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Comparative Pharmacokinetics of Two Tablet Formulations of Losartan: Bioequivalence Assessment

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ABSTRACT: The pharmacokinetic profiles of two brands of losartan 50 mg tablets were compared in 24 healthy adult volunteers after a single oral dose in a randomized cross-over study. The study was conducted at the ACDIMA Center for Bioequivalence & Pharmaceutical Studies, Amman, Jordan. The reference (Cozaar, MSD, The Netherlands) and test (Blosart, Julphar, UAE) products were administered to fasting volunteers. Blood samples were collected at specified time intervals, and the plasma separated and analysed for losartan and its active metabolite (losartan carboxylic acid) using a validated HPLC method with fluorescence detection. Pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, elimination rate constant, *MRT*, Cl/*F* and *V*ss/*F* were determined from plasma concentration-time profiles of both formulations and found to be in good agreement with reported values. Three parameters (AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}) were compared statistically to evaluate the bioequivalence between the two brands, using statistical modules recommended by the FDA. Analysis of variance (ANOVA) did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range (80%–125%) for bioequivalence. Based on these statistical inferences it was concluded that the two formulations exhibited comparable pharmacokinetic profiles and that Julphar's Blosart is bioequivalent to Cozaar of MSD, The Netherlands. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: Losartan; bioequivalence; pharmacokinetics; HPLC; Julphar

Introduction

Losartan potassium, the first of a new class of antihypertensive agents, is an angiotensin II receptor (type AT_1) antagonist [1,2]. It is chemically described as 2-butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt.

Losartan is an effective, synthetic, orally active angiotensin II receptor antagonist [3,4]. Losartan and its principal active metabolite block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor found in many tissues (e.g. vascular smooth muscle, adrenal gland). Losartan differs from ACE inhibitors in that it does not inhibit ACE (kininase II), the enzyme that degrades bradykinin [5].

Losartan undergoes substantial first-pass metabolism by cytochrome P450 enzymes. It is converted, in part, to an active carboxylic acid metabolite (E-3174) that is responsible for most of the angiotensin II receptor antagonism that follows losartan treatment [4,5]. Following oral administration, losartan is well absorbed with a systemic bioavailability of approximately 33% [6–8]. The mean peak plasma concentrations of losartan and its metabolite are reached in 1 h and in 3–4 h, respectively [5,7,9]. While maximum plasma concentrations of losartan and its metabolite are approximately equal, the *AUC* of the

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metabolite is about 4 times as great as that of losartan [5,9]. Food slows the absorption of losartan and metabolite and decreases their C_{max} but has only minor effects on their *AUC*s [5]. Both losartan and its metabolite are highly (98.7%) bound to plasma proteins [8]. Upon oral administration, about 4% of the dose is excreted unchanged in the urine and about 6% is excreted in urine as active metabolite. Biliary excretion contributes to the elimination of losartan and its metabolites. Following oral ^{14}C-labelled losartan, about 35% of radioactivity is recovered in the urine and about 60% in the faeces [5,7,10]. The terminal half-life of losartan is about 2 h and that of the metabolite is about 6–9 h [5,8,10].

Objectives

The purpose of this study was to determine the bioequivalence of a new tablet formulation of losartan (Blosart 50 mg tablets) produced locally in United Arab Emirates by Gulf Pharmaceutical Industries-Julphar, in comparison with Cozaar from MSD, The Netherlands.

Materials and Methods

Study products

Test Product:	Blosart-Losartan 50 mg tablets
Batch No.:	0004 Manufacturing date: 06/00; Expiry date: 06/02
Manufacturer:	Gulf Pharmaceutical Industries-Julphar, UAE
Reference Product:	Cozaar-Losartan 50 mg tablets
Batch No.:	HM 68860 Manufacturing date: 10/00; Expiry date: 10/02
Manufacturer:	MSD, The Netherlands

Study design

Twenty-four healthy male volunteers completed this comparative pharmacokinetic study at Jordan Hospital, Amman, Jordan. Their mean age was 30.8 $\pm$ 5.9 years with a range of 21–40 years and the mean body weight was 73.6 $\pm$ 6.2 kg with a range of 60–85 kg. On the basis of medical history, clinical examination and laboratory investigation (haematology, blood biochemistry and urine analysis), no subject had evidence of hepatic, renal, gastrointestinal or haematological disorders or any acute or chronic diseases or drug allergy to sulfonylureas. The consumption of alcohol or beverages and food, containing methylxanthines was not permitted for the volunteers 48 h prior to the study and after drug administration until the last blood sample was collected in the respective study phase. Subjects were instructed to abstain from taking any medication for at least 1 week prior to and during the study period. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. The study protocols were approved by the Institutional Review Board (IRB) of Jordan Hospital.

Drug administration and sample collection

This study was based on a single dose, randomized, two treatments, two periods cross-over design. In the morning of phase I, after an overnight fasting (12 h) volunteers were given a single dose of either formulation (reference or test) of losartan 50 mg with 240 ml of water. No food was allowed until 4 h after dose administration. Water intake was allowed 2 h after the dose; water, lunch and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by Jordan Hospital Staff throughout the confinement period of the study. They were not being permitted to lie down or sleep for the first 4 h after the dose. Approximately 5 ml of blood for losartan assay was drawn into heparinized tubes through an indwelling cannula before (0 h) and at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12, 16, 24, 30 and 36 h after dosing. Blood samples were centrifuged at 4000 rpm for 10 min, the plasma was separated and kept frozen at -20°C until assayed. After a washout period of 7 days the study was repeated in the same manner to complete the cross-over design.

Measurement of losartan and its metabolite in the plasma samples

The concentrations of losartan and its carboxylic acid metabolite in human plasma were

determined by an HPLC method developed in the ACDIMA BioCenter Laboratories. After spiking 1 ml samples with 200 ng/ml of valsartan as internal standard, the drug was extracted using 10 ml *t*-methyl butyl ether and hexane mixture. The organic layer was separated and transferred into another tube containing 0.05 M NaOH. 100 µl of the aqueous fraction was injected into the HPLC column (Zorbax CN 5 µm; 250 × 4.6 mm) using a Waters autosampler (Milford, MA, USA).

The mobile phase was prepared by mixing 0.015 M phosphoric acid and acetonitrile (72:28, v/v), and a flow rate of 1.25 ml/min was found to give adequate resolution. Separations and determinations were monitored employing a Waters 4872 fluorescence detector operating at an excitation wavelength of 250 nm and an emission wavelength of 370 nm. The method was validated by following international guidelines [12].

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of a model independent method using a Kinetica™ 2000 computer program [13]. The elimination rate constant (λ_Z) 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 ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. 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 $AUC_{0-t}+C_t/\lambda_Z$, where C_t is the last measurable concentration. The mean residence time (*MRT*) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. $AUMC_{0-\infty}$ was calculated by standard methods. The *Cl*/*F* (oral plasma clearance was calculated as Dose/$AUC_{0-\infty}$. The volume of distribution (*V*ss/*F*) was calculated as $Cl \times MRT/F$.

Statistical analysis

For the purpose of bioequivalence analysis AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary variables. Two-way analysis of variance (ANOVA GLM procedure; Kinetica™ 2000 computer program [13]) for cross-over design was used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for *p*-value equal to or less than 0.05. Parametric 90% confidence intervals [14] based on the ANOVA of the mean test/reference (T/R) ratios of *AUC* and C_{max} were computed.

Results and discussion

Losartan was well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who started the study continued to the end and were discharged in good health.

The described analytical method was proven sensitive and accurate for the determination of losartan and its metabolite in plasma. Retention times were 14.5, 25 and 30 min for losartan, active metabolite and valsartan (internal standard), respectively. Under the conditions described, the limit of quantitation for losartan and metabolite was 1.0 ng/ml and the relationship between concentration and peak area ratio was found to be linear within the range 1–400 ng/ml. A stability study showed that drug and metabolite are stable in plasma for 9 weeks when stored at −20°C.

Both formulations were readily absorbed from the gastrointestinal tract and losartan was measurable at the first sampling time (0.33 h) in almost 50% of volunteers, while active metabolite was detectable after 0.67 h in some volunteers only while it was detectable in all volunteers after 1.67 h. The mean concentration-time profile for losartan for the two formulations is shown in Figure 1, while Figure 2 shows the mean concentration-time profile for active metabolite. Both figures indicate that the mean plasma concentration profiles of the two brands were closely similar and superimposable on the basis of parent drug as well as active metabolite. ANOVA was applied on the concentration attained at individual time intervals for both formulations, indicating no significant difference ($p > 0.05$). Peak concentrations of 233.7 ng/ml and 252.6 ng/ml for losartan were attained at

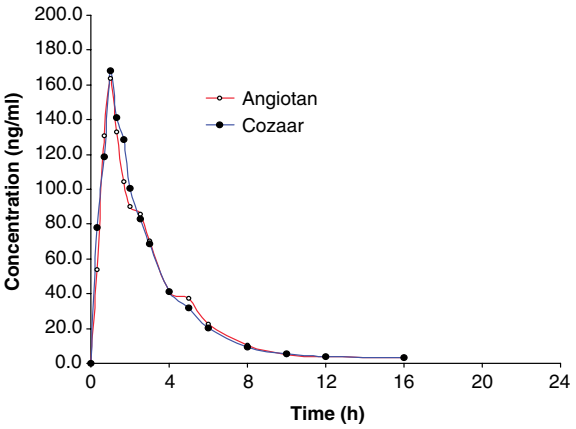

Figure 1. Mean plasma concentration of Losartan (parent drug) after oral administration of a single dose of two brands to 24 healthy human volunteers

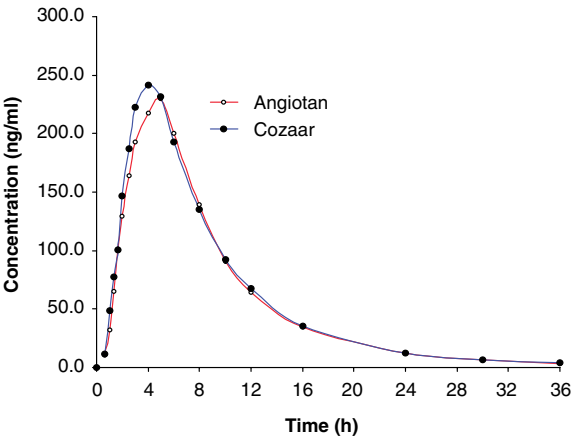

Figure 2. Mean plasma concentration of Losartan metabolite after oral administration of a single dose of two brands to 24 healthy human volunteers

1.36 and 1.34 h after drug administration and then declined rapidly and remained detectable until 16 h. Peak concentrations of 268.54 ng/ml and 283.2 ng/ml for active metabolite were attained at 4.4 and 3.8 h after drug administration and then declined rapidly but were detectable until 36 h. All calculated pharmacokinetic parameters were in good agreement with the published studies conducted in Western and Japanese populations [11,15,16].

Table 1 shows the pharmacokinetic parameters of losartan and metabolite for the two brands. The extent of absorption is a key characteristic of drug formulation, and therefore *AUC* is an important parameter for comparative bioavailability studies [17]. However, the other two parameters, C_{max} and T_{max}, are also important features of the plasma level profile and could affect the therapeutic use of a drug [18] and hence were also considered in the study.

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 [19]. To demonstrate bioequivalence certain limits should be set depending on the nature of the drug, patient population and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as *AUC* and C_{max}, the standard equivalence range is 0.8–1.25 [14,17]. The results of statistical analysis are shown in Table 2.

Table 1. Pharmacokinetic parameters of losartan and active metabolite (mean ± standard deviation; $n = 24$)

Pharmacokinetic parameter	Losartan (parent drug)		Active metabolite	
	Blosart (test)	Cozaar (reference)	Blosart (test)	Cozaar (reference)
AUC_{0-t} (ng/ml.h)	462.8 ± 144.3	480.8 ± 162.6	2078.3 ± 836.1	2148.2 ± 853.5
$AUC_{0-\infty}$ (ng/ml.h)	472.0 ± 144.0	490.5 ± 163.6	2113.1 ± 840.5	2181.8 ± 865.2
C_{max} (ng/ml)	233.7 ± 111.9	252.6 ± 102.6	268.4 ± 119.3	283.2 ± 127.1
T_{max} (h)	1.36 ± 0.71	1.34 ± 0.65	4.40 ± 1.40	3.80 ± 1.20
$T_{1/2}$ (h)	2.78 ± 1.26	2.84 ± 1.14	6.05 ± 0.94	6.01 ± 0.94
K_{elim} (h/h)	0.311 ± 0.156	0.283 ± 0.106	0.117 ± 0.019	0.119 ± 0.025
MRT (h)	3.48 ± 1.37	3.30 ± 1.29		
Cl/F (ml/min)	1929.5 ± 610.5	1933.6 ± 845.6		
Vss/F (l)	391.7 ± 152.9	360.2 ± 132.6		

Table 2. Statistical analysis of ln-transformed data

Pharmacokinetic parameter	ANOVA		90% CI	
	Losartan (parent drug)	Metabolite	Losartan (parent drug)	Metabolite
AUC_{0-t}	0.4071 (0.0523)	0.1919 (0.7511)	93.1–102.5% (100.0–111.1%)	92.1–101.1% (96.3–105.6%)
$AUC_{0-\infty}$	0.3819 (0.050)	0.2450 (0.8286)	93.4–102.2% (100.98–110.5%)	92.5–101.4% (96.1–105.3%)
C_{max}	0.0889 (0.7434)	0.2276 (0.9916)	80.5–99.6% (91.7–113.5%)	87.1–102.3% (92.2–108.3%)

Parenthesis values indicate analysis for periods.

For parent drug and active metabolite mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the blood profiles generated by Blosart are comparable to those produced by Cozaar. 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 or formulations, with p values greater than 0.05. 90% confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ or C_{max} of the two formulations lie within the FDA acceptable range of 80–125%.

For losartan, the absolute difference in T_{max} (test−reference) was −0.02 h, within the acceptance limits, while for the metabolite the difference was 0.6 h and again within the limits ($\pm$ 20% of reference mean).

Summary and Conclusion

Based on the statistical analysis (ANOVA and 90% CI) for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} it is concluded that Blosart, manufactured by Gulf Pharmaceutical Industries, UAE is bioequivalent to Cozaar, manufactured by MSD, The Netherlands, and that both products can be considered equally effective and safe in medical practice.

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