Relative Bioavailability Study of 20-mg Enalapril Tablets in Healthy Male Volunteers

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Abstract

The pharmacokinetic and relative bioavailability studies of 20-mg enalapril tablets, the test product manufactured by Biolab, Thailand compared to the reference product (Merck Sharp & Dohme, USA) was conducted in 14 healthy Thai male volunteers following a single dose, two-period, crossover design. Each subject received 20-mg enalapril tablets of both formulations with a 1-week washout period. Plasma samples collected over a 24-h period after administration were analyzed by LC/MS/MS. Pharmacokinetic parameters were determined by using non-compartmental analysis. Regarding bioequivalence testing, the 90 per cent confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios (test/reference) of enalapril were 101.8-134.9 per cent and 105.9-121.4 per cent and those of enalaprilat were 104.2-122.3 per cent and 104.5-118.1 per cent. Based on the European bioequivalence guideline, the 90 per cent confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios of both parent and metabolite forms were within the acceptable ranges of 70-143 per cent and 80-125 per cent, respectively. It was concluded that the test formulation was bioequivalent to the reference formulation and both formulations can be used interchangeably in clinical practice.

Key word : Enalapril, Enalaprilat, Pharmacokinetics, Bioequivalence, Relative Bioavailability

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Enalapril maleate is an ethylester of a long-acting angiotensin converting enzyme (ACE) inhibitor and enalaprilat. It is widely used for the treatment of hypertension, congestive heart failure and asymptomatic left ventricular dysfunction. Enalapril itself is a prodrug with little pharmacological effect. Following oral administration, 55-75 per cent of administered enalapril is rapidly absorbed without food effect. The time to reach maximal concentration (T_{max}) of enalapril is 0.5-1.5 h. Approximately 60 per cent of enalapril is hydrolysed by liver enzymes to enalaprilat, a specific inhibitor of angiotensin converting enzyme which is important for the formation of angiotensin II resulting in artery relaxation and blood pressure reduction. It also increases the renal blood flow and reduces the aldosterone secretion. The half-lives of enalapril and enalaprilat are 4.0 and 3.9 h, respectively. Both parent and metabolite forms are primarily excreted in urine (60-78%) and feces (33%)[1-4].

The initial dose of enalapril for treatment of hypertension is 5 mg/day for 1-2 weeks and gradually increased to 10-40 mg/day. Patients with congestive heart failure should begin with 2.5 mg/day for 1-2 weeks and increased to 5-80 mg/day[1-3]. For treatment of asymptomatic left ventricular dysfunction, the initial dose is 2.5 mg/day and gradually increased to 20 mg/day. The most common adverse effect of enalapril is a dry cough. Angioedema was reported in the long-term treatment[5-8]. It should be used with caution in patients with impaired renal function, cardiovascular diseases and hyponatremia and contraindicated in hypersensitive patients.

The objectives of this study were to assess pharmacokinetic parameters of enalapril and enalaprilat in healthy Thai male volunteers and the average bioequivalence of two formulations of 20-mg enalapril tablets : Enaril® manufactured by Biolab, Thailand as the test formulation and Renitec® manufactured by Merck Sharp & Dohme, USA as the reference formulation, in healthy Thai male volunteers. The clinical protocol was reviewed and approved by the Ethics Committee of the Ministry of Public Health, Thailand.

MATERIAL AND METHOD

Enalapril preparations

Reference preparation: Renitec® 100 tablets (Merck Sharp & Dohme, USA) containing 20 mg enalapril per tablet (Lot no. Y6093, Mfg. date 12/99).

Test preparation: Enaril® 100 tablets (Biolab, Thailand) containing 20 mg enalapril per tablet (Lot no. ER20/3, Mfg. date 11/00).

Study design and healthy volunteers

Fourteen healthy Thai male volunteers aged between 18-45 years (19.4 ± 1.3 years) with a body mass index between 18-24 (20.3 ± 1.4) participated in a randomized, single dose, fasting, two-period, two-sequence, crossover study with a 1-week washout period. All the healthy volunteers provided written informed consent before enrollment, after details and purpose of the study had been explained to them. The volunteers were non-smoking, non-alcoholic and free from significant cardiac, hepatic, renal, gastrointestinal, and hematological diseases, as assessed by physical examination and the following laboratory tests: complete blood count, total bilirubin, serum creatinine, blood nitrogen urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and hepatitis B surface antigen.

During each period, the volunteers were admitted to the Bioequivalence Test Center, Naresuan University at 6.00 p.m. and had an evening meal before 9.00 p.m. After an overnight fast, they received a single 20 mg enalapril dose (1 x 20-mg tablet) of each of the formulations at 7.00 a.m. along with 240 ml of water. They remained seated for at least 30 min and fasted for 2 h. A standard lunch and an evening meal were provided at 4 and 9 h after dosing. No other food was permitted during the study period. Liquid consumption was allowed *ad libitum* after lunch, however, xanthine-containing and acidic beverages were prohibited. Blood pressure in the volunteers was monitored regularly throughout the study. After each period of the study, the volunteers were investigated by a physician.

Drug analysis

Seven milliliters of each blood sample was collected into a lithium-heparinized containing tube by catheterized venupuncture at the forearms before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 24 h after drug administration. The blood samples were centrifuged at 4°C (2,000 g, 10 min) and the plasma samples were separated within 10 min after collecting blood. All encoded plasma samples were kept at -80°C until transportation in dry ice to the Pharmakin GmbH Gesellschaft fur Pharmakokinetik, Germany for enalapril and enalaprilat chemical analysis.

As described earlier by Lohitnavy O et al (9), plasma enalapril and enalaprilat were measured by LC-MS-MS in the combined ESI(-)/ESI(+) mode with the lower limits of quantitation of 0.56 and 0.59

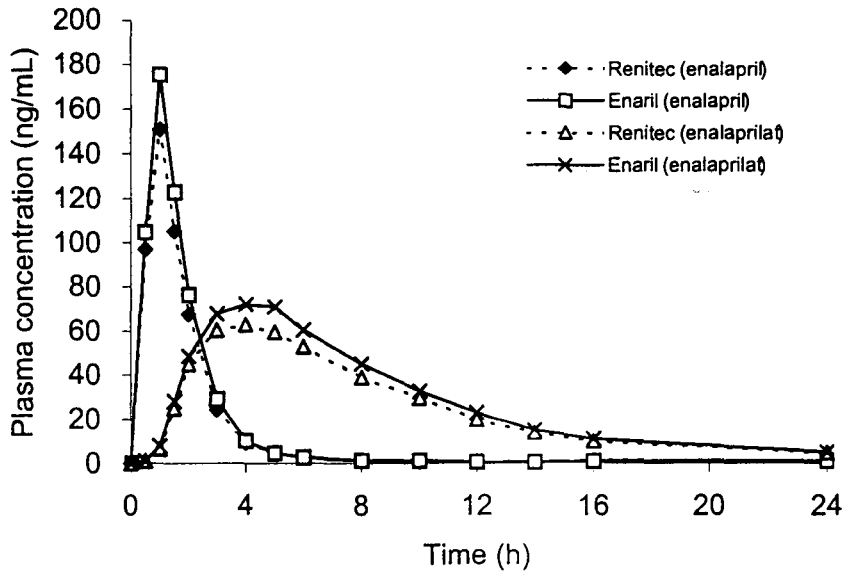

Fig. 1. Plasma concentration-time curve of enalapril and enalaprilat after 20-mg single dose administrations of Renitec® (reference) and Enaril® (test) in 14 healthy Thai male volunteers.

Table 1. Summary data of pharmacokinetic parameters of enalapril and enalaprilat after 20-mg single dose administrations of reference and test formulations in healthy Thai male volunteers (n = 14, mean ± SD).

Pharmacokinetic parameters	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng.h/ml)	$t_{1/2}$ (h)
Enalapril				
Test	1.0 ± 0.2	185.39 ± 62.20	319.39 ± 89.85	1.1 ± 0.5
Reference	1.0 ± 0.2	154.48 ± 46.37	278.93 ± 77.54	1.2 ± 0.5
Enalaprilat				
Test	4.3 ± 0.7	74.56 ± 18.41	701.48 ± 158.74	4.9 ± 0.8
Reference	4.2 ± 1.0	65.73 ± 15.45	623.06 ± 98.44	5.0 ± 1.0

ng/ml, using a validated method described in the full report of the laboratory. Subsequently the analytical data were sent directly to the principle investigator for further pharmacokinetic and statistic data analysis.

Pharmacokinetic and statistical analysis

A non-compartmental pharmacokinetic method was employed to determine the pharmacokinetic parameters of enalapril and enalaprilat. The time to peak plasma concentration (T_{max}) and the peak concentration (C_{max}) were obtained directly from the plasma concentration results. The area under the concentration-time curve ($AUC_{0-\infty}$) and half-life ($t_{1/2}$) were determined by linear trapezoidal protocol by using WinNonlin Standard (version 3.0).

The analysis of variance (ANOVA) was conducted on C_{max} and $AUC_{0-\infty}$, using general linear models (GLM) procedures, in which sources of variation were sequence, subjects within sequence, period, and formulation. Then the 90 per cent confidence intervals of the test/reference ratios for C_{max} and $AUC_{0-\infty}$ (log transformed) were determined. The average bioequivalence between two formulations can be concluded when the 90 per cent confidence inter-

Table 2. ANOVA table of C_{max} and $AUC_{0-\infty}$ (log transformed) of enalapril and enalaprilat following 20-mg single dose administrations of reference and test formulations in 14 healthy Thai male volunteers.

	Enalapril					
	Source of Variation	DF	SS	MS	F	P
A. C_{max}	Total	27	3.033			3.033
	Sequence	1	0.127	0.127	0.711	0.127
	Subject (sequence)	12	2.145	0.179	4.092	2.145
	Formulation	1	0.177	0.177	4.042	0.177
	Period	1	0.061	0.061	1.394	0.061
	Error	12	0.524	0.044		0.524
B. $AUC_{0-\infty}$	Total	27	2.365			
	Sequence	1	0.167	0.167	1.054	0.325
	Subject (sequence)	12	1.902	0.159	15.230	0.000
	Formulation	1	0.110	0.110	10.605	0.007
	Period	1	0.060	0.060	5.787	0.033
	Error	12	0.125	0.010		
	Enalaprilat					
	Source of Variation	DF	SS	MS	F	P
A. C_{max}	Total	27	1.323			
	Sequence	1	0.119	0.119	1.539	0.238
	Subject (sequence)	12	0.931	0.078	5.486	0.003
	Formulation	1	0.102	0.102	7.247	0.020
	Period	1	0.001	0.001	0.055	0.819
	Error	12	0.170	0.014		
B. $AUC_{0-\infty}$	Total	27	1.193			
	Sequence	1	0.010	0.010	0.125	0.730
	Subject (sequence)	12	1.004	0.084	10.150	0.000
	Formulation	1	0.078	0.078	9.414	0.010
	Period	1	0.002	0.002	0.227	0.642
	Error	12	0.099	0.008		

vals for the difference in the means of the log transformed C_{max} and $AUC_{0-\infty}$ of both parent and metabolite forms of the two products were within 70-143 per cent and 80-125 per cent, respectively.

RESULTS AND DISCUSSION

Enalapril was well-tolerated. Although the blood pressure decreased in certain subjects at 3-4 h after dosing, no volunteer was withdrawn from this study and no serious adverse event was observed during the entire study.

Pharmacokinetics of enalapril and enalaprilat

Fig. 1 depicts the average plasma concentration-time curves of enalapril and enalapriat over a 24-h period following oral administrations of 20-mg enalapril tablets. Pharmacokinetic parameters of enalapril and enalaprilat are summarized in Table 1. Maximal plasma levels of enalapril for both formulations were observed at 1.0 h whereas, those of enalaprilat were achieved at 4.3 h for the test formulation and 4.2 h for the reference formulation. The peak concentrations of both enalapril and enalaprilat for the test product were slightly higher than those for the reference product (185.39 *vs* 154.48 ng/ml and 74.56 *vs* 65.73 ng/ml, respectively) as well as the area under the concentration-time curves ($AUC_{0-\infty}$) (319.39 *vs* 278.93 ng.h/ml and 701.48 *vs* 623.06 ng.h/ml, respectively). The mean half-lives of enalapril and enalaprilat for the test and the reference formulation were 1.1 *vs* 1.2 h and 4.9 *vs* 5.0 h, respectively. The relative bioavailability of test/reference ratios in terms of enalapril and analaprilat were 1.1 and 1.1 respectively.

Bioequivalence testing of enalapril and enalaprilat

Regarding statistical analysis of enalapril pharmacokinetic parameters, the 90 per cent confidence intervals of test/reference ratio for C_{max} and

Table 3. The mean and 90 per cent confidence intervals of C_{max} and $AUC_{0-\infty}$ (log transformed) of enalapril and enalaprilat after 20 mg administrations of reference and test formulations in healthy Thai male volunteers (n = 14).

	Mean ± SD		SEM[a]	90% CI[b] (Test/referenc)	Acceptable range
	Test	Reference			
Enalapril					
C_{max}	5.16 ± 0.38	5.00 ± 0.27	0.079	101.8-134.9	70-143
$AUC_{0-\infty}$	5.72 ± 0.32	5.60 ± 0.26	0.039	105.9-121.4	80-125
Enalaprilat					
C_{max}	4.29 ± 0.23	4.16 ± 0.20	0.045	104.2-122.3	70-143
$AUC_{0-\infty}$	6.53 ± 0.24	6.42 ± 0.17	0.034	104.5-118.1	80-125

[a] $SEM = \sqrt{EMS*(1/N_A+1/N_B)}$, [b] 90% $CI = (\bar{X}_B - \bar{X}_A) \pm (t_{12, 0.1}*SEM)$

$AUC_{0-\infty}$ were 101.8-134.9 per cent and 105.9-121.4 per cent, respectively. When the plasma enalaprilat pharmacokinetics was assessed, the 90 per cent confidence intervals of test/reference ratio for C_{max} and $AUC_{0-\infty}$ were 104.2-122.3 per cent and 104.5-118.1 per cent (Table 2 and 3).

SUMMARY

The average bioequivalence of 20-mg enalapril tablets of the test formulation (Biolab, Thailand) compared to the reference formulation (Merck Sharp & Dohme, USA) was studied in healthy Thai male volunteers. Regarding the pharmacokinetic study, both formulations were equivalent in terms of rate and extent of absorption for both parent form and its active metabolite, enalaprilat. Based on the European bioequivalence guideline, 90 per cent confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios of enalapril and its active metabolite of these two preparations were in the acceptable range of 70-143 per cent and 80-125 per cent, respectively. Consequently, the test product containing 20 mg of enalapril per tablet was equivalent to the reference product containing 20 mg enalapril per tablet and can be used interchangeably.

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การศึกษาการเอื้อประโยชน์สัมพัทธ์ของยาเม็ดอีนาลาพริลขนาด 20 มิลลิกรัมในอาสาสมัครชายสุขภาพดี

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จากการศึกษาเภสัชจลนศาสตร์ของยาอีนาลาพริลขนาด 20 มิลลิกรัม ระหว่างยาสามัญซึ่งผลิตโดยบริษัทไบโอแล็บ ประเทศไทย จำกัด เปรียบเทียบกับยาต้นแบบ ซึ่งผลิตโดยบริษัท Merck Sharp & Dohme ประเทศสหรัฐอเมริกา ในอาสาสมัครชายไทยจำนวน 14 คน ซึ่งศึกษาแบบสุ่มข้ามสลับ 2 ระยะ ห่างกัน 1 สัปดาห์ แต่ละระยะ อาสาสมัครได้รับยาเม็ดอีนาลาพริล 20 มิลลิกรัมครั้งเดียว และเก็บตัวอย่างพลาสมาภายใน 24 ชั่วโมงหลังจากได้รับยา เพื่อส่งไปวิเคราะห์หาระดับยาอีนาลาพริลและอีนาลาพริแลต โดยใช้เครื่องมือ LC/MS/MS และหาตัวชี้วัดทางเภสัชจลนศาสตร์ โดยใช้ non-compartmental analysis ผลการศึกษาพบว่า ร้อยละ 90 ของระดับความเชื่อมั่นของระดับยาสูงสุดในพลาสมา และพื้นที่ใต้โค้งระหว่างความเข้มข้นในพลาสมากับเวลาของยาอีนาลาพริล (ยาสามัญ/ยาต้นแบบ) เท่ากับร้อยละ 101.8-134.9 และร้อยละ 105.9-121.4 ตามลำดับ และค่าดังกล่าวสำหรับอีนาลาพริแลต (ยาสามัญ/ยาต้นแบบ) เท่ากับร้อยละ 104.2-122.3 และร้อยละ 104.5-118.1 ซึ่งอยู่ในช่วงที่ยอมรับได้ตามหลักเกณฑ์การทดสอบชีวสมมูล ดังนั้นยาเม็ดอีนาลาพริลขนาด 20 มิลลิกรัมทั้งสองตำรับจึงมีชีวสมมูลซึ่งกันและกัน และสามารถใช้แทนกันได้ในทางคลินิก

คำสำคัญ : อีนาลาพริล, อีนาลาพริแลต, เภสัชจลนศาสตร์, ชีวสมมูล, การเอื้อประโยชน์สัมพัทธ์

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