positive pregnancy test women were ineligible for enrollment. Exclusion criteria included an allergy to the study drug. Volunteers who used any drugs, food supplements, vitamins, mineral, herbal remedies, and/or hormonal contraceptives within 14 days before study participation were also excluded.

Sample size was calculated to yield a power of 80% and using an alpha level of 0.05. Assuming the % intra-subject CV for C_{max} and AUC was 21%,[11,14] the 90% confidence interval (CI) indicated that a total of 20 subjects would be sufficient for the study. In order to account for possible dropouts, 24 subjects were included in the study.

Study design

This bioequivalence study was conducted as an open-label, randomized sequence, single dose, two-period crossover study. The volunteers were randomized and assigned equally into two groups using a pre-printed randomization table generated by Microsoft Excel. Volunteers in Test-Reference (TR) group received a single dose of test product at the first period and then crossed over to receive reference product at the second period after a 7-day washout period. Volunteers in Reference-Test (RT) group received the reference product first and then the test product with the same washout period. In each period, volunteers were admitted on the night before administration day and were confined to the research ward for 14 hours after dosing. A single dose of either test or reference product was administered with 220 mL water under fasting condition for at least 10 hours. Standard meals were provided at 4, 8 and 12 hours after dosing, and post-dose meals were identical for all periods. A total of 14 blood samples were collected within 1 hour prior to dosing and 0.167, 0.333, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8 and 14

hours after dosing. The clinical part was conducted at Siriraj Clinical Research Center and the bio-analytical part was done at Siriraj Bioequivalent Center, Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University. The protocol had been approved by the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si.351/2012) on October 9, 2012. The study was performed in accordance with the Declaration of Helsinki for biomedical research involving human subjects and the Guideline for Good Clinical Practice.

Analysis of 2-pyridyl acetic acid (2-PAA) in plasma

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was developed for the determination of 2-PAA in humanplasma. Imipramine was used as an internal standard.[10-11,15] 2-PAA was extracted by liquid-liquid extraction technique (LLE), using ethyl acetate and isopropanol. Chromatographic separation was carried out on LC-MS/MS with Kinetex HILIC column (1.7 µm, 50 x 2.1 mm). A mobile phase consisting of acetonitrile and 2% formic acid in milli-Q water (75:25, v/v) was delivered with a flow rate of 0.2 mL/min. Mass spectra were obtained using a Quattro Premier XE mass spectrometer (Micromass Technologies, UK) equipped with electrospray ionization (ESI) source. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode.

Sample introduction and ionization were electrospray ionization in the positive ion mode. The mass transition ion-pair for quantification and confirmation of 2-PAA were selected as m/z 138.19 to 92.37 and 138.19 to 120.32. The data acquisition was ascertained by Masslynx 4.0 software. Validation of this method was performed as recommended by the USFDA guidelines.[16] The relative standard deviation (RSD) of quality control should be within 15% of the actual value, except at the lower limit of quantitation, where RSD should be within 20%. The assay was linear over a range of concentrations of 5 to 1,000 ng/mL ($r^2 \geq 0.995$), with a lower limit of quantitation of 5 ng/mL. No interfering peaks were observed in the validation process. The recoveries of 2-PAA were 61.22, 61.55 and 64.43% for the low quality control (LQC), medium quality control (MQC), and high quality control (HQC) samples, respectively. Precision was expressed as percent coefficient of variation (% CV), while accuracy was measured as percent of the nominal value (% nominal). Both precision and accuracy were acceptable for intraday and interday assessments in the lower limit of quantification (LLOQ), LQC, MCQ and HQC samples, and the data are presented in Table 1.

TABLE 1. The precision and accuracy for the analysis of 2-PAA in human plasma

2-PAA concentration	Day	Intraday Mean ± SD (ng/mL)	Intraday Accuracy (%norminal)	Intraday Precision (%CV)	Interday Mean±SD (ng/mL)	Interday Accuracy (%norminal)	Interday Precision (%CV)
LLOQ	1	4.96 ± 0.51	99.28	10.37	4.88 ± 0.39	97.53	7.94
(5 ng/mL)	2	5.06 ± 0.22	101.16	4.25			
	3	4.61 ± 0.25	92.17	5.49			
LQC	1	15.33 ± 0.82	102.18	5.33	15.70 ± 0.83	104.69	5.26
(15 ng/mL)	2	16.28 ± 0.57	108.50	3.51			
	3	15.51 ± 0.85	103.37	5.46			
MQC	1	472.36 ± 18.63	104.97	3.94	479.45 ± 18.93	106.54	3.95
(450 ng/mL)	2	494.45 ± 14.48	109.88	2.93			
	3	471.54 ± 15.99	104.79	3.39			
HQC	1	988.56 ± 10.97	109.84	1.11	961.19 ± 45.81	106.8	4.77
(900 ng/mL)	2	988.71 ± 33.43	109.86	3.38			
	3	906.29 ± 21.75	100.70	2.40			

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of 2-PAA were calculated by non-compartmental methods using WinNonlin® software version 3.1 (Scientific Consulting Inc., Apex, North Carolina). For the purpose of bioequivalence analysis, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were considered as the primary variables and T_{max}, $T_{1/2}$ and λ_z were considered as the secondary variables. C_{max} and T_{max} of 2-PAA were taken directly from the concentration-time data. The AUC_{0-t} was calculated using the log-linear trapezoidal approach. The $AUC_{0-\infty}$ was calculated by the formulation $AUC_{0-\infty} = AUC_{0-t} + (C_{t\,last} / \lambda_z)$, where $C_{t\,last}$ was the last detectable concentration. The λ_z was the elimination rate constant calculated from the log (ln) transformation of concentration-time curves. The plasma concentration half-life ($T_{1/2}$) was calculated by using the formulation $T_{1/2} = 0.693/ \lambda_z$.

Two-way analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for p-value equal to or less than 0.05. The 90% CI for the ratios of geometric mean Test/Reference (T/R) for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were calculated based on least squares means from the ANOVA of log-transformed data. A non-parametric statistical analysis, Friedman's test using Kinetics 2000 software was performed on T_{max} and considered significant difference between test and reference formulations when $p<0.05$. The bioequivalence between the two products would be accepted if the 90% CI of the log transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of test fell within 80-125% of the reference product.

RESULTS

Demographic data

There was no significant differences in demographic characteristics between the 2 groups of volunteers. The mean age of volunteers in the TR group (5 males and 7 females) was 31.7 years and the mean body mass index (BMI) was 22.33 kg/m^2. For the RT group (7 males and 5 females), the mean age was 32.5 years, and the mean BMI was 22.37 kg/m^2. One male volunteer in the RT group dropped out before taking any products due to personal reasons. Hence, 23 volunteers completed the entire study and were included in the pharmacokinetic analysis. Demographic data of volunteers are summarized in Table 2.

Pharmacokinetic parameters

The geometric means of Cmax, AUC_{0-t},

TABLE 2. Demographic Data and Baseline Characteristics of Volunteers

	Mean ± SD	
Characteristics	**TR Group (n=12)**	**RT Group (n=12)**
Gender		
Male	5	7
Female	7	5
Age (years)	31.7 ± 5.2	32.5 ± 4.0
Weight (kg)	58.6 ± 9.6	64.3 ± 9.6
Height (cm)	161.3 ± 8.3	165.5 ± 8.4
Body mass index (kg/m^2)	22.3 ± 1.9	23.37 ± 1.81
Vital signs		
Temperature (ºC)	36.4 ± 0.3	36.3 ± 0.3
Pulse (beats/minute)	76.5 ± 9.3	74.9 ± 9.8
Respiratory rate (time/minute)	19.0 ± 1.0	19.3 ± 0.9
Systolic blood pressure (mmHg)	110.7 ± 10.8	113.3 ± 9.8
Diastolic blood pressure (mmHg)	66.1 ± 7.1	64.6 ± 8.3

TR = Test-Reference; RT = Reference-Test

$AUC_{0-\infty}$, T_{max} and T1/2 of 2-PAA are summarized in Table 3. The geometric means of plasma concentration-time profiles are also presented in Fig 1.

Bioequivalence analysis

ANOVA of the log-transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ obtained from the test and reference products demonstrated no significant period, sequence and treatment effects ($p>0.05$). However, the significant subject nested in the sequence effect was solely significant ($p<0.05$) for all parameters which is normally seen in small sample size bioequivalence studies (Data not shown). The statistical analysis of 2-PAA obtained from the present study (n = 23) showed that the point estimate (90% confidence interval) of the geometric mean ratios (test/reference) of the log transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were within the equivalence criteria (80.00-125.00%) which were 96.44% (89.79-105.44%) for C_{max} ratios and 96.99% (89.11-107.36%) for AUC_{0-t} ratios and for $AUC_{0-\infty}$ was 94.56% (86.91 - 105.27%) with the power more than 80% (Table 3). The median (range) T_{max} of 2-PAA from the test and reference formulations were 0.5 (0.33-1.5) and 0.75 (0.33-1.25) hours, respectively. There was no statistically significant difference between these values ($p>0.05$).

Tolerability

Adverse events (AEs) were monitored and recorded in case report forms based on volunteer interview and physical examination. Treatments were well tolerated. Only 2 AEs were reported in 1 volunteer, which were common cold after administration of the reference product and dysmenorrhea after administration of the test product. Based on the documented AEs, these AEs were mild and not related to the study drug. No serious AEs were observed throughout the study.

DISCUSSION

The bioequivalence study of 2 different betahistine dihydrochloride 24-mg tablet formulations was conducted in 23 healthy Thai volunteers between a generic product (Stei®) and the reference product (Serc®). The analytical method (LC-MS/MS) utilized to determine the concentra-

TABLE 3. Summary of the pharmacokinetic parameters

Pharmacokinetic Parameters	Test product (Stei®)	Reference product (Serc®)
C_{max} (ng/mL)	367 (28.9)	380 (24.2)
AUC_{0-t} (ng.h/mL)	1,351 (31.8)	1,389 (25.4)
$AUC_{0-\infty}$ (ng.h/mL)	1,456 (33.0)	1,538 (32.1)
T_{max} (h)	0.5 (0.33-1.5)	0.75 (0.33-1.25)
λ_z (h-1)	0.206 (25.8)	0.203 (51.6)
$T_{1/2}$ (h)	3.37 (25.8)	3.41 (51.6)

AUC = area under plasma concentration-time curve; C_{max} = maximal plasma concentration; T_{max} = time for the maximal plasma concentration; T1/2 = half-life; λ_z = elimination rate constant. Data shown as geometric mean (CV%) and median (range) for T_{max}.

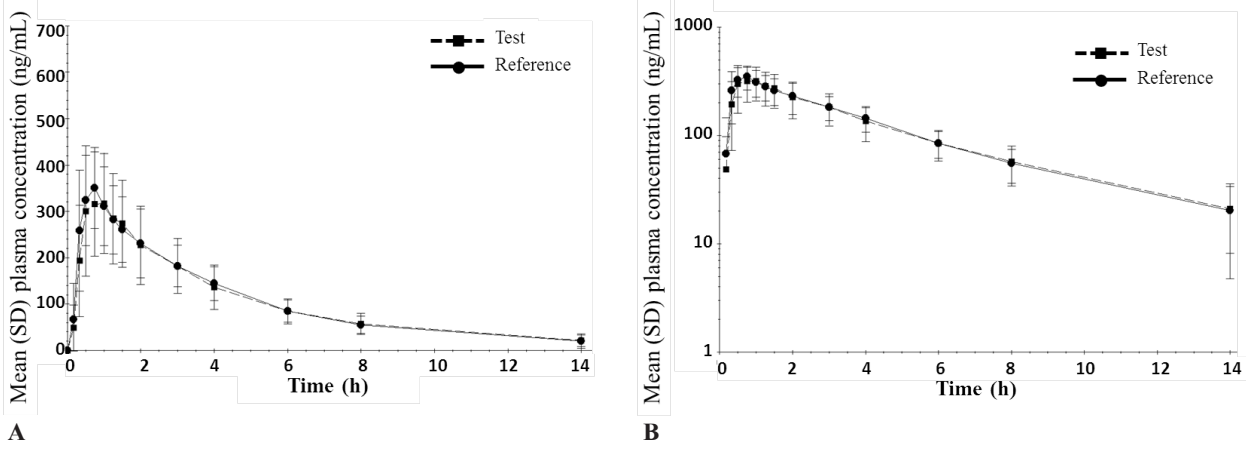

Fig 1. Geometric mean (SD) of plasma concentration-time profile of 2-PAA in a test (Stei®) and reference (Serc®) products of 24 mg betahistine dihydrochloride after a single administration in 23 healthy Thai volunteers. Lower limit of quantitation = 5 ng/mL; normal plot (A) and semi logarithmic plot (B)

TABLE 4. Statistical summary of the comparative bioavailability data (n = 23)

Dependent	Geometric mean ratio (T/R)	90% confidence interval (CI)	Power	Intra-subject coefficient of variation (%CV)
Ln (C_{max})	96.44	89.79-105.44	0.9914	17.69
Ln (AUC_{0-t})	96.99	89.11-107.36	0.9739	20.21
Ln ($AUC_{0-\infty}$)	94.56	86.91-105.27	0.9613	21.37

T = test product (Stei®); R = reference product (Serc®)

tions of 2-PAA, a major metabolite of betahistine in plasma demonstrated good precision and accuracy. The present study design and sample size are considered most appropriate and standard for this type of study. The results showed that both products were well tolerated. The 90% confidence intervals for log-transformed geometric mean test/reference formulation ratios of primary parameters including C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were entirely within 80.00%-125.00%. There was no statistically significant difference of T_{max} between the reference and the test products (p>0.05).

CONCLUSION

From the present study, both reference and test products of 24-mg betahistine dihydrochloride tablets were bioequivalent regarding rate and amounts of drug absorption.

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Potential conflicts of interest

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