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Bioequivalence of Two Oral Tablet Formulations of Betahistine 24 Mg: Single-Dose, Open-Label, Randomized, Two-Period Crossover Comparison in Healthy Individuals

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Abstract

A bioequivalence study of betahisitine tablets was conducted. Thirty two healthy Mexican mestizo volunteers received each test (T) and reference (R) formulations of betahistine at a dose of 24 mg in a 2 × 2 cross-over study. There was a three-day washout period between the two formulations. Plasma concentrations of betahistine were monitored by an ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC/MS/MS) for over a period of 24 h after the administration. AUC_{0-t} (the area under the plasma concentration–time curve from time 0 to last sampling time) and AUC $_{0-\infty}$ (that from time 0 to infinity) were calculated by the linear-log trapezoidal rule method. C_{max} (maximum plasma drug concentration) and T_{max} (time to reach C_{max}) were compiled from the plasma concentration–time data. Analysis of variance was carried out using logarithmically transformed AUC and C_{max}, and untransformed T_{max}. The mean of AUC_{0-t} was 7139.8 ng mL^{-1} h^{-1} (test medication) and 6714.4 ng mL^{-1} h^{-1} (reference medication) and that of $AUC_{0-\infty}$ were 7660.2 (test) and 6850.3 ng mL^{-1} h^{-1} (reference). C_{max} values of 1716.2 and 1677.3 ng mL^{-1} were achieved for the test and the reference medication, respectively. T_{max} were determined at 0.86 h for the test and 0.87 h for the reference formulations. The 90% confidence intervals for AUC_{0-t}, AUC_{0}-∞ and C_{max} were 0.994-1.102, 0.994-1.131 and 0.969-1.069, respectively, satisfying the bioequivalence criteria of the Mexican Comisión Federal Para la Protección Contra Riesgos Sanitarios, the European Committee for Proprietary Medicinal Products and the US Food and Drug Administration Guidelines. These results indicate that the two medications of betahistine are bioequivalent and, thus, may be prescribed interchangeably.

Keywords: Bioequivalence; Betahistine; Ménière's disease; Pharmacokinetics; Healthy volunteers

Introduction

Ménière's disease and related disease of the vestibular system are common and debilitating. Current therapy is multi-modal and includes drug therapy and lifestyle adaptations. Unfortunately many of the drugs used in treatment are sedative and hamper the process of vestibular compensation. Although betahistine is the mainstay of drug treatment in these illnesses, its efficacy has not, until recently, been evaluated to modern standards. Betahistine dihydrochloride (CAS 5579-84-0) (Figure 1) is an analog of histamine with weak agonist properties at histamine H1 receptors and more potent anatgonistic effects at histamine H3 receptors. Growing evidence suggests that the mechanism of action of betahistine lies in the central nervous system and in particularly in the neuronal systems involved in the recovery from process after vestibular loss. The histaminergic neurones of the tuberomamillary and vestibular nuclei are implicated. In recent years the clinical efficacy of betahistine has been demonstrated in double-blind, randomized, placebo, and active controlled studies in adequate numbers of patients [1-7]

The expiration of the patent of the brand name betahistine (Serc®, BetaSerc®; Solvay Pharmaceuticals) allows the production of generic formulations of the drug. Generic drugs are important options that allow greater access to health care for all individuals; however, the generic drug manufacturer must prove its drug is bioequivalent to the brand name drug [8]. In this study, a generic formulation of 24 mg of betahistine was compared to the brand name formulation in healthy subjects, and found to be bioequivalent and, thus, may be prescribed interchangeably

Material and Methods

Test and reference medications

The test medication, Microser®, lot no. 120702, Grünenthal S.A. C.V.) and the reference medication, (Serc®, lot no. 18027MC Italmex S.A.) were supplied as tablets.

Subjects and methods

Thirty-two healthy Mexican volunteers, from both sexes, ranging in age from 18 to 54 years (Median of 27), averaging in weight 65.81 ± 7.76 kg, and 164.53 ± 7.59 cm in height, completed the study. The sample size of n = 32 subjects was sufficient to ensure a power of ≥90% for correctly concluding bioequivalence under the following assumptions: $\alpha = 0.05$, $0.80 < \mu T/\mu R < 1.25$ and an intrasubject coefficient of variation of 25.0% [8]. Volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests (blood analysis; hemoglobin, hematocrit, WBC, platelet, differential counting of WBC, blood urea nitrogen, cholesterol, glucose fasting,

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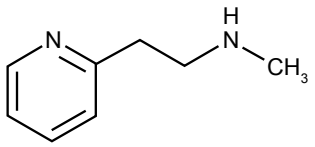

Figure 1: Structure of Betahistine dihydrochloride.

sGOT and sGPT and urine analysis; specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, and cast), electrocardiogram and simple chest X-ray. Volunteers were excluded if they were possibly sensitive to this type of medication, had a history of any illness of hepatic, renal, or cardiovascular systems, or had taken alcohol or other medications for a long period of time. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications. All volunteers avoided using other drugs for at least one week prior to the study and until after its completion. They also refrained from alcoholic beverages, and xanthine-containing foods and beverages 48 h prior to each dosing and until the collection of the last blood sample. At the time of going into each of the periods, the investigation of drugs of abuse in urine and pregnancy test were performed Each volunteer received an oral dose of 24 mg (1 tablet) of betahistine dihydrochloride in a standard 2 × 2 cross-over model in a randomized sequence. There was a three day washout period between the doses. This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and therules of Good Clinical Practice. The protocol of this study was approved by the Ethical Committee of Laboratorios Clinicos de Puebla de Bioequivalencia (Conbioética No. 21CEI00120130605, Comisión Federal para la Protección Contra Riesgos Sanitarios [COFEPRIS] No. 13 CEI 21 114 126), and registered in the Mexican National Registry of Clinical Studies. All participants signed a written informed consent after they had been informed of the nature and details of the study in accordance with COFEPRIS Guidelines for Bioequivalence Tests [9-14].

Subjects were entered at the Clinical Unit of Laboratorios Clínicos de Puebla de Bioequivalencia at 17:00 h 1 day before each study period and fasted 10 h before each drug administration and 2 h after. At 06:45 h, the median cubital vein was cannulated (BD Saf-T-Intima™), and 0.5 mL of heparinized normal saline injectable solution (20 units mL^{-1}) was flushed into the cannula to prevent blood clotting. The doses were taken at 8:00 a.m. of each dosing day with 250 mL of tap water. At 2 h after oral administration, all subjects were given standarized meals. Subjects were not allowed to remain in a supine position or to sleep until 8 h after oral administration. Approximately 6 mL blood samples were collected via the cannula at the following times; predose, 0.16, 0.33, 0.50, 0.66, 0.82, 1.00, 1.25, 1.50, 2.00, 4, 8, 12 and 24 h after the administration. The heparinized normal saline injectable solution, 0.5 mL, was flushed after each blood sampling. The blood sample was centrifuged immediately, and plasma sample was frozen at −196°C in liquid nitrogen tanks until the ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) analysis.

UPLC/MS/MS assay of betahistine in plasma

The concentrations of betahistine (acid-2-pyridyl acetate) in plasma were measured by a method that was developed and validated for this purpose [15-17]. Briefly, a 30 µL aliquot of internal standard (D-6 acid-2-pyridyl-acetate 5000 ng/mL), and a 330 µL aliquot of 4% phosphoric acid were added to a 300 µL aliquot of plasma sample in a test tube. After vigorous vortexing, 300 µL of this mixture were transferred to one well of an Oasis® MCX pre-conditioned solid phase extraction plate, whixh was then washed twice with 2% formic acid and 100% methanol. Three hundred µL of methanol:water (80:20 v/v) with 5% ammonium hydroxide and constant vacuum were used to elute the contents of the wells to a collection plate that was sealed and introduced to the autosampler of an ultra-performance liquid chromatography system coupled to tandem mass spectrometer.

The mobile phase, a mixture of ammonium acetate: acetonitrile (20:80 v/v) was run at a flow rate of 0.15 mL min^{-1}. The column effluent was monitored using tandem mass spectrometry in a positive electrospray ionization mode, using 18eV cone voltage and 10eV colliding energy, to detect the following transitions 138.00>119.65 and 141.95>95.60 for detection of acid-2-pyridyl acetate and the deuterated isotope respectively. The full system consisted of an Acquity UPLC™ system (Waters, Milford, MA, USA), a Quattro Premier XE Mass Spectrometer (Waters), Waters MassLynx™ software and an Acquity UPLCTM BEH Shield RP18 column, 130Å, 1.7 µm, 2.1 × 100 mm column (Waters).

Pharmacokinetic analysis

Non-compartmental pharmacokinetic characteristics were derived by standard methods. The maximum plasma concentration, C_{max}, and the time of its occurrence, T_{max}, were compiled from the concentration–time data. The AUC_{0-t} was calculated using the linear trapezoidal rule and was extrapolated to infinity according to the relationship

$$AUC_{0-\infty} = AUC_{0-t} + Ct / \beta \quad (1)$$

where $AUC_{0-\infty}$ is the area under the plasma concentration–time curve from 0 to time infinity, Ct is the last concentration evaluated in plasma greater than the limit of quantification (LOQ) and β is elimination rate constant at terminal phase.

Statistical analysis of data

The following tests or procedures have been carried out for AUC, C_{max}, and T_{max} ANOVA was performed using logarithmically transformed AUC and C_{max}, and original scaled values of T_{max}. The Schuirmann's two one-sided t-tests (i.e. for logarithmically transformed AUC and C_{max}) were conducted to test the bioequivalence of the pharmacokinetic characteristics between the medications. The range of bioequivalence for parametric analysis was set to the commonly accepted 80–125% obtained from ln-transformed parameters from the reference medication, and the range of equivalence for non-parametric analysis was set to the 20% of the reference mean. All statistical comparisons were made using the Phoenix™ WinNonlin® software version 6.3 program.

Results

UPLC/MS/MS analysis

In this UPLC/MS/MS method, no interferences were observed in human plasma. The retention time for pyridyl acetic acid and D6 pyridyl acetic acid (internal standard) was approximately 1.4 min for both. The calibration curve was reproducible and linear over the range of 10-1000 ng mL^{-1}. The LLOQ for betahistine in human plasma was 10 ng mL^{-1} based on a signal-to-noise ratio ≥ 39. For the intra-batch assay, the precision ranged from 3.90% to 5.53%, and the accuracy ranged from 94.93% to 104.75%. Inter-batch precision and accuracy ranged from 2.11% to 4.97% and 100.70% to 106.60%, respectively.

Clinical observations

The tolerability of both betahistine medications was good. Clinically relevant or drug-related side effects were not observed in any of the 32 volunteers [18].

Pharmacokinetic characteristics

The plasma betahistine pharmacokinetic parameters from the Serc® (reference, manufactured by ITALMEX) tablets and Microser® (test, manufactured by GRÜNENTHAL) tablets are summarized in Table 1, and the mean plasma betahistine concentration–time profiles are shown in Figure 2.

Almost identical plasma betahistine concentration profiles were obtained from both formulations. The mean terminal half-life for test and reference medications was 3.7 ± 0.6 h and 3.3 ± 0.2 h, respectively which were very similar to other studies.

Standard bioequivalence analysis

As shown in Table 2, no significant sequence or period effects were found for all the three bioavailability parameters C_{max}, AUC_0-t and AUC_0-∞, indicating that the cross-over design was properly performed.

Table 2 summarizes the bioequivalence statistics of the Ln-transformed parameters C_{max}, AUC_0-t and AUC_0-∞. As shown, the

	Test, Microser ® 24 mg Betahistine						Reference, Serc ® 24 mg Betahistine					
	Cmax	tmax	t ½	AUC	AUC∞	MRT	Cmax	tmax	t ½	AUC	AUC∞	MRT
	(ng/mL)	(h)	(h)	(ng*h/mL)	(ng*h/mL)	(h)	(ng/mL)	(h)	(h)	(ng*h/mL)	(ng*h/mL)	(h)
Mean	1716.2	0.9	3.7	7139.8	7660.2	4.6	1677.3	0.9	3.3	6714.4	6850.3	4.6
St Dev	307.5	0.3	3.1	2284.3	4636.9	1.3	252.6	0.3	1.3	1413.2	1622.8	1.1
St Error	54.4	0.0	0.6	403.8	819.7	0.2	44.7	0.1	0.2	249.8	286.9	0.2
CV	17.9	30.6	84.3	32.0	60.5	28.6	15.1	38.3	38.1	21.0	23.7	24.2
Median	1772.8	0.8	3.3	6971.8	7081.4	4.5	1742.5	0.8	3.3	6363.6	6427.7	4.5

MRT= Mean residence time; St Dev = Standard deviation; St Error = Standard error; CV = Coefficient of variation expressed as percent

Table 1: Values of pharmacokinetic parameters of betahistine in two oral formulations.

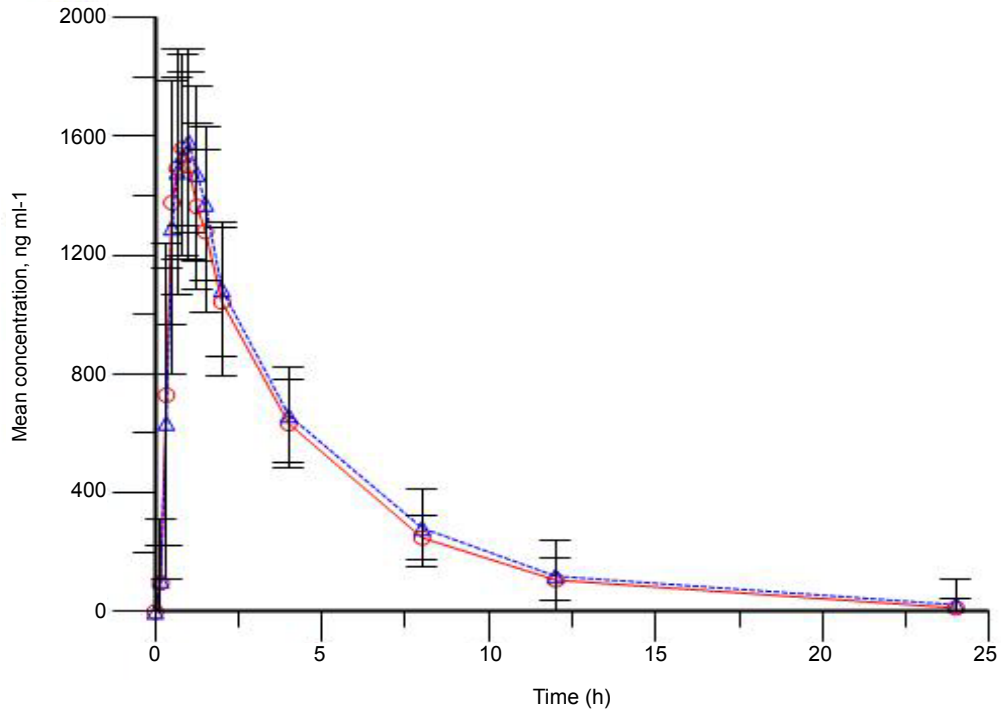

Figure 2: Mean plasma concentration-time plot of the kinetics of betahistine after single oral administration of 24 mg tablets of two formulations (test △, and reference O, vertical bars depict standard deviation).

	Ratio B/A	90% Confidence Interval		Westlake Interval		Shuirmann´s P value	
Ln(Cmax)	101.79	96.94	106.89	94.19	105.81	0.0000	0.0000
Ln(AUCt)	104.68	99.42	110.22	91.07	108.93	0.0000	0.0000
Ln(AUC∞)	106.02	99.35	113.14	88.53	111.47	0.0000	0.0001

Ratio B/A = Ratio of mean values of parameters obtained from the test (B) and reference (A) formulations

Table 2: Summary of Bioequivalence Statistics.

90% confidence interval, the Westlake interval, and the two-sided Shuirmann's probability test, all indicate that the test formulation of betahistine is bioequivalent to the reference formulation and hence, they may be prescribed interchangeably.

Discussion

Bioequivalence studies are cross-over studies in which each subject acts as its own control. This model, (*in vivo* healthy volunteers) is regarded as adequate to detect formulation differences. The results obtained allow extrapolation to populations in which the reference product is approved. Bioequivalence studies usually involve single doses of a medicine. It is theoretically possible that excipients used in the generic formulation (preservatives, pH adjusters, thickening agents, etc.) could affect the absorption and metabolism at steady state without producing these differences from a single dose. However this is extremely unlikely and would normally be apparent from differences observed in the bioequivalence study. Any difference that may exist is negligible compared to the variability of the conditions in the gastrointestinal tract and its effect on absorption.

A crucial issue on bioequivalence studies is the number of healthy subjects that should be used to demonstrate equivalence or in equivalence of two formulations. Several statistical approaches have been recommended, and the prevalent criteria have evolved during the last years [19-21]. Currently, most regulatory bodies worldwide agree that unnecessary exposure of healthy subjects to any drug, particularly if it is toxic or potentially hazardous- is to be avoided and therefore, the number of subjects should be limited to those that are needed but suffice to fulfill the statistical criteria to demonstrate bioequivalence.

According to the results of this study and current criteria, the intra-subject coefficient of variation in Mexican mestizos is 11.56% ($C_{máx}$) and, following the formula of Chow [21], the study could have been conducted in 14 healthy volunteers. When this particular study was conducted, the recommended criterion in México was based on the inter-subject coefficient of variation, which explains why the sample size was 32 subjects.

Genetic differences may account for the intra-subject variability of a given formulation and hence, the number of subjects that need to be recruited in a Bioequivalence trial might change from one ethnic group to another. Pharmacogenomic selection of volunteers might prove a useful approach to understand and harmonize inter-ethnic variability and also to reduce the number of volunteers needed in bioavailability and bioequivalence studies. Reduction of intra and inter subject coefficients of variation might result in increased stringency of bioequivalence statistics.

The plasma betahistine pharmacokinetic parameters from the Serc® (reference, manufactured by ITALMEX) tablets and Microser® (test, manufactured by GRÜNENTHAL) tablets demonstrated almost identical plasma betahistine concentration profiles. All indicate that the test formulation of betahistine is bioequivalent to the reference formulation and hence, they may be prescribed interchangeably.

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