Korean J. of Pharmacology
Vol. 26, No. 2, pp. 219-226, 1990

Comparison of Enalapril Maleate Tablets on Bioavailability and the Time Course of Inhibition of Plasma Angiotensin-Converting Enzyme

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ABSTRACT

Enalapril maleate tablets of two different producers were tested for bioequivalence. Enalapril is rapidly metabolized to an active metabolite, enalaprilat which inhibits angiotensin-converting enzyme (ACE). The pharmacokinetics of enalapril maleate and the time course of inhibition of plasma ACE activity after administration of the drugs were studied. Two single doses of 10mg each of enalapril maleate were administered orally to twelve male volunteers in a balanced, randomized, two-way crossover investigation. Plasma enalaprilat concentrations were determined over a 23-hour after the dose by enzyme inhibition assay and enalapril by the same method following *in vitro* hydrolysis. Urinary recoveries of enalapril and enalaprilat were determined for the calculation of renal clearance. Plasma ACE activity was determined by an enzyme assay. Peak plasma levels of enalapril were observed about 1 hour after the doses, and practically all enalapril had disappeared from plasma within 6 hour. Peak enalapril concentrations of both formulations were almost identical (Vasotec®, 61.38 ng/ml; Beartec®, 64.27 ng/ml). The values of the pharmacokinetic parameters of enalaprilat computed for Vasotec® and Beartec® tablets are presented in that order; area under the curve = 330.63:320.96 ng·hr/ml; peak concentration = 38.63:39.43 ng/ml; time to peak = 3.83:4.08 hour; elimination half-life = 3.95:3.92 hours. No statistically significant difference was detected when area under the curve and all other parameters were compared. Using criteria of 95% confidence interval for the comparison of these parameters, only the upper limits of area under the curve and time to peak of enalapril were over 120%. All the parameters of enalaprilat were acceptable. Percent inhibition of plasma ACE to plasma enalaprilat concentration showed the sigmoid concentration-inhibition relationship. Time courses of plasma ACE inhibition after the administration of both formulations were quite similar.

The formulations were found to be equivalent when compared on the premise that no significant difference was detected when pharmacokientic parameters and inhibition of ACE activity were compared, based on the confidence limits analysis.

Key Words: Enalapril, Enalaprilat, Bioequivalence, Bioavailability, Angiotensin-Converting Enzyme Activity, Pharmacokinetics

INTRODUCTION

The concept of bioavailability is 45 years old. It first appeared in 1945 when researchers at US War Food Administration discussed that various multivitamin preparations did not deliver the same amount of their content *in vivo*. Thereafter, Interest had been increased on the topic of bioequivalence of generic products as a indirect evidence of therapeutic equivalence. However, the regulations of the drug products; Bioequivalence requirement and

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in vivo Bioavailability Procedures took effect on February, 1977 in America (Fed Reg, 1977). These regulations specify that all NDAs and ANDAs must demonstrate *in vivo* bioavailability of the drug product that is the subject of the application followed by an *in vitro* test. After then, most developed and developing countries including Korea followed the bioequivalence regulation of United States.

Enalapril maleate, a prodrug of the angiotensin converting enzyme (ACE) inhibitor, is hydrolyzed *in vivo* to enalaprilat, which is an extremely potent inhibitor of converting enzyme (Hymann, 1980, Williams, 1985). Enalapril maleate is an effective antihypertensive and can be also useful in the treatment of congestive heart failure. Enalapril maleate is rapidly absorbed, but extensively hydrolyzed to enalaprilat by 1st pass-effect. The average urinary recovery of enalaprilat and total drug (enalaprilat plus enalapril) is known to be approximately 40% and 60% of the administered enalaprilat equivalent, respectively (Johnston *et al.*, 1983).

Two enalapril maleate products are approved for marketing in Korea. One is the new generic product from his own synthetic procedure developed in Korea and the other product linsenced from original manufacturer. The above findings, high 1st-pass effect and incomplete bioavailability, has been suggested that altered bioavailability may likely to occur with different formulation, and lead us to compare the bioavailability as well as pharmacological effect of Korean generic product of enalapril maleate with the reference product, to be sure the bioequivalence or therapeutic equivalence of the new generic product. The purpose of this study also include to analyze the relationship of plasma enalaprilat concentration-ACE inhibition-hypotensive effect after single oral administration of enalapril maleate.

METHODS

Subjects and study design

Subjects: Twelve healthy male volunteers (age, 22 ± 2 years; weight, 64.8 ± 4.4kg), participated in the open balanced randomized cross-over study after screening by history, physical examination, urinalysis, ECG and routin laboratory test of hematology and serum biochemistry to ensure good health. The study protocol was approved by the Institutional Review board of Seoul National University Hospital and written informed consent was obtained from each subject before starting the study.

Study design: After a drug free period of at least one week, subjects were randomly assigned to receive either a single 10mg dose of Beartec® (Boryung Pharmaceutical Co., 10mg tablet) or Vasotec® (Merck Sharp & Dohm, 10mg tablet) in a cross-over two period study after a 12 hour fast. There was a seven-day washout period between medications. Water (200ml) was taken with both mediations and again two hours after mediation. Food intake was permitted 4 hours after the dose.

Blood samples (7ml) were collected through intravenous heparin-locked (100 unit/ml) catheter at 0, 0.25, 0.5, 1,1.5, 2, 3, 4, 5, 6, 8, 10, 12, 23 hours after adminisration of the dose. The plasma of the collected blood were seperated in refrigerated centrifuge at 2,000 rev/min for 10 min and stored at $-20°C$ until the mearement of drug concentration and the plasma ACE activity. Urine was collected upto 12 hours after dosing and stored at $-20°C$ until the assay of drug level.

Subjects were observed in the research unit for at least 12 hours after drug dosing and were monitored for the changes of blood pressure, heart rate, respiration rate, and adverse reactions. Blood pressure and heart rate in sitting position were measured in triplicate and the average values were recorded.

Measurement of enalapril and enalaprilat level in biological fluid

The concentration of enalaprilat in plasma and urine was determined by the degree of inhibition of angiotensin-converting enzyme described by Tocco *et al.* (1982). Spectrophotometric assay was that of Makato *et al.* (1978). Converting enzyme was isolated from rabbit lung by the method of Cushman and Cheung (1971) and diluted with 0.2M phosphate buffer (pH 8.0) to obtain 50% substrate hydrolysis at 30 min in controls.

Biological samples (0.1ml) were diluted with 0.4ml of methanol to precipitate endogenous ACE and other proteins, then centrifuged at high speed (>10,000g) for 2 min in Eppendorf centrifuge. For the simultanous mesurement of enalaprilat and enalapril level in the biological fluid, the supernatants were either directly diluted with assay buffer or first subjected to treatment with 0.5N NaOH (2:1 mixture of sample and NaOH incubated for 1 hour at 37°C), then neutralized with HCl prior to dilution. Diluted samples and drug standards (0.1ml) mixed with 0.1ml phosphate buffer (pH 8.0) were preincubated at 37°C for 5 minutes after adding 0.1ml of

rabbit lung ACE solution. After then, the reaction was started by adding 0.15ml of the substrate solution, 6.7mM Hippuryl-Histidyl-Leucin (50mM HEPES buffer; pH 8.3 in 0.3M NaCl). After 30 min incubation, the reaction was stopped by transfering the reaction tube into the boiling water bath for 10 min. Phase seperation was completed by centrifugating the tubes at 3000 rev/min for 5 min after vertexing with 3ml of 0.2M phosphate buffer (pH 8.0) and 1.5ml of 3% 2,4,6-trichloro-s-triozine. The absorbance of the supernatant was measured at 380nm. Detection threshold of the method was 2 ng/ml and coefficients of variation at the concentration of 10 ng/ml and 40 ng/ml were 6.3 and 4.1%, respectively.

Measurement of plasma angiotensin-converting enzyme activity

Plasma ACE activity was quantified using the technique described by Makato *et al.* (1978), using hippuryl-histidyl-leucine (HHL) as a substrate for antigotensin-converting enzyme. The interassay coefficient of variation of this method was 6.8%. The activity of ACE was expressed as in unit, whears 1 unit correspond to the activity to hydrolyze 1 nmole HHL per mililiter of plasma for 1 minute. For each plasma sample, the percentage of ACE inhibition relative to the activity before drug administration was calculated as follows;

$$\left[1 - \frac{ACE_t}{ACE_o}\right] \times 100$$

in which ACE_t is ACE activity measured in each sample and ACE_o is the activity measured in plasma sample before the dose administration.

Pharmacokinetic and statistical analyses

The bioequivalency decision of the test product based on the following pharmacokinetic parameters; area under the plasma concentration-time curve (AUC), peak concentration (Cmax) and time to peak concentration (Tmax). The terminal slope (β) was estimated by linear regression on the log-linear plot of plasma drug concentration-time course and elimination half-life ($t_{1/2}\beta$) was calculated by $0.693/\beta$. The calculation of AUC was done according to the linear/log-linear trapezoidal rule. The peak concentration and time to peak concentration was read from the respective observed values of each subjects. Total clearances (CL/F) of enalapril was calcualted as follows;

$$CL/F = Dose/AUC.$$

, where F is the term of bioavailability

Renal clearance of the drug was obtained by dividing the cumulative 12 hour excreted amount of the drug with the area under the concentration-time curve from zero time to 12 hours after the dosing. The decision to accept the bioequivalence of test preparation to reference drug was based on over all considerations for the results of analysis of variance, 95% confidence limit analysis of each pharmacokinetic parameter.

Pharmacodynamic comparison between test and reference drugs was made by assessing the area under the ACE percent inhibition curve (Lecocq *et al.*, 1990) and the time course of hypotensive effect after administration of both compounds. Student T-test and repeated analysis of variance method (Cole and Grizzle, 1966) using SAS program were applied to test the difference of ACE inhibition and time dependent hypotensive effect, respectively. As a pharmacodynamic index of ACE inhibition after drug dosing, area under the percentage inhibition-time course (AUCI) from zero time to 23 hours were calculated by linear trapezoidal rule.

For the analysis of the realtion between plasma enalaprilat concentration-ACE inhibition, sigmoid dose-response model described by Hill's equation was applied, where r is the slope function and IC_{50} is the enalaprilat concentration producing 50% inhibition of plasma ACE activity.

$$\%\ \text{ACE inhibition} = \frac{100 \times [\text{enalaprilat}]^r}{[IC_{50}]^r + [\text{enalaprilat}]^r}$$

IC_{50} and slope function(r) were estimated by least square non-linear regression using NONLIN program (Metzler, 1984).

RESULTS AND DISCUSSION

No adverse symptoms and signs attributable to the drug were observed after single dose of 10mg administration of both products.

Pharmacokinetics of enalapril maleate and assessment of bioequivalence

Mean plasma enalapril and enalaprilat concentrations versus time values after single oral administration of 10mg enalapril maleate are displayed in Fig. 1. Peak plasma levels of enalapril were observed

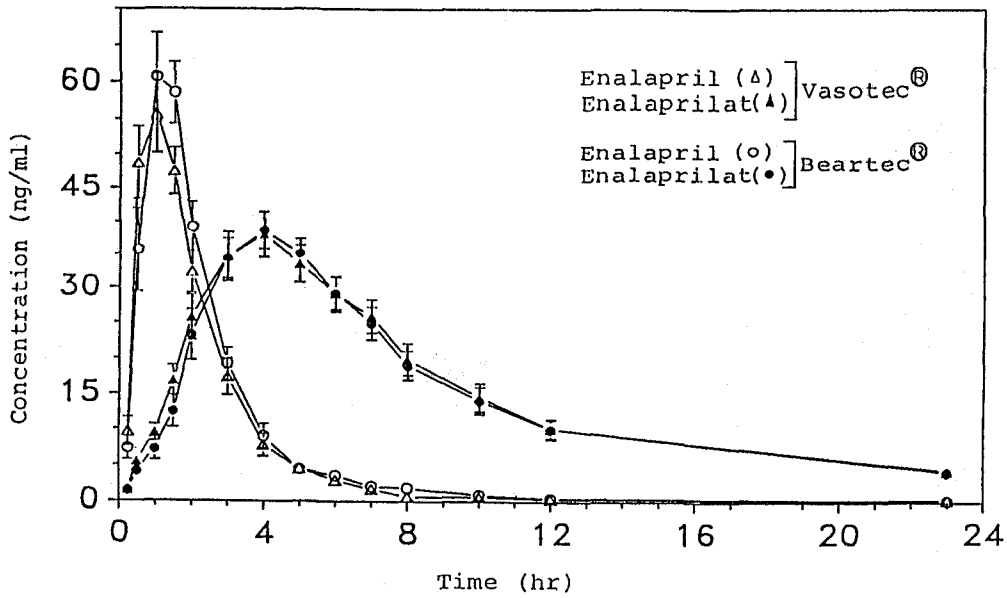

Fig. 1. Plasma enalapril and enalaprilat concentration-time data after single oral administration of 10mg enalapril maleate tablets to twelve normal subjects. Mean ± S.E. are plotted.

Table 1. Pharmacokinetic parameters of two enalapril maleate tablets and 95% confidence interval for the parameters (Mean ± S.D.)

Pharmacokinetic parameters	Reference compound	Test compound	Confidence limit (%)	Statistics (ANOVA)
Ennalapril				
$AUC_{0-\infty}$ (ng·hr/ml)	130.31 ± 36.47	144.28 ± 38.68	89.6 - 131.9	NS
Cmax (ng/ml)	61.38 ± 12.79	64.27 ± 17.99	94.1 - 115.3	NS
Tmax (hr)	0.96 ± 0.40	1.21 ± 0.26	91.4 - 160.4	NS
$t_{1/2\beta}$ (hr)	0.96 ± 0.20	1.15 ± 0.44	—	NS
$A_{eo\text{-}12\ hr}$ (μg)	1470.45 ± 517.44	1452.93 ± 364.92	—	NS
Cl_r (L/hr)	11.57 ± 3.23	11.1 ± 4.61	—	NS
Cl/F (L/hr)	57.72 ± 14.48	63.25 ± 17.33	—	NS
Enalaprilat				
$AUC_{0-\infty}$ (ng·hr/ml)	330.63 ± 105.86	320.96 ± 112.49	82.1 - 111.9	NS
Cmax (ng/ml)	38.63 ± 12.13	39.43 ± 9.57	88.7 - 115.4	NS
Tmax (hr)	3.83 ± 0.58	4.08 ± 0.51	93.3 - 119.7	NS
$t_{1/2\beta}$ (hr)	3.95 ± 1.57	3.92 ± 1.27	—	NS
$A_{eo\text{-}12\ hr}$ (μg)	3945.3 ± 837.4	4240.2 ± 813.8	—	NS
Cl_r (L/hr)	16.45 ± 0.55	18.06 ± 5.84	—	NS

about 1 hour after the dose in both formulations (Vasotec®, 0.9 ± 0.40 hr; Beartec®, 1.21 ± 0.26 hr). Peak plasma enalapril level of the test tablet (64.27 ± 17.99 ng/ml) was slightly higher than that of the reference tablet (61.88 ± 12.79 ng/ml). The terminal plasma half-life of enalapril of both compounds were almost identical and practically all enalapril could not be detected in 6 hour samples after the dose. Enalaprilat, active metabolite, showed peak level around 4 hours after dose. Mean peak

enalaprilat concentration of both compounds were almost identical (Vasotec®, 38.63 ± 12.13 ng/ml; Beartec®, 39.43 ± 9.57 ng/ml).

Mean pharmacokinetic parameters of enalapril and enalaprilat determined from analysis of single dose data are presented in table 1. Peak plasma concentrations, times to peak, areas under the curve (AUCs), and other kinetic parameters of enalapril and enalaprilat after single oral administration of 10mg reference tablet were comparable to the findings of the previously reported pharmacokinetic studies in Caucacian and Orientals (Biollaz *et al.*, 1982, Ohnishi *et al.*, 1989). Terminal half-lifes and clearance values of enalapril and enalaprilat were almost identical in both formulations. Relative bioavailability of the test tablet compared to the reference tablet were determined as the ratio of AUCs of enalapril and enalaprilat after the dose. AUC values of enalapril averaged 144.28 ± 38.68 ng·hr/ml for test tablet and 130.31 ± 36.47 ng·hr/ml for reference tablet. Mean AUC values of enalaprilat for test and reference tablets were quite similar, 39.43 ± 9.47 and 38.63 ± 12.13 ng·hr/ml, respectively. There were no statistically significant difference between formulations. The relative bioavailability, as measured by the ratios of the AUCs of enalapril and enalaprilat for test tablet with respect to reference tablet, averaged 1.02 ± 0.26 and 0.99 ± 0.20, respectively. The 95% confidence intervals of the relative bioavailability values for test tablet were from 82.1 to 111.9% and from 89.6 to 131.9% in terms of enalaprilat and enalapril concentration, respectively. Peak plasma enalapril and enalaprilat concentrations and time to the peak values of both formulations did not show any statistical difference. In the 95% confidence limit analysis, only time to peak level of enalapril showed out of the upper limit.

Where analysis of variance and confidence limit analysis were applied as the different perspectives for the evaluation of absorption extent and rate in this study, mean value of AUC, peak concentration and time to peak of test tablet would be quite acceptable. On the other hand, the results of 95% confidence interval analysis of the parameters showed slight deviation from upper limit in AUC and time to peak for enalapril, if less than + 20% difference from the reference value is used as acceptance criterion. However, enalapril level after administration of enalapril maleate will not represent the therapeutic efficacy because enalapril itself does not show pharmacological activity. Therefore, it seems to be reasonable to give more attention to the parameters of enalaprilat, active metabolite, after enalapril maleate dose in the judgement of bioequivalence. Moreover, the tolerance range could not be strictly applied to the evaluation of time to peak, since the parameter of time to peak is not direct measure for the absorption rate (Jackson and Chen, 1987). Concise resolution of the situation may require a combination of statistial analysis of the pharmacokinetic parmeters as well as the clinical significance of the parameter, in order to reach an informed conclusion. In this regard, the test formulation of enalapril maleate could be declared acceptable, especially considering the pharmacodynamic results discussed in the next part.

Comprison of ACE inhibition and hypotensive effects

The mean baseline (or predose) values of plasma ACE activity for both formulations were not different. Plasma ACE activities were significantly inhibited during whole periods of this experiment after the administration of both drugs (Fig. 2); Peak inhibition at 4 hour, and about 50% inhibition at 23 hours after the dose. The magnitude of the ACE inhibitions were fairly comparable between the two formulations: the maximum post dose percentages of inhibition from the baseline were 91.9 ± 0.64% for the reference tablet, and 92.1 ± 0.73% for the test tablet, which did not show statistically significant difference. The areas under the percentage inhibition time curve (AUCI) for 23 hour after the dose of the test and the reference tablets were 1685.0 ± 71.3 and 1655.6 ± 63.8%·hr, respectively. The pharmacodynamic activity ratio (AUCItest/AUCIref) was 1.02 ± 0.05, which indicate no significant difference between both formulations (Table 2).

No difference was detected between the test and reference tablets in the sensitivity of plasma angiotensin converting enzyme to enalaprilat in plasma (Fig. 3). The plasma concentrations of enalaprilat to produce 50% inhibition of plasma ACE activity (IC_{50}) and slope function of sigmoid concentration-inhibition relationship was about 5.8 ng/ml and 2, respectively.

Systolic and diastolic pressure fell significantly ($p<0.05$) from the respective predose level from 2 hours after administration of the both formulations, whereas heart rates were not significantly changed by the drug administration. Fell in systolic pressure showed statistical significance only up to 4 hours after dose in both formulations. By contrast to this, fell in diastolic pressure sustained up to 8 hours after the

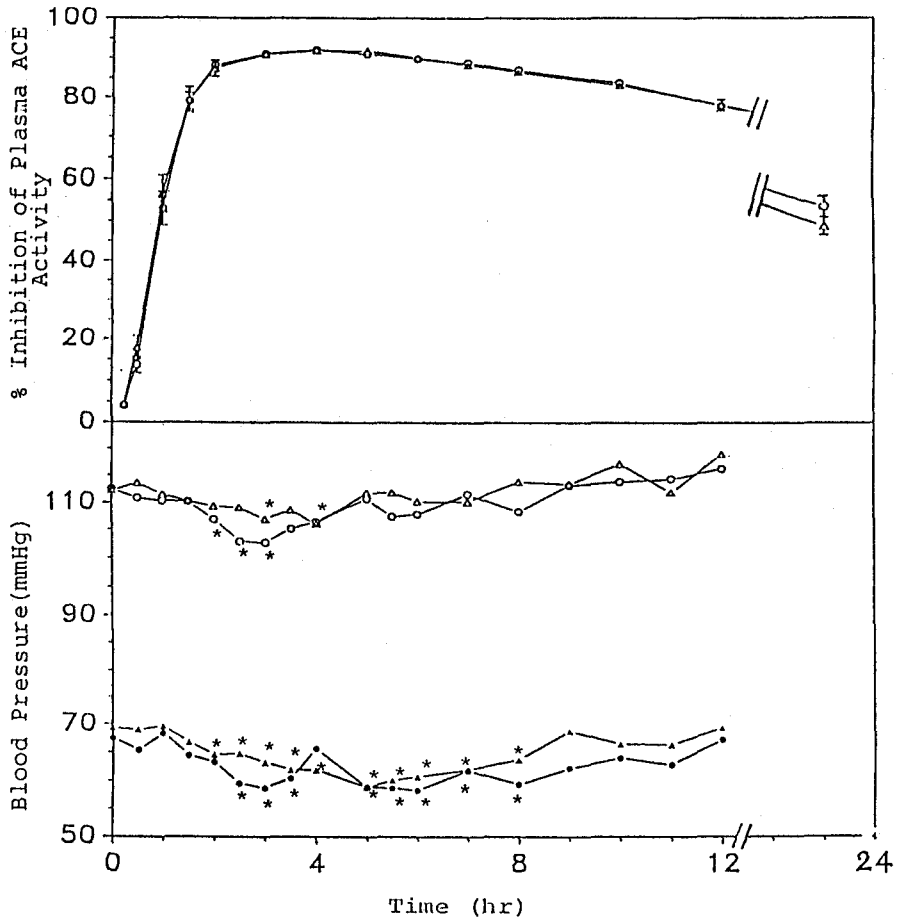

Fig. 2. Percent inhibition-time data (Mean ± SE) of plasma ACE activity and changes of systolic (open symbol) and diastolic (closed symbol) blood pressure after single oral administration of 10mg enalapril maleate talbets. Circles and triangles indicate values for Beartec® and Vasotec®, respectively.
*; statistical significance between the corresponding and predose values ($p<0.05$)

Table 2. Relationship between serum enalaprilat concentration and percentage of inhibition of plasma ACE activity (Mean ± S.D.)

	IC_{50} (ng/ml)	Slope function(r)	AUCI (%·hr)
Reference compound	6.2 ± 3.0	2.1 ± 0.6	1655.6 ± 63.8
Test compound	5.3 ± 2.3	2.3 ± 0.7	1685.0 ± 71.3

dose. Over all hypotensive effect of both formulations did not show statistical significance comparing by repeated analysis of variance (Fig. 2). From the above results, it was suggested that the pharmacologic responsiveness based on the ACE inhibition and hypotensive effect does not seem to be different between the two formulations.

In conclusion, the results of comparison in pharmacokinetics, ACE inhibition and hypotensive effect after single oral dose administration provide strong evidence that the tested generic product of enalapril maleate would be biologically and therapeutically

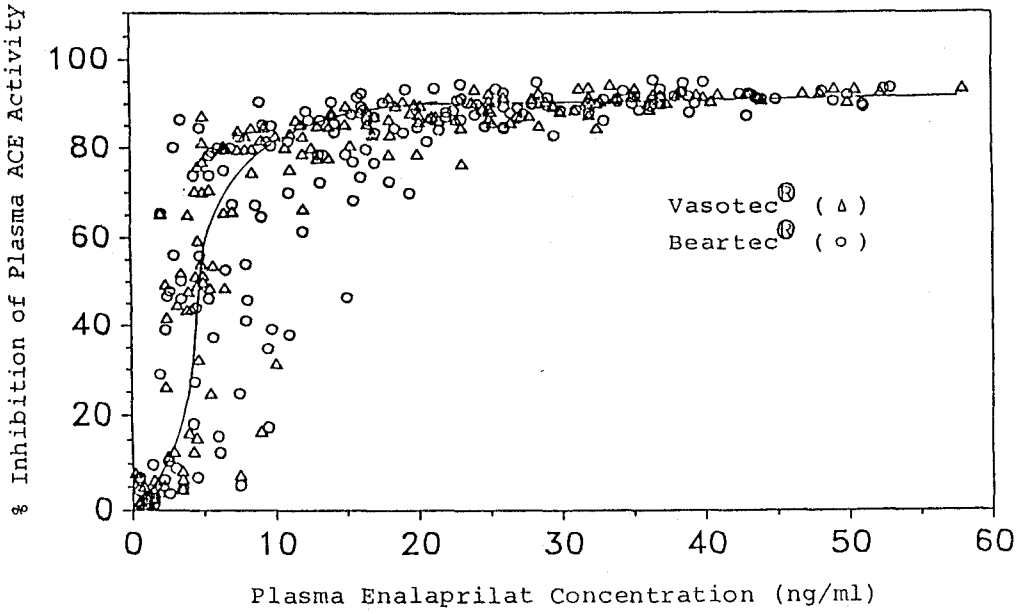

Fig. 3. Relationship between plasma enalaprilat concentration and percent inhibition of plasma ACE activity.

equivalent to the reference product.

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= 국문초록 =

Enalapril Maleate 정제의 동등성에 관한 연구 ; 약동학적 성상 및 혈장 ACE 활성도 억제 효과

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국내 생산 enalapril maleate 10mg 제제(Beartec®)의 생물학적 동등성을 검토키위해, 원 제조원인 Merck사의 Vasotec®을 기준 제제로하여 12명의 건강한 남성지원자를 대상으로 10mg 1회 경구 투여 교차시험후 약동학적 성상, ACE 활성억제의 경시적 변화 및 혈압 변동을 검토한 결과는 다음과 같다.

1. 혈장 enalapril 및 활성형 대사물인 enalaprilat의 생체이용율 지표들(AUC, Tmax 및 Cmax)의 평균치는 시험제제에서 enalapril의 최고 혈장농도 도달시간(Tmax)이 약 27%(0.21시간)지연되었을 뿐 타 지표는 대조제제에 대한 백분율 차이에 있어 ±20%내외였다.

2. 혈장 enalapril 및 enalaprilat의 생체 이용율 지표들은 분산 분석에 의해 두 제제간에 차이를 인지할 수 없었다.

3. 시험제제의 생체이용율 지표들은, 대조제제에 대한 백분율을 95% 신뢰구간 검정시, enalapril의 AUC 및 Tmax를 제외한 enalapril 및 enalaprilat의 모든 지표는 ±20% 내외의 결과를 보였다.

4. 두제제 투여후 ACE 활성도는 enalaprilat 혈장농도 5-6ng/ml에서 50%의 억제를 보였으며, 투약 23시간까지의 활성억제 AUC는 차이가 없었다.

5. 두 제제 투여후 수축기 및 이완기 혈압은 투약 2시간 이후 유의한 감소를 보였으며 혈압 변동은 두제제간에 차이를 인지할 수 없었다.

이상의 실험 결과로 enalapril maleate의 국내 생산 generic product는 기준제제인 Vasotec®과 동등한 생물학적 동등성을 지니며 치료적 등가성을 보이는 제제로 판단하였다.