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COMPARATIVE BIOAVAILABILITY OF TWO LOSARTAN FORMULATIONS IN HEALTHY HUMAN VOLUNTEERS AFTER A SINGLE DOSE ADMINISTRATION

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ABSTRACT

The aim of this study was to evaluate the bioequivalence of test and reference losartan potassium 50 mg formulations in healthy volunteers. This open label, balanced, single-dose, randomized, 2-period, crossover oral bioequivalence study was conducted in 54 healthy human adult subjects under fasting condition. Subjects received losartan 50 mg of either test or reference formulation with a washout period of 7 days. After study drug administration, serial blood samples were collected over a period of 48 hours. The plasma concentrations of losartan and its active metabolite were determined by a validated method using LC/MS/MS. Pharmacokinetic parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, and k_{el}, were determined for both the formulations. The formulations were to be considered bioequivalent if the log-transformed ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125%. A total of 54 subjects were enrolled. No significant differences were found based on analysis of variance. The mean values and 90% confidence intervals (CI) of test/reference ratios for these parameters of losartan as follows: C_{max} 274.90 Vs 286.93 ng/mL (83.95 -113.69); AUC_{0-t} 434.67 Vs 438.68 ng.hr/mL (93.30 -104.03); and $AUC_{0-\infty}$ 463.23 Vs 464.71 ng.hr/mL (94.65 -104.80) and for its active metabolite (losartan carboxylic acid) the mean values and 90% CI of test/reference ratios for these parameters as follows: C_{max} 572.63 Vs 543.82 ng/mL (96.87-109.37); AUC_{0-t} 3987.89 Vs 4051.07 ng.hr/mL (93.48-104.22); and $AUC_{0-\infty}$ 4215.58 Vs 4271.67 ng.hr/mL (94.84-105.26). This study shows that the test formulation is bioequivalent to the reference formulation for losartan and its main active metabolite.

Key Words: Bioequivalence, Losartan, losartan carboxylic acid, Pharmacokinetics.

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INTRODUCTION

Losartan was the first orally active AT1 receptor antagonist available in the market, and it is the antagonist with which the greatest clinical experience has been accumulated[1]. Losartan and its active metabolite block the vasoconstrictor and aldosterone secreting effects of angiotensin II by type AT1 receptor blockage. Following oral administration, losartan is rapidly absorbed, reaching maximum concentrations 1–2 hour post administration[2].

Losartan undergoes substantial first-pass metabolism by cytochrome P450[3,4]. The biotransformation results in a major active metabolite, losartan carboxylic acid (EXP3174), which is 10 to 40 times more potent than parent losartan in blocking the angiotensin II–induced responses in vascular smooth muscle cells and is responsible for most of the agent's pharmacologic activity[3,5,6]. After oral administration of a losartan, bioavailability is approximately 33% and approximately 14% of Losartan dose is converted to the pharmacologically active carboxylic acid metabolite (E-3174) by action of CYP2C9, CYP3A4 and CYP2C10[3,7,8]. The terminal half life of losartan is about 2 hours and of the metabolite is about 6 to 9 hours. Losartan and its metabolite are excreted by the kidney and in bile[1].

The primary objective of study was to evaluate the bioequivalence of Zyltan 50 (Containing 50 mg Losartan Potassium) tablets of Troikaa Pharmaceuticals Ltd., India with Cozaar (containing 50mg Losartan Potassium) tablet of Merck Sharp & Dohme Ltd, U.S.A. in 54 healthy human adult subjects under fasting condition.

MATERIAL AND METHODS

The study was carried out at the Auriga Research Ltd., New Delhi. All the subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was approved by the Independent Ethics Committee and was conducted in accordance with good clinical practice and the declaration of Helsinki.

Study Subjects

The study population consisted of 54, adult, healthy subjects with mean BMI 22.78 ± 2.51 Kg/m^2, a mean age of 27.98 ± 4.57 years, mean weight of 62.24 ± 7.69 kg and a mean height of 165.27 ± 5.84 cm.

Design

The study was designed as open label, balanced, single-dose, randomized, 2-period, crossover oral bioequivalence study with 7 days washout period. The volunteers were administered one of the two study drugs after an overnight fast. The dose administration was performed as per the

randomization schedule. Subjects received single oral dose of Zyltan 50 (Containing 50 mg Losartan Potassium) tablet of Troikaa Pharmaceuticals Ltd., India and Cozaar (containing 50mg Losartan Potassium) tablet of Merck Sharp & Dohme Ltd, U.S.A.

Blood sampling

A total 38 blood samples were drawn during the study per subject. Blood samples were collected in pre-labeled, pre-chilled K_3EDTA vacutainers. From each volunteer 5 mL of blood samples were withdrawn at pre-dose (00.00 hr) and 00.25, 00.50, 00.75, 01.00, 01.33, 01.67, 02.00, 03.00, 03.50, 04.00, 04.50, 05.00, 06.00, 08.00, 12.00, 24.00, 36.00 and 48.00 hrs following drug administration in each period. After centrifugation, plasma separated from blood samples were stored in a freezer at –-80°C or colder until completion of analysis.

Method of analysis

Drug analysis

Losartan, losartan carboxylic acid and the internal standard Ketoconazole were extracted from plasma samples by protein precipitation method. An aliquot of that was analyzed by combined reversed phase liquid chromatography with tandem mass spectrometry in negative ion electrospray ionization using multiple reaction monitoring (MRM). Briefly, pipette out 200 ul of human plasma sample in to ria vial followed by internal standard (50 -ul of ketoconazole 10.0 ug/ml). After vortex-mixing for 10 sec, add 0.750 ml of methanol and vortex for 10 minutes. Centrifuge at 14000 rpm for 10 minutes. Transfer the layer in to injector vials and inject on LC/MS/MS system.

Chromatographic conditions

The compounds were eluted by pumping the mobile phase (10mM ammonium formate and 0.1% formic acid in methanol) at a flow rate of 0.8 ml/ min. Under these conditions, typical standard retention times were 0.92 min for losartan and its active metabolite, and 0.92 min for ketoconazole. The temperature of the auto - sampler was kept at 5 ºC and the run-time was 2.5 min.

Mass-spectrometric conditions

The mass spectrometer (API3000 LC/MS/MS System) was equipped with an electrospray ion source running in positive (ES+), and set up in multiple reaction monitoring (MRM), monitoring the transitions 423.04 > 207.10 and 436.93 > 234.90 for losartan and losartan carboxylic acid, and 531.20 > 489.30 for ketokonazole, respectively. In order to optimize all the MS parameters, working solutions (1 ug/ml) of the analytes and internal standard were in fused (at 10 ul/min)

into mass spectrometer. For each losartan, losartan carboxylic acid, and the internal standard, the following optimized parameters were obtained: the dwell time and the collision gas (nitrogen) were 0.1 sec and 5.0, respectively. The collision energy was 31 eV for losartan, 23 eV for losartan carboxylic acid, and 42 eV for ketoconazole. Data acquisition and analysis were per formed using the software Analyst Software 1.4.2.

Pharmacokinetic and Statistical Analysis

Based on the plasma concentrations of losartan and its active metabolite losartan carboxylic acid, the pharmacokinetic parameters were calculated by using "Non-compartmental model" and ANOVA statistics. All pharmacokinetic analysis carried out by using WinNonlin Version 5.2. The elimination rate constant (k_{el}) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($t_{½}$) was calculated as 0.693/ k_{el}. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant.

The variance model included sequence, subjects nested in sequence, period, and product as factors. A 5% level of significance used for all comparisons (period, product and sequence). Inconsistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the ratios of means was calculated for both untransformed and log-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for losartan and its active metabolite losartan carboxylic acid. The formulations were to be considered bioequivalent if the log transformed ratios (test/reference) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125%.

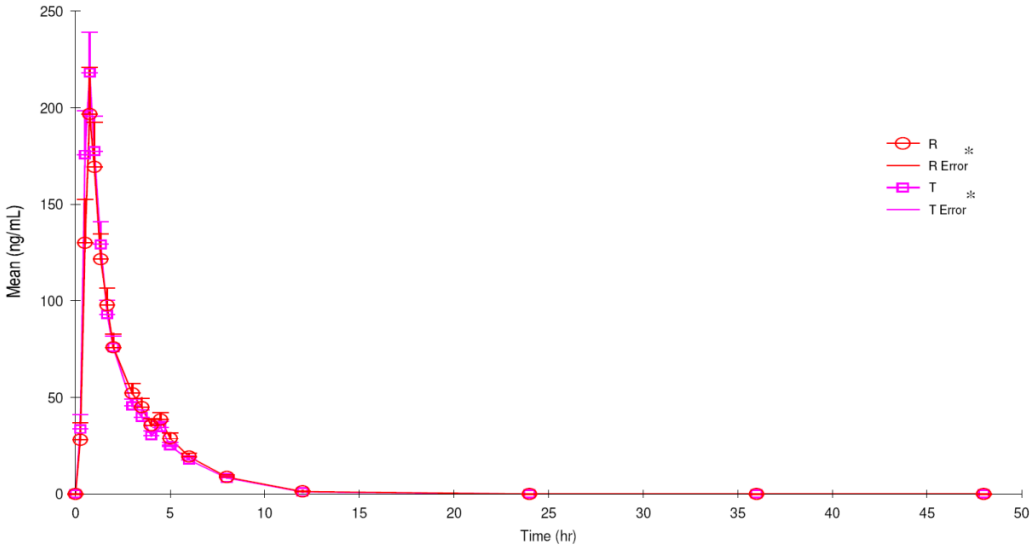
Safety and tolerability

General clinical safety was assessed via medical examinations, vital signs and clinical laboratory test conducted at screening and at the end of the study. Clinical laboratory tests and ECGs were also conducted as per protocol. Adverse events were assessed for severity and relationship to treatment throughout the study.

RESULT AND DISCUSSION

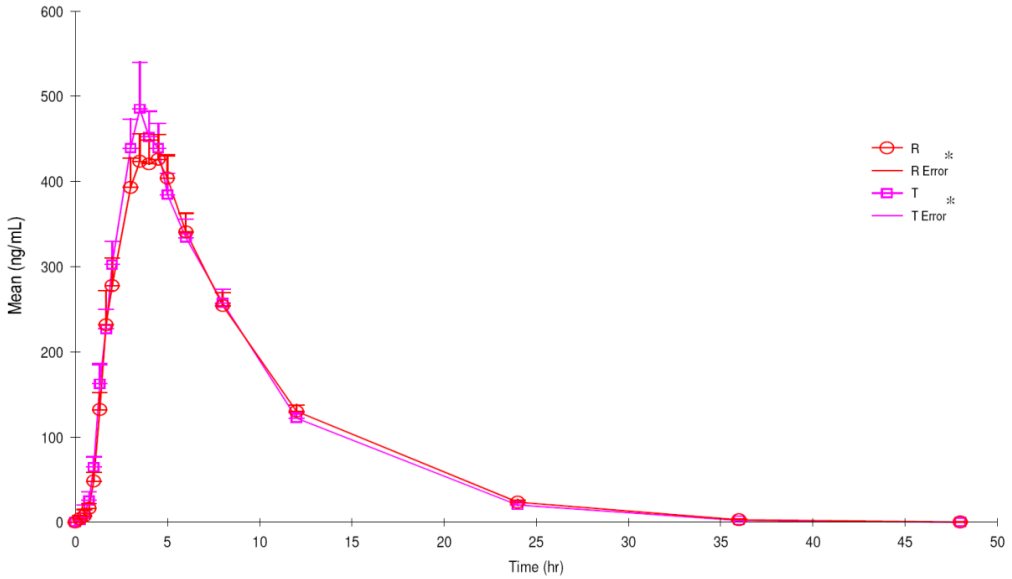
52 subjects had successfully completed both the study periods. There was one dropout and one volunteer was withdrawn from the study before dosing on Period-II due to viral fever. The data obtained from dropped out and withdrawn subjects were not included in pharmacokinetic and statistical analysis.

The mean concentration–time curves of test and reference formulations for losartan and losartan carboxylic acid are shown in the Figure 1 and 2 respectively.



Error * = Standard Error of Mean

Figure 1. Linear Plot of Mean Plasma Concentration of losartan (ng/ml) versus Time (hr)



Error * = Standard Error of Mean

Figure 2. Linear Plot of Mean Plasma Concentration of losartan carboxylic acid (ng/ml) versus Time (hr)

The plasma drug concentration-time curves show that the mean concentrations of losartan and losartan carboxylic acid were similar for the two formulations over 48 hours sampling period. The primary and secondary mean pharmacokinetic parameters for losartan and losartan carboxylic acid after administration of test and reference formulations are listed in Table 1 and 2 respectively.

Table 1. Primary and secondary Pharmacokinetic Parameters for losartan after administration of Test and Reference Product

Products	Test						Reference					
Parameters	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	K_{el} (hr^{-1})	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	K_{el} (hr^{-1})
Mean	274.90	434.67	463.23	0.92	2.14	0.35	286.93	438.68	464.71	1.14	1.93	0.40
Minimum	69.20	121.39	144.56	0.50	1.02	0.18	59.69	156.40	167.30	0.25	0.95	0.16
Maximum	825.82	1146.25	1162.36	4.50	3.89	0.68	958.80	1159.56	1187.71	4.50	4.46	0.73
SD	169.04	212.30	212.79	0.64	0.62	0.12	185.00	209.13	213.49	0.85	0.67	0.13
CV%	61.49	48.84	45.94	69.43	28.89	32.68	64.48	47.67	45.94	74.27	34.85	32.81

Table 2. Primary and secondary Pharmacokinetic Parameters for losartan carboxylic acid after administration of Test and Reference Product

Products	Test						Reference					
Parameters	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	K_{el} (hr^{-1})	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	K_{el} (hr^{-1})
Mean	572.63	3987.89	4215.58	3.99	4.65	0.17	543.82	4051.07	4271.67	3.84	4.99	0.16
Minimum	74.61	503.39	516.77	0.50	0.77	0.07	71.50	306.71	323.34	0.50	0.84	0.05
Maximum	2844.50	7359.32	7426.99	8.00	9.61	0.90	1785.65	7729.18	7850.89	8.00	14.68	0.83
SD	398.84	1717.62	1675.33	1.27	1.47	0.11	283.62	1710.88	1737.11	1.22	1.96	0.10
CV%	69.65	43.07	39.74	31.92	31.63	64.74	52.15	42.23	40.67	31.62	39.24	62.46

For losartan, the mean C_{max} (274.90 Vs 286.93 ng/mL), AUC_{0-t} (434.67 Vs 438.68 ng.hr/mL) and $AUC_{0-\infty}$ (463.23 Vs 464.71 ng.hr/mL) values of test formulation were similar to reference formulation. These mean pharmacokinetic parameter values were comparable to the values reported by Jing-Ying Jia et al for Losartan 50 tablets[3]. The mean T_{max} (0.92 Vs 1.14 hr) values of test and reference formulation were closure to those reported in literature[3,9]. The mean elimination half-life $t_{1/2}$ (2.14 Vs 1.93 hr) of test and reference formulation was in the range of values (1.5 to 2.5 hrs) reported in literature[7,10].

For losartan carboxylic acid, the mean C_{max} (572.63 Vs 543.82 ng/mL), AUC_{0-t} (3987.89 Vs 4051.07 ng.hr/mL), $AUC_{0-\infty}$ (4215.58 Vs 4271.67 ng.hr/mL) and mean T_{max} (3.99 Vs 3.84) values of test formulation were similar to reference formulation.

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent[11]. To demonstrate bioequivalence certain limits should be set depending on the nature of drug, patient population, and clinical end points. It is generally accepted that the 90% confidence interval for the ratio of averages of logarithmically transformed AUC and C_{max} should lie within the range of 80 to 125 %[11,12].

Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, treatment or sequence, with *p* value greater than 0.05. The 90% confidence intervals were completely contained within the predefined bioequivalence criteria of 80% to 125% for all the primary pharmacokinetic parameters of losartan and losartan carboxylic acid and thus test and reference formulations are bioequivalent for the rate and extent of absorption. For losartan, the 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 83.95 -113.69, 93.30 - 104.03 and 94.65 -104.80 meeting the predetermined criteria for bioequivalence. Similarly for losartan carboxylic acid, the 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were meeting the predetermined criteria for bioequivalence. The 90% confidence intervals for losartan and losartan carboxylic acid are listed in Table 3.

Both formulations were well tolerated by the subjects, with no major side effects and no clinically significant differences in safety profiles. One volunteer was withdrawn from the study before dosing on Period-II due to viral fever. As per the study investigator, the relationship with the study products was unlikely.

Table 3. 90 % Confidence Interval for losartan and losartan carboxylic acid

Pharmacokinetic Parameter	90 % Confidence Interval for losartan		90 % Confidence Interval for losartan carboxylic acid	
	Lower	Upper	Lower	Upper
C_{max} (ng/ml)	83.95	113.69	96.87	109.37
AUC_{0-t} (ng hr/ml)	93.30	104.03	93.48	104.22
$AUC_{0-\infty}$ (ng hr/ml)	94.65	104.80	94.84	105.26

CONCLUSIONS

Based on the results, it is concluded that the Zyltan 50 (Containing 50 mg Losartan Potassium) tablet of Troikaa Pharmaceuticals Ltd., India is bioequivalent to Cozaar (containing 50mg Losartan Potassium) tablet of Merck Sharp & Dohme Ltd, U.S.A.

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REFERENCE

1. Michel Burnier. Angiotensin II Type 1 Receptor Blockers. Circulation. 2001; 103: 904-912.
2. Budi Prasaja, Lucy Sasongko, Yahdiana Harahap, Hardiyanti, Windy Lusthom, Matthew Grigg. Simultaneous quantification of losartan and active metabolite in human plasma by liquid chromatography–tandem mass spectrometry using irbesartan as internal standard. J Pharm Biomed Anal 2009; 49(3): 862-7.
3. Jing-Ying Jia, Meng-Qi Zhang, Yan-Mei Liu et al. Pharmacokinetics and Bioequivalence Evaluation of Two Losartan Potassium 50-mg Tablets: A Single-Dose, Randomized-Sequence, Open-Label, Two-Way Crossover Study in Healthy Chinese Male Volunteers. Clin Ther 2010; 32(7): 1387-95.
4. Tamimi JJ, Salem II, Mahmood Alam S et al. Comparative pharmacokinetics of two tablet formulations of losartan: Bioequivalence assessment. Biopharm Drug Dispos 2005; 26: 205–210.
5. Kolocouri F, Dotsikas Y, Apostolou C, et al. Simultaneous determination of losartan, EXP-3174 and hydrochlorothiazide in plasma via fully automated 96-well-formatbased

solid-phase extraction and liquid chromatographynegative electrospray tandem mass spectrometry. Anal Bioanal Chem 2007; 387: 593–601.

6. Oliveira CH, Medeiros Silva R, Santagada V, et al. Comparative bioavailability of two losartan formulations in healthy human volunteers after a single dose administration. Int J Clin Pharmacol Ther 2006; 44: 142–148.
7. Tausif Ahmed, Sivacharan Kollipara, Anirudh Gautam, Radhika Gigras et al Bioavailability and Interaction Potential of Atorvastatin and Losartan on Co-administration in Healthy Human Subjects. J Bioequiv Availab 2009; 1(1): 18-27.
8. Lo MW, Goldberg MR, McCrea JB, Lu H, Furtek CI, et al. Pharmacokinetics of Losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. Clin Pharmacol Ther 1995; 58 (6): 641-649.
9. Sica DA, Gehr TW, Ghosh S. Clinical pharmacokinetics of losartan. Clin Pharmacokinet 2005; 44(8): 797-814.
10. Nakashima M, Umemura K. The clinical pharmacology of losartan in Japanese subjects and patients. Blood Press Suppl 1996; 2: 62-6.
11. Dhaneshwar Shep, Ankit Shah, Asmita Nimkar, Rajen Shah, Vijaya Jaiswal. Comparative bioavailability study with two amiodarone tablet formulations in healthy subjects. Int J Res Pharm Sci 2010; 1: 481-485.
12. Hassan Y, Alfadly SO, Azmin MN, Peh KK, Tan TF, Noorizan AA, Ismail O. Bioequivalence evaluation of two different formulations of ciprofloxacin tablets in healthy volunteers. Singapore Med J 2007; 48: 819-823.