PHARMACODYNAMICALLY-EVALUATED BIOEQUIVALENCE OF TWO PREPARATIONS OF ENALAPRIL MALEATE

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ABSTRACT

The bioequivalence of two preparations of enalapril maleate (20 mg tablets) manufactured in Iran has been exploited in reference to a standard preparation (Xanef 20 tablets, MSD, Germany) in 14 healthy volunteers. Following oral dosing of a single tablet of each of test and standard products, as a randomized crossover design with 10-day washout intervals, the blood samples were collected in predetermined time points and using a synthetic substrate, Hippuryl-Histidy-Leucine (HHL), the release of hippuric acid from the substrate was determined as Angiotensin-Converting-Enzyme (ACE) activity of serum fractions. The percent of ACE inhibition in each sample was calculated and plotted against time, from which three pharmacodynamic parameters, i.e. E_{max}, t_{max} and AUC_{0-24}were derived. The results of statistical comparison of these parameters showed that both of the test preparations are bioequivalent with reference standard preparation.

Key words: Enalapril maleate, Bioequivalence, ACE, ACE inhibitors, Clinical study

INTRODUCTION

Nowadays, the cardiovascular diseases are prevalent remarkably and, as a result, the importance of efficient drug therapy for the management of these diseases is clear. Angiotensin converting enzyme (ACE) inhibitors are a group of effective antihypertensive drugs with unique mechanism of action (1). In addition, these drugs have proven to be useful in management of congestive heart failure (CHF) (1,2). Enalapril is a prodrug of enalaprilat, which can not be used orally (3). In contrast, enalapril has an acceptable degree of GI absorption (about 60%) (4, 5) which is not influenced by the presence of food (6) and once absorbed, is rapidly changed to an active metabolite, enalaprilat, mainly by liver esterases (3). Because of the some physicochemical properties, determination of enalapril in biological fluids which is necessary for it's pharmacokinetic studies, is very problematic (7) and, therefore, the activity of ACE in serum which has a remarkable degree of correlation with serum concentration of drug (3, 8, 9, 10), can be used as an indirect measure of serum concentrations of the drug. In the present study, the bioequivalence of two preparations of enalapril maleate produced in Iran has been evaluated using serum ACE inhibition as a quantitative pharmacodynamic parameter.

MATERIALS AND METHODS

Materials

Enalapril maleate (Farmhispania Co., Spain) and enalapril maleate USP reference standard (U.S.P.C. Inc., MD, USA) were kindly donated by Dr. Abidi Pharmaceutical Co. (Tehran, Iran). Enalapril maleate 20 mg tablets (Dr. Abidi Pharmaceutical Co., Tehran, Iran, batch no LO3-10-76) and Sobhan Pharmaceutical Co., Rasht, Iran, batch no 001) and Xanef 20 mg tablets (MSD, Germany, batch no HJ 65610) were available locally. Hippuric acid (Sigma Co., Art. no. H-6375) and Hippuryl-L-Histidyl-L-Leucine (Sigma Co., Art no. H-1635) were purchased from Sigma Co. Other chemicals and solvents were of chemical laboratory or HPLC grades and were available locally.

Preparation of buffered solution of HHL

94 mmoles of boric acid and 714 mmoles of KCl were dissolved in 400 ml of distilled water and the pH of solution was adjusted to 8.3 (optimal pH of ACE activity) by addition of 1M KOH solution and the volume was adjusted to 500 ml by the addition of distilled water. In 1 ml of the prepared buffered solution, 4 µmoles (approximately 1.75 mg) of HHL was dissolved.

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Table 1. The demographic data of 14 healthy volunteers

Subject no.	Age (year)	Weight (kg)	Height (cm)	Serum ACE Activity (U/L)
	33	63	172	15.16
2	27	80	175	21.66
3	30	67	180	12.50
4	26	65	171	10.08
5	26	68	170	7.75
6	27	57	164	7.50
7	24	63	172	13.08
8	32	76	176	6.58
9	22	73	182	11.66
10	28	59	160	15.33
11	32	68	176	13.16
12	23	60	174	15.83
13	23	60	172	12.66
14	33	78	177	10.41
Mean (SD)	**27.6 (3.9)**	**67 (7.4)**	**173 (5.8)**	**12.38 (4.0)**

Determination of ACE Activity

ACE activity of the serum samples were determined using a synthetic, substrate Hippuryl Histidyl Leucine (HHL). To 20 µl of serum, was added 100 µl of buffered solution of HHL and the resulting mixture was vortexed for15 Sec. After incubation at 37 °C for 30 min, the reaction was terminated by the addition of 10 µl of perchloric acid (60%) and the mixture was vortexed for 10 sec and was centrifuged at 3000g for 5 min. Finally, 20 µl of the supernatant was injected to chromatograph in order to determine the released hippuric acid, and 1 µmole of hippuric acid which was produced in 1 min under the reaction condition was defined as a unit of ACE activity.

HPLC method

A reversed-phase HPLC method was used for determination of the hippuric acid released upon action of ACE on HHL. The mobile phase consisted of a mixture of the aqueous solution of potassium dihydrogen phosphate (10 mM) and methanol (80:20) with a final pH of 3 and was pumped by a double-reciprocating pump (Waters, USA, model 600) at a flow rate of 2 ml/min. A C_{18} Column (µ-Bondapack, 300 x 3.9 m.m., waters, USA) was used for separation at room temperature. The detection was made by a UV-detector (Waters, USA, model 486) at wavelength of 228 nm. A rheodyne injection device (Rheodyne, USA) with a 20 µl loop was used for injection and the peak heights were determined as the method response. A set of experiments were performed for validation of the method using solutions of hippuric acid with the known concentrations of 0.025, .05, 0.1, 0.2, 0.4, 0.8 and 1 mM in buffered solution instead of the substrate buffered solution.

Subjects

14 male non-smoker volunteers were participated in the study after passing the medical examinations and clinical laboratory tests and completing the informed consent. They had not taken any medications for at least one week before and during the study. The demographic data of the subjects are shown in table 1.

Study design

The bioequivalence study was performed as a double-blind, three-treatment, three-period, six-sequence randomized crossover design with 10-day washout periods. Single 20 mg tablets of test or reference preparations were given to overnight fasted subjects. Then, at 0,0.5, 1,1.5, 2, 2.5, 3, 3.5, 4, 6, 9, and 24 hours following the drug administration, 0.5 ml blood samples were withdrawn by venipuncture and the serum faction was used for determination of ACE activity.

Pharmacodynamic parameters

The percent of ACE inhibition which was calculated as follows, was used as a quantitative effect (E) of the drug and was plotted against time:

%ACE inhibition = [1- (ACE activity in the given time / ACE activity in the zero time) X 100]

Then, the values of maximum ACE inhibition (E_{max}) the time corresponding to the percent of inhibition

(t_{max}) and the area under the resulting 'effect-time' curve (AUC) were determined as three pharmacodynamic parameters in each subject for each product.

Statistical tests

As a standard protocol, using the "GraphPad Instat tm V.2" statistical software (Dr. Soliman, Florida A&M University, USA) ANOVA test was used to evaluate the bioequivalency of three preparations. For this purpose, each of three pharmacodynamic parameters obtained after administration of preparations to individual subjects were compared statistically for significance of the differences between the corresponding mean values at the significance level of 5%. In addition, the statistical 90% confidence intervals of the mean F values (relative bioavailability) of each parameter were determined using "Microsoft Excel 97"

RESULTS AND DISCUSSION

The results of the validation tests for the analysis of the method are summerized in table 2. From these data, it is obvious that the used method has a considerable degree of the precision, accuracy and a desirable range of applicability. The average serum ACE inhibition-time curves for test and reference preparations in 14 normal subjects are shown in Fig.1. Due to the difficulties in direct determination of biological concentrations of enalapril and its active metabolite, enalaprilat, (7) the ACE activity in biological fluids was determined using a synthetic substrate, HHL, as a suitable indicator for active metabolite concentration. This pharmacodynamic parameter which has a significant correlation with enalaprilat concentration in serum (3, 8-10) can be used successfully for evaluation of the pharmacokinetics of the drug. As it is obvious from Fig.1, the percent of serum ACE inhibition increased up to about 2 hours following drug intake which corresponds to more than 90% inhibition. A plateau in ACE inhibition-time curve was seen between 2 and 10 hours followed by a decrease in trend up to the final sampling time, i.e. 24 hours. The inter-subject variations in ACE activity were remarkable and were thought to be due to the individual differences in GI absorption o f the drug, the inter-subject variations in the ACE activity, the drug tissue distribution, substrate binding, kinetic behaviour and recons-titution of the activity of the enzyme. As it can be seen in Fig.1, the percent of ACE inhibition is relatively high (about 75%) even after 24 hours of drug intake. This finding is consistent with the other reports (3, 9) and shows that due to the relatively long terminal half-life of enalaprilat, recons-titution of serum ACE activity takes place several hours following drug administration. In addition, because of the unpredictable trend of the curve beyond the sampling period, calculation of the AUC by extrapolation of the terminal descending phase may be erratic and, as a result, only AUC between zero and 24 hours was used in this study.

Table 2. The summarized results of the validations tests carried out on the HPLC method for determination of hippuric acid

Test	Result
Linearity range (r^2)	0.025-0.1 mM (0.998)
Limit of detection	0.0125 mM
Absolute recovery*	93.09 ± 6.12%
Within-day variations*	5.92 ± 1.98%
Between-day variations*	5.76 ± 2.16%

* The values in the table are the mean of 6 measurements made throughout the whole linear range

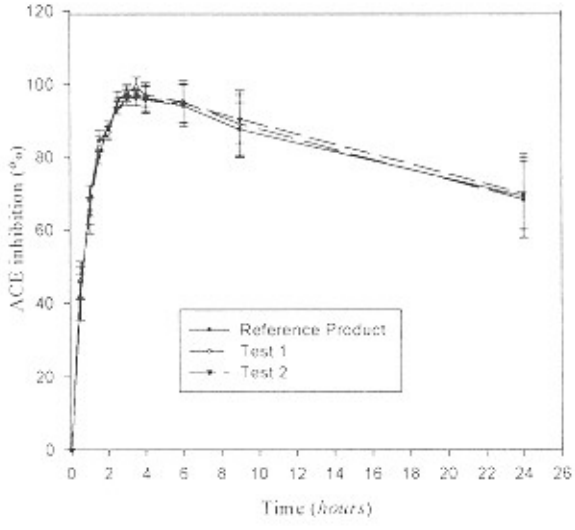

Figure 1. Average serum ACE inhibition-time profiles in 14 healthy volunteers upon administration of a single 20 mg tablet of test and reference preparations. Each point represents mean±SD of 14 samples.

The pharmacodynamic parameters of enalapril following administration of both tests and reference preparation in all subjects are shown in table 3. The relatively high inter-subject variations in drug pharmacodynamics, as previously discussed, also can be concluded from this table (see S.D. values). The results of the statistical ANOVA test showed no significant difference between the preparations

Table 3. Pharmacodynamic parameters of enalapril maleate following administration of test and reference preparations to 14 healthy volunteers

Subject no.	E_{max} (%)			t_{max} (hours)			AUC_{0-24} (%hours)		
	Test1	Test2	Ref.	Test1	Test2	Ref.	Test1	Test2	Ref.
1	93.80	94.01	96.45	3.0	3.0	4.0	2106.30	1904.22	1980.77
2	96.00	99.15	98.22	2.5	2.0	2.0	1983.68	2026.65	1536.92
3	95.30	96.99	89.73	3.0	3.0	3.0	2043.07	1904.69	1887.36
4	97.80	100.00	100.00	3.0	2.0	2.5	2077.46	2260.02	2198.87
5	92.90	100.00	100.00	2.5	1.5	2.0	2009.09	1487.55	1528.79
6	100.00	89.84	97.20	4.0	6.0	6.0	1911.08	1879.09	1994.28
7	91.00	100.00	100.00	3.0	2.0	2.5	1758.87	2028.97	1673.95
8	95.40	98.71	95.06	4.0	6.0	4.0	1853.17	1997.03	1953.38
9	99.20	100.00	100.00	2.0	1.5	1.5	2007.33	1988.14	2179.16
10	98.10	94.38	91.58	6.0	4.0	4.0	1992.74	2057.36	1816.72
11	97.20	100.00	96.06	3.0	4.0	6.0	1951.54	2079.50	1897.76
12	88.70	96.82	98.55	4.0	2.5	4.0	1880.04	1553.39	1984.28
13	97.20	92.24	96.95	3.0	2.5	3.0	1882.21	1964.15	1989.80
14	96.60	97.25	97.70	3.0	3.0	3.0	1907.24	1689.90	1975.59
Mean	**95.66**	**97.10**	**96.96**	**3.29**	**3.07**	**3.39**	**1954.56**	**1915.76**	**1899.83**
S.D.	**3.15**	**3.31**	**3.13**	**0.97**	**1.47**	**1.38**	**95.08**	**209.76**	**202.72**

Table 4. Relative Pharmacodynamic parameters (F values) of enalapril maleate following administration of test and reference preparations to 14 healthy volunteers

Subject no.	FE_{max}		Ft_{max}		$FAUC_{0-24}$	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	0.97	0.98	0.75	0.75	1.06	0.96
2	0.98	1.01	1.25	1.00	1.29	1.32
3	1.06	1.08	1.00	1.00	1.08	1.01
4	0.98	1.00	1.20	0.80	0.94	1.03
5	0.93	1.00	1.25	0.75	1.31	0.97
6	1.03	0.92	0.67	1.00	0.96	0.94
7	0.91	1.00	1.20	0.80	1.05	1.21
8	0.90	1.04	1.00	1.50	0.94	1.02
9	0.99	1.00	1.33	1.00	0.92	0.91
10	1.07	1.03	1.50	1.00	1.10	1.13
11	0.91	1.04	0.50	0.67	1.03	1.10
12	0.90	0.98	1.00	0.63	0.95	0.78
13	1.00	0.95	1.00	0.83	0.95	0.99
14	0.99	0.99	1.00	1.00	0.97	0.86
Mean	**0.97**	**1.00**	**1.05**	**0.91**	**1.04**	**1.02**
S.D.	**0.06**	**0.04**	**0.27**	**0.22**	**0.12**	**0.14**
CI* 90%	**0.94-1.00**	**0.98-1.02**	**0.93-1.17**	**0.81-1.01**	**0.99-1.09**	**0.96-1.08**

*. Confidence Interval 90%

(P>0.4 in all of cases). In addition, the F values for three parameters in each subject and the corresponding 90% confidence intervals for three parameters are shown in table 4. By referring to these data one can conclude that since the 90% confidence intervals of all of the pharmacodynamic parameters complies with the FDA requirements, i.e. 0.8-1.2, both test preparations are bioequivalent with reference preparations.

CONCLUSION

Two preparations of enalapril maleate (20 mg tablets) produced in Iran were tested for bioequivalency with respect to a standard preparation. As a result of the lack of a common method for direct determination of enalapril and/or its active metabolite, enalaprilat, in biological fluids, on the basis of FDA guideline an acute and quantifiable pharmacodynamic effect of this drug, i.e. percent of ACE inhibition, was determined by a HPLC method and was used for indirect evaluation of bioavailability of drug from three preparations. Statistical evaluation of E_{max}, T_{max} and AUC_{0-24} of drug following administration of preparations showed that both of test preparations are bioequi-valent with reference product.

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REFERENCES

1. Jackson, E.K., Garrison, J.C. (1996) Renin and angiotensin. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon,R.W. (eds.) The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, pp. 733-758.
2. Opie, L.H (1992)., ACE inhibitors for congestive heart failure. In: Angiotensin-Converting Enzyme Inhibitors: Scientific Basis for Clinical Use, Willey-Liss, New York, pp. 107-136.
3. Kubo, S.H., Cody, R.J. (1985) Clinical pharmacokinetics of the Angiotensin Converting Enzyme inhibitors. Clin. Pharmacokinet. 10: 377-391.
4. Vertes, V., Haynie, R. (1992) Comparative pharmacokinetics of captopril, enalapril, and quinapril. Am. J. Cardiol. 69: 8C-16C.
5. Irvin, J.D., Till, A.E., Vlasses, P.H., Hickens, M., Rotmensch, H.H., Harris, K.E., Merrill, D.D., Ferguson, M.D. (1984) Bioavailability of enalapril maleate. Clin. Pharmacol. Ther. 33: 248-252
6. Swanson, B.N., Vlasses, P.H., Ferguson, P. K., Bergquist, P. A., Harris, K. (1984) Influence of food on the bioavailability of enalapril. J. Pharm. Sci. 73: 1655-1657.
7. Ip, D.P., Brenner, G.S. (1987) Enalapril maleate. In: Florey, K. (ed.) Analytical Profiles of Drug Substances. Vol.16., Academic Press, London. , pp. 207-243.
8. Belz, G.G., Kirch, W., Kleinbloesem, C.H. (1988) Angiotensin-Converting Enzyme inhibitors. Clin. Pharmacokinet. 15: 295-318.
9. Biollaz, J., Schelling, J.L., Jacot Des Comes, B., Brunner, D.B., Desponds, G., Brunner, H.R. (1982) Enalapril maleate and a lysine analogue (MK- 521) in normal volunteers: relationship between plasma drug levels and the renin angiotensin system. Brit. J. Clin. Pharmacol. 14: 363-368.
10. Millar, J.A., Derkx, F.H.M., McLean, K., Reid, J.L. (1982) Pharmacodynamics of converting enzyme inhibition: the cardiovascular, endocrine and autonomic effects of MK 421 (enalapril) and MK 521. Brit. J. Clin. Pharmacol. 14: 347-355.