

ORIGINAL ARTICLE

# Pharmacokinetics and pharmacodynamics profiles of enalapril maleate in healthy volunteers following determination of enalapril and enalaprilat by two specific enzyme immunoassays

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## SUMMARY

**Background and objectives:** Most of the pharmacokinetic (PK) parameters for enalapril and enalaprilat were established following determination of the drug and its metabolite, using angiotensin converting enzyme (ACE) inhibition assays. In these methods, enalapril has to be hydrolysed to enalaprilat first and then assayed. The purpose of this study was to re-estimate the PK parameters of enalapril and enalaprilat in healthy volunteers using two specific enzyme immunoassays for enalapril and enalaprilat.

**Methods:** The rate and extent of absorption of enalapril and enalaprilat from a 10-mg dose of two enalapril maleate commercial brands (Renetic® and Enalapril®) were estimated using a two-way-cross over design with 1-week washout period. Blood pressure was also measured at specified time intervals and correlated to enalaprilat plasma concentrations.

**Results:** For enalapril, the  $AUC_{0 \rightarrow \infty}$  values (Mean  $\pm$  SD) were  $450.0 \pm 199.5$  and  $479.6 \pm 215.6$  ng h/mL,  $C_{max}$  values were  $313.5 \pm 139.6$  and  $310.1 \pm 186.6$  ng/mL,  $T_{max}$  values were  $1.06 \pm 0.30$  h and  $1.13 \pm 0.22$  h, and  $t_{1/2}$  ranged between  $0.3$  to  $6.1$  h ( $1.6 \pm 1.5$ ) and  $0.40$  to  $5.05$  h ( $1.3 \pm 1.0$ ), for the two brands. For enalaprilat, the  $AUC_{0 \rightarrow \infty}$  values were  $266.9 \pm 122.7$  and  $255.9 \pm 121.8$

ng h/mL,  $C_{max}$  values were  $54.8 \pm 29.5$  and  $57.2 \pm 29.0$  ng/mL,  $T_{max}$  values were  $4.6 \pm 1.6$  h and  $4.3 \pm 1.45$  h, and  $t_{1/2}$  ranged between  $1.1$  to  $10.5$  h ( $4.5 \pm 2.9$ ) and  $0.6$  to  $9.4$  h ( $3.5 \pm 2.5$ ) for the two brands.

**Conclusions:**  $C_{max}$  values for enalapril are about 10 times those published in the literature and the rate and extent of absorption of the two brands of enalapril and their deesterification to enalaprilat following the administration of either brand were bioequivalent. Secondly, enalaprilat concentrations at 12–24 h following a single oral dose of enalapril in healthy volunteers were lower than those reported in the literature. The values reported here correlated with the return of blood pressure to predose level. Thirdly, enzyme immunoassays for enalapril and enalaprilat are better than ACE inhibition assays and can be used in bioequivalence assessment of enalapril and enalaprilat and for therapeutic drug monitoring in a clinical laboratory setting.

**Keywords:** EIA, enalapril, enalaprilat, pharmacodynamics, pharmacokinetics

## INTRODUCTION

Enalapril maleate, N-(N-S-1-ethoxycarbonyl-3-phenylpropyl)-L-alanyl-L-proline hydrogen maleate, is an anti-hypertensive prodrug, which is deesterified to an active diacid form enalaprilat. Enalaprilat is an angiotensin converting enzyme (ACE) inhibitor (1). As oral absorption of

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enalapril is superior to that of enalaprilat, it is preferred in oral dosage forms. Several review articles give a detailed account of the history, design, chemistry and pharmacology of the drug (2–4). The pharmacokinetic (PK) and metabolism of enalapril following oral administration has been the subject of a number of investigations and reviews (4–6). These PK studies were based on determining enalapril in plasma by converting it to enalaprilat by alkaline hydrolysis and then, assaying the latter form by a fluorometric (5), radio-enzymatic (7) ACE inhibition assays or radioimmunoassay (RIA) (8). Newer methods for determining directly enalapril and enalaprilat are now available such as, chemiluminescence using flow injection (9), GC/MS (10) and LC/MS/MS (11). These latter methods, however, need sample preparation and extraction, which are tedious and time-consuming. Therefore, we have developed two specific enzyme immunoassays for enalapril and enalaprilat. These two methods are rapid, simple, highly sensitive and less expensive for accurately measuring enalapril and enalaprilat in biological fluids without any cross-reactivity between the two antibodies (12).

It has been shown that ACE inhibition assay gave lower concentration values of enalaprilat than RIA during the first 45 min and higher values after 45 min of intravenous administration of enalapril into rabbits (13). In addition, our preliminary data from EIA and, others using LC/MS/MS methods revealed that  $C_{max}$  for enalapril was significantly higher than those reported in the literature (11, 12). Therefore, in this study, we sought to re-evaluate the PK and pharmacodynamics (PD) of enalapril and enalaprilat in humans. We assessed the rate and extent of absorption of enalapril and enalaprilat from two commercial brands in a two-way cross-over study on healthy volunteers using specific enalapril and enalaprilat EIA methods. The differences between PK parameters following 10- and 20-mg doses of enalapril were estimated using the same analytical method. Furthermore, the acute decrease in blood pressure, a PD parameter, was evaluated in normotensive volunteers. Finally, we comment on the possible use of such methods for therapeutic drug monitoring (TDM) of enalapril and enalaprilat. Enalapril

accumulation especially in patients with congestive heart or chronic renal failures may require reducing doses to avoid adverse effects (14).

## MATERIAL AND METHODS

### *Drug supplies*

A sample of a commercial size batch of 10-mg tablet Enalapril® (Lot: P003) was tested against one of Renitec® (Batch No. HJ57620). In addition, 20-mg tablet of Renitec® (Batch No. HJ01860) was used. The *in vivo* study was carried out at Al Mowasah and at Jordan Red Crescent Hospitals in Amman – Jordan.

### *Study design*

For the 10-mg dose study, 24 healthy male volunteers aged 18–43 years (mean  $28.3 \pm 6.1$ ) and weighing 60–90 kg ( $76.7 \pm 9.0$ ), received 10 mg of enalapril on two occasions, in a two way cross over design, with a 1 week washout period between doses. For the 20-mg dose study, 24 healthy male volunteers aged 19–43 years (mean  $30.0 \pm 7.4$ ) and weighed 60–90 kg ( $76.1 \pm 8.9$ ), received 20 mg of enalapril.

Volunteers from either study, stayed at the hospital for the whole period watching television and reading. A comprehensive checkup including physical examination, clinical chemistry/haematology evaluation and urine analysis revealed no evidence of any kind of disease. The project was subject to ethics review, and each subject gave his signed informed consent. The study was approved by the Ethics Committee of both hospitals and was in accordance with the relevant articles of the Declaration of Helsinki (1964) as revised in Tokyo (1975), Venice (1983), Hong Kong (1989), and Somerset West, RSA (1996). Prior to the study the participants were asked not to take any medication for 2 weeks before or during the course of the investigation. One tablet containing 10 mg (or 20 mg) of enalapril was administered in the morning with a 240 mL of water following 10-h fasting. A light breakfast was given to the volunteers, 4 h after drug intake, and a standard lunch was given to all participants after 8 h. Blood samples (4–5 mL) were collected in heparinized tubes at 0:0, 0:25, 0:50, 0:75, 1:00, 1:25, 1:50, 1:75, 2:00, 2:50, 3:00, 3:50, 4:00, 5:00, 6:00,

7:00, 8:00, 10:00, 12:00 and 24:00 h. Plasma was separated directly by centrifugation and was stored at  $-20^{\circ}\text{C}$  until date of analysis.

### Analytical methods

Two specific EIA methods for the quantification of enalapril and enalaprilat concentrations in human plasma were employed. The following chemicals were purchased from Sigma Chemicals Co. (St Louis, MO, USA): bovine serum albumin (BSA), ovalbumin (OVA), avidin, glutaraldehyde (25%), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC), N-hydroxysuccinimide ester, goat anti-rabbit alkaline phosphatase conjugate, p-nitrophenyl phosphate, Tween-20, sodium azide and diethanolamine. Tris buffer and sodium chloride reagents were purchased from Acros Organics (Fisher Chemicals, Fairlawn, NJ, USA). Biotin-X-hydrazide was purchased from Calbiochem Co., UK. Microtitre 96-well flat bottom plates were purchased from Greiner, Labor-technik, Germany. Wellscan plate reader and Wellwash were obtained from Denley Co. (Billinghurst, UK).

**Enalapril analysis.** The reagents for EIA was prepared as previously described (12, 15–17). Briefly, anti-enalapril antibodies were raised in rabbits (White New Zealand) against BSA-enalapril conjugate. The conjugate was made by purifying enalapril from maleic acid by HPLC, and then linked it to BSA via carbodiimide followed by purification by gel chromatography. The antisera was tested for binding with enalapril, enalaprilat, proline, phenylalanine, alanine-proline, and captopril and revealed no binding with any of those compounds except enalapril.

Enalapril was linked to biotin via its carbohydrate group using long chain biotin (biotin-x-hydrazide). The biotin was conjugated to enalapril using carbodiimide and N-hydroxysuccinimide ester. The complex was purified by HPLC and tested for binding with the anti-enalapril antisera.

Flat bottomed 96-well microtitre plates were coated with  $5\ \mu\text{g}/\text{mL}$  of avidin ( $0.1\ \text{mL}/\text{well}$ ) in  $0.05\ \text{M}$  carbonate buffer, pH 9.6. Wells were washed three times with  $25\ \text{mM}$  Tris-HCl buffer, pH 7.3, containing 0.05% Tween-20,  $0.15\ \text{M}$  NaCl, and 0.02% sodium azide, blocked with the same washing buffer for 30 min, and washed twice. Biotin-

enalapril conjugate ( $1 : 8000$ ) was added ( $0.1\ \text{mL}/\text{well}$ ), incubated for 30 min, and wells then were washed twice. In duplicates, plasma standards or samples were added ( $10\ \mu\text{L}/\text{well}$ ) followed by the addition of anti-enalapril antisera ( $0.1\ \text{mL}$ ) diluted ( $1 : 6000$ ) in assay buffer ( $50\ \text{mM}$  Tris-HCl buffer, containing 1% BSA, 0.05% Tween-20,  $0.15\ \text{M}$  NaCl,  $20\ \text{mM}$  EDTA and 0.02% sodium azide). Plates were left incubating at  $22^{\circ}\text{C}$  for 1 h with orbital shaking to allow competitive binding between the bound enalapril to biotin-avidin complex and the enalapril in the sample. Unfixed antigen-antibody complexes are removed by washing, leaving the bound enalapril-antibody complex in the well. The wells were then washed three times, and anti-rabbit antibody alkaline phosphatase conjugate, diluted in assay buffer without EDTA, was added in  $0.1\ \text{mL}/\text{well}$  and left incubating for 1 h at  $22^{\circ}\text{C}$  with orbital shaking. The wells were washed four times, and  $0.1\ \text{mL}/\text{well}$  of  $1\ \text{mg}/\text{mL}$  of p-nitrophenyl phosphate in 10% diethanolamine, pH 9.8, containing  $1\ \text{mM}$   $\text{MgCl}_2$  and 0.02% sodium azide was added. After 30 min, colour development was stopped by the addition of  $2\ \text{M}$  NaOH ( $0.1\ \text{mL}/\text{well}$ ), and absorbance was read at 405 and 600 nm using a differential filter. Enalapril concentrations were calculated by averaging the mean absorbance of standards after subtracting the absorbance of the chromogen blank from all the means. The corrected mean absorbance obtained was then divided by the corrected mean absorbance of the zero standard to get the ratio binding (i.e. when the ratio binding is close to 1, no enalapril is present in the sample). The standard curve conducted by plotting the logit binding [ $\log_{10}(\text{Ratio Binding})/(1-\text{Ratio binding})$ ] vs. the respective enalapril concentrations on a semilog graph and the unknown enalapril concentrations in the samples were determined by interpolation from standard curves using a suitable program. The standard curves constructed exhibited good linearity ( $r^2 = 0.989\text{--}0.995$ ). The intra- ( $n = 10$ ) and inter- ( $n = 8$ ) assay accuracy (% recovery) ranged from 90.2–110.5 and 98.9–102%, respectively, over 1–1000 ng/mL range of enalapril in human plasma. The intra- and inter-assay precision (% CV) ranged from 1.06 to 9.98 and 5.06 to 7.09%, respectively, over 1–1000 ng/mL range of enalapril in human plasma. The quality control samples (low, medium and high) showed a range of 93.5–103.0% for recovery and 2.5–13.3% for precision.

**Enalaprilat analysis.** Antisera specific for enalaprilat were produced by immunization with lisinopril-BSA conjugate. Lisinopril has a similar structure to enalaprilat with the exception that lisinopril has a side chain amino group, conjugated to BSA (12, 15). This antibody detects only enalaprilat (and lisinopril) and shows no cross-reactivity with enalapril.

Flat bottomed 96-well microtitre plates were coated with 12.6 µg/mL of OVA-lisinopril (0.1 mL/well) in 0.05 M carbonate buffer, pH 9.6. Wells were washed three times with 25 mM Tris-HCl buffer, pH 7.3, containing 0.05% Tween-20, 0.15 M NaCl, and 0.02% sodium azide, blocked with the same washing buffer for 30 min, and washed twice. In duplicates, plasma standards or samples were added (50 µL/well) followed by the addition of anti-lisinopril antisera (1 : 1000, 50 µL/well) diluted in assay buffer (50 mM Tris-HCl buffer, containing 1% BSA, 0.05% Tween-20, 0.15 M NaCl, 20 mM EDTA and 0.02% sodium azide). The wells were left incubating at 22 °C for 1 h with orbital shaking. The wells were then washed three times, and anti-rabbit antibody alkaline phosphatase conjugate, diluted in assay buffer without EDTA, was added in 0.1 mL/well and left incubating for 1 h at 22 °C with orbital shaking. The wells were washed four times, and 0.1 mL/well of 1 mg/mL of p-nitrophenyl phosphate in 10% diethanolamine, pH 9.8, containing 1 mM MgCl<sub>2</sub> and 0.02% sodium azide was added. After 30 min, colour development was stopped by the addition of 2 M NaOH (0.1 mL/well), and absorbance was read at 405 and 600 nm differential filter. The standard curves constructed (as above) exhibited good linearity ( $r^2 = 0.988-0.996$ ) following transformation of the absorbance values to logit binding and plotted vs. concentration of enalaprilat on a semilog graph. The intra- ( $n = 9$ ) and inter- ( $n = 9$ ) assay accuracy (% recovery) ranged from 80.7–117.3 and 87.6–111.0%, respectively, over 1–500 ng/mL range of enalaprilat in human plasma. The intra- and inter-assay precision (% CV) ranged from 1.87 to 9.87 and 1.89 to 7.47%, respectively, over 1–500 ng/mL range of enalaprilat in human plasma. The quality control samples (low, medium and high) showed a range of 95.5–105.9% for recovery and 2.8–12.92% for precision.

### Data analysis

Non-compartmental PK calculations were used to estimate the area under the plasma concentration–time profile from time zero to time  $t$ , ( $AUC_t$ ), zero to infinity ( $AUC_{0 \rightarrow \infty}$ ) and elimination half-life ( $t_{1/2}$ ) (18). Briefly,  $AUC_t$  was calculated using linear trapezoidal rule, where  $t$  is the last measurable time point, which begins at time 0 and finishes at the last quantifiable point.  $AUC_{0 \rightarrow \infty}$  was calculated by adding  $AUC_t$  to the value of dividing the last measurable concentration at time  $t$  over elimination rate constant. The elimination  $t_{1/2}$  was calculated from the slope of the semi-logarithmic of the last plasma concentration points vs. time. The maximum plasma drug concentration ( $C_{max}$ ) and time to reach the maximum drug concentration ( $T_{max}$ ) were calculated by averaging the highest plasma concentration and its corresponding time of the drug or metabolite in each individual. The MRT was calculated by dividing the area under a moment curve from time 0 to infinity ( $AUMC_{0 \rightarrow \infty}$ ) over  $AUC_{0 \rightarrow \infty}$ .

A PD model for evaluating the decrease in blood pressure with plasma enalaprilat concentrations was performed using a regression analysis model. The PD equation model was

$$\%DBP = \%DBP \text{ predose} + S^*C$$

where % DBP is the per cent decrease in blood pressure, % DBP predose (which is close to zero),  $S$  is the slope of the effect–concentration curve and  $C$  is the plasma drug concentration. The slope represents change in % DBP per unit change of concentration (ng/mL).

### Statistical analysis

The assessment of bioequivalence between the test and reference product was based on the ratios of the main PK characteristics,  $T_{max}$ ,  $C_{max}$  and  $AUC_{0 \rightarrow \infty}$ . Additive effects because of subjects (with  $n - 1$  d.f.), error (with  $n - 2$  d.f.) and period (with 1 d.f.) have been assessed by detailed analysis of variance. Logarithmically transformed values of  $C_{max}$  and  $AUC$  and linear values of  $T_{max}$  have been used for such analysis. The two one-sided test procedures were used to conclude the existence of bio(in)equivalence between the test and the reference products. The decision rule, that

both products are bio-in-equivalent was equated with the classical null hypothesis. Bioequivalence was concluded if either tail probability did not exceed 5% or if the 90% confidence interval was completely contained in the 0.80–1.25 range. A non-parametric test procedure based on the Wilcoxon's Sign Rank test (Tukey Modification) was also used.

Pearson correlation test with ANOVA and two-sided significance was used to evaluate the association that exist between the change in blood pressure and enalaprilat and enalapril plasma concentrations. In addition, two-sided *t*-test was used to compare the PK parameters obtained at the 10- and 20-mg doses and the differences in blood pressure at each time point.

## RESULTS

### Pharmacokinetics

The mean plasma concentrations of enalapril for the 24 healthy volunteers and the individual PK parameters,  $C_{max}$ ,  $T_{max}$  and AUC for each brand are shown in Fig. 1 and Table 1. The  $AUC_{0 \rightarrow \infty}$  values were  $450.0 \pm 199.5$  and  $479.6 \pm 215.6$  ng h/mL for Enalapril® and Renitec®, respectively. The  $C_{max}$  values were  $313.5 \pm 139.6$  and  $310.1 \pm 186.6$  ng/mL for Enalapril® and Renitec®, respectively. In addition,  $T_{max}$  values were  $1.06 \pm 0.30$  and

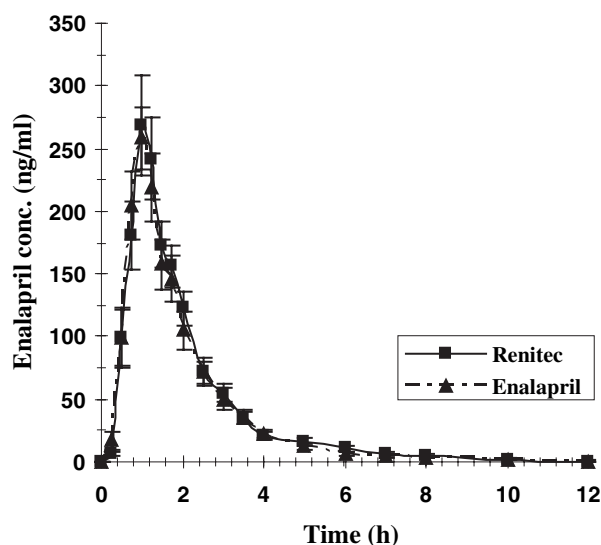


Fig. 1. Plasma enalapril concentrations profile after 10-mg oral administration of Enalapril® and Renitec®. Error bars represent  $\pm$  SE.

**Table 1.**  $C_{max}$ ,  $T_{max}$  and AUC of enalapril following the administration of Enalapril® (Test) and Renitec® (Reference) tablets, each containing 10-mg enalapril

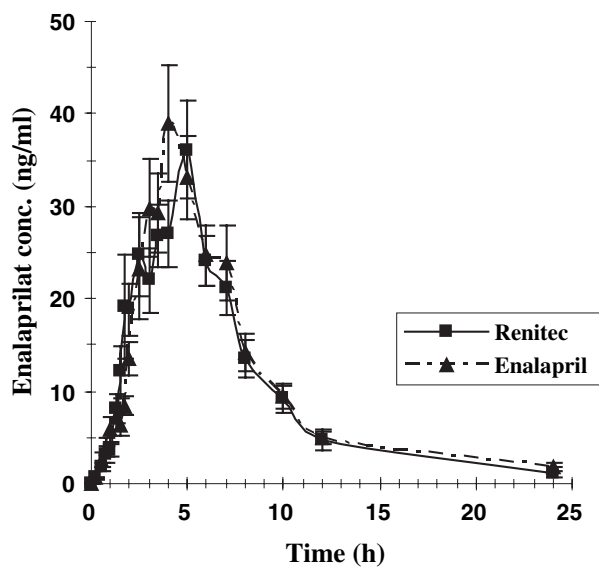
Vol. no.	$C_{max}$	$T_{max}$	$AUC_{0-00}$	$AUC_{0-t}$
Renitec® (reference)				
1	300	1.25	549.4	527.8
2	768	1.25	789.4	788.4
3	156	1.25	288.4	280.4
4	143	1.00	272.0	270.1
5	246	1.75	380.7	376.9
6	426	1.00	708.4	695.8
7	210	1.00	676.5	654.6
8	175	1.25	261.4	260.0
9	627	1.00	755.1	754.0
10	280	1.00	649.0	598.9
11	127	1.25	249.6	248.3
12	408	1.00	737.8	734.8
13	549	1.00	710.7	708.9
14	293	1.00	384.6	382.9
15	238	1.00	489.8	488.9
16	250	0.75	469.9	467.9
17	322	1.00	330.7	326.4
18	157	1.00	227.2	225.3
19	97	1.00	207.7	202.1
20	94	1.25	82.4	81.5
21	359	1.50	602.6	558.5
22	252	1.00	381.1	379.8
23	257	1.50	493.3	470.3
24	709	1.00	812.1	807.5
Mean	310.1	1.13	479.6	470.4
SD	186.6	0.22	215.6	211.9
Enalapril® (test)				
1	173	1.50	406.1	406.8
2	378	1.00	779.9	778.0
3	215	0.75	228.3	219.8
4	194	1.00	304.7	303.1
5	287	1.75	619.4	618.0
6	565	1.00	542.4	541.0
7	299	1.00	607.3	597.6
8	185	1.50	316.6	315.3
9	339	1.00	352.4	347.8
10	229	0.75	478.5	469.8
11	201	1.00	212.1	207.8
12	567	0.50	582.1	580.1
13	331	1.25	538.5	534.4
14	484	1.00	750.1	742.1
15	524	1.25	628.9	626.6
16	367	0.75	466.3	464.8
17	222	0.75	243.7	240.0
18	189	1.00	166.1	165.1
19	128	1.00	190.3	189.9

**Table 1.** Continued

Vol. no.	$C_{max}$	$T_{max}$	$AUC_{0-00}$	$AUC_{0-t}$
20	69	1.25	124.0	120.9
21	422	1.25	664.3	652.8
22	364	1.00	306.8	306.0
23	475	1.50	617.7	601.0
24	316	0.75	671.1	653.3
Mean	313.5	1.06	450.0	445.1
SD	139.6	0.30	199.5	197.5

1.13 ± 0.22 h for Enalapril<sup>®</sup> and Renitec<sup>®</sup>, respectively. Enalapril half-life in the present study ranged between 0.3 to 6.1 h (1.6 ± 1.5) for Enalapril<sup>®</sup> and 0.40 to 5.05 h (1.3 ± 1.0) for Renitec<sup>®</sup>. No significant difference between the two brands was established for any of the parameters tested at the 90% confidence interval and therefore, indicates that the rate and extent of absorption of both brands were comparable.

The mean plasma concentrations of enalaprilat for the 24 healthy volunteers and the individual PK parameters  $C_{max}$ ,  $T_{max}$  and AUC for each brand are shown in Fig. 2 and Table 2. The  $AUC_{0-\infty}$  values were 266.9 ± 122.7 and 255.9 ± 121.8 ng h/mL for Enalapril<sup>®</sup> and Renitec<sup>®</sup>, respectively. The  $C_{max}$  values were 54.8 ± 29.5 and 57.2 ± 29.0 ng/mL for



**Fig. 2.** Plasma enalaprilat concentrations profile after 10-mg oral administration of Enalapril<sup>®</sup> and Renitec<sup>®</sup>. Error bars represent ± SE.

**Table 2.**  $C_{max}$ ,  $T_{max}$  and AUC of enalaprilat following the administration of Enalapril<sup>®</sup> and Renitec<sup>®</sup> tablets, each containing 10-mg enalapril

Vol. no.	$C_{max}$	$T_{max}$	$AUC_{0-00}$	$AUC_{0-t}$
Renitec <sup>®</sup> (reference)				
1	39.0	4.0	198.0	187.0
2	37.4	3.5	321	278.8
3	42.0	5.0	238.8	189.1
4	67.4	5.0	223.8	197.0
5	97.4	5.0	370.0	365.1
6	58.2	5.0	249.4	241.0
7	57.1	5.0	157.7	155.6
8	97.6	5.0	351.8	346.1
9	38.3	3.0	323.6	269.4
10	54.6	5.0	151	147.8
11	43.8	2.5	94.8	88.9
12	23.5	5.0	152.1	141.9
13	53.9	3.0	87.4	85.8
14	25.8	6.0	281.6	267.5
15	36.3	3.5	202.7	198.9
16	32.4	2.5	165.0	154.1
17	67.5	2.5	405.3	378.5
18	24.0	4.0	183.4	173.9
19	80.2	4.0	466.5	394.7
20	99.0	2.5	281.5	276.2
21	30.5	7.0	203.9	200.1
22	141	1.75	186.1	184.3
23	65.0	7.0	612.6	505.0
24	61.5	6.0	233.3	226.2
Mean	57.2	4.3	255.9	235.5
SD	29.0	1.5	121.8	102.5
Enalapril <sup>®</sup> (test)				
1	44.1	3.5	167.3	165.2
2	29.0	4.0	158.7	145.9
3	84.7	5.0	448.0	422.7
4	67.6	3.0	394.4	388.5
5	107	4.0	268.2	266.3
6	63.6	4.0	296.6	239.2
7	32.2	4.0	121.1	110.5
8	39.9	3.5	183.7	165.3
9	28.0	3.5	201.7	194.1
10	64.1	5.0	222.6	215.0
11	33.1	4.0	255.7	213.7
12	27.8	3.5	169.5	146.1
13	36.5	3.0	173.5	147.4
14	24.0	10	225.1	198.1
15	53.9	7.0	255.7	252.9
16	36.7	3.5	124.1	118.7
17	52.3	6.0	300.9	294.5
18	69.4	5.0	416.7	352.6
19	60.7	5.0	334.3	329.5

Table 2. Continued

Vol. no.	$C_{\max}$	$T_{\max}$	$AUC_{0-00}$	$AUC_{0-t}$
20	26.3	4.0	158.9	147.2
21	36.3	3.0	146.3	144.0
22	144	4.0	454.7	448.0
23	94.5	7.0	591.0	538.2
24	60.0	6.0	337.3	314.9
Mean	54.8	4.6	266.9	248.3
SD	29.5	1.6	122.7	115.7

Enalapril<sup>®</sup> and Renitec<sup>®</sup>, respectively. In addition,  $T_{\max}$  values were  $4.6 \pm 1.6$  and  $4.3 \pm 1.45$  h for Enalapril<sup>®</sup> and Renitec<sup>®</sup>, respectively. Enalaprilat elimination half-life ranged between 1.1 to 10.5 h ( $4.5 \pm 2.9$ ) hours for Enalapril<sup>®</sup> and 0.6 to 9.4 h ( $3.5 \pm 2.5$ ) for Renitec<sup>®</sup>. No significant difference was established between the two brands for any of the parameters tested at the 90% confidence interval and therefore, indicates that the rates and extents of deesterification of enalapril to enalaprilat following the administration of either brand were comparable.

#### Ten vs. 20 mg of enalapril

The  $AUC_{0 \rightarrow \infty}$  values for 10- and 20-mg doses of enalapril were  $480 \pm 216$  and  $832 \pm 325$  ng h/mL, respectively ( $P < 0.0001$ ). The  $C_{\max}$  values for enalapril were  $310 \pm 187$  and  $481 \pm 185$  ng/mL for 10- and 20-mg doses, respectively ( $P = 0.0026$ ). In addition,  $T_{\max}$  values were  $1.13 \pm 0.22$  and  $1.09 \pm 0.33$  h for 10- and 20-mg doses of enalapril, respectively. Furthermore, the elimination  $t_{1/2}$  values were  $1.31 \pm 0.99$  and  $0.93 \pm 0.37$  h and MRT values were  $2.4 \pm 1.0$  and  $2.0 \pm 0.4$  h, for the 10- and 20-mg doses of enalapril, respectively.

The  $AUC_{0 \rightarrow \infty}$  for enalaprilat were  $256 \pm 122$  and  $383 \pm 158$  ng h/mL, for 10- and 20-mg enalapril doses, respectively ( $P = 0.0032$ ). The  $C_{\max}$  values for enalaprilat were  $57 \pm 29$  and  $72.9 \pm 33.6$  ng/mL for 10- and 20-mg enalapril doses, respectively ( $P = 0.023$ ). In addition,  $T_{\max}$  values were  $4.28 \pm 1.45$  and  $4.05 \pm 1.22$  h for 10- and 20-mg doses of enalapril, respectively. Furthermore, the elimination  $t_{1/2}$  values were  $3.47 \pm 2.47$  and  $3.95 \pm 2.48$  h and MRT values were  $7.4 \pm 2.8$  and  $7.9 \pm 3.2$  h for the 10- and 20-mg doses of enalapril, respectively.

#### Blood pressure and its correlation with enalapril and enalaprilat concentration

Both brands of enalapril caused a comparable and significant decrease in systolic and diastolic blood pressures (Fig. 3a,b). The maximum decrease in systolic (6–7.7 and 7.6–8.4%) and diastolic (11.3–12.5 and 12.3–13.6%) blood pressure was between 4 and 6 h post-dosing with both enalapril brands.

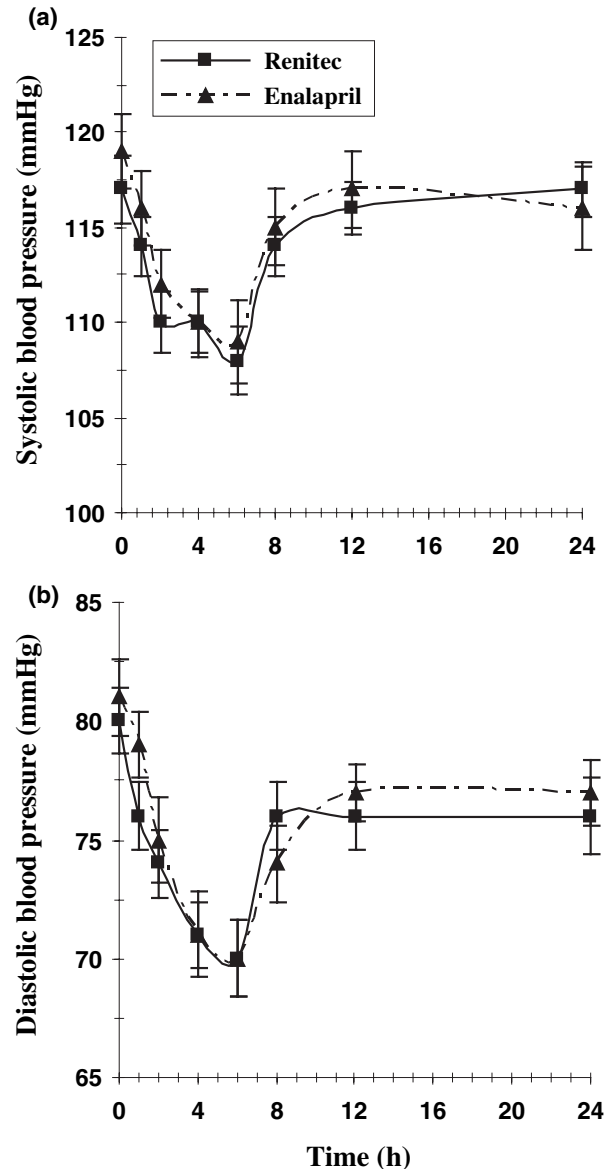
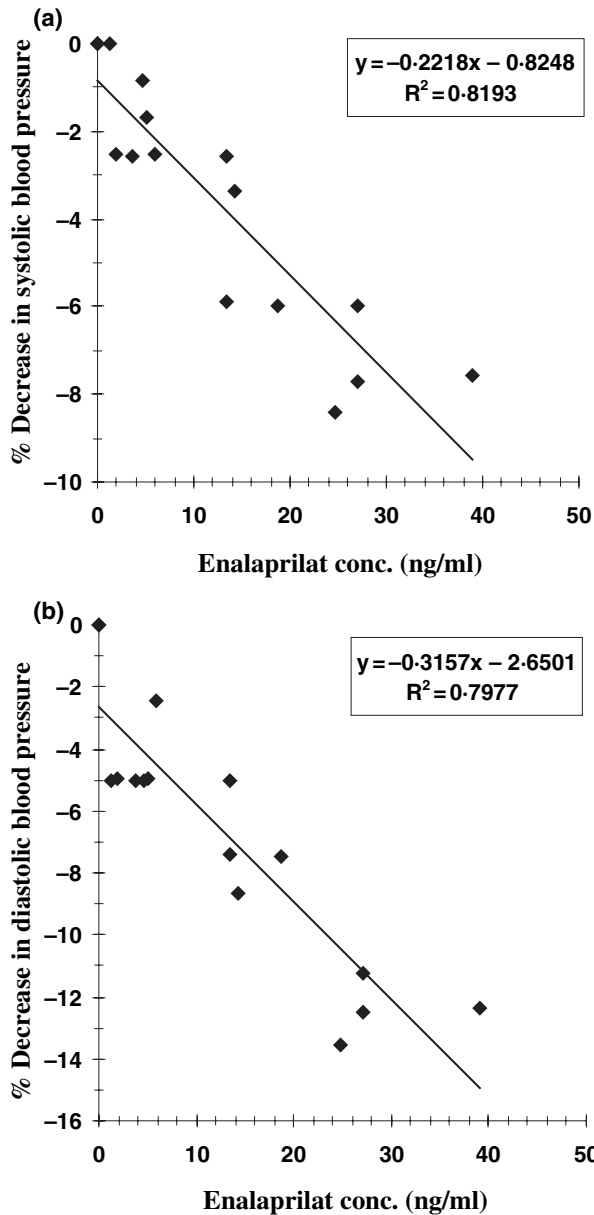


Fig. 3. (a) Systolic blood pressure after 10-mg oral administration of Enalapril<sup>®</sup> and Renitec<sup>®</sup>. Error bars represent  $\pm$  SE. (b) Diastolic blood pressure after 10-mg oral administration of Enalapril<sup>®</sup> and Renitec<sup>®</sup>. Error bars represent  $\pm$  SE.



**Fig. 4.** (a) Correlation between the percent decrease in systolic blood pressure and the plasma enalaprilat concentrations following 10-mg oral administration of Enalapril<sup>®</sup> and Renitec<sup>®</sup>. (b) Correlation between the percent decrease in diastolic blood pressure and the plasma enalaprilat concentrations. Each point represents the mean of blood pressure to all individuals at time *t* with its corresponding enalaprilat concentration.

This drop in systolic and diastolic blood pressures was statistically significant ( $P < 0.0001$ ). In addition, there was a significant correlation between plasma enalaprilat concentrations and the decrease in diastolic blood pressure ( $r = 0.88$  and  $P < 0.0037$  vs.  $r = 0.90$  and  $P < 0.0019$  for Enalapril<sup>®</sup> and

Renitec<sup>®</sup>, respectively), and systolic blood pressure ( $r = 0.88$  and  $P < 0.004$  vs.  $r = 0.95$  and  $P < 0.0003$  for Enalapril<sup>®</sup> and Renitec<sup>®</sup>, respectively). Figure 4 shows the combined correlation for the two drugs giving a slope of  $-0.22$  for systolic and  $-0.32$  for the diastolic blood pressure. However, no significant correlation was observed between plasma enalapril concentrations and the decrease in blood pressure.

Both doses (10 and 20 mg) of enalapril resulted in a significant decrease ( $P < 0.001$ ) in the blood pressure (systolic and diastolic). The maximum range of decrease in the systolic (6–7.6 and 11–12%) and diastolic (11.3–12.5 and 17.2–18.5%) blood pressures were at 4–6 h post-dosing of 10- and 20-mg doses of enalapril, respectively. In addition, a significant correlation between enalaprilat concentrations in plasma and the decrease in systolic ( $r = 0.946$ ,  $P < 0.001$ ) and diastolic ( $r = 0.954$ ,  $P < 0.001$ ) blood pressure was observed. No correlation was seen between the decrease in blood pressure and enalapril concentrations.

## DISCUSSION

The present work shows that  $C_{max}$  values for enalapril are  $\sim 300$  and  $\sim 500$  ng/mL following 10- and 20-mg oral dose, respectively, which are higher  $\sim 10$  times than for those values obtained by alkaline hydrolysis followed by ACE-inhibition assays. Secondly, enalaprilat concentrations at 12–24 h following a single 10- and 20-mg oral dose of enalapril in healthy volunteers were lower than those reported in the literature but the values here correlated with the return of blood pressure to predose level. Thirdly, enzyme immunoassay for enalapril is a better method for measuring of enalapril concentrations than the two step approach using alkaline hydrolysis followed by ACE inhibition assays. The method is appropriate for bioequivalence assessment of enalapril and enalaprilat and for TDM in a clinical laboratory setting.

Using RIA, Worland and Jarrott (13) have shown that enalaprilat concentrations were  $\sim 2$  times higher than those concentrations obtained by an ACE inhibition assay, especially those between 0 and 0.75 h post-intravenous administration of enalapril to rabbits. It was postulated that enalaprilat concentrations were approaching those required to maximally inhibit the enzyme and therefore, the full extent of ACE inhibition is

relatively slow in onset. This in part explains why in our EIA the  $C_{\max}$  enalapril results were much higher than those in the literature. It has been reported also that using LC/MS/MS,  $C_{\max}$  values for enalapril were higher than those using ACE-inhibition assays (11). On the contrary, enalaprilat concentrations following 0.75 h were found to be higher using ACE inhibition assay than RIA (13). In our study, enalaprilat concentrations at 12 and 24 h were lower than those in the literature, which were determined by ACE inhibition assay, and were also similar to values determined by LC/MS/MS (11). Other reviews mentioned that younger age and better health increase clearance of enalaprilat (4). It has been shown that the apparent clearance of enalapril after oral administration and the clearance of enalaprilat after intravenous administration were about 30% lower in elderly than in young individuals.

The maximum decrease in blood pressure in normotensive individuals was higher following 20-mg than 10-mg oral dose of enalapril. The maximum decrease in systolic blood pressure was 12% compared with 7% for 20 and 10 mg, respectively, and the maximum decrease in diastolic blood pressure was 18% compared with 12% for 20 and 10 mg, respectively. Furthermore, when plasma enalaprilat concentrations from the same number of volunteers following 10- and 20-mg doses were combined and correlated with the decrease in systolic and diastolic blood pressure the  $r$  values ( $r = 0.946$  and  $0.954$  respectively) became higher than following 10-mg dose only ( $r = 0.819$  and  $0.798$ , respectively). This is because of a higher decrease in blood pressure when higher plasma enalaprilat concentrations were reached following a 20-mg dose of enalapril.

In conclusion, this study confirms that  $C_{\max}$  of enalapril is about 10 times higher than those reported in the literature. This is because EIA measures the concentration of enalapril directly. In addition, in young normotensive subjects the clearance of enalaprilat might be faster than in aged hypertensive individuals. Finally, as EIA is simple, accurate and sensitive, it may be useful for TDM of enalapril and enalaprilat in patients with congestive heart failure or chronic renal insufficiency because enalapril accumulation may lead to adverse events.

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