

A SINGLE-DOSE RANDOMIZED TWO SEQUENCES, OPEN-LABEL CROSSOVER STUDY OF TWO DIFFERENT FORMULATIONS OF DUTASTERIDE CAPSULE IN HEALTHY INDIAN ADULT, HUMAN MALE VOLUNTEERS UNDER FASTING CONDITIONS.

GANESAN M*, ASHOK.P,RAGUNATH M P, KATHIRAVAN S,SUDHA A , ELANGO VAN N, PUNITHA T, VENKATRAO K.,

Micro Therapeutic Research Labs Private Limited , No.6, Kamarajar Salai, Selaiyur, East Tambaram, Chennai - 600 059, Tamil Nadu, India.
Email : mpraghunath@microtheraps.com

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ABSTRACT

Studies to establish bioequivalence (BE) of a drug are important elements in support of drug applications and thus require (BE) strategy to introduce generic equivalents of brand name to lower the cost of medication through proper assessment as directed by the international regulatory authorities. A typical BE study was conducted as a single dose, randomized, 2-period crossover design in 52 healthy, adult, male human volunteers under fasting conditions to compare 0.5 mg capsules of two different Dutasteride formulations. The drug was given as a single dose of 0.5 mg and blood samples were collected up to 72 hour period after drug administration. Dutasteride levels in plasma were determined by using a validated LCMS/MS method. The pharmacokinetic variables of AUC_{0-72 hr}, C_{max} and T_{max} was calculated. The results of this study suggest that the two formulations are bioequivalent.

Keywords: Dutasteride, Bioequivalence, Pharmacokinetics, Cross over study.

INTRODUCTION

Dutasteride is indicated for the treatment of symptomatic benign prostatic hyperplasia (BPH) in men with an enlarged prostate and to reduce the risk of acute urinary retention (AUR). The maximum effect of daily doses of dutasteride on the reduction of DHT is dose dependent and is observed within 1 to 2 weeks. The drug is nearly 60-90% absorbed from the gastrointestinal tract after oral administration, and serum concentrations are usually reached within 2-3 hours. Dutasteride is highly bound to plasma albumin (99.0%) and alpha-1 acid glycoprotein (96.6%). It is metabolized by the CYP3A4 and CYP3A5 isoenzymes. Both of these isoenzymes produced the 4'-hydroxydutasteride, 6-hydroxydutasteride, and the 6,4'-dihydroxydutasteride metabolites. In addition, the 15-hydroxydutasteride metabolite was formed by CYP3A4. Dutasteride is not metabolized in vitro by human cytochrome P450 isoenzymes CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. Only trace amounts of unchanged dutasteride were found in urine (<1%). The terminal elimination half-life of dutasteride is approximately 5 weeks at steady state. Hence a truncated AUC was adopted due to the fact that terminal elimination rate constant cannot be calculated accurately^{9, 10}. The aim of the study was to compare 0.5mg capsules of two different formulations of Dutasteride.¹

SUBJECTS AND METHODS

Fifty -two healthy male human volunteers between 18 and 45 years with a body mass index (BMI) range between 18.50 kg/m² and 29.90 kg/m² participated in the study. All subjects were nonsmokers and denied use of illicit drugs which was also verified during screening and before check-in of both the periods; All subjects underwent a screening procedure (performed within 21 days) prior to the start of the study. Medical history and detailed demographic data were recorded. Each subject underwent a complete general physical examination (including but may not be limited to an evaluation of the cardiovascular, gastrointestinal, respiratory, and central nervous systems), vital sign assessments, 12-lead electrocardiogram (ECG), X-ray (obtained within 6 months) and clinical laboratory assessments. Further, the subjects were selected on the basis of predefined inclusion and exclusion criteria as per the protocol. Subjects laboratory testing included (complete blood count; biochemistry; urine analysis; and antibody testing for the human immunodeficiency virus, hepatitis B surface antigen, and hepatitis C virus)^{2,3,4,5}

STUDY DESIGN

In this randomized, two-way, single-dose, crossover study, each subject initially received a single dose of 0.5 mg capsules of test or reference formulation on two occasions with a washout period of 45 days between the treatments. No beverages containing alcohol and no food or beverages containing xanthines (eg, caffeine) were allowed for 24 hours before the study or during the study. No medications were to be taken for 1 week before the study. Subjects were fasted for atleast 10 hours before a high fat high calorie breakfast at 30 minutes before drug administration and remained fasted from 4 hours after drug administration. The study was approved by an Independent Ethics committee. All subjects provided written informed consent before entering the study.

In each period, 23 blood samples will be collected. The pre-dose (00.00) blood sample (08 mL) was collected within 75 minutes prior to dosing. The post-dose blood samples (06 mL each) was collected at 00.33, 00.67, 01.00, 01.33, 01.67, 02.00, 02.33, 02.67, 03.00, 03.50, 04.00, 04.50, 05.00, 06.00, 08.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours in labeled K₂EDTA via an indwelling cannula placed in one of the forearm veins of the subjects. The samples were centrifuged 4000 ± 50 RPM for 10 minutes at 2°C - 8°C to separate plasma. The separated plasma were transferred to pre-labeled polypropylene tubes into two aliquots. The resulting two aliquots were stored upright in a freezer at a temperature of -30°C ± 10°C for interim storage at the clinical site until transferred to analytical site. From then the samples were stored at -70°C ± 20°C until analysis.

DETERMINATION OF DUTASTERIDE PLASMA CONCENTRATION

Dutasteride was extracted by solid phase extraction technique. The thawed plasma samples were Vortexed to ensure complete mixing of contents and 50 µL of internal standard (10 ng/mL of Dutasteride 13 C6) was added to all labeled RIA vials containing 400 µL of plasma samples except blank. 200 µL of HPLC water was added and vortexed. The MCX30 mg/mL cartridge with 1.0 mL of Acetone followed by 1.0 mL of HPLC water. The samples were loaded into the cartridge and washed twice with 1 mL of HPLC water. The samples were eluted with 0.5 mL of mobile phase. The samples are transferred to the respective labeled auto-injector vials and loaded to process the samples in to LC-MS/MS

Chromatographic separation was performed using C18 column (100 × 2.0) mm, 2.5 µm. The mobile phase consisted of Acetonitrile/5 mM Ammonium Formate with 0.1% Formic acid (95:05, v/v). The flow rate of mobile phase and the column oven temperatures were set at 0.350 mL/min and 40°C respectively.

The HPLC system was coupled to an API 4000 triple quadrupole mass spectrometer equipped with a turbo ion spray ionization interface operated in positive mode. The mass spectrometer was operated at a unit resolution for both Q1 and Q3 in multiple reactions monitoring mode, with a dwell time of 300 milliseconds in each transition. The transition was monitored at 529.19 > 461.11 for Dutasteride and 535.15 > 467.13 for internal standard (Dutasteride 13 C6). The mean (SD) retention times were 1.11 min for both analyte and Internal Standard. The method was validated for specificity, linearity, recovery, lower limit of quantification (LLOQ) stability, accuracy and precision⁶. Data analysis was performed using mass lynx software 4.1 SCN843 (Waters India Limited).

PHARMACOKINETIC AND STATISTICAL ANALYSES

A total of 52 subjects participated and 50 subjects completed the study. The samples from the 50 subjects who completed the study were analyzed to determine the concentration of Dutasteride. Pharmacokinetic and statistical analysis was performed on the data obtained from 50 subjects who completed the study¹¹. All concentration values below the lower limit of quantification (LLOQ) were set to "zero" for all pharmacokinetic and statistical calculations. Dutasteride plasma concentrations were analysed as a function of time. The following pharmacokinetic parameters were obtained for each formulation; C_{max}, AUC_{0-72hr} and T_{max} were calculated using non compartmental model of WinNonlin[®] version 5.3 of Pharsight Corporation, USA. Statistical analysis was performed on PK data of subjects by using SAS statistical software (Version 9.2 or higher, SAS[®] Institute Inc., USA). The Ln-transformed data of C_{max} and AUC 0-72hr was evaluated statistically using the PROC GLM from SAS[®] for difference due to group treatment, period and sequence as a fixed effects and subject within sequence as a random effect. An F-test was performed to determine the statistical significance of the effects involved in the model at a significance level of 5 % (α = 0.05). Consistent with two one-sided tests for bioequivalence, the 90 % confidence intervals for the ratio of least squares mean between drug formulations was calculated, for Ln-transformed data of C_{max}, and AUC_{0-72hr} for Dutasteride. Ratio of least squares means for Dutasteride of test and reference formulations was computed and reported for Ln-transformed pharmacokinetic parameters C_{max}, and AUC_{0-72hr}. The power of ANOVA test to detect a

20% mean difference between test and reference formulations is reported for Dutasteride. The criteria to establish bioequivalence

used was a 90% confidence interval⁸ of the relative mean C_{max}, and AUC_{0-72hr} of the test to reference formulation for Ln-transformed data should be within 80.00 – 125.00 %.

RESULTS

There was only one adverse event reported during the study which was mild in intensity unrelated to the study drug and resolved without any sequelae. Hence the test and reference product were comparable in safety and well tolerated at the selected dose level of Dutasteride. The mean plasma concentration of both test and reference products are presented in Figure. 1. The mean, standard deviation (SD), geometric mean, coefficient of variation (CV %), minimum, median, maximum were calculated for C_{max}, AUC_{0-72hr} and t_{max} and presented in the table 1. Peak or maximal plasma concentration (C_{max}) was calculated for each subject with respect to treatment and the results are presented in mean values (± SD) of C_{max} for test product (T) and for reference product (R). Area under the concentration-time curve from time zero to 72 hr (AUC_{0-72hr}) was calculated for each subject with respect to treatment and the results are presented in mean values (± SD) of AUC_{0-72hr} for test product (T) and for reference product (R). The ANOVA was performed on the Ln-transformed C_{max} and AUC_{0-72hr} parameters. The least-square mean ratio, the 90% confidence intervals and intra-subject CVs were also determined for C_{max} and AUC_{0-72hr}. These results are summarized in table 2. Hence the results suggest that both the test and reference formulation are bioequivalent under the conditions used in the study.

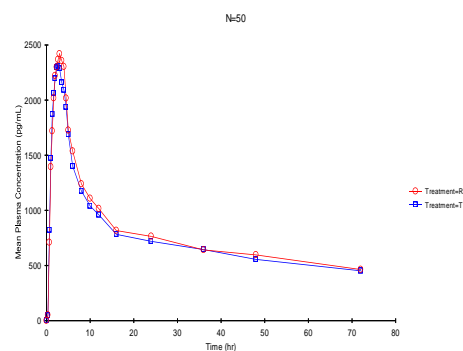


Figure 1: Linear Plot of Mean Plasma Dutasteride Concentrations versus Time (N=50)

Table 1: Summary of Pharmacokinetic Parameters of Test Product-T and Reference Product -R

Untransformed Data (Mean ± SD)			
PK Parameter	N	Test Product (R)	Reference product (T)
C _{max} (pg/mL)	50	2751.2124 ± 931.6568	2675.8821 ± 825.8926
AUC ₀₋₇₂ (pg.hr/mL)	50	56835.9510 ± 24991.7404	54155.7307 ± 23760.4681
*t _{max} (hr)	50	2.56 ± 0.97	2.49 ± 0.93

* Expressed as median and range.

Table 2: Statistical Results of Test Product-T versus Reference Product-R for Dutasteride

Anti Log of Least Square Mean					
PK Parameter	Test Product (T)	Reference Product (R)	(T/R) Ratio	90% Confidence Intervals	Intra subject CV %
C _{max} (pg/mL)	2618.0312	2560.9581	97.82	94.45% to 101.31%	10.48 %
AUC _{0-72hr} (pg.hr/mL)	51543.3224	49020.1820	95.10	91.81% to 98.52%	10.52 %

DISCUSSION

The test and reference formulation of Dutasteride exhibited overlapping plasma profiles especially in the elimination phase, which indicated that the formulations were similar in both

absorption and elimination. To ensure a reliable estimate of the extent of absorption a collection period of ≥ 3 t_{1/2} is recommended by USFDA⁷ and for drugs which have a long half life the collection of blood samples time can be truncated¹⁰. These requirements were

fulfilled, and the mean extrapolated area was well below 20% for both formulations, indicating that extraction period was adequate to fully characterize the PK properties of Dutasteride. The AUC values obtained with the test and reference formulations were not significantly different which reflects the similar PK characteristics of the 2 formulations particularly during the elimination phase. The 90% CIs constructed around the ratio of expected geometric means for AUC after administration of each formulation was 91.81%

to 98.52%. Because this was well within the bioequivalence range of 0.8 to 1.25 the formulation can be considered bioequivalent according to US FDA definition of bioequivalence with respect to the extent of absorption.

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