



Pharmacokinetics of 2-pyridyl acetic acid, a major betahistine metabolite in healthy Indian volunteers

Manoj Singh Tomar^{1†}, Anil Patni¹, Sudershan Kumar¹, Nageshwar Rao Thudi², Sunil Kumar Dubey¹, Arshad Hussain Khuroo¹, Simrit Reyar¹, Rakesh Jain¹ and Tausif Monif¹

¹Department of Clinical Pharmacology and Pharmacokinetics, Ranbaxy Laboratories Ltd., GP 5, HSIDC Sector-18, Old Delhi-Gurgaon Road, Gurgaon, Haryana, India.

²Ranbaxy Pharmaceuticals Canada Inc., Suite 2010, 2680 Matheson Blvd., Mississauga, Ontario, Canada.

Abstract

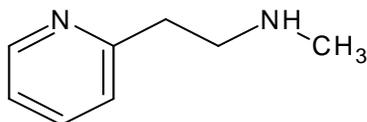
Betahistine dihydrochloride is a vasodilator and most commonly used to treat the symptoms of Ménière's disease, vertigo and tinnitus. Due to the rapid metabolism of betahistine in humans, its estimation in human plasma is not possible. The only major metabolite detected in urine and plasma is 2-pyridyl acetic acid. The results of pharmacokinetic parameters in Indian volunteers have not been published before, and are presented and discussed for the first time in this article. A randomized, single dose, crossover bioequivalence study was conducted in healthy Indian volunteers to assess pharmacokinetics of 2-pyridyl acetic acid, by administering betahistine dihydrochloride tablet (24 mg) under fasting condition. 2-pyridyl acetic acid, was determined by a validated liquid chromatography/mass spectrometry method (LC-MS/MS) in human plasma with standard curve range 3.48 to 1279.12 ng/mL. The test betahistine dihydrochloride (24 mg) tablet formulation showed bioequivalence with the respective reference betahistine dihydrochloride (24 mg) tablet formulation. However, on comparison of the pharmacokinetic parameters of the Indian volunteers with the results of previous study done on Chinese volunteers, Indian volunteers showed quite high C_{max} (approximately twice) and AUC (approximately thrice) and also the deviation observed in both pharmacokinetic parameter was quite low as compared to Chinese volunteers.

Key Words: Betahistine, 2-pyridyl acetic acid, pharmacokinetics, LC-MS/MS, Bioequivalence,

Introduction

Betahistine dihydrochloride [*N*-methyl-2-(pyridin-2-yl)ethanamine dihydrochloride] is a vasodilator and most commonly used to treat the symptoms of Ménière's disease, vertigo and tinnitus [1]. The mode of action of betahistine was believed to be a direct stimulating (agonistic) effect on H₁ receptors located on blood vessels in the inner ear. This would give rise to local vasodilation and increased permeability, which would help reverse the underlying problem of endolymphatic hydrops. Various studies have underlined the efficacy of betahistine in Ménière's disease and vertigo [2-7]. The recommended daily dose for betahistine is 24–48mg divided in two or three single doses. Betahistine is available as scored or un-scored tablets containing 8, 16 or 24mg of betahistine and as a solution for oral administration containing 8 mg/mL of betahistine [8]. The drug (Figure 1) is mainly metabolized in the liver and undergoes almost complete first-pass metabolism in healthy volunteers. Due to the rapid metabolism of betahistine in humans it is not possible to estimate the same in human plasma. The only metabolite detected in urine and plasma is 2-pyridyl acetic acid (Figure 2). The histamine metabolism – a ring methylation and an oxidative deamination – is not influenced by betahistine [8]. Pharmacokinetics of 2-pyridyl acetic acid from betahistine mesylate 24 mg tablet in Chinese volunteers has been previously reported in literature [9], however no literature was available regarding pharmacokinetic characterization of betahistine dihydrochloride and its major metabolite 2-pyridyl acetic acid in Indian volunteers.

The results of pharmacokinetic parameters of 2-pyridyl acetic acid in Indian volunteers have not been published before, and are presented and discussed for the first time in this article.

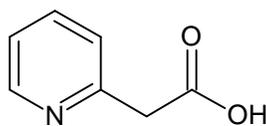


Molecular Formula = C₈H₁₂N₂

Formula Weight = 136.19428

Betahistine

Figure 1: Structure of Betahistine



Molecular Formula = C₇H₇NO₂

Formula Weight = 137.14

2-pyridyl acetic acid

Figure 2: Structure of 2-pyridyl acetic acid

Materials and Methods

Volunteers

In the study, healthy human adult volunteers in the age range of 18-55 years with the BMI range of 18 – 25 Kg/m² were enrolled. Volunteers with hypersensitivity to betahistine or related group of drugs, clinically significant abnormal hematology, biochemistry or urinalysis tests; a history or current condition of gastrointestinal, cardiovascular, hepatic, hematopoietic, renal or respiratory disease were excluded. Other exclusion criteria included exposure to other investigational drugs, recent ingestion of alcohol or drug abuse, heavy smoking, recent donation of blood, or history of HIV infection. Women with child bearing potential were required to have a negative serum and urine pregnancy test and be practicing an effective method of contraception. Study was conducted at Fortis Clinical Research Limited, Faridabad, India and was approved by the Sentinel Independent Ethics Committee (SIEC), New Delhi, India. The study was conducted in accordance with the Basic Principles defined in US 21 CFR Part 320, the International Conference of Harmonization (ICH) 'Guidance for Good Clinical Practice' and the principles enunciated in the Declaration of Helsinki, and written, informed consent was obtained from all volunteers prior to participation in the study.

Study Design

Randomized crossover study design was selected over non-crossover design because of two advantages. First, the influence of covariates was reduced because each crossover subject serves as his or her own control. Secondly, optimal crossover designs are statistically efficient and require fewer volunteers than non-crossover designs. Pharmacokinetics and bioavailability for 2-pyridyl acetic acid was assessed when betahistine dihydrochloride tablet (24 mg) was administered under fasting condition.

Study : Pharmacokinetics and Bioavailability

The study employed an open –label, randomized, single-dose, two period crossover design conducted on 12 healthy adult Indian human volunteers. A single oral dose of test or reference formulation, betahistine dihydrochloride (24 mg) tablet, was administered under low light conditions with 200 mL of drinking water after an overnight fast of at least 10 hrs. The dose was administered under the supervision of trained study personnel. Formulation to be dosed in each period was determined by the randomisation schedule generated with the help of Statistical Analysis System (SAS) software for Windows, Version 9.1.3 (SAS Institute Inc., USA). All volunteers were fasted overnight after admission for at least 10 hours before the morning dose and for 4 hrs post-dose. They received standard meals – lunch, snacks and dinner at 4, 9 and 13 hours, respectively, after drug administration. Meal plans were identical for both the periods. Volunteers withdrawn and/or dropped out subsequent to dosing were not replaced. Data was presented on all the volunteers who completed the study. There was a washout period of 5 days between the periods.

Sample Collection and Analysis

The blood samples were collected pre-dose and at 0.167, 0.250, 0.333, 0.500, 0.833, 1.000, 1.250, 1.500, 1.750, 2.000, 2.500, 3.000, 3.500, 4.000, 4.500, 5.000, 6.000, 8.000, 10.000, 12.000, 16.000 and 24.000 hours post dose in each period. The pre-dose blood samples were

collected within a period of 1.5 hours before dosing and the post-dose samples were generally within ± 2 minute of the scheduled time. The plasma was separated from each blood sample and stored at -15°C or below, pending assay for 2-pyridyl acetic acid, the major metabolite of betahistine by a validated liquid chromatography/mass spectrometry method (LC-MS/MS).

Analysis: 2-pyridyl acetic acid Assay

Separation of 2-pyridyl acetic acid was achieved on a Hypersil Gold C18, 100×4.6 mm, 5μ column using mobile phase (methanol:water:0.1% ammonia- 90:10:0.1 v/v/v). Plasma was spiked with internal standard (acyclovir). Solid phase extraction of 2-pyridyl acetic acid was carried out on Oasis MCX (30mg/1CC) extraction cartridges with methanol, evaporated under nitrogen and reconstituted in methanol: water (90:10 v/v).

2-pyridyl acetic acid, the major metabolite of betahistine was quantified by a validated liquid chromatography/mass spectrometry method (LC-MS/MS).

Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated for 2- pyridyl acetic acid by a non compartmental method using WinNonlin Pharmacokinetic PK Software, Version 5.0.1. C_{max} , maximum measured plasma concentration over the time span specified and T_{max} , the time of observed peak concentration, were determined for each subject and for each treatment. AUC_{0-t} , the area under the plasma concentration versus time curve, from time zero to the last measurable concentration, was calculated by the linear trapezoidal method. $AUC_{0-\infty}$, The area under the plasma concentration versus time curve, from time zero to Infinity. $AUC_{0-\infty}$, was calculated as the sum of AUC_{0-t} plus the ratio of the last measurable plasma concentration (C_t) to the apparent first-order terminal elimination rate constant (K_{el}). Apparent first-order terminal elimination rate constant(K_{el}) was calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-square regression analysis using the maximum number of points in the terminal log-linear phase (e.g. three or more non-zero plasma concentrations). $T_{1/2}$, the apparent first-order terminal elimination half-life was calculated as $0.693/K_{el}$.

Statistical Analysis

The log-transformed pharmacokinetic parameters (C_{max} , AUC_{0-t} and $AUC_{0-\infty}$) were analyzed using a mixed effects analysis of variance (ANOVA) model using Type III sum of squares, with the main effects of sequence, period and formulations as fixed effects and volunteers nested within sequence as random effect. Each analysis of variance included calculation of least-squares means, the difference between the adjusted formulation means and the standard error associated with the difference. The above analyses were done using the PROC GLM SAS[®] Version 9.1.3 procedure. Bioequivalence would be concluded if for 2- pyridylacetic acid the 90% confidence interval (CI) for the ratio of test and reference product of AUC_{0-t} an $AUC_{0-\infty}$ was between 80% and 125% for the log transformed data and 90% confidence interval for the ratio of test and reference product of C_{max} was between 75% and 133% for the log transformed data. T_{max} was analyzed as an individual difference (Test-Reference) building a 90% confidence interval, using a non parametric test.

Safety Assessments

Safety was assessed by monitoring vital signs, electrocardiographs, and clinical laboratory tests. Volunteers were monitored throughout the study period for adverse events. Volunteers were informed to bring to the notice of the nurse or the doctor any adverse event that may occur during their stay at the site of investigation. Volunteers were also specifically asked about any adverse events on admission, pre-dose and once every 4 hrs during post-dose hours of in-house stay and at discharge. Adverse events monitoring were done within ± 45 min of the scheduled time.

Results and Discussion**Analysis: 2-pyridyl acetic acid Assay**

The lower limit of quantitation (LLOQ) was established at 3.48 ng/mL, and the standard curve range was 3.48 to 1279.12 ng/mL. Best fit calibration lines of chromatographic response versus

Table 2: Summary of the LC-MS/MS Method Validation Parameters and results

Validation Parameters	2-pyridyl acetic acid
Within Batch accuracy (%)	92.6-107.5
Between Batch accuracy (%)	100.3-105.3
Within Batch precision (%CV)	2.3-9.1
Between Batch precision (%CV)	4.1-6.6
Extended Precision and Accuracy Batch	
Accuracy (%)	96.5-99.3
Precision (%CV)	3.0-4.7
% Recovery	
LQC	74.8
M1QC	76.6
MQC	76.9
HQC	80.7
% Stability (Mean)	
Bench-top (17.83 hours)	103.5-105.7
In-injector (46.32 hours)	102.8-106.5
Stock solution stability	
At room temperature (in hours)	98.9 (27.02)
Dilution integrity	
2 times dilution	
Accuracy (%Nominal)	93.7
Precision (% CV)	5.3
4 times dilution	
Accuracy (%Nominal)	96.2
Precision (% CV)	1.6
Matrix effect (% CV)	3.3-3.6
Ruggedness	
Within Batch accuracy (% Nominal)	93.9-102.5
Within Batch precision (%CV)	2.4-6.4

concentration determined by weighted least square regression analysis with weighing factor of $1/\text{concentration}^2$. The R^2 was greater than 0.9978. The overall between and within run variability

of the analytical quality control (QC) samples was less than 7% of their coefficient of variation (CV). The mean observed concentrations of the analytical QC samples in plasma deviated less than 8% from the nominal values. Summary of the LC-MS/MS Method Validation Parameters and results are presented in Table 2.

Study: All 12 enrolled volunteers completed the study. A summary of pharmacokinetic and statistical parameters is provided in Table 3 for 2-pyridyl acetic acid after oral administration of a betahistine dihydrochloride tablet (24 mg). Mean plasma concentration-time curves for the fasted study are illustrated in Figure 3 for 2-pyridyl acetic acid. The mean $T_{max} \pm$ standard deviation (SD) was 0.69 ± 0.30 hours. The mean plasma concentration-time profiles for 2-pyridyl acetic acid were well superimposable from both test and reference formulation.

Table 3: 2-Pyridyl Acetic acid Pharmacokinetic and Statistical Parameters under fasting condition

Treatment	Parameter	Mean \pm SD	Ratio of Means	
			Point Estimate	90% Confidence Interval
Betahistine dihydrochloride tablet (24 mg)	C_{max} (ng/mL)	786.625 ± 154.104	1.10	(1.04, 1.15)
	AUC_{0-t} (ng.h/mL)	3495 ± 799.495	1.04	(0.95, 1.13)
	$AUC_{0-\infty}$ (ng.h/mL)	3539 ± 807.536	1.03	(0.95, 1.13)

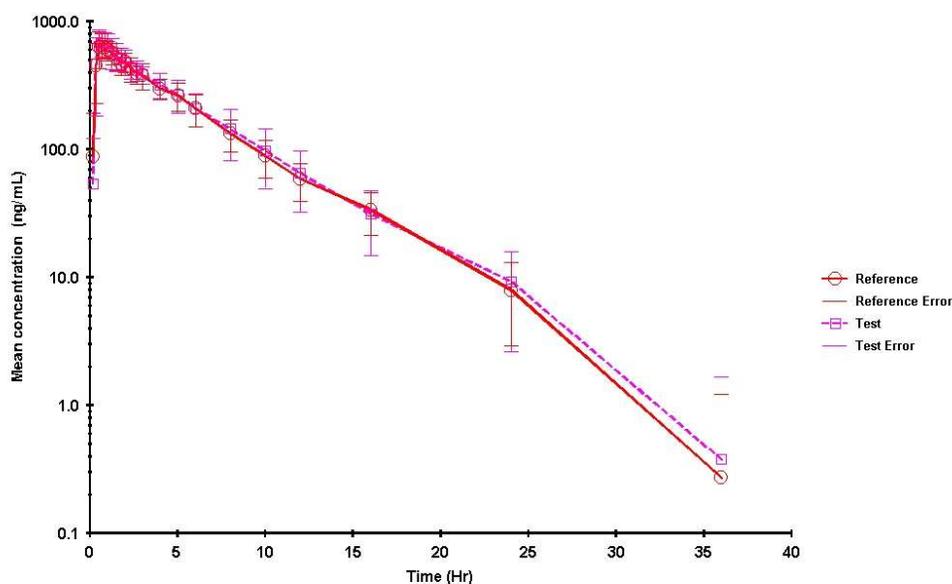


Figure 3: Mean plasma concentration-time curve of 2-pyridyl acetic acid under fasting condition

Safety

The 24 mg single dose of betahistine dihydrochloride tablet was well tolerated by the volunteers, and no adverse event was reported during the study.

As betahistine is rapidly metabolised in humans, its estimation in human plasma is not possible. The only major metabolite detected in urine and plasma is 2-pyridyl acetic acid. So, a sensitive LC-MS/MS method used for the quantification of 2-pyridyl acetic acid was established with the lower limit of quantitation (LLOQ) 3.48 ng/mL, and the range of the standard curve was kept large (3.48 to 1279.12 ng/mL) so as to cover the varying concentration of the 2-pyridyl acetic acid in the plasma in different volunteers.

In healthy volunteers, betahistine dihydrochloride (24 mg) tablet formulation showed bioequivalence with the respective strength reference betahistine dihydrochloride tablet when administered to healthy Indian volunteers under fasting condition. For 2-pyridyl acetic acid the C_{max} (ng/mL) observed was 786 and extent of 2-pyridyl acetic acid absorption AUC_{0-t} and $AUC_{0-\infty}$ (ng.h/mL) was 3495 and 3524. After oral administration of a single dose of 24 mg betahistine mesylate to 20 healthy Chinese volunteers, reported C_{max} for 2-pyridyl acetic acid was 339.4 ng ml^{-1} (range $77.3-776.4 \text{ ng ml}^{-1}$) and the AUC_{0-t} for 2-pyridyl acetic acid obtained was $1153.5 \text{ ng ml}^{-1} \text{ h}$ (range $278.5-3150.8 \text{ ng ml}^{-1} \text{ h}$) [9]. This difference in the results of pharmacokinetic parameters of Indian and Chinese volunteers may be attributed to ethnic factors. Significant ethnic differences can exist in the four pharmacokinetic phases and mainly in metabolism. The most common situations causing these variabilities are differences in drug metabolism. Both genetic and environmental factors can lead to ethnic differences in drug metabolism to a varying extent, depending on the ethnic groups and substrates [10-16].

Conclusion

2-pyridyl acetic acid, the major metabolite of betahistine was successfully determined by a validated liquid chromatography/mass spectrometry method (LC-MS/MS) in human plasma with standard curve range 3.48 to 1279.12 ng/mL. For 2-pyridyl acetic acid the C_{max} observed was (786 ng/mL) and extent of 2-pyridyl acetic acid absorption AUC_{0-t} and $AUC_{0-\infty}$ (ng.h/mL) was 3495 and 3524 in healthy Indian volunteers. Indian healthy volunteers showed quite high C_{max} (approximately twice) and AUC (approximately thrice) in comparison to Chinese volunteers and also the deviation observed in both pharmacokinetic parameters was quite low as compared to Chinese volunteers.

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