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

Xiaogao Zhang and Shengjun Zhang*

The first Affiliated Hospital of Zhengzhou University, Henan Province, Zhengzhou, Henan, China

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 AUC0-48, AUC0-inf, and Cmax 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: Cmax were 308.8 ± 108.8 and 339.9 ± 114.3 ng/mL, tmax were 2.670 (1.0–6.0) and 2.330 (1.0–6.0) h, AUC0-48 were 1832 ± 781.8 and 1702 ± 616.2 ng∙h/ml, AUC0-inf were 1848 ± 785.2 and 1720 ± 616.7 ng∙h/mL, t1/2 were 4.742 ± 1.129 and 4.608 ± 1.120 h. The 90% confidence intervals (CIs) of Cmax, AUC0-48 and AUC0-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 V2 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, poliuria 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 Samscsa , 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 meal) and high carbohydrate meal (approximately 30% of total caloric content of the meal) was served 1 hour before each tolvaptan dose was administered.

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

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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, 240 ng/ml), 200 μl of 0.1 M NaOH solution and 2 ml of methyl-butyll 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 Cologrum -Synerg, Polar-Rp, 50 μm, 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 2-demethyl 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 (AUC0-τ, AUC0-∞ and Cmax) of tolvaptan and these procedures were performed by Analyst I.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 (AUC0-τ, AUC0-∞, Cmax, tmax, t1/2 and K0) of tolvaptan and these 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 (AUC0-τ, AUC0-∞ and Cmax).

Analysis of variance (ANOVA) were used to analyze the bioequivalence of Test and Reference study drug which were determined based on AUC0-τ, AUC0-∞ and Cmax 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 AUC0-τ, AUC0-∞ and Cmax 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 a random effect model. The analysis of variance was performed on the log transformed data. The ln-transformed concentrations were used to calculate the geometric mean ratios of the two formulations as well as the 90% CIs on the log scale. The CIs on the ratio were calculated on the log scale. The CIs on the ratio were calculated on the inverse scale of the log transformation.
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/kg². 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 (AUCₜₐ, AUCₜₐₜ, Cₘₐₓ, tₘₐₓ, tₜₐₜ, and Kₑ) was evaluated with a non-compartmental model using Win Nonlin Version 6.2.1 (Pharsight Corporation, Mountain View, California, USA). The

<table>
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<tr>
<th>Parameter</th>
<th>Test Product N=50</th>
<th>Reference Product N=50</th>
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<tr>
<td>Age (Years)</td>
<td>Mean(SD)</td>
<td>23.1 (1.71)</td>
</tr>
<tr>
<td>Range</td>
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<td>20-26</td>
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<tr>
<td>Age Groups n (%)</td>
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<td>&lt;18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-40</td>
<td>50 (100.0)</td>
<td>50 (100.0)</td>
</tr>
<tr>
<td>41-64</td>
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<td>0</td>
</tr>
<tr>
<td>65-75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;75</td>
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<tr>
<td>Sex n (%)</td>
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<td></td>
</tr>
<tr>
<td>M</td>
<td>30 (60.0)</td>
<td>30 (60.0)</td>
</tr>
<tr>
<td>F</td>
<td>20 (40.0)</td>
<td>20 (40.0)</td>
</tr>
<tr>
<td>Race n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
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<td>50 (100.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean (SD)</td>
<td>22.19 (2.466)</td>
</tr>
<tr>
<td>Range</td>
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<tr>
<td>Other Factors</td>
<td>N/A</td>
<td>N/A</td>
</tr>
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</table>

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
Table 3: Summary statistics of pharmacokinetic parameters for the PK population (N=50).

<table>
<thead>
<tr>
<th>PK Parameter (unit)</th>
<th>Statistics</th>
<th>Test (N = 50)</th>
<th>Reference (N = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>Mean (SD)</td>
<td>308.8 (108.8)</td>
<td>339.9 (114.3)</td>
</tr>
<tr>
<td>AUC0-t (ng hr/mL)</td>
<td>Mean (SD)</td>
<td>1832 (781.8)</td>
<td>1702 (616.2)</td>
</tr>
<tr>
<td>AUC0-inf (ng hr/mL)</td>
<td>Mean (SD)</td>
<td>1848 (785.2)</td>
<td>1720 (616.7)</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>Mean (SD)</td>
<td>4.742 (1.129)</td>
<td>4.608 (1.120)</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>Mean (SD)</td>
<td>2.670 (1.0-6.0)</td>
<td>2.330 (1.0-6.0)</td>
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<tr>
<td>Kel (1/hr)</td>
<td>Mean (SD)</td>
<td>0.1545 (0.0367)</td>
<td>0.1599 (0.0411)</td>
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</table>

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

The pharmacokinetic parameters Cmax 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 pharmacology of tolvaptan.

Conclusions

The test product is 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.

References

1. Samsca (tolvaptan tablets for oral use).