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Bioequivalence Study of Two Formulations of Enalapril, at a Single Oral Dose of 20 mg (Tablets): A Randomized, Two-Way, Open-Label, Crossover Study in Healthy Volunteers

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ABSTRACT

Background: Enalapril maleate is the monoethyl ester prodrug of enalaprilat, an angiotensin-converting enzyme inhibitor indicated in the management of essential and renovascular hypertension, and in the treatment of congestive heart failure and in asymptomatic patients with left ventricular dysfunction and an ejection fraction of ≥35%. Enalapril has little pharmacologic activity until hydrolyzed in vivo to enalaprilat.

Objective: The aim of the present study was to compare the bioavailability and tolerability of 2 commercial brands (test and reference formulations) of enalapril tablets (20 mg), described as the rate and extent of absorption of the active moiety, to assess their bioequivalence.

Methods: This single-dose, randomized, 2-way, open-label, crossover study in healthy volunteers aged 18 to 40 years was conducted at the Clinical Pharmacology Study Unit, Hospital Clínico San Carlos (Madrid, Spain). Subjects were randomized to receive (under fasting conditions) either the test or reference formulation of enalapril (20-mg tablet) at study period 1 and the opposite formulation at study period 2. Study periods were separated by a washout period of at least 7 days. During each study period, 15 plasma extractions were made to determine enalapril and enalaprilat plasma concentrations and to calculate the pharmacokinetic (PK) properties (maximal plasma drug concentration [C_{max}], time to C_{max} [T_{max}], area under the plasma concentration–time curve [AUC] to the last measurable concentration [AUC_t], AUC from time 0 to infinity [$AUC_{0-\infty}$], mean residence time, and elimination half-life [$t_{1/2}$]) of both. Physical examination, subject interview, laboratory analyses, electrocardiogram, and blood pressure (BP) were used to assess tolerability.

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Results: Twenty-four subjects were included in the study (12 men, 12 women; mean [SD] age, 22.8 [2.2] years [range, 19–30 years]). Of these, 1 subject (4.2%) withdrew from the study for personal reasons; thus, PK and statistical analyses included results from 23 subjects. No statistically significant sequence or period effect was found. T_{max} was not statistically different between the 2 formulations, and the 90% CI calculated for T_{max} for the difference of the medians was within the predefined range. The 90% CIs of the logarithmically transformed concentration–derived parameters (C_{max}, AUC_t, and $AUC_{0-\infty}$) also were within the predefined range; thus, the 2 formulations are considered bioequivalent. For both formulations, systolic and diastolic BPs showed significant reductions compared with baseline values ($P < 0.05$). Seven adverse effects were recorded, all of them transient and none of severe intensity.

Conclusions: In this study of 2 commercial brands (test and reference formulations) of enalapril in healthy subjects, designed and conducted under Good Clinical Practice guidelines, a similar rate and extent of absorption for both formulations were found to be bioequivalent. Both formulations produced a significant decrease in BP values and were generally well tolerated. (*Curr Ther Res Clin Exp.* 2004;65:34–46) Copyright © 2004 Excerpta Medica, Inc.

Key words: bioequivalence, bioavailability, pharmacokinetics, enalapril, enalaprilat, healthy volunteers, clinical trial, blood pressure.

INTRODUCTION

The use of generic drugs is of increasing importance, in terms of efficiency, in the selection of therapeutic alternatives. But their use in clinical practice depends not only on their "essential similarity" (in terms of formulation, composition, and bioequivalence as considered by regulatory agencies), but mostly on the conviction of their interchangeability with their reference counterparts. Thus, the publication of the comparative bioavailabilities of test and reference formulations is significant for the knowledge and appropriate assessment by the scientific community of what they are dealing with. When 2 formulations of the same drug present similar bioavailabilities to the extent that they are considered bioequivalent by certain criteria (ie, those described by the Committee for Proprietary Medicinal Products[1] [CPMP]), it is assumed that when administered in the same molar dose, they will provide the same therapeutic effect, or they will be therapeutically equivalent.[2]

Enalapril maleate is the monoethyl ester prodrug of enalaprilat, an angiotensin-converting enzyme (ACE) inhibitor, that acts by decreasing plasma angiotensin II and aldosterone levels, consequently decreasing blood pressure (BP) by decreasing peripheral vascular resistance.[3,4] It is indicated in the management of all grades of essential and renovascular hypertension, as well as in the treatment of congestive heart failure and in asymptomatic patients with left ventricular dysfunction and an ejection fraction of $\geq$35%.[4]

Enalapril has little pharmacologic activity until hydrolyzed in vivo to enalaprilat. Unlike enalaprilat, the prodrug is well absorbed after oral administration, with an oral absorption of 55% to 75% compared with 3% to 12% for enalaprilat.[3,4] The maximal plasma drug concentration (C_{max}) for the prodrug is reached within ~1 hour after oral administration, whereas C_{max} for enalaprilat is somewhat delayed (3–4 hours after oral administration).[3,4] Plasma enalaprilat concentrations are reportedly linearly related to the administered dose over the therapeutic range (2.5–40.0 mg).[3] Enalaprilat is ~50% bound to plasma proteins.[3] Renal excretion is the primary route of elimination. The elimination half-life ($t_{1/2}$) of unchanged enalapril is >2 hours in healthy subjects.[3] For enalaprilat, polyphasic elimination kinetic properties have been reported, with an initial $t_{1/2}$ of ~5 hours and a reported terminal half-life of 30 to 35 hours[3] or, in other cases,[4] 35 to 38 hours (range, 30–87 hours), probably reflecting its binding to the high-affinity, low-capacity binding site of circulating serum ACE.[4] Some evidence shows a correlation between plasma enalaprilat concentrations and plasma ACE activity and a possible correlation between these plasma concentrations and decreases in BP.[5]

Adverse effects (AEs) that occur with enalapril therapy usually are mild and transient, occurring in <10% of patients.[6] The most typical AEs associated with enalapril use include headache, dizziness, fatigue, diarrhea, nausea and/or vomiting, rash, cough, and hypotension.[6] In 3% to 6% of patients, therapeutic discontinuance occurs as a consequence of dry, persistent cough, followed by rare cases (<1%) of angioedema, hypotension, hyperkalemia, or acute renal failure.

In the present study, the bioequivalence of 2 commercial brands (test and reference) of enalapril tablets (20 mg) was assessed by comparison of their pharmacokinetic (PK) properties that describe the rate and extent of absorption—area under the plasma concentration–time curve (AUC), C_{max}, and time to C_{max} (T_{max}).

SUBJECTS AND METHODS

Study Design

This single-dose, randomized, 2-way, open-label, crossover study was conducted at the Clinical Pharmacology Study Unit, Hospital Clínico San Carlos (Madrid, Spain). The protocol was approved by the hospital Ethical Committee and was authorized by the Spanish Ministry of Health. It was developed according to the revised principles of the Declaration of Helsinki (World Medical Association revised Somerset West, 1996) and the Good Clinical Practice Guidelines (International Conference on Harmonization, 1996).

Subjects

Subjects were selected from a panel of healthy volunteers recruited by the Clinical Pharmacology Study Unit. Inclusion criteria were age 18 to 40 years, body weight 50 to 100 kg, and body mass index 18 to 27 kg/m^2. All subjects were

examined to verify their healthy status; these examinations included medical history taking, vital sign measurements, electrocardiography (ECG), blood sample analysis (basic profile, complete blood cell count, prothrombin time, viral serology), and urinalysis (sediment, drugs, pregnancy test). Subjects with relevant clinical, analytical, or ECG abnormalities were excluded from the trial. Additional exclusion criteria were as follows: smoking; history of alcohol or other drug abuse; high consumption (>8 cups/d) of stimulating beverages; consumption of medication that could affect the drug under study (eg, antacids, antidepressants); regular consumption of any medication in the 2 weeks before enrollment; consumption of any enzyme inhibitors or inducers in the month before enrollment; participation in a clinical trial in the 2 months before enrollment or 4 times in the year before enrollment; history of clinically important illness or major surgery in the 3 months before enrollment; inability to relate to and/or cooperate with the investigators; medication allergy; illnesses or disorders that could affect the absorption, distribution, metabolism, and/or excretion of drugs (eg, malabsorption, edemas, renal and/or hepatic failure); a history of positive serology for hepatitis B or C (not due to immunization) or HIV; blood loss or donation >200 mL in the 3 months before enrollment; blood or blood-derivative transfusion in the 6 months before enrollment; and exhausting physical exercise in the 72 hours before enrollment. Pregnant, possibly pregnant, or breastfeeding women were excluded from the study. Women of childbearing age were required to use an effective method of birth control (except oral anovulatory drugs) throughout the study. All eligible subjects provided written informed consent to participate.

Methods

Subjects were admitted to the study unit at ~8 PM on each of the 2 evenings before study drug administration. Clinical entry controls (physical examination and subject interview) were performed, and all subjects received a standard dinner (balanced composition with ~25% of daily calories). Subjects fasted for at least 10 hours before and 4 hours after study drug administration.

Subjects were randomized, according to a computer-generated randomization table of sequences, to receive either the test* or reference† formulation of enalapril (20-mg tablet) at study period 1 and the opposite formulation at study period 2. Study periods were separated by a washout period of at least 7 days. Drug administration started at 8 AM, and volunteers received the medication with 150 mL of water at room temperature by a nurse.

Drug Analysis

Blood samples of ~10 mL each were drawn by a nurse at 15 time points: baseline (immediately before study drug administration); 30, 45, 60, 75, 105, 135, 180,

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195, and 225 minutes; and 5, 8, 12, 24, and 36 hours after study drug administration. Samples were centrifuged at 3000 rpm for 10 minutes (at 4°C), and the obtained plasma samples were separated in two 1.5-mL aliquots and stored in suitably labeled, tightly sealed tubes at –30°C. After 24 hours, the samples were transferred to –80°C. Volunteers remained under medical supervision at the study center until 12 hours after study drug administration, and returned at 24 and 36 hours after administration for the last extractions.

Analytical determination of plasma drug and metabolite concentrations were performed by MCC Analítica S.A. (Barcelona, Spain) and followed an acid solid–liquid extraction with a reversed-phase high-pressure liquid chromatography separation and mass spectrometry detector. Lisinopril was used as an internal standard. The method of analysis was validated under the principles of Good Laboratory Practice through the following parameters: linearity, precision, intra-assay and interassay accuracy, limit of quantification (LOQ), validation of the dilution factor, specificity, stability, and recovery. The analytical part of the study was developed blindly.

Pharmacokinetic Analysis

PK properties were calculated by a noncompartmental approach from plasma concentrations of enalapril and enalaprilat, using WinNonlin Pro software version 2.1 (Pharsight Corporation, Mountain View, California). C_{max} was estimated directly from observed concentrations, and T_{max} as the corresponding time point at which C_{max} occurred. AUC_t was calculated by the linear trapezoidal method until the last measurable plasma drug concentration, and AUC from time 0 to infinity ($AUC_{0-\infty}$) was calculated as:

$$AUC_{0-\infty} = AUC_t + C_t/K_e,$$

where C_t is the plasma drug concentration at time t and K_e is the elimination rate constant. The mean residence time ($MRT_{0-\infty}$) and $t_{1/2}$ also were calculated and presented for descriptive purposes. Given the inactive nature of enalapril, the PK properties of enalaprilat were defined as primary assessment criteria. AUC is considered the most representative parameter of bioequivalence.

Tolerability Assessment

For tolerability assessment, clinical controls (vital constants, eg, BP, temperature, or heart rate, and the question on AE) were performed at 5, 12, 24, and 36 hours after study drug administration. Laboratory analyses and ECG also were performed at prestudy and poststudy times. Tolerability of the 2 formulations was the secondary assessment criterion; thus, only descriptive statistics were foreseen and performed.

Statistical Analysis

The sample size needed for >90% power was determined using PC-SIZE software version 1.0 (StatTools™, Palisade Corporation, Newfield, New York) and variability data from a previously published study.[7]

Concentration-derived parameters were analyzed (WinNonlin, Pharsight Corporation, Mountain View, California) using a parametric method, conducted by means of analysis of variance (ANOVA), using as dependent variables the logarithmic transformations (Ln) of the test/reference ratios of the parameters $AUC_{0-\infty}$, AUC_t, C_{max}, and $C_{max}/AUC_{0-\infty}$. In the analysis, the following model effects were considered: form, period, sequence, and volunteer (sequence). The 90% CIs were calculated for these ratios and used as bioequivalence-assessment criteria. Acceptance criteria for the 90% CIs were prospectively defined in the study protocol as 80% to 125% for Ln AUC ratios and 70% to 143% for Ln C_{max} ratios. The T_{max}s from both formulations were compared using a nonparametric method (CI of the median of the differences by the Wilcoxon signed rank test), and the acceptance criterion for this parameter was 70% to 130%.

RESULTS

Subjects

Twenty-four white healthy volunteers were included in the study (12 men, 12 women; mean [SD] age, 22.8 [2.2] years [range, 19–30 years]; mean [SD] body weight, 65.1 [12.4] kg [men, 75.3 (9.1) kg; women, 54.9 (4.6) kg]; mean [SD] height, 173.0 [10.2] cm [men, 182.7 (4.6) cm; women, 163.9 (3.6) cm]). One subject (4.2%) withdrew between study periods for personal reasons. The results of 23 subjects were included in the PK analysis.

Analytical Assay

Assessment of PK parameters determined the adequacy of the analytical method for the determination of plasma drug and metabolite concentrations. The LOQ was set at 1 ng/mL for both enalapril and enalaprilat.

Mean plasma drug concentrations of enalapril and enalaprilat for both the test and reference formulations are presented in **Figure 1**. No predose detectable levels were found in any of the subjects in either treatment period.

Pharmacokinetic Analysis

The PK properties of enalapril and enalaprilat are summarized in **Table I** and **Table II**. Mean (SD) C_{max}s of 118.58 (52.38) ng/mL and 134.63 (56.28) ng/mL (enalapril) and 70.02 (31.90) ng/mL and 73.60 (30.31) ng/mL (enalaprilat) for test or reference formulations, respectively, were attained at median T_{max}s of 0.96 and 0.90 hours (enalapril) and 3.25 and 3.39 hours (enalaprilat) (respectively, for test and reference formulations). Results of $MRT_{0-\infty}$ and $t_{1/2}$ are shown in Tables I and II. All subjects (100.0%) presented an $AUC_t/AUC_{0-\infty}$ ratio >80%, and the ANOVA of Ln C_{max}, AUC_t, and $AUC_{0-\infty}$ showed no statistically significant sequence or period effect. The obtained 90% CIs for the parameter ratios are presented in **Table III**.

The ratios and their 90% CIs calculated for the Ln-transformed parameters for enalapril were Ln C_{max}, 88.90% (77.35%–102.16%); Ln AUC_t, 94.08% (87.14%–

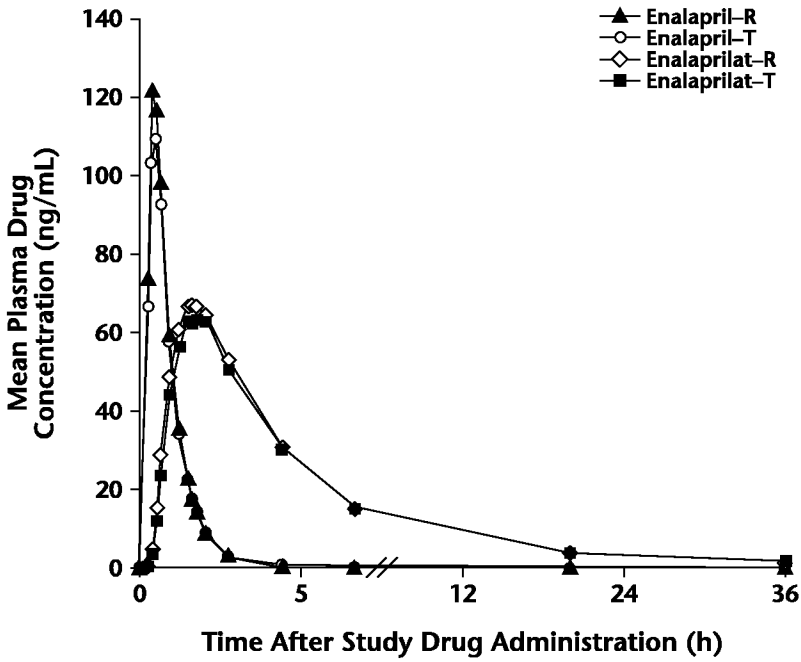

Figure 1. Plasma drug concentrations of the test (T; trademark: Enalapril Farmoz®, Tecnimede Sociedade Técnico-Medicinal S.A., Prior Velho, Portugal) and reference (R; trademark: Renitec®, Merck Sharp & Dohme, Lda., PaÇo de Arcos, Portugal) formulations of enalapril (prodrug) and enalaprilat (active metabolite) (N = 23 subjects).

101.58%); and Ln $AUC_{0-\infty}$, 94.19% (87.42%–101.49%). The respective values for enalaprilat were 92.98% (83.56%–103.46%) for Ln C_{max}, 95.87% (88.60%–103.74%) for Ln AUC_t, and 96.27% (89.20%–103.91%) for Ln $AUC_{0-\infty}$. All of the calculated intervals were within the predefined bioequivalence ranges; also, 90% CIs obtained for the median of differences of T_{max} were within predefined ranges for both enalapril (86.14%–113.86%) and enalaprilat (88.90%–100.00%).

Tolerability

All 24 subjects were included in the tolerability assessment. During the course of the study, 7 AEs were reported. Five possibly treatment-related AEs were recorded (dizziness, 2 subjects [8.3%]; fatigue, headache, and somnolence, 1 subject each [4.2%]). One subject (4.2%) experienced an AE with doubtful treatment relationship (diarrhea), and 1 subject (4.2%) experienced an AE that was not treatment related (dizziness). All AEs were mild except 1 case of dizziness, which was moderate. All AEs resolved completely and spontaneously. Six AEs occurred during administration of the reference formulation, and 1 occurred during administration of the test formulation. No safety concerns arose.

Table I. Pharmacokinetic properties of enalapril (prodrug) from the test* and reference† (Ref) formulations (N = 23 subjects).

	C_{max}, ng/mL		T_{max}, h		AUC_t, ng/mL · h		$AUC_{0-\infty}$, ng/mL · h		$MRT_{0-\infty}$, h		$t_{1/2}$, h	
Parameter	Test	Ref	Test	Ref	Test	Ref	Test	Ref	Test	Ref	Test	Ref
Mean	118.58	134.63	0.96	0.90	189.40	200.65	192.07	203.62	1.67	1.61	0.87	0.82
SD	52.38	56.28	0.34	0.26	85.52	79.98	85.50	81.02	0.33	0.34	0.33	0.22
Minimum	48.28	48.43	0.50	0.50	102.67	90.70	104.53	91.92	1.23	1.07	0.59	0.54
Median	108.95	132.51	1.00	0.75	171.48	198.81	175.08	201.58	1.61	1.60	0.79	0.74
Maximum	291.94	251.54	2.25	1.75	483.86	390.77	487.01	395.07	2.60	2.85	1.76	1.37
CV%	44.17	41.81	35.81	28.63	45.15	39.86	44.51	39.79	19.71	21.27	37.47	26.37
Geometric mean	109.67	122.98	0.91	0.87	175.37	185.87	178.18	188.68	1.64	1.58	0.83	0.80
Harmonic mean	101.97	111.37	0.87	0.85	164.45	171.89	167.29	174.54	1.61	1.56	0.79	0.78

C_{max} = maximum plasma drug concentration; T_{max} = time to maximal plasma drug concentration; AUC_t = area under the plasma concentration–time curve to the last measurable concentration; $AUC_{0-\infty}$ = area under the plasma concentration–time curve from time 0 to infinity; $MRT_{0-\infty}$ = mean residence time; $t_{1/2}$ = elimination half-life; CV% = coefficient of variation.

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Table II. Pharmacokinetic properties of enalaprilat (active metabolite) from the test* and reference† (Ref) formulations of enalapril (prodrug) (N = 23 subjects).

	C_{max}, ng/mL		T_{max}, h		AUC_t, ng/mL · h		$AUC_{0-\infty}$, ng/mL · h		$MRT_{0-\infty}$, h		$t_{1/2}$, h	
Parameter	Test	Ref	Test	Ref	Test	Ref	Test	Ref	Test	Ref	Test	Ref
Mean	70.02	73.60	3.25	3.39	563.48	588.69	587.10	610.07	9.64	9.37	6.06	5.98
SD	31.90	30.31	0.71	0.82	226.50	234.00	237.95	241.38	3.10	2.91	2.04	2.01
Minimum	21.42	17.89	2.25	1.75	233.41	252.28	249.23	269.81	4.91	4.28	2.54	2.13
Median	73.99	69.62	3.00	3.25	483.34	529.65	523.09	549.87	9.16	9.27	5.52	6.01
Maximum	152.57	136.88	5.00	5.00	1056.22	1089.09	1099.65	1155.58	16.12	15.90	9.93	9.81
CV%	45.56	41.18	21.88	24.23	40.20	39.75	40.53	39.57	32.14	31.08	33.65	33.69
Geometric mean	62.51	67.03	3.18	3.30	524.19	545.60	546.44	566.51	9.17	8.93	5.70	5.60
Harmonic mean	54.57	59.41	3.12	3.20	488.94	504.52	510.84	525.63	8.70	8.46	5.33	5.16

C_{max} = maximum plasma drug concentration; T_{max} = time to maximal plasma drug concentration; AUC_t = area under the plasma concentration–time curve to the last measurable concentration; $AUC_{0-\infty}$ = area under the plasma concentration–time curve from time 0 to infinity; $MRT_{0-\infty}$ = mean residence time; $t_{1/2}$ = elimination half-life; CV% = coefficient of variation.

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Table III. Differences in pharmacokinetic properties of enalapril (prodrug) and enalaprilat (active metabolite) between the test* and reference† formulations (N = 23 subjects).

Property	Enalapril	Enalaprilat
Ln C_{max}		
Difference, mean (SE)	−0.12 (0.08)	−0.07 (0.06)
Ratio, %	88.90	92.98
90% CI, %	77.35 to 102.16	83.56 to 103.46
Ln AUC_t		
Difference, mean (SE)	−0.06 (0.04)	−0.04 (0.05)
Ratio, %	94.08	95.87
90% CI, %	87.14 to 101.58	88.60 to 103.74
Ln $AUC_{0-\infty}$		
Difference, mean (SE)	−0.06 (0.04)	−0.04 (0.04)
Ratio, %	94.19	96.27
90% CI, %	87.42 to 101.49	89.20 to 103.91
T_{max}‡		
Differences, median, h	0.0	−0.125
90% CI, difference, h – decimal	−0.125 to 0.125	−0.375 to 0.0
90% CI, difference, % (with respect to mean of reference form)	86.14 to 113.86	88.90 to 100.00

Ln = logarithmically (e-base) transformed; C_{max} = maximal plasma drug concentration; AUC_t = area under the plasma concentration–time curve to the last measurable concentration; $AUC_{0-\infty}$ = area under the plasma concentration–time curve from time 0 to infinity; T_{max} = time to maximal plasma drug concentration.
*Trademark: Enalapril Farmoz® (Tecnimede Sociedade Técnico-Medicinal S.A., Prior Velho, Portugal).
†Trademark: Renitec® (Merck Sharp & Dohme, Lda., Paço de Arcos, Portugal).
‡No significant differences were found between the test and reference formulations.

Laboratory analysis for tolerability revealed some modifications in the biochemical and hematologic results, namely: (1) decreased red blood cell count (9 subjects [37.5%]), (2) slight elevations in a biochemical parameter (total bilirubin, 2 subjects [8.3%]), (3) reductions in other biochemical parameters (alkaline phosphatase, 1 subject [4.2%]; uric acid, 4 [16.7%]; and total cholesterol, 3 [12.5%]), and (4) presence of urine sediment anomalies (oxalates, 2 subjects [8.3%]; and white blood cells, 1 [4.2%]). Symptoms were not detected in any of these cases, and all of these laboratory abnormalities were considered clinically nonsignificant.

The mean values obtained for systolic and diastolic BP are presented in **Figure 2**. After 5 hours, the mean values obtained for both formulations were significantly lower than baseline values (both $P < 0.05$), corresponding to a minimum in the systolic and diastolic BPs, with mean (SD) systolic BP values of 102 (18) mm Hg for the reference formulation and of 101 (11) mm Hg for the test formulation.

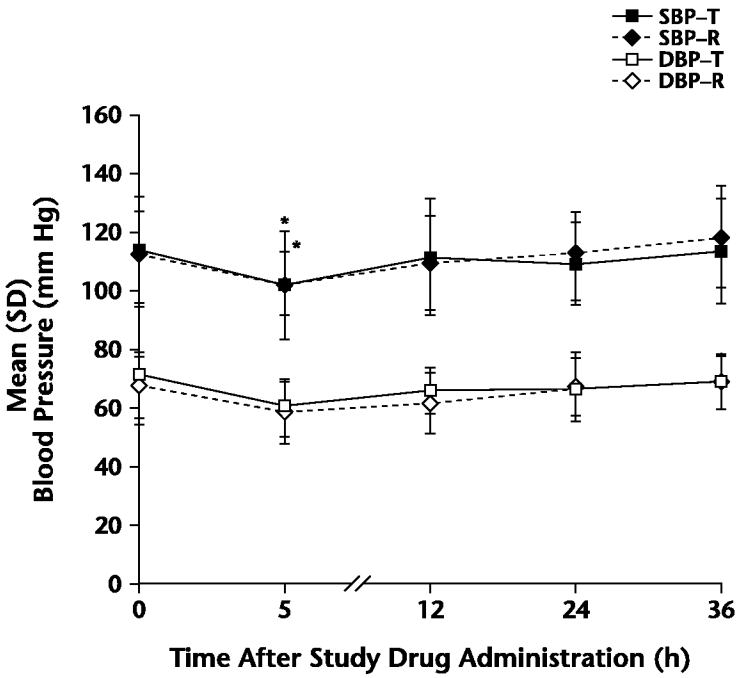

Figure 2. Systolic and diastolic blood pressures (SBP and DBP, respectively) of the test (T; trademark: Enalapril Farmoz®, Tecnimede Sociedade Técnico-Medicinal S.A., Prior Velho, Portugal) and reference (R; trademark: Renitec®, Merck Sharp & Dohme, Lda., PaÇo de Arcos, Portugal) formulations of enalapril (N = 23 subjects). *$P < 0.05$ 5 hours versus baseline.

DISCUSSION

Both the test and reference formulations of enalapril exhibited overlapping plasma profiles. The fact that no model effects (period or sequence) or detectable predose plasma drug concentrations were found indicates the adequacy of the proposed study design and of the study conduction.

To ensure a reliable estimate of the extent of absorption, a collection period of at least 3 $t_{1/2}$s is recommended by US Food and Drug Administration[8] and CPMP[1] guidelines. This requisite was fulfilled, and the mean extrapolated area was well below 20% for both formulations, indicating that the extraction period was adequate to fully characterize the PK properties of the prodrug and its active metabolite.

Taking into account the PK characteristics of the prodrug and of its active metabolite, a single-dose PK study was deemed as appropriate. The chosen dose falls within the range of clinically administered doses. The study was conducted under fasting conditions to reduce the possible interference of food over absorption kinetics.

Because the assessed variables were PK properties, it was regarded unnecessary to use a blinded design. Nevertheless, as mentioned earlier, the analytical part of the study was conducted blindly.

The design we chose complies with European[1] and US[8] guidelines on bioavailability/bioequivalence studies. The study design is suited to its objectives, and the study was carried out with no significant deviations from the protocol.

The calculated PK parameters are similar to those previously described: T_{max} for enalapril and enalaprilat were in accordance with previously reported values.[3] In our study, the $t_{1/2}$ for enalapril was relatively short for both formulations (medians: test, 0.79 hour; reference, 0.74 hour); for enalaprilat, the median $t_{1/2}$s were 5.52 and 6.01 hours for the test and reference formulations, respectively. Although polyphasic elimination kinetic properties have been described for enalapril, with mean terminal half-lives ~30 hours, similar half-lives for enalaprilat have been reported in a separate bioequivalence study by Ribeiro et al.[7] This difference in the estimated half-lives is probably related to the sampling period (in our case, up to 36 hours after study drug administration, and in the study by Ribeiro et al,[7] up to 24 hours after administration).

Although the CPMP guidelines[1] recommend that bioequivalence should be assessed by comparing the bioavailability of the parent compound administered, in this case, given that the parent compound is inactive and its metabolite is in fact the compound exhibiting pharmacologic activity, it was considered appropriate to compare the bioavailability of the metabolite in both formulations.

All 90% CIs obtained for enalapril (the prodrug) and enalaprilat (the active metabolite) were within the predefined ranges of bioequivalence acceptance; thus, the 2 formulations are considered bioequivalent. Although a relatively high variability was found for some parameters of each formulation, that was not the case in the comparison of the intrasubject differences and ratios.

In general, enalapril was well tolerated. No unexpected AEs occurred, and the reported possibly treatment-related AEs could be explained by the hypotensive effect of the drug. The hematologic changes in the red blood cell count were attributable to the blood extraction during the study. No problems concerning safety of the formulation were detected.

Results for BP were collected to assess the tolerability of the formulations and not their pharmacodynamic properties. Accordingly, the assessment times and the study design did not allow for a closer monitoring of the BP as would be necessary for a pharmacodynamic assessment. However, unlike what was reported by Ribeiro et al[7] on the absence of an evaluable effect (BP) of enalapril in healthy volunteers, we found significant reductions in mean BPs ($P < 0.05$), which returned to baseline values at the following measurement (assessed at 12 hours after study drug administration). This finding is in accordance with the reported reduction in BP found in another study[4] in normotensive individu-

als, and suggests that, for this group of drugs (hypotensives), a more detailed assessment of pharmacodynamic properties could be performed in healthy subjects using a more specific study design (ie, BP monitoring).

CONCLUSIONS

In this study of 2 commercial brands (test and reference formulations) of enalapril in healthy subjects, designed and conducted under Good Clinical Practice guidelines, a similar rate and extent of drug absorption for both formulations were found to be bioequivalent. Both formulations produced a significant decrease in BP values and were generally well tolerated.

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