Journal of
Bioequivalence & Bioavailability
ISSN: 0975-0851

Zhang and Zhang, J Bioequiv Availab 2014, 6:6
DOI: 10.4172/jbb.1000201

Research Article Open Access

Bioequivalence Study of Two 30 Mg Tolvaptan Tablets Formulations in Healthy Chinese under Fed Condition

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Abstract

Objective: The purpose of this study was to compare the bioavailability between the two 30 mg tolvaptan tablets formulations and to evaluate the bioequivalence of Reference and Test formulations of tolvaptan tablets 30 mg in healthy adult male and female subjects under fed condition.

Method: 50 healthy Chinese male and female subjects were enrolled in a single-center, randomized, open-label, single-dose, two-treatment, two-sequence, two-period, crossover study. The plasma of tolvaptan were determined by a validated LC- MS/MS method. The bioequivalence of Test and Reference will be determined based on AUC_{0-t}, AUC_{0-inf} and C_{max} of tolvaptan in plasma.

Results: All the 50 subjects completed the study and the main pharmacokinetic parameters for test and reference preparations were as follows: C_{max} were 308.8 ± 108.8 and 339.9 ± 114.3 ng/mL, t_{max} were 2.670 (1.0–6.0) and 2.330 (1.0–6.0) h, AUC_{0-48} were 1832 ± 781.8 and 1702 ± 616.2 ng·h/ml, AUC_{0-inf} were 1848 ± 785.2 and 1720 ± 616.7 ng·h/ml, $t_{1/2}$ were 4.742 ± 1.129 and 4.608 ± 1.120 h. The 90% confidence intervals (CIs) of C_{max}, AUC_{0-48} and AUC_{0-inf} on the ratio of test to reference formulation were 82.83%-97.61%, 99.55%-112.91% and 99.44%-112.66%, respectively. The results of two one-side t test and variance analysis showed that there was no significant difference between the main parameters of the two preparations (P>0.05).

Conclusion: This study shows that two tolvaptan tablets 30 mg preparations are bioequivalent in Chinese adult healthy volunteers under fed condition.

Keywords: Tolvaptan; Pharmacokinetics; Bioequivalence; LC-MS/MS

Introduction

Tolvaptan (INN) is a selective, competitive vasopressin receptor 2 antagonist used to treat hyponatremia (low blood sodium levels) associated with congestive heart failure, cirrhosis, and the syndrome of inappropriate antidiuretic hormone (SIADH) [1]. Tolvaptan was developed by Otsuka Pharmaceutical Co. and approved by the U.S. Food and Drug Administration in 2009. And it is the first and acquire V2-receptor antagonist in Chinese market [2]. Tolvaptan vasopressin antagonist role by increasing the excretion of urine sleep, enhance free water clearance, reduced urine osmolality, and increased serum sodium values, while not changing the urine and serum potassium, sodium and potassium secretion content. Tolvaptan is mainly metabolized by CYP3A4 [3]. The most common adverse reactions were thirst, dry mouth, asthenia, constipation, pollakiuria or polyuria and hyperglycemia. The most commonly reported adverse event (AE) among the tolvaptan-treated subjects oral tolvaptan administered in 15- to 60-mg single doses to healthy Korean men was thirst, which is associated with the pharmacological action of tolvaptan as an aquaretic agents [4].

Currently, there are several studies to evaluate the pharmacokinetics and bioequivalence of tolvaptan [4-6], or to assess relative bioavailability of tolvaptan administered via nasogastric tube and tolvaptan tablets swallowed intact [7]. Also in China there are some studies [8,9] has been report the bioequivalence between new formulation tolvaptan and Samsca , but they were conduct it in small samples [9] or single sex volunteers [8]. And now a new generic formulation of tolvaptan 30 mg tablets (Test, [Zhejiang Huahai Pharmaceutical Co., LTD. for Prinston Pharmaceutical Inc., Lot No.637B13003]) has been developed. So we expend the mount of samples to design a crossover trail in 50 healthy Chinese male and female subjects under Fed condition to assess their bioequivalence. The reference formulation was Samsca 30 mg tablets (Reference [Otsuka America Pharmaceutical, Inc., Lot No.1k76TB1S]). The aim of this study is to make the new formulation tolvaptan to market and to instruct application of the tolvaptan reasonably.

Methods

Study design and procedures

The trail was a single-center, randomized, open-label, single- dose, two period, crossover study to assess the bioequivalence of test (T) and reference (R) formulation of tolvaptan tablets 30 mg in healthy Chinese subjects under Fed condition reviewed by the Institutional Review Board of The First Affiliated Hospital of Zhengzhou University. Fifty subjects were enrolled into the trail and all the subjects singed informed consent forms before conducting the trail. Randomly, subjects were divided into two groups and each subject will be randomized to one of two treatment sequences (T-R, R-T) according to a randomization schedule prepared prior to the start of the study. On session 1, day 1, After an overnight fast of 10 hours, in accordance with the United States (U.S.) Food and Drug Administration (FDA) requires [10], the following high fat (approximately 50% of total caloric content of the

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Received September 23, 2014; **Accepted** October 26, 2014; **Published** November 01, 2014

Citation: Zhang X, Zhang S (2014) Bioequivalence Study of Two 30 Mg Tolvaptan Tablets Formulations in Healthy Chinese under Fed Condition. J Bioequiv Availab 6: 181-185. doi:10.4172/jbb.1000201

meal), high calorie (approximately 800-1000 calories) breakfast (One boiled egg, One grilled chicken sandwich, Two fried wings, 240 ml whole milk) will be ingested starting 30 minutes prior to dosing. The breakfast should be completed at approximately 5 minutes prior to the study drug dose. All the subjects received a signal oral dose (T or R) of tolvaptan tablets 30 mg with approximately 240 ml room temperature water. Subjects were discharged from the study center after 24 hours post-dose and return on an out-patient basis for collection of the 36 and 48 hour post-dose samples. Following a washout period of 7 days, subjects were crossed over to the alternate treatment and the same procedures were performed.

Blood samples for PK analysis were taken at pre dose and 0.5, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36 and 48 hours post-dose. Blood samples were collected into chilled blood collection tubes containing K2EDTA.

Subjects

This trail was conducted in healthy Chinese male and female subjects (30 male, 20 female) between 18 and 45 years of age and the body mass index (BMI) between18 and 30 kg/m^2 and a negative pregnancy test result for female. One subjects was excluded if he/she was a current smoker or user of any tobacco products, had no ability of the patient to sense or appropriately respond to thirst, had a history of hypersensitivity to tolvaptan or any other component of the tolvaptan tablets, had used of any recreational drugs within the past year or a previous history of drug abuse, had used any prescription drug therapy (especially Concomitant use of strong CYP3A inhibitors such as clarithromycin, ketoconazole, itraconazole, ritonavir, indinavir, nelfinavir, saquinavir, nefazodone, and telithromycin) within 14 days prior to receiving study drug, donated blood (>450 mL) within 30 days or plasma within 7 days prior to receiving study drug, had used any grapefruit or grapefruit-containing juices within 72 hours prior to receiving study drug for each period, had used any OTC drugs for therapeutic purposes or dietary or herbal supplements or megavitamin supplements within 48 hours prior to receiving study drug for each period, consumed of any caffeine or beverages or alcohol within 24 hours prior to receiving study drug for each period and had any clinically significant abnormality based on medical history, physical examination and laboratory analysis.

Sample collection and processing

Tolvaptan blood samples were obtain at Time 0 (within 60 minutes pre-dose), 0.5, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36 and 48 hours post-dose. Blood samples (about 5 ml) were collected into blood collection tubes containing K2EDTA, immediately chilled on crushed ice and centrifuged for 5 minutes in a refrigerated centrifuge (4°C) at 3500 rpm within 30 minutes after collection. Plasma was transferred into two polypropylene tubes and plasma samples were stored at -70°C freezer.

Tolerability

The safety and tolerability of tolvaptan were based on the incidence of treatment-emergent adverse events, study discontinuation information, clinical laboratory test results, physical examination findings and vital signs. The vital signs (blood pressure, pulse, and temperature) were measurement at time 0, 6, 24, and 48 hrs post dose in each session. All the subjects were received a complete physical examination including vital signs evaluation (sitting blood pressure, pulse rate, and temperature), resting 12-lead electrocardiogram (ECG), clinical laboratory tests [chemistry, hematology, urinalysis, Hepatitis B & C diagnostic profile and pregnancy (females only)] within 28 days prior to receiving study drug. And an abbreviated physical examination and sitting vital signs (blood pressure, pulse and temperature) were measured; blood and urine were obtained for clinical safety laboratory tests (Chemistry, hematology and urinalysis) at the end of the study.

All AEs that occurred during the study including the washout intervals were recorded whether or not they were considered related to the investigational drug.

Determination of plasma tolvaptan

Tolvaptan concentrations in plasma were analyzed using a validated liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) method. Plasma samples were extracted using liquid-liquid extraction. Plasma (200 µl) was mixed with 20 µl of diluents of methanol and water (50:50, v/v) for double blank, 20 µl of internal standard (IS) spiking solution (2-demethyl tolvaptan, 20 ng/ml), 200 µl of 0.1 M NaOH solution and 2 ml of methyl-butyl as the extracting solvent. Then the samples were vortexed for 10 min followed be centrifuged at 3500 rpm for 5 min. Transferred the upper organic layers to clean test tubes and evaporated to dryness at 40°C under nitrogen flow. Taken 200 µl solution containing methanol and water (50:50,v/v) to reconstitute the samples and taken a 50 µl samples injected to the LC-MS/MS system-Sciex API 4000 coupled to Shimadzu LC pump and auto-sampler with Colum -Synergi, Polar-Rp, 50 ×3.0 mm, 4 um for determination of tolvaptan concentration. The product ion transition of m/z 449.2→252.1 for tolvaptan, and m/z 435.2→238.1 for internal standard. The LLOQ of tolvaptan was set at 1 ng/ml and the range of detectable concentration is 1-500 ng/ml. Data analysis was performed with Analyst 1.4.2 software package.

Pharmacokinetic and statistical methods

Tolvaptan Plasma concentrations for each subject were summarized by treatment at each time point using descriptive statistics (mean, standard deviation, coefficient of variation, median, minimum and maximum values) with the non-compartmental methods. The Non-compartmental methods was also used to determine the pharmacokinetic parameters (AUC_{0-t}, AUC_{0-inf}, C_{max}, t_{max}, $t_{½}$ and K_{el}) of tolvaptan and this procedures were performed by WinNonlin Version 6.2.1 (Pharsight Corporation, St. Louis, MO, USA). In addition to the descriptive statistics listed above, geometric means were reported for the pivotal pharmacokinetic endpoints (AUC_{0-t}, AUC_{0-inf}, and C_{max}).

Analysis of variance (ANOVA) were used to analyze the bioequivalence of Test and Reference study drug which were determined based on AUC_{0-t}, AUC_{0-inf} and C_{max} of tolvaptan in plasma. To demonstrate bioequivalence, the 90% CIs on the ratio of test to reference formulations were have to lie within a range of 80.00-125.00%. Statistical calculations was done by SAS software (Version 9.3, SAS Institute, Cary, North Carolina, USA). Log-transformed pharmacokinetic parameters AUC_{0-t}, AUC_{0-inf}, and C_{max} were analyzed by analysis of variance (ANOVA) model including terms for sequence, study treatment, and period as fixed effects, and subject nested within sequence as a random effect. Sequence was tested using subject nested within sequence as the error term. A CI on the ratio of untransformed pharmacokinetic parameters was derived through reverse transformation of the 90% CI for the difference in the log scale to the 90% CI for the ratio in the original scale.

Results

Demographic data

In this study, there were 50 healthy Chinese subjects enrolled in and all the subjects completed the study were included in the pharmacokinetic analysis. The mean (SD) age of subjects is 23.1 (1.71) years, and the mean (SD) BMI was 22.19 (2.466) m/kg^2. All the subjects are Asian. The demographic characteristics of the study were presented in Table 1.

Safety assessments

Single oral doses of tolvaptan 30 mg were generally safe and well-tolerated in this healthy Chinese adult male and female population.

Treatment-emergent adverse events reported during the study were mild in intensity and consistent with those reported previously. Twenty-nine (58.0%) subjects reported at least one treatment-emergent adverse event following administration of test study drug and thirty (60.0%) subjects reported at least one treatment-emergent adverse event following administration of reference study drug. The most common adverse events reported were dry mouth reported by twenty-eight (56.0%) subjects after receiving test and twenty-five (50.0%) subjects after receiving reference study drug. All other AEs were reported by two or less subjects in each treatment group. There were no serious adverse events reported during the study. All AEs were resolved prior to discharge from the study.

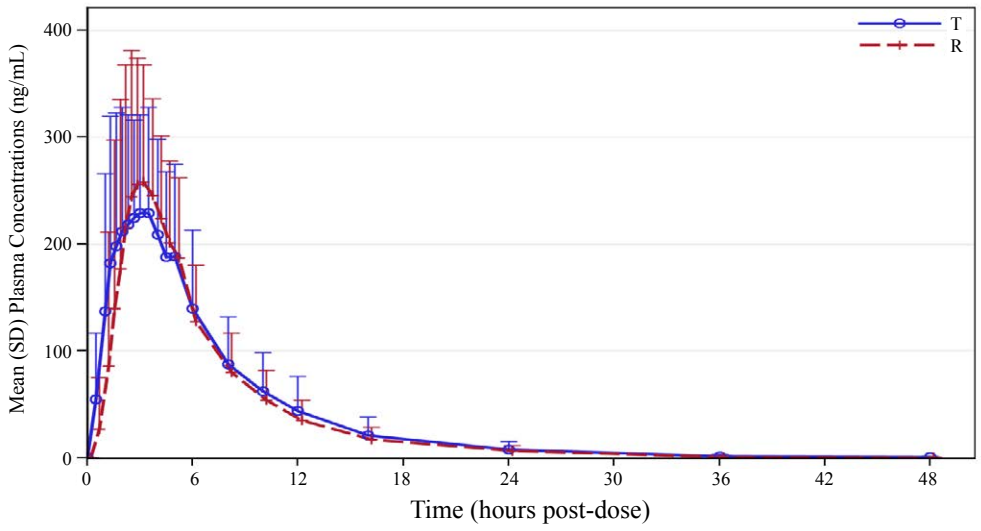
There were no overall clinically meaningful or significant changes noted for clinical safety parameters or vital sign assessments.

Pharmacokinetic analysis

Linear and semi-log plots of mean tolvaptan concentration-time profiles after administration of a single 30 mg oral dose of test or reference formulations to 50 healthy Chinese subjects under fed condition were presented in Figure 1. Pharmacokinetic analysis of the primary parameters (AUC_{0-t}, AUC_{0-inf}, C_{max}, t_{max}, $t_{½}$ and K_{el}) was evaluated with a non-compartmental model using Win Nonlin Version 6.2.1 (Pharsight Corporation, Mountain View, California, USA).The

Parameter		Treatment Groups	
		Test Product N=50	Reference Product N=50
Age (Years)	Mean(SD)	23.1 (1.71)	23.1 (1.71)
	Range	20- 26	20- 26
Age Groups n (%)	<18	0	0
	18 - 40	50 (100.0)	50 (100.0)
	41 - 64	0	0
	65 - 75	0	0
	>75	0	0
Sex n (%)	M	30 (60.0)	30 (60.0)
	F	20 (40.0)	20 (40.0)
Race n (%)	Asian or Pacific Islander	50 (100.0)	50 (100.0)
BMI (kg/m^2)	Mean (SD)	22.19 (2.466)	22.19 (2.466)
	Range	18.0- 29.7	18.0- 29.7
Other Factors		N/A	N/A

Table 1: Subject Demographics and Baseline Characteristics (N=50).



Note: T=Test formulation - Tolvaptan Tablets 30 mg.
R=Reference formulation - Samsca® (Tolvaptan) Tablets 30 mg.

Figure 1: Mean (SD) Tolvaptan Plasma Concentration -Time profile (Linear Scale) PK Population

Parameter (Unit)	Statistics	Test (N = 50)	Reference (N = 50)
Cmax (ng/mL)	Mean (SD)	308.8 (108.8)	339.9 (114.3)
AUC0-t (ng·hr/mL)	Mean (SD)	1832 (781.8)	1702 (616.2)
AUC0-inf (ng·hr/mL)	Mean (SD)	1848 (785.2)	1720 (616.7)
T1/2 (hr)	Mean (SD)	4.742 (1.129)	4.608 (1.120)
Tmax (hr)	Mean (SD)	2.670 (1.0–6.0)	2.330 (1.0–6.0)
Kel (1/hr)	Mean (SD)	0.1545 (0.0367)	0.1599 (0.0411)

Note: T=Test formulation - Tolvaptan Tablets 30 mg.
R=Reference formulation - Samsca® (Tolvaptan) Tablets 30 mg.

Table 2: Summary statistics of pharmacokinetic parameters for the PK population (N=50).

PK Parameter (unit)	Geometric Mean		Ratio of Geometric Means	90% CI	Inter-Subject	Intra-Subject
	T	R	T/R	T/R	CV%	CV%
Cmax (ng/mL)	290.0	322.5	89.91	(82.83 - 97.61)	25.07	24.85
AUC0-t (ng·hr/mL)	1697	1601	106.02	(99.55 - 112.91)	32.35	18.94
AUC0-inf (ng·hr/mL)	1714	1619	105.84	(99.44 - 112.66)	32.19	18.77

Note: T=Test formulation - Tolvaptan Tablets 30 mg.
R= Reference formulation - Samsca® (Tolvaptan) Tablets 30 mg.

Table 3: Tolvaptan Plasma Pharmacokinetic Parameters Summarized by Treatment (N=50).

data of this study was summarized in Table 2, respectively. After log-transformation of the data, the mean (SD) C_{max} of plasma tolvaptan following administration of test and reference study drug were 308.8 (108.8) and 339.9 (114.3) ng/mL, respectively; The mean (SD) AUC_{0-t} of plasma tolvaptan following administration of test and reference study drug were 1832 (781.8) and 1702 (616.2) ng·hr/mL, respectively; The mean (SD) AUC_{0-inf} of plasma tolvaptan following administration of test and reference study drug were 1848 (785.2) and 1720 (616.7) ng·hr/mL, respectively; The mean (SD) $t_{1/2}$ of plasma tolvaptan following administration of test and reference study drug were 4.742 (1.129) and 4.608 (1.120) hr, respectively.

Bioequivalence analysis

The bioequivalence of Test and Reference formulations was determined based on AUC_{0-t}, AUC_{0-inf} and C_{max} of tolvaptan in plasma. The BE analysis of the three PK parameters (AUC_{0-t}, AUC_{0-inf} and C_{max}) is shown in Table 3. The 90% confidence interval for tolvaptan for T : R for AUC_{0-t} was ((99.55 - 112.91); The 90% confidence interval for T : R of AUC_{0-inf} was (99.44 - 112.66); The 90% confidence interval for T:R of C_{max} was (82.83 - 97.61). ANOVA analysis among these parameters showed that there is no significant difference between the two formulations (P>0.05). The 90% confidence intervals for tolvaptan for T:R for AUC_{0-t}, AUC_{0-inf} and C_{max} were contained within the range of 80-125%.

Geometric means are least square means derived from mixed models which including terms for sequence, study treatment, and period as fixed effects and subject nested within sequence as a random effect.

Discussion

According to the previous literature [4,5,11], we used the LC-MS/MS method to determine the tolvaptan concentrations in plasma and the lower limit of quantitation (LLOQ) was set at 1 ng/ml. It was an accurate, rapid and sensitive method for determining the tolvaptan concentrations in plasma and was suitable for pharmacokinetic study.

The pharmacokinetic parameters C_{max} and AUC of this study is higher than Li and Xia's study. Although their study was conducted in Chinese volunteers, but it was conducted under fasting state. This is consistent with Shoaf's report [6]. So the food have an effect on the pharmacokinetics of tolvaptan.

Compared with the study conducted in other ethnicity, the pharmacokinetic parameters of tolvaptan in Chinese have difference from other race. Another report of Blair [12] analysis on EVEREST was also show that tolvaptan have different place pharmacokinetic parameters in different race. We all know that tolvaptan is mainly metabolized by CYP3A4 and have study proved that tolvaptan is a sensitive CYP3A4 substrate with no inhibitory activity [3]. The genotype of CYP3A4 may be have an effect on metabolize of tolvaptan.

For this study, we only understand the pharmacokinetic and evaluated the bioequivalence of two formulations tolvaptan under fed condition. In order to better understand the metabolism of tolvaptan in Chinese, we need to conduct a further study to investigate the effect of the food and gene polymorphism of CYP3A4 on the pharmacokinetic pharmacy of tolvaptan.

Conclusion

Base on the results, the tolvaptan manufactured by Huahai Pharmacy (Test product) and Samasca manufactured by Otsuka Pharmaceutical Co. (Reference product) can be considered bioequivalent.

Acknowledgements

The test product is manufactured by Huahai Pharmacy in China. The authors wish to acknowledge the support of Huahai Pharmacy.

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