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Bioequivalence Evaluation of Two Brands of Enalapril 20 mg Tablets (*Narapril* and *Renitec*) in Healthy Human Volunteers

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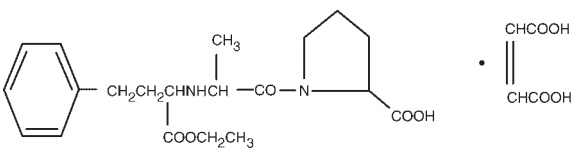
ABSTRACT: The bioequivalence of two brands of enalapril 20 mg tablets was demonstrated in 24 healthy human volunteers after a single oral dose in a randomized cross-over study, conducted at IPRC, Amman, Jordan. Reference (Renitec, MSD, Netherlands) and test (Narapril, Julphar, UAE) products were administered to fasted male volunteers; blood samples were collected at specified time intervals, plasma separated and analysed for enalapril and its active metabolite (enalaprilat) using a validated LC-MS/MS method at Cartesius Analytical Unit, Institute of Biomedical Sciences, USP, Sao Paulo, Brazil. The pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$ and elimination rate constant were determined from plasma concentration-time profile for both formulations and were compared statistically to evaluate bioequivalence between the two brands, using the statistical modules recommended by FDA. The analysis of variance (ANOVA) did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range for bioequivalence. Based on these statistical inferences it was concluded that the two brands exhibited comparable pharmacokinetic profiles and that Julphar's Narapril is bioequivalent to Renitec of MSD, Netherlands. Copyright © 2003 John Wiley & Sons, Ltd.

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

Introduction

Enalapril is an angiotensin converting enzyme inhibitor. In the liver it is converted to enalaprilat by the hydrolysis of the ethyl ester [1]. Enalapril maleate is the maleate salt of enalapril, the ethyl ester of a long-acting angiotensin converting enzyme inhibitor, enalaprilat. Enalapril maleate is chemically described as (*S*)-L-[*N*-[L-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (*Z*)-2-butenedioate salt (1:1). Its empirical formula is $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$, having the following structural formula [2].



Enalapril is a weak angiotensin-converting enzyme inhibitor; however, hepatic conversion of enalapril leads to enalaprilat, which is more potent than the parent drug. In its active form enalapril is a competitive inhibitor of angiotensin-converting enzyme (ACE; also known as

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kininase II) in human tissues with high affinity. ACE is a peptidyl dipeptidase that catalyses the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decreased aldosterone secretion. The isomerized enzyme–inhibitor complex has a slow rate of dissociation, which results in high potency and a long duration of action [3,4].

Enalapril maleate is well absorbed (53%–73%) from the gastrointestinal tract [4–6], while enalaprilat is poorly absorbed following oral administration [7]; food does not affect the oral absorption of enalapril [4,8]. Following oral doses of 5, 10, 20, and 40 mg of enalapril maleate, peak serum enalaprilat concentrations of 15, 37, 71 and 123 ng/ml, respectively, were observed between 3.2 and 4.8 h after administration. Peak concentrations of enalapril following these doses were similar to concentrations for enalaprilat, and occurred within the first hour after administration [9–11]. Enalapril binds moderately (50%–60%) with plasma proteins [12]. It is metabolized in the liver to enalaprilat in the first 4 h following oral administration [4,8,11,12]; first-pass metabolism of enalapril to enalaprilat is approximately 18% [12]. It is excreted mainly (60%) in the urine, 18% in the form of parent drug and 43% as enalaprilat [4,11,13]; faecal elimination is 6% as enalapril and 27% as enalaprilat [11]. The elimination half-life of enalapril is 1.3 h [13] while enalaprilat has a half-life of 11 h [4]. Although the pharmacokinetics of enalapril has been reported in many studies, very few of them focused on the bioequivalence issue. Therefore in the present work the bioequivalence was demonstrated of two brands of enalapril based on the plasma concentration of parent drug and its metabolite.

Objectives

The purpose of this study was to determine the bioequivalence of a new tablet formulation of enalapril (Narapril 20 mg tablets) produced in United Arab Emirates by Gulf Pharmaceutical Industries-Julphar, in comparison with Renitec from MSD, Netherlands.

Materials and Methods

Study products

The test product was Narapril (enalapril 20 mg tablets); batch no.: 0004; manufacturing date: 09/00; expiry date: 09/02; manufacturer: Gulf Pharmaceutical Industries, Julphar, UAE.

The reference product was Renitec (enalapril 20 mg tablets); batch no.: HN01020; manufacturing date: 09/00; expiry date: 03/03; manufacturer: Merck Sharp & Dhome (MSD), Netherlands.

Study design

Twenty-four healthy adult male volunteers participated in this comparative study at Al-Istiklal Hospital, Amman, Jordan, as a joint venture with the International Pharmaceutical Research Center (IPRC), Amman, Jordan. Their mean age was 23.25 ± 4.55 years with a range of 18–37 years; mean body weight was 73.38 ± 9.39 kg with a range of 54–89 kg and mean height was 175.96 ± 7.66 cm with a range of 162–193 cm. The volunteers did not have any significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal or haematological diseases, as determined by their medical history, physical examination and routine laboratory tests (haematology, blood biochemistry and urine analysis) and were negative for hepatitis B antigen. Subjects were instructed to abstain from taking any drug including over-the-counter (OTC) for 2 weeks prior to and during the study period. They were informed about the aim and risks of the study by the clinical investigator, based on which they signed a written informed consent statement before entering the study. The study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practices. Before the start of the study the protocol was approved by the Institutional Review Board (IRB) of Al-Istiklal Hospital.

Drug administration and sample collection

The study was designed as a single dose, randomized, two treatment, two-period crossover. In the morning of phase I, after an

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overnight fasting (10 h) volunteers were given a single dose of either formulation (reference or test) of enalapril 20 mg with 240 ml of water. No food was allowed for 5 h before the dose administration. Water intake was allowed from 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 Al-Istiklal Hospital Staff throughout the confinement period of study. They were not permitted to lie down or sleep for the first 5 h after the dose. Approximately 10 ml blood samples were drawn into heparinized tubes through indwelling cannula before (0 h) and at 0.25, 0.50, 0.75, 1.0, 1.33, 1.66, 2.0, 2.50, 3.0, 3.5, 4.0, 6.0, 8.0, 10, 12, 16, 24 and 48 h after dosing. Blood samples were centrifuged at 3500 rpm for 10 min, 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 crossover design.

Analysis of Enalapril and Enalaprilat

Sample preparation for HPLC injection. 50 µl of internal standard (mixture of enalapril-phenyl-d5/enalaprilat-phenyl-d5 at 0.20 µg/ml for both) was added to 500 µl plasma sample and vortexed for 15 s. 400 µl of washing solution (0.01 M hydrochloric acid) was added to the sample and then the sample was subjected to a solid phase extraction cartridge, washed five times with 3 ml washing solution, and then the sample was eluted with 0.5 ml of elution solution (10 mM formic acid in 80% acetonitrile: 20% water). The eluant was transferred to an autosampler vial and 40 µl was injected to a X-Terra C_8 (150 × 4.6 mm, 3.5 µm) HPLC column, on which enalapril, enalaprilat and the internal standards were separated from endogenous plasma substances.

Chromatographic conditions. Plasma samples were analysed for enalapril and enalaprilat by a validated LC-MS-MS method. All solvents used were of HPLC grade and were purchased from Merck, Germany; while other chemicals and reagents were of analytical grade. Enalapril and enalaprilat were obtained from USP, USA, while both internal standards were obtained from CDN isotopes, Canada.

The LC-MS-MS consisted of a liquid chromatograph, Agilent 1100 series, Model G1312A, degasser, Agilent 1100 series, Model G1322A, an auto-injector CTC Analytics, Model MXY01-01B, column oven, Shimadzu, Model CTO 10Avp and a Micromass Quattro LC with an electroscopy source mass spectrometer (Micromass, UK); integration was done using Masslynx software version 3.5 (Micromass, UK). Chromatographic separation was performed using an X-Terra C_8 (3.5 µm) (150 × 4.6 mm) column from Waters, USA. The mobile phase consisted of 10 mM formic acid in 60% acetonitrile and 40% water and eluted at a flow rate of 0.5 ml/min; the oven temperature was set at 35°C. Detection was done at MRM of 377.36>239.23 for enalapril, MRM of 349.32>206.24 for enalaprilat, MRM of 282.26> 239.23 for enalapril-phenyl-d5 and MRM of 354.28>211.26 for enalprilat-phenyl-d5. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Masslynx software. The method was validated by following international guidelines [14].

Pharmacokinetic analysis. Pharmacokinetic analysis was performed by means of a model independent method using a Kinetica™ 2000 computer program [15]. 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.

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; KineticaTM 2000 Computer program [15], for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. The difference between two related parameters was considered to be

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statistically significant for a *p*-value equal to or less than 0.05. Parametric 90% confidence intervals[16] based on the ANOVA of the mean test/reference (T/R) ratios of *AUC*s and C_{max} were computed.

Results and Discussion

Enalapril was well tolerated by all 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.

Under the conditions described, the lower limit of quantitation from 500 µl plasma was 0.50 ng/ml for enalapril and enalaprilat. The relationship between the concentration and peak area ratio was found to be linear within the range 0.50–400 ng/ml for both parent drug and metabolite. The intra-day accuracy of the method for enalapril ranged from 94.86% to 102.1%, while the intra-day precision ranged from 2.21% to 12.04%. The inter-day accuracy for enalapril ranged from 96.00% to 101.53%, while the inter-day precision ranged from 3.01% to 8.98%. The intra-day accuracy for enalaprilat ranged from 96.20% to 98.80%, while the intra-day precision ranged from 2.30% to 11.13%. The inter-day accuracy for enalaprilat ranged from 98.51% to 99.44%, while the inter-day precision ranged from 3.73% to 9.86%. Absolute recoveries were 96.29% and 93.56% for enalapril and enalaprilat; relative recoveries ranged from 98.05% to 101.30% for enalapril, and from 97.15% to 98.04% for enalaprilat. The stability study showed that both parent drug and active metabolite were stable in plasma for 6 months when stored at −20°C. Different methods have been reported for the determination of enalapril and enalaprilat in pharmacokinetics studies [17,18], and pharmacologic response (ACE inhibition) has been used as a marker for serum concentration of the drug[19]. The method used in this study was found to be reliable, accurate, sensitive and rapid for detecting plasma levels of enalapril and enalaprilat simultaneously.

Both formulations were readily absorbed from the gastrointestinal tract and enalapril was measurable at the first sampling time (0.25 h) in all volunteers, while the active metabolite was detectable after 0.75 h. The mean concentration-time profile of enalapril and enalaprilat for the two formulations is shown in Figure 1. The figure indicates that the mean plasma concentration profiles of the two brands were closely similar and superimposable. Peak concentrations of 123.99 ng/ml and 121.28 ng/ml for enalapril were attained at 0.86 and 0.96 h respectively, after drug administration and then declined rapidly and was detectable up to 12 h only. Peak concentrations of 54.41 ng/ml and 53.87 ng/ml for enalaprilat were attained at 3.92 and 3.71 h, respectively, after drug administration and then declined slowly and was detectable up to 48 h.

Table 1 shows the pharmacokinetic parameters of enalapril and enalaprilat for two brands. The extent of absorption is a key characteristic of drug formulation and, therefore *AUC* is an important parameter for comparative bioavailability studies [20]. However, the other two parameters, C_{max} and T_{max}, are also important features and could affect the therapeutic behaviour of a drug [21] and hence were also considered in the study. The relative bioavailability of Narapril on the basis of the parent drug was 103.38% for AUC_{0-t}, 103.31% for $AUC_{0-\infty}$ and 105.34 for C_{max}. On the basis of the active metabolite the relative bioavailability was

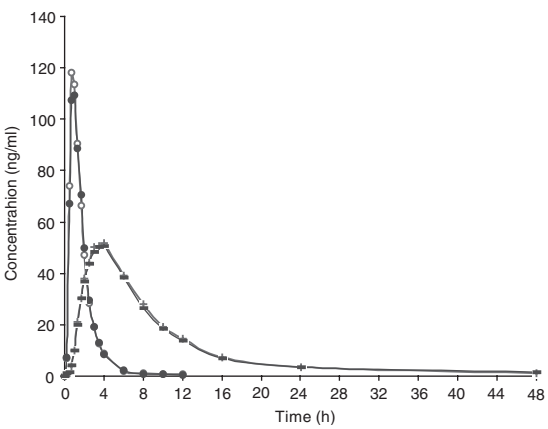

Figure 1. Mean plasma concentration of enalapril and enalaprilat (active metabolite) after oral administration of single dose of two brands to 24 healthy human volunteers —○— narapril parent drug, —●— renitec parent drug, —+— narapril active metabolite, —■— renitec active metabolite

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Table 1. Pharmacokinetic parameters of enalapril and enalaprilat for two brands (mean ± standard deviation, n=24)

Pharmacokinetic parameter	Narapril 20 mg tablets (test)		Renitec 20 mg tablets (reference)	
	Enalapril	Enalaprilat	Enalapril	Enalaprilat
AUC_{0-t} (ng/ml.h)	199.96 ± 67.77	492.94 ± 148.77	196.78 ± 63.57	476.89 ± 117.47
$AUC_{0-\infty}$ (ng/ml.h)	201.72 ± 67.68	518.36 ± 148.91	198.51 ± 63.32	505.54 ± 115.97
C_{max} (ng/ml)	123.99 ± 43.77	54.41 ± 23.50	121.28 ± 37.54	53.87 ± 24.12
T_{max} (h)	0.86 ± 0.16	3.92 ± 1.21	0.96 ± 0.30	3.71 ± 0.91
$T_{1/2}$ (h)	1.28 ± 0.72	11.79 ± 4.83	1.24 ± 0.50	12.35 ± 5.66
λ_Z (h)	0.65 ± 0.26	0.07 ± 0.04	0.65 ± 0.24	0.07 ± 0.04

Values are given as ± standard deviation.

Table 2. Statistical analysis of Ln-transformed data of enalapril and enalparilat

Statistical analysis	AUC_{0-t}		$AUC_{0-\infty}$		C_{max}	
	Enalapril	Enalaprilat	Enalapril	Enalaprilat	Enalapril	Enalaprilat
ANOVA GLM (p-value)	0.8386	0.7160	0.8353	0.8396	0.8965	0.7513
90% CI Lower	94.43	94.27	94.51	93.69	91.81	93.26
Upper	107.57	109.55	107.49	108.64	110.48	110.72

104.84% for AUC_{0-t}, 103.79% for $AUC_{0-\infty}$ and 105.71% for C_{max}.

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 [22]. 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 for basic pharmacokinetic characteristics, such as AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} the standard equivalence range is 0.8–1.25 [16]. The results of statistical analysis are shown in Table 2.

For the parent drug and its active metabolite, mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two formulations did not differ significantly, suggesting that the blood profiles generated by Narapril are comparable to those produced by Renitec. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations, with a p value greater than 0.05. 90% confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} of the two formulations lie within the FDA acceptable range of 80%–125%.

In the case of enalapril the absolute difference in T_{max} (test – reference) was 0.04 h, and found to be within the acceptance limits ± 20% of the reference mean; for the active metabolite the T_{max} difference was 0.21 h (within ± 20% of reference mean).

Plasma levels may be used as surrogate parameters for clinical activity; therefore the results of this study suggest equal clinical efficacy of the two brands of enalapril.

Summary and Conclusion

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for enalapril and enalaprilat clearly indicated no significant difference between Narapril and Renitec tablets in any of the assessed pharmacokinetic parameters. The confidence intervals for the ratio of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} indicated that these values were entirely within the bioequivalence acceptance range (using log-transformed data). Based on the above it can be concluded that Narapril, manufactured by Gulf Pharmaceutical Indus-

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tries, UAE is bioequivalent to Renitec, manufactured by MSD, Netherlands, and that both products can be considered equally effective in medical practice.

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