Mini Review

Bioequivalence Study of Trazodone: Single Oral Dose under Fasting Condition in Healthy Human Subjects

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Abstract

Objective: To assess the bioequivalence of single dose trazodone hydrochloride USP 100 mg tablets administered as an oral dose under fasting condition.

Methods: This study was an open-label, balanced, randomized, two-sequence, two-treatment, two-period, single oral dose, crossover bioequivalence study in healthy, adult, human subjects under fasting conditions. After an overnight fast of at least 10 hours, a single oral dose (100 mg) of either the test or the reference product was administered to the subjects with 240mL of drinking water at ambient temperature in sitting posture. The primary pharmacokinetic parameters, maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve (AUC) from time zero to last measurable concentration (AUC_{0-t}) and extrapolated to infinity (AUC_{0-}) were compared by an analysis of variance using log-transformed data. Bioequivalence was concluded if the 90% confidence intervals (CIs) of the adjusted geometric mean (gMean) ratios for C_{max} and AUC were within the predetermined range of 80%-125%, in accordance with regulatory requirements.

Results: For the test formulation, the trazodone gMean C_{max} was 2172.2 ng/mL (vs. 2031.2 ng/mL for reference), AUC_{0-t} was 16631.6 ng·h/mL (vs. 16342.9 ng·h/mL) and AUC_{0-} was 17460.6 ng·h/mL (vs. 17270.1 ng·h/mL). The 90% CIs for the ratio (test/reference) were 101.2-113.0% for C_{max}, 98.5-105.1% for AUC_{0-t} and 97.7-104.5% for $AUC_{0-\infty}$. There were no deaths or serious adverse events during the conduct of the study.

Conclusion: Test product when compared with the Reference product meets the bioequivalence criteria with respect to the rate and extent of absorption of Trazodone under fasting condition.

INTRODUCTION

Trazodone Hydrochloride is an antidepressant chemically unrelated to tri-cyclic, tetra-cyclic, or other known antidepressant agents. The mechanism of trazodone hydrochloride's antidepressant action in man is not fully understood [1]. Trazodone is not a monoamine oxidase inhibitor and, unlike amphetamine-type drugs, does not stimulate the central nervous system.

Trazodone is rapidly and almost completely absorbed from the GI tract following oral administration. The rate and extent of absorption are affected by the presence of food [2]. When trazodone is taken shortly after the ingestion of food, there may be a slight increase (up to 20%) in the amount of drug absorbed, a decrease in peak plasma concentration of the drug, and a lengthening of the time to reach the peak plasma concentration. Peak plasma concentrations of trazodone occur approximately 1 hour after oral administration when drug is taken on an empty stomach or 2 hours after oral administration when taken with food.

Distribution of trazodone into human body tissues and fluids has not been determined. Following oral administration of trazodone in animals, the drug and its metabolites are distributed mainly into the liver, kidneys, small intestine, lungs, adrenal glands, and pancreas, with lower concentrations being distributed into adipose tissue, heart, and skeletal muscle [3].

Plasma concentrations of trazodone decline in a biphasic manner. The half-life of trazodone in the initial phase is about 3-6 hours and the half-life in the terminal phase is about 5-9 hours.

The clearance of trazodone from the body shows wide inter-individual variations. It is said that the drug may accumulate in plasma in some individuals. Trazodone is extensively metabolized in the liver via hydroxylation, oxidation, N-oxidation, and splitting of the pyridine ring. Approximately 70-75 % of an oral dose of trazodone is excreted in urine within 72 hours of administration, principally as metabolites [4].

There is a literature available on the dose proportionality study of Trazodone under fasting condition over a range of 75 mg to 300 mg dose.

The aim of the study was to compare the bioavailability and assess the pharmacokinetic profile of the test formulation Trazodone Hydrochloride tablets, USP 100 mg, Manufactured by Intas Pharmaceutical Limited, India in comparison with the reference formulation.

SUBJECTS AND METHODS

This was a single-center (Lambda Therapeutic Research Ltd, Ahmedabad, India), randomized, single-dose, open-label, 2-treatment, 2-period, 2-sequence, crossover trial conducted in healthy volunteers between 01 December 2013 and 24 December 2013 [5]. The Independent Ethics Committee reviewed the study Protocol, Informed consent Form, Curriculum Vitae of Investigators and Product information. The study was conducted in accordance with local regulations and the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all subjects before performing any trial-related activities. For this bioequivalence study, a crossover design was planned as per the USFDA requirements. A crossover study was conducted on 56 subjects under fasting conditions.

Subjects

All subjects willing to participate in the study were screened prior to their enrolment, in order to assess their eligibility by satisfying all of the inclusion and exclusion criteria [6]. During screening, the medical history of the subjects was elicited and they underwent a general clinical examination, measurement of blood pressure, heart rate, oral body temperature, respiratory rate, 12-lead ECG, clinical laboratory evaluations, chest X-ray and immunological tests for HIV, HBsAg, and HCV. This procedure was conducted within 21 days prior to the first dose of IMP administration. Healthy human subjects aged 18-45 years with a body mass index (BMI) of 18.5-27.5 calculated as kg/ height in m^2 were considered for the study [7]. Additional exclusion criteria included any history or presence of asthma or nasal polyp or other non-steroidal anti-inflammatory drugs (NSAID) -induced urticaria, smokers, who smoked >10 cigarettes per day or donation of blood (350 ml), or receipt of an investigational medicinal product or participation in a drug research study within 90 days prior to receiving the first dose of study medicine.

Study design and treatments

In this study the screening phase was carried out within 21 days prior to the scheduled dosing day of Period-I. The duration of the clinical part of the study was about 14 days (11 hours prior to the dose administration in Period-I until the last pharmacokinetic sample in Period-II). Based on available

and data and statistical analysis and elimination half-life of Trazodone, a wash out period of 10 days was kept in between the dosing days of the study periods. In this study with a crossover design, each subject received both the treatments (test drug and reference drug) during the study [8]. Hence, every subject acted as his own control and no separate group of subjects was required to act as the control group. The sequence of administration was determined by the randomization schedule.

After an overnight fast of at least 10 hours, a single oral dose (100 mg) of either the test or the reference product was administered to the subjects. The IMP was administered in sitting posture with 240mL of drinking water at ambient temperature.

This activity was followed by a mouth check to assess the compliance to dosing. The subjects were in supine position or comfortable recumbent position within 15-30 minutes of dosing and remained in the same position until 06 hours post-dose. During each period subjects remained in the study center until blood samples had been taken 48h after dosing.

Pharmacokinetic evaluation

Blood samples were collected through an indwelling intravenous cannula (Venflon) placed in a forearm vein of the subjects. A total of 20 blood samples, each of 05mL were collected from each subject in each period at pre-dose (0.000 hours) and at 0.250, 0.500, 0.750, 1.000, 1.250, 1.500, 1.750, 2.000, 2.500, 3.000, 4.000, 6.000, 8.000, 10.000, 12.000, 16.000, 24.000, 36.000 and 48.000 hours following drug administration. Immediately after collection of blood, the collection tube (vacutainer) was inverted gently several times to ensure the mixing of tube contents (i.e. anticoagulant K2EDTA). The blood samples were centrifuged at 3000rcf for 5 minutes at 2ºC to separate plasma. The separated plasma was stored in a freezer below 55ºC.

The plasma samples of subjects were analyzed using a validated LC-MS/MS method for trazodone at the Bioanalytical facility of Lambda Therapeutic Research Ltd., Ahmedabad, India. Calibration curves using an 8-point calibration curve standards for Trazodone, with concentration ranging from 5.203μg/mL to 3025.166μg/mL, were used to determine the concentration of Trazodone in the samples of various subjects.

Plasma concentrations of trazodone were analyzed using liquid chromatography mass spectrometry procedures, which were fully validated and developed at Lambda Therapeutic Research Ltd, Ahmedabad, India. Briefly, trazodone plasma concentrations were measured following protein precipitation extraction (internal standard trazodone-d6) by liquid chromatography mass spectrometry (LC-MS/MS) using a Eclipse XBD C8 150 X 4.6 mm, 5μm column, (mobile phase 70% methanol and 30% 2mM Ammonium Format buffer pH 3.0). The limit of quantification was 5.203μg/mL. Assay performance was assessed by back-calculation of calibration standards, tabulation of the standard curve fit function parameters and measurement of quality control samples. Validation data documented adequate accuracy, precision and specificity of the liquid chromatography mass spectrometry assays employed for the study.

Safety evaluation

All the subjects underwent a pre-enrolment laboratory

parameters evaluation including tests for haematology, biochemistry, and immunology and urine analysis. The post-study safety assessments included haematology and biochemistry (except random glucose, sodium, potassium and chloride). Sitting blood pressure and radial pulse were measured during each clinical examination, prior to administration of study drug and at approximately 02 and 11 hours after administration of investigational medicinal product in each period. Subjects were questioned for well-being at the time of clinical examinations and at the time of recording of vital signs in each period. Adverse events were collected during each study period with severity (mild, moderate or severe) and investigator assessment of the relationship to the study medication (definite, possible, doubtful or none).

Pharmacokinetic analyses

The pharmacokinetic parameters were derived individually for each analyzed subject from the concentration vs. time profiles of Trazodone in plasma. The primary variables were the area under the plasma concentration-time curve from time 0 to the last quantifiable data point (AUC_{0-t}), from time zero extrapolated to infinity ($AUC_{0-\infty}$) and C_{max}. Time of maximum exposure (t_{max}) was a secondary variable. Non-compartmental analysis of plasma concentration-time data was performed using WinNonlin® Professional software (Version 5.3, Pharsight Corporation, and USA). Actual time points of the sample collection were used for the calculation of pharmacokinetic parameters. All values below the limit of quantification were considered as zero for pharmacokinetic analysis.

Statistical analysis

Descript statistics were computed and reported for primary and secondary pharmacokinetic parameters for Trazodone. Analysis of variance was performed using PROC MIXED (SAS®, version 9.3, SAS Institute Inc., and USA) for ln-transformed pharmacokinetic parameters $AUC_{0-\infty}$, AUC_{0-t} and C_{max} for trazodone. The ANOVA model included sequence, period and formulation as fixed effects and subject (sequence) as a random effect. Using two one-sided tests for bioequivalence, 90% confidence intervals for the ratio of geometric least squares means between drug formulations were calculated for ln-transformed pharmacokinetic parameters AUC_{0}, AUC_{0-t} and C_{max} for trazodone bioequivalence was concluded if the 90% CIs were within the range 80 to 125%. For all other parameters, descriptive statistics were presented. The power of the study to detect 20% difference between the test and reference formulations was computed and reported for Trazodone.

RESULTS

A total of 58 subjects were enrolled and checked in for the study. As per the protocol 56 subjects were randomized (mean {standard deviation} 29.6 ± 6.47 years, BMI 22.123 ± 2.7397 kg/m^2) in period-I of the study. One subject was withdrawn from the study on medical grounds in Period-I. One subject was withdrawn from the study on the grounds of emesis, two subjects were withdrawn from the study on their own accord and one subject was withdrawn from the study due to protocol deviation in Period-II. In all, 51 subjects completed the clinical phase of the study successfully.

The trazodone plasma concentration-time profiles are shown in Figure 1 and pharmacokinetic parameters are summarized in Tables 1 and 2.

Trazodone was rapidly absorbed, with a median t_{max} of 0.5 hour (Table 1). The plasma concentration-time curves of trazodone showed a parallel decline in distribution and elimination phases (Figure 1). The gMean values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for trazodone were comparable for test and reference formulations. Bioequivalence was demonstrated as the 90% CIs of the ratios of point estimates (test/reference) for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were within the range of 80 to 125%.
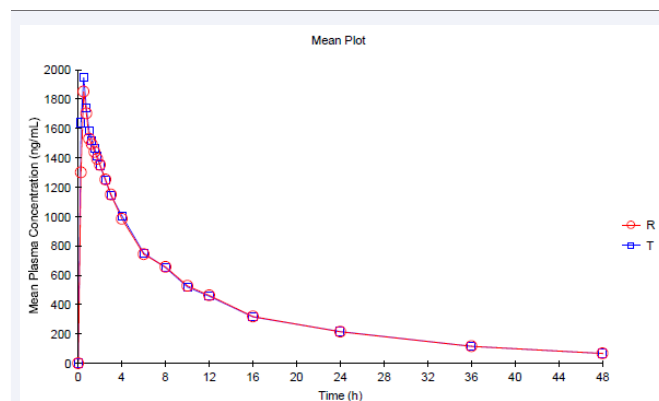
Safety results

In general, the clinical portion of the study was completed with two (02) significant adverse events. The investigational products were well tolerated by healthy subjects, as a single dose administration. Three (03) adverse events (AEs) were reported by two (02) subjects during the conduct of the study. All the AEs were mild in nature. The subjects were treated accordingly and were followed up until resolution of their AEs. The causality assessment was judged as possible for one (01) AE and as unlikely for two (02) AEs.

There were no deaths or serious adverse event during the conduct of the study. There were no clinically significant findings

Table 1: Descriptive Statistics of Formulation Means for Trazodone.

Parameters (Units)	Mean ± SD (un-transformed data)	
	Test Product-T	**Reference Product-R**
T_{max} (h)*	0.500 (0.250 – 4.000)	0.500 (0.250 - 4.000)
C_{max} (ng /mL)	2233.688 ± 535.1674	2089.418 ± 499.3017
AUC_{0-t} (ng.h /mL)	17394.571 ± 5188.4775	17245.887 ± 5765.5373
$AUC_{0-\infty}$ (ng.h /mL)	18282.497 ± 6004.6095	18446.459 ± 6895.7530
$t_{1/2}$ (h)	12.239 ± 3.1850	12.531 ± 3.5462

*T_{max} is represented as median (min-max) value.

Figure 1 Mean Plasma concentration vs. Time Curve for Trazodone (Test Product – T and Reference Product – R).

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Table 2: Relative Bioavailability Results for Trazodone.

Parameters	Geometric Least Squares Means		Ratio (T/R)%	90% Confidence Interval	Power (%)
	Test Product-T	Reference Product-R			
lnC_{max}	2172.221	2031.229	106.9	101.21 – 113.00	100.00
$lnAUC_{0-t}$	16631.683	16342.959	101.8	98.53-105.11	100.00
$lnAUC_{0-\infty}$	17460.679	17270.105	101.1	97.76 – 104.56	100.00

in the vital signs assessment or the laboratory tests in any of the subjects.

DISCUSSION

The ratio of geometric least squares means of Test product and Reference Product for ln-transformed pharmacokinetic parameter, C_{max} was 106.9%. The 90% confidence interval for the ratio of geometric least squares means was found to be 101.21 – 113.00%. The ratio of geometric least squares means of Test product and Reference Product for ln-transformed pharmacokinetic parameter, AUC_{0-t} was 101.8%. The 90% confidence interval for the ratio of geometric least squares means was found to be 98.53 – 105.11%. The ratio of geometric least squares means of Test product and Reference Product for ln-transformed pharmacokinetic parameter, $AUC_{0-\infty}$ was 101.1%. The 90% confidence interval for the ratio of geometric least squares means was found to be 97.76 – 104.56%. These intervals were within the acceptance limits of 80.00 – 125.00%, required for the conclusion of bioequivalence as per criteria set in the protocol.

Upon conclusion of the clinical portion of the study, the results from all subjects, who completed post-study procedures including laboratory tests and vital signs measurements, confirmed the absence of significant changes in the subject's state of health.

In summary, Test Product when compared with the Reference product meets the bioequivalence criteria with respect to the rate and extent of absorption of Trazodone under fasting conditions as per criteria set in the protocol.

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Ronak Patel was responsible for the design of the study. The author was responsible for analytical analysis and the preparation of the manuscript. Editorial assistance was provided by Ms. Devangi Soni.

Conflicts of interest

This study was sponsored by Intas Pharmaceuticals Limited, Ahmedabad, India. Prashant Kale is an employee of Lambda Therapeutic Research Ltd, which was contracted by Intas Pharmaceuticals Limited as CRO for this study, and has received financial support for its services.

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