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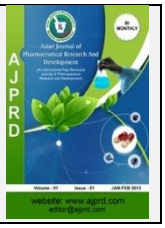

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Research Article

Randomized two way crossover open label study to compare the bioequivalence of LOSARDIL 100 TM (Drug International Ltd, Bangladesh) and COZAAR TM (Merck Sharp & Dhome Ltd, UK) in Bangladeshi normal male volunteers

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A B S T R A C T

Objectives: A crossover-randomized bioequivalence study of two oral formulations of losartan (100 mg) tablets was carried out in 16 healthy male Bangladeshi volunteers. The test and reference formulations were LOSARDIL 100™ (Drug International Ltd, Bangladesh) and COZAAR™ (Merck Sharp & Dohme Ltd, UK), respectively.

Methods: Each tablet was administered with 150 mL of water to subjects after whole night fasting condition on two therapy days distinct by 1 week washout period. After administration, blood samples were accumulated periodically for 24 hours. The plasma concentrations of losartan were evaluated using a validated HPLC method. The pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and K_{el} were determined.

Results: The mean (± SD) $AUC_{0\rightarrow 24h}$ for losartan of test drug LOSARDIL 100™ for 16 volunteers was 3310 ± 1165 ng.hr/mL whereas it was 3545 ± 1251 ng.hr/mL for losartan of COZAAR™ . The relative bioavailability (LOSARDIL 100™/COZAAR™ ratio) was 93%. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan of test drug were 1855 ± 675 ng/mL, 0.77 ± 0.39 hours, 4.69 ± 1.17 hour and 0.15 ± 0.04 respectively. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan of reference drug were 2254 ± 944 ng/mL, 0.87 ± 0.29 hours, 4.13 ± 1.41 hour and 0.20 ± 0.04 respectively.

Conclusion: Depend on the statistical interpretation the 90% CI for the test and reference drugs were observed within the acceptance range of 80-125%. In conjecture, LOSARDIL 100™ is bioequivalent to COZAAR ™ in terms of absorption.

Keywords: Bioequivalence, Losartan potassium, Hypertension, HPLC, Pharmacokinetics, Drug International Ltd.

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INTRODUTION:

Hypertension is a chronic medical situation having the blood pressure in the arteries endures above the normal delegated range. It conducts to the improvement of cerebrovascular disease, ischemic heart disease, cardiac and renal failure. By the treatment of hypertension the risk factor of stroke will reduce 40% and 15% reduction in the risk of myocardial infarction accomplished [1]. The molecular formula of Losartan potassium (LP) is $C_{22}H_{22}ClKN_6O$. LP act as angiotensin II receptor (type AT1) antagonist. Its chemical name is 2-

butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl] imidazole-5-methanol monopotassium and also a non-peptide molecule [2]. Angiotensin II is an important vasoconstrictor plays an potent role to cause hypertention. LP is a reversible competitive inhibitor of the ATI receptor. LP and its metabolite Losartan carboxylic acid block the vasoconstrictor and adrenaline secretion effect of angiotensin II to the ATI receptors present in many tissues. In clinical trials it is found that LP lowers the blood pressure effectively compare to the other first line antihypertensive drugs [3]. It is rapidly absorbed and metabolized by CYP450 to form an active metabolite EXP-3174 [4]. This active metabolite provides an enormous antihypertensive effect about last 24 hours after administered [5]. In pharmacokinetics (the study of the time course of drug absorption, distribution, metabolism, and excretion) the term **Bioequivalence (BE)** is utilize to evaluate the anticipated *in vivo* biological equivalence of two exclusive preparations of a drug. Two products are said to be bioequivalent if it means that they would be expected to be, for all intents and purposes, the same. According to the FDA the Bioequivalence study means the rate and extent to which the active ingredients become available at site of the drug action when administered in the same molar doses under similar condition [6]. BE documentation play an vital role in new drug development. Bioequivalence mentions to the quality of a locally produced drug by comparison with reference drug of internationally highly regarded company. The hike price of medicine increases the overall expense of health care. Generic equivalents of branded or enovator drugs decline the expense of medications [7]. A regular fashion for a bioequivalence study requires administration of the test and reference products to healthy volunteers periodically in patients by open label, two way two sequences cross-over and randomized fashion, with each administration separated by a washout period. The washout period is chosen to ensure that drug given in one treatment is entirely eliminated prior to administration of the next treatment i.e.10 times of elimination half- life. Just prior to administration, plasma samples are collected at regular time intervals and evaluated for parent drug concentration and one or more metabolites. The rise and fall of these drug concentrations over time in each subject in the study provide an estimate of how the drug substance is absorbed into the test and reference products and released from the body. Occasionally, blood concentration levels are neither attainable nor possible to contrast the two products (e.g. inhaled corticosteroids), then pharmacodynamics endpoints are used for comparison rather than pharmacokinetic endpoints. To allow comparisons between the two products, these blood (to include plasma or serum) and urine concentration time curves are used to calculate certain bioequivalence metrics of interest. These metrics are calculated for each subject in the study and the resulting values are compared statistically. If the pharmacokinetic parameters such as AUC_{0-t}, C_{max}, $t_{1/2}$, K_{el} etc. for the new drug coexist with the reference properties lie in the range between confidence interval (CI) 80-125%, then the test drug will said to be bioequivalent with the reference drug. BE also requires the similar bio availabilities, efficacy and safety of the two preparation in the above mentioned range (80-125%). In BE study the comparison of C_{max} and AUC_{0-t} are the main key features which are most frequently used in-vivo studies to construct BE. Those values give information of concentration-time profile in the plasma for a single dose of drug administration, either the test or reference one. The statistical analysis of the observed data generates an estimated value, called confidence interval (CI) and it propounds a range of likely values of an unacquainted metrics.

The availability of test LOSARDIL 100™ offers a more economical surrogate for patients requiring β-blocker therapy. That's why BE study is require to ensure the quality of a locally manufactured drug by comparison with standard drug of internationally reputed company.

The purpose of the study: To assess the bioequivalence of a test product LOSARDIL 100™ (losartan 100 mg per tablet; Drug International Ltd, Bangladesh) with a reference product COZAAR™ (losartan 100 mg per tablet; Merck Sharp & Dohme Ltd, UK) by measurement of plasma concentrations by HPLC and calculation of bioequivalence parameters.

Protocol: Bioequivalence study was carried out as randomized, open-label, two-way crossover method with washout period of more than 7 days. There was no utmost deflection made from the accepted protocol.

MATERIALS AND METHODS:

Study subjects:

Volunteers were collected from the volunteer bank which was made by counseling with every individual. Total number of subjects was sixteen in this study and the average age of the volunteers with standard deviation was 26.82 ± 3.97 years; the average height with standard deviation was 165.5 ± 4.11 cm; the average weight was 57.87 ± 5.79 kg during screening examination. The blood samples were cumulated by two phases after drug dosing. This was in Phase I: 29/05/2013 to 30/05/2013 and 05/06/2013 to 06/06/2013 and in Phase II**:** 19/06/2013 to 20/06/2013 and 26/06/2013 to 27/06/2013. The blood samples were evaluated from: 05/08/2013 to 15/10/2013

Study medication:

Treatment A (test formulation): LOSARDIL 100™ Batch No 03, Manufacturing date: March 2013, Expiry date: February 2015; Manufacturer: Drug International Ltd, Bangladesh.

Treatment B (reference formulation): COZAAR™ Lot No. R1231, Expiry date: 18 March 2014; Manufacturer: Merck Sharp & Dohme Ltd, UK.

Study Design

Every healthy individual secured as a single dose treatment in accordance with crossover randomized fashion (according to the protocol) having more than seven days washout period. Test and reference product denoted by T and R. A crossover design can be represented as (TR, RT), where TR is the first succession of treatments and RT represents the second succession of treatments. Under the (TR, RT) design, healthy qualified subjects who are randomly allocated to succession 1 (TR) will receive the test product (T) first and then cross-over to receive the reference product (R) after more than seven days of wash-out period. Similarly, subjects who are randomly allocated to succession 2 (RT) will receive the reference product (R) first and then cross-over to receive the test product (T) after the wash-out period.

The Institutional review board: The protocol and the ethical feature of this study were approved by the Institutional review board of Khwaja Yunus Ali Medical College Hospital. This encompasses of seven members committee including a local religious leader (Imam), a lawyer and a woman representatives. The protocol was ratified with insignificant mitigation.

This study was regulated in accordance with the International Conference of Harmonization (ICH) Good Clinical Practice (CGP) guidelines arrogated by the European Agency for the assessment of Medicinal products (EMEA).

Hospital admission: The person who has result in normal range of some clinical screening for example CBC, Urine R/E, RBS, kidney function test, liver function test etc. selected as a volunteer. Volunteers were admitted into twelve bedded bioequivalence ward in the hospital one day before starting the study. The ward was equipped with 24 inch color TV for watching television programs and carom board for playing by the volunteers in their free time. At a time eight normal individuals were admitted in the ward in this study.

Informed consent: Before starting the treatment the motive of the study was explicated to every volunteer in regional language (Bengali) by medical officer. When volunteer was acknowledged to engage in the study after careful reading the consent form written informed consent was only taken from him. If any question elevated by the volunteer was explained details with the medical officer. A copy of written informed consent was attached into the protocol.

Drug dosing and Sample collection

In this treatment every volunteer was given a single dose of test or reference product of metoprolol with 250 millilitre water after whole night fasting. Breakfast was supply to the volunteers after 4 hour of the drug dosing. Volunteers were permit to consume water after 2 hour of the dosing and then breakfast, lunch and dinner were given according to the time schedule. Volunteers were under direct medical observation at the study place. The blood samples were taken immediately before (2 mL in each time) and at 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 hours after dosing metoprolol. The blood samples were accumulated in EDTA tube and were centrifuged at 4000 rpm for 10 min. The plasma was separated and kept frozen at –80°C in eppendrof tube. The same method was repeated to finish the crossover study after seven days.

Chromatographic condition for drug analysis

HPLC with UV-Visible detector was utilized to exploration metoprolol drug. HPLC grade solvents and analytical grade reagent and chemicals were used in this study. The HPLC method was flourish and approved before the study by following international guidelines [8].

Agilent Germany 1200 series reverse-phase high performance liquid chromatography (HPLC) was used for the determination of metoprolol which comprised solvent reservoir, degasser, solvent delivery binary pump, auto sampler, column and diode array detector. ChemStation software was utilized to integrate the signal. C18 column (4.6 mm×150 mm) with particle size 5 µm (Sigma Aldrich) was employed for chromatographic separation. The mobile phase was used for analysis consisted of 35% acetonitrile (HPLC grade; E. Merck, Germany) and 65% sodium di-hydrogen phosphate buffer (pH 4.3, 50mmol, adjusted with o-phosphoric acid) was delivered at a rate of 0.5 mL/min. The phosphate buffer was prepared freshly on each day of experiment and filtered using 0.45 µm nylon filters. The wavelength was set at 220 nm (bandwidth 2 nm). Separations were achieved at 30°C. Injection of sample (20 µL) was done using an auto sampler. The peak with retention time and area were defined using software.

The retention time of losartan was 4.9 min. The plasma assay procedures were validated. The limit of detection (LOD) was 57 ng/mL (3SD) whereas limit of quantification (LOQ) was 190 ng/mL (10SD).

The extent of absorption was determined by $AUC_{0\rightarrow 24h}$ of losartan. The rate of absorption was determined by C_{max} and t_{max}. The half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan was used to further characterize the pharmacokinetic outcome of this study.

Sample preparation for the HPLC injection

The plasma which was collected from this volunteer was stayed few minutes for liquefaction. One hundred microliter

plasma was taken from each eppendrop tube then it was added 800 µL acetonitrile and it was vortexed for one minute for liquid-liquid extraction. The solution was centrifuged at 10,000 rpm for 2 minutes separated the organic phase, the process was continued again then the organic phase was evaporated with nitrogen gas. After evaporation the residue was reconstituted with 400µL mobile phase (acetonitrile : buffer = 35 : 65) and a volume of 20µL was injected to the HPLC system.

Statistical analysis

The pharmacokinetic parameters maximal plasma concentration (C_{max}), time for the maximal plasma concentration (T_{max}), half-life ($t_{½}$), area under the curve ($AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$,) and the elimination rate constant (K_{el}) for the two formulation (test and reference products) were calculated by two-way analysis of variance (ANOVA) procedures using Thermo Kinetica 2000 software[9]. The 90% confidence interval was evaluated by applying online software.

Pharmacokinetic analysis

The pharmacokinetic parameter C_{max}, T_{max}, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and K_{el} was determined. The mean (± SD) $AUC_{0\rightarrow 24h}$ for losartan of test drug **LOSARDIL** 100™ for 16 volunteers was 3310 ± 1165 ng.hr/mL whereas it was 3545 ± 1251 ng.hr/mL for losartan of **COZAAR™** . The relative bioavailability (**LOSARDIL** 100™/**COZAAR**™ ratio) was 93%. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan of test drug were 1855 ± 675 ng/mL, 0.77 ± 0.39 hours, 4.69 ± 1.17 hour and 0.15 ± 0.04 respectively. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan of reference drug were 2254 ± 944 ng/mL, 0.87 ± 0.29 hours, 4.13 ± 1.41 hour and 0.20 ± 0.04 respectively.

Table I: Pharmacokinetic parameters following oral administration of LOSARDIL 100™ (test) and COZAAR™ (reference)

Parameters	LOSARDIL 100™		COZAAR™	
	Mean ± SD	90% CI	Mean ± SD	90% CI
Losartan				
C_{max}	1855 ± 675 ng/mL	85.01 to 114.93	2254 ± 944 ng/mL	82.74 to 117.21
T_{max}	0.77 ± 0.39 hours	79.22 to 120.77	0.87 ± 0.29 hours	86.20 to 113.79
$AUC_{0\rightarrow 24h}$	3310 ± 1165 ng.hr/mL	85.49 to 114.47	3545 ± 1251 ng.hr/mL	85.47 to 114.49
$t_{1/2}$	4.69 ± 1.17 hour	89.76 to 110.23	4.13 ± 1.41 hour	85.95 to 114.04
K_{el}	0.15 ± 0.04	86.67 to 113.33	0.20 ± 0.04	90.00 to110.00

C_{max} = maximal plasma concentration; t_{max} = time for the maximal plasma concentration; $AUC_{0\rightarrow 24h}$ = Area under the plasma concentration–time curve from zero hours to 24 hours; $t_{1/2}$ = half-life; K_{el} = elimination rate constant.

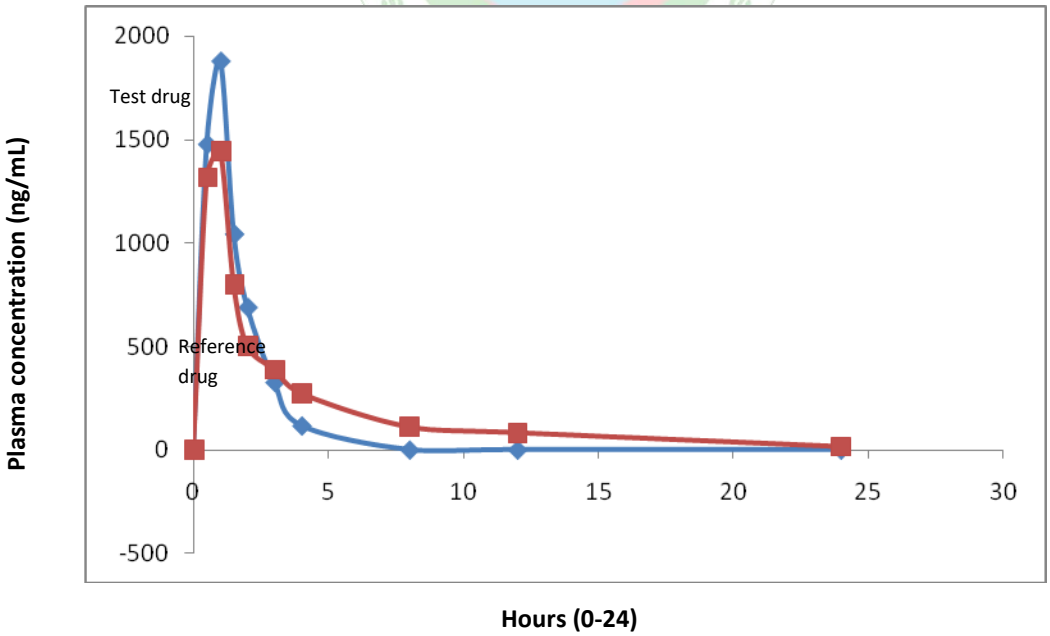

Figure 1: Mean plasma concentrations of Losartan Potassium in 16 human volunteers

Tolerance: Single dose of losartan (100 mg) of both products was well tolerated by the volunteers. Since blood pressure was reduced to 10-15 mmHg in both systolic and diastolic blood pressure but no patient complaint anything abnormal

RESULT AND DISCUSSION:

An randomized, crossover way in-vivo bioequivalence studies were executed between a test product LOSARDIL 100™ (losartan 100 mg per tablet; Drug International Ltd, Bangladesh) with a reference product COZAAR™ (losartan 100 mg per tablet; Merck Sharp & Dohme Ltd, UK) by measurement of plasma concentration using HPLC and estimation of pharmacokinetic parameters. There is no clinically significant difference in the rate and extent to bioequivalence of any local test product with reference innovator product at which the agile body of the drug becomes available at the site of drug operation. Healthy male individuals were involved in this research and a dose with single manner under the fasting situation was delivered to every volunteer. There was no unobtainable incidence during the study that could have changed the study findings. The present study had some restrains that should be contemplated. The volunteers who take part in the study resume to the end and were allow to leave from the study room with good health. The reported analytical method was demonstrated tactful and accurate for the estimation of losartan in plasma. The plasma assay procedures were validated. The retention time of losartan was 4.9 min. The plasma assay procedures were validated. The limit of detection (LOD) was 57ng/mL (3SD) whereas limit of quantification (LOQ) was 190ng/mL (10SD). Our study examined the pharmacokinetic properties and bioequivalence of two formulations of losartan in healthy Bangladeshi male volunteers. The pharmacokinetic parameters calculated for both the test and reference formulations were not significantly different, which reflects the comparable pharmacokinetic characteristics of two formulations [10]. Losartan was readily absorbed for both formulations from the gastrointestinal tract and losartan was quantifiable at the first sampling time (0.77 h) for most of the volunteers. The mean concentration-time profiles of the study is shown in the Figure 1 and indicating that the mean plasma drug concentration profiles of the two formulations were nearly similar. Peak concentrations maximum were achieved at 2.5-3.0 h after drug administration and then diminished reasonably but the losartan was noticeable until the last blood sample. All calculated pharmacokinetic parameters were in good concurrence with reported values. Table 1 shows the pharmacokinetic parameters for two studies. The extent of absorption is a key characteristic of a drug formulation, and therefore AUC, Cmax and Tmax are important parameters for bioequivalence study and could affect the therapeutic use of a drug [11]. The mean (± SD) $AUC_{0\rightarrow 24h}$ for losartan of test drug **LOSARDIL** 100™ for 16 volunteers was 3310 ± 1165 ng.hr/mL whereas it was 3545 ± 1251 ng.hr/mL for losartan of **COZAAR™** . The relative bioavailability (**LOSARDIL** 100™/**COZAAR**™ ratio) was 93%. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan of test drug were 1855 ± 675 ng/mL, 0.77 ± 0.39 hours, 4.69 ± 1.17 hour and 0.15 ± 0.04 respectively. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of losartan of reference drug were 2254 ± 944 ng/mL, 0.87 ± 0.29 hours, 4.13 ± 1.41 hour and 0.20 ± 0.04 respectively. The mean and standard deviation of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$, Cmax, Tmax, $t_{1/2}$ and Kel of the two products did not differ remarkably; recommend that the blood profiles produced by Losardil are comparable to those produced by Losartan potassium. Analysis of variance (ANOVA) for these parameters, showed no statistically remarkebly difference between the two formulations. The 90% confidence intervals also revealed that the ratios of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$, Cmax, Tmax, $t_{1/2}$ and Kel of the two formulations lie within the FDA acceptable range of 80%–125%.

Table-2: Demographic data for bioequivalence study of losartan among 16 volunteers

		Range
Age (years)	26.82 ± 3.97	18-35
Height (cm)	165.50 ± 4.11	160-180
Weight (Kg)	57.87 ± 5.79	50-75
BMI (kg/m^2)	21.13± 1.56	18-35
Gender	Male	
Race	Asian	

CONCLUSION:

The 90% confidence intervals for the LOSARDIL 100™ (test) and COZAAR™ (reference) were demonstrated within the acceptance range of 80–125%.

LOSARDIL 100™ (test) is bioequivalent to COZAAR™ (reference) in terms of absorption and can be used interchangeably in clinical setting.

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REFERENCES

1. Whitworth JA, World Health Organization, International Society of Hypertension Writing Group. WHO/ISH Statement on management of Hypertension. J Hypertens 2003;21:1983-92
2. Losartan Potassium Tablet Prescribing information. Lupin Pharmaceuticals, Inc. Revised: 02/2011 Available from: http://www.dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=e5886220-43b7-46e1-9034-5242ba245bd1).[Last revised on 2011 Feb 28]
3. Michael W. Clinical Safety and Tolerability of Losartan. Clin Ther 1977; 4:604-16.
4. Aghera NJ, Shah SD, Vadalia KR. Formulation and Evaluation of Sublingual Tablets of Losartan Potassium. Asian Pacific J Tropical Disease 2012; 2:S130-5.
5. Koytcheva R, Ozalpb Y, Erenmemisogluc A, Van der Meerd MJ, Alpanb RS. Combination of Losartan and Hydrochlorothiazide: In vivo Bioequivalence. Arzneimittelforschung 2004;54:611-7
6. Center for Drug Evaluation and Research (2003). "Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations" (PDF). United States Food and Drug Administration.
7. Midha KK, McKay G. Bioequivalence; Its History, Practice, and Future. AAPS J 2009; 11:664-70.
8. Shah VP, Midha KK, Dighe S, et al. Conference report: Analytical method validation: Bioavailability, bioequivalence and pharmacokinetic studies. Eur J Drug Metab Pharmacokinet 1991; 16: 249–255.
9. Kinetica TM2000. Version 3.0, Innaphase, User Manual, 1999.
10. Uesugi T. FDA. Bioanalytical method validation. Food and drug administration. Guidance for Industry. 2001:1–22;
11. Westlake WJ. Bioavailability, bioequivalence of pharmaceutical formulations. In Biopharmaceutical Statistics for Drug Development, Peace KE (ed.). Marcel Dekker: New
12. York, 1988; 329–352.