Bioequivalence Study of Enalapril Tablets in Healthy Thai Male Volunteers

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Abstract

The bioequivalence study of 5-mg enalapril tablets, Enaril (Biolab, Thailand) compared to Renitec (Merck Sharp & Dohme, USA) was conducted in 14 healthy Thai male volunteers following a single dose, two-period, crossover design. Each subject received 4 tablets of 5-mg enalapril tablets of both formulations with a 1-week washout period. Plasma samples collected over a 24-hour 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 (Enaril/Renitec) of enalapril were 86.3 - 126.1 per cent and 93.0 - 118.5 per cent and those of enalaprilat were 86.4 - 124.1 per cent and 90.3 - 116.8 per cent. Based on the European bioequivalence guideline, the 90 per cent confidence interval of C_{max} and $AUC_{0-\infty}$ ratios of both parent and metabolite forms were within acceptable ranges of 70 - 143 per cent and 80 - 125 per cent, respectively. It was concluded that Enaril 5 mg tablet was bioequivalent to Renitec 5 mg tablet.

Key word : Bioequivalence, Enalapril, Enalaprilat

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Enalapril maleate is an ethyl ester of a long-acting angiotensin converting enzyme (ACE) inhibitor, 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 hours. 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 hours, respectively. Both parent and metabolite forms are primarily excreted in the 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 increase the dose to 5-80 mg/day [1-3]. For treatment of asymptomatic left ventricular dysfunction, the initial dose is 2.5 mg/day and gradually increases 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 objective of this study was to assess the average bioequivalence of two formulations of 5-mg enalapril tablets : Enaril from Biolab, Thailand as the test formulation and Renitec from 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 preparations: Renitec 100 tablets (Merck Sharp & Dohme, USA) containing 5 mg enalapril per tablet (Lot no. A5007, Mfg. date 11 May 2000).

Test preparations: Enaril 100 tablets (Biolab, Thailand) containing 5 mg enalapril per tablet (Lot no. ER5/11, Mfg. date 10/2000).

Study design and clinical protocol

Fourteen healthy Thai male volunteers with aged between 18-45 years (20.4 ± 1.2 years) and body mass index between 18-24 (20.3 ± 1.0) participated in a randomized, single dose, fasting, two-period, two-sequence, crossover study with a 1-week washout period. The volunteers were non-smoking, non-alcoholic and had no cardiac, hepatic, renal, gastrointestinal, and hematological diseases assessed by physical examination and the following laboratory tests: complete blood count, total bilirubin, serum creatinine, blood urea nitrogen, AST, ALT, alkaline phosphatase and hepatitis B surface antigen. After explaining the detail and purpose of the study, all healthy volunteers provided written informed consent before participation.

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 dose (4 x 5-mg tablets) of one of both formulations at 7.00 a.m. with 240 ml of water. They were then in the seated position at least 30 minutes and fasted for 2 hours. A standard lunch and an evening meal was provided at 4 and 9 hours after dosing. No other food was permitted during the study period. Liquid consumption was permitted *ad libitum* after lunch. Acidic beverages containing xanthine were prohibited. Blood pressure was periodically monitored throughout the study. After each period, the volunteers were re-examined by a physician.

Drug analysis

Seven milliliters of each blood sample was collected in a lithium-heparinized containing tube by catheterized venupuncture at forearms before dosing (t=0) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 24 hours after oral administration of each enalapril formulation. The blood samples were centrifuged at 4°C (2,000 g, 10 minutes) and the plasma samples were separated within 10 minutes after collecting blood. All samples were kept at -80°C until transportation to the Pharmakin GmbH Gesellschaft fur Pharmakokinetik, Germany for drug

analysis. Plasma enalapril and enalaprilat were measured by a validated LC/MS/MS method in the combined ESI(-)/ESI(+) mode with the lower limits of quantitation of 0.57 and 0.59 ng/ml, respectively.

Pharmacokinetic and statistical analysis

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

An analysis of variance (ANOVA) was performed on C_{max} and $AUC_{0-\infty}$, using general linear model (GLM) procedures, in which sources of variation were sequence, subjects within sequence, period, and preparation. Based on the European bioequivalence guideline, bioequivalence between two formulations could be concluded when the 90 per cent confidence intervals for the differences in the means of the log transformed C_{max} and $AUC_{0-\infty}$ of both parent and metabolite forms of 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 hours after dosing, no volunteer was withdrawn from this study and no serious adverse events occurred.

Pharmacokinetics of enalapril and enalaprilat

The plasma concentration-time curves of enalapril and enalapriat over a 24-hour period following oral administration of 20 mg (4 x 5 mg) enalapril is demonstrated in Fig. 1. Pharmacokinetic parameters of enalapril and enalaprilat are summarized in Table 1. Maximal levels of enalapril for both formulations were observed at 0.9 hours, whereas, those of enalaprilat were achieved at 3.6 hours for Enaril and 3.9 hours for Renitec. The peak concentrations of both enalapril and enalaprilat between Enaril and Renitec were comparable (154.47 *vs* 147.02 ng/ml and 74.64 *vs* 71.71 ng/ml, respec-

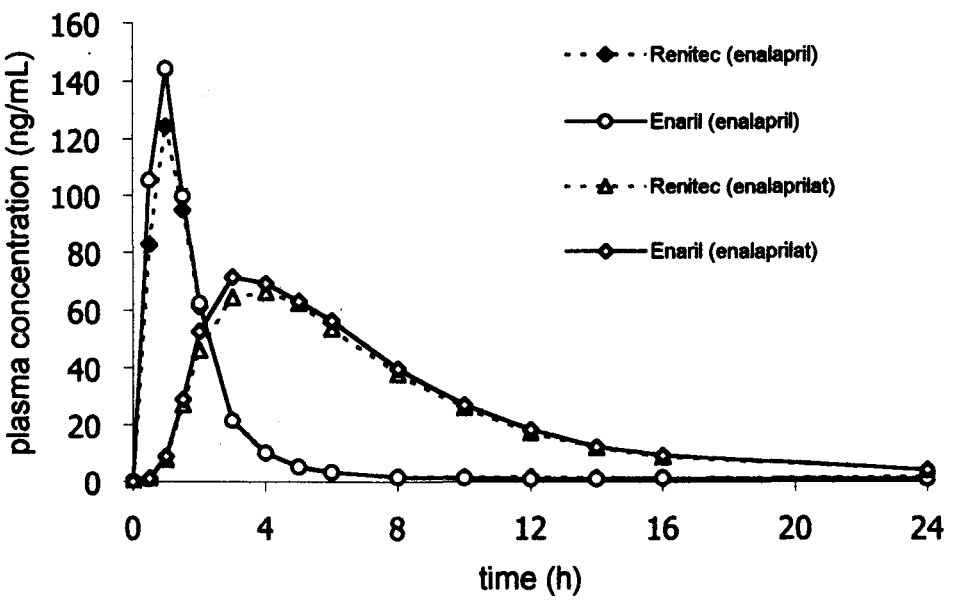

Fig. 1. Plasma concentration-time curve of enalapril and enalaprilat after 20-mg (4 x 5-mg tablets) single dose administrations of Renitec (reference) and Enaril (test) in 14 healthy Thai male volunteers.

Table 1. Summary data of pharmacokinetic parameters of enalapril and enalaprilat following oral administration of 20 mg (4 x 5 mg tablets) of Renitec (reference) and Enaril (test) in healthy Thai male volunteers (n = 14, mean ± SD).

Pharmacokinetic parameters	T_{max} (hours)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng.h/ml)	$t_{1/2}$ (hours)
Enalapril				
Renitec	0.9 ± 0.3	147.02 ± 50.26	266.41 ± 79.20	2.9 ± 2.0
Enaril	0.9 ± 0.3	154.47 ± 57.37	279.67 ± 88.31	1.2 ± 0.5
Enalaprilat				
Renitec	3.9 ± 0.7	71.71 ± 23.49	619.64 ± 160.68	4.7 ± 0.8
Enaril	3.6 ± 0.8	74.64 ± 24.20	637.85 ± 177.71	4.6 ± 0.7

Table 2. ANOVA table of C_{max} and $AUC_{0-\infty}$ (log transformed) of enalapril and enalaprilat after 20 mg (4 x 5-mg tablets) administrations of Renitec (reference) and Enaril (test) in 14 healthy Thai male volunteers.

	Source of Variation	DF	SS	MS	F	P
Enalapril						
A. C_{max}	Total	27	3.317			
	Sequence	1	0.324	0.324	1.950	0.188
	Subject (sequence)	12	1.996	0.166	2.095	0.107
	Formulation	1	0.012	0.012	0.156	0.700
	Period	1	0.031	0.031	0.390	0.544
	Error	12	0.953	0.079		
B. $AUC_{0-\infty}$	Total	27	2.607			
	Sequence	1	0.158	0.158	0.931	0.354
	Subject (sequence)	12	2.042	0.170	5.246	0.004
	Formulation	1	0.017	0.017	0.509	0.489
	Period	1	0.000	0.000	0.015	0.903
	Error	12	0.953	0.032		
Enalaprilat						
A. C_{max}	Total	27	3.253			
	Sequence	1	0.104	0.104	0.549	0.473
	Subject (sequence)	12	2.272	0.189	2.620	0.054
	Formulation	1	0.008	0.008	0.117	0.739
	Period	1	0.001	0.001	0.014	0.908
	Error	12	0.867	0.072		
B. $AUC_{0-\infty}$	Total	27	2.097			
	Sequence	1	0.066	0.066	0.496	0.495
	Subject (sequence)	12	1.589	0.132	3.634	0.017
	Formulation	1	0.005	0.005	0.134	0.721
	Period	1	0.000	0.000	0.005	0.944
	Error	12	0.437	0.036		

tively). The area under the concentration-time curves ($AUC_{0-\infty}$) of both parent and metabolite forms for Enaril were slightly higher than those for Renitec (279.67 *vs* 266.41 ng.h/ml and 637.85 *vs* 619.64 ng.h/ml, respectively). The mean half-lives of enalapril and enalaprilat for Enaril and Renitec were 1.2 *vs* 2.9 hours and 4.6 *vs* 4.7 hours, respectively. The relative bioavailability of Enaril/Renitec was 1.08.

Bioequivalence testing of enalapril and enalaprilat

Regarding statistical analysis of enalapril pharmacokinetic parameters, the 90 per cent confi-

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 (4 x 5 mg tablets) administrations of Renitec (reference) and Enaril (test) in 14 healthy Thai male volunteers.

	Mean ± SD		SEM[a]	90% CI[b] (Enaril/Renitec)	Acceptable range
	Enaril	Renitec			
Enalapril					
C_{max}	4.98 ± 0.37	4.94 ± 0.35	0.106	86.3 - 126.1	70 - 143
$AUC_{0-\infty}$	5.59 ± 0.31	5.54 ± 0.32	0.068	93.0 - 118.5	80 - 125
Enalaprilat					
C_{max}	4.26 ± 0.37	4.22 ± 0.34	0.102	86.4 -124.1	70 - 143
$AUC_{0-\infty}$	6.42 ± 0.29	6.39 ± 0.28	0.072	90.3 - 116.8	80 - 125

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

dence intervals of Enaril/Renitec ratio for C_{max} and $AUC_{0-\infty}$ were 86.3 - 126.1 per cent and 93.0 - 118.5 per cent, respectively. When the plasma enalaprilat pharmacokinetics was assessed, the 90 per cent confidence intervals of Enaril/Renitec ratio for C_{max} and $AUC_{0-\infty}$ were 86.4 - 124.1 per cent and 90.3 - 116.8 per cent (Table 2 and 3).

SUMMARY

The bioequivalence of 5-mg enalapril tablets of Enaril (Biolab, Thailand) compared to Renitec (Merck Sharp & Dohme, USA) were studied in 14 healthy Thai male volunteers. Regarding pharmacokinetics study, both formulations were equivalent in terms of rate and extent of absorption. 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, enalaprilat, of these two preparations were in the acceptable range of 70 - 143 per cent and 80 - 125 per cent, respectively. Consequently, Enaril 5 mg tablet was equivalent to Renitec 5 mg tablet and can be used interchangeably.

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การศึกษาชีวสมมูลของยาเม็ดอีนาลาพริล ในอาสาสมัครชายไทยสุขภาพดี

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จากการศึกษาชีวสมมูลของยาเม็ดอีนาลาพริลขนาด 5 มิลลิกรัม ระหว่างยาสามัญ (Enaril ผลิตโดยบริษัท ไบโอแล็บ จำกัด) และยาต้นแบบ (Renitec ผลิตโดยบริษัท Merck Sharp & Dohme ประเทศสหรัฐอเมริกา) ในอาสาสมัครชายไทยสุขภาพดีจำนวน 14 คน ซึ่งศึกษาแบบสุ่มข้ามสลับ 2 ระยะ ห่างกัน 1 สัปดาห์ แต่ละระยะ อาสาสมัครได้รับยาเม็ดอีนาลาพริลขนาด 5 มิลลิกรัม จำนวน 4 เม็ด ครั้งเดียว และเก็บตัวอย่างพลาสมาภายใน 24 ชั่วโมงหลังจากได้รับยา เพื่อนำไปวิเคราะห์หาระดับยาอีนาลาพริล และอีนาลาพริแลต โดยใช้เครื่องมือ LC/MS/MS และหาตัวชี้วัดทางเภสัชจลนศาสตร์ โดยใช้ non-compartmental analysis ผลการศึกษาพบว่า ร้อยละ 90 ของระดับความเชื่อมั่นของระดับยาสูงสุดในพลาสมา และพื้นที่ใต้โค้งระหว่างความเข้มข้นกับเวลาของยาอีนาลาพริล ของยาทั้งสองตำรับเท่ากับร้อยละ 86.3–126.1 และร้อยละ 93.0–118.5 ตามลำดับ และค่าดังกล่าวสำหรับอีนาลาพริแลต เท่ากับร้อยละ 8.4–124.1 และ 90.3–116.8 ตามลำดับ ซึ่งอยู่ในช่วงที่ยอมรับได้ตามหลักเกณฑ์การทดสอบชีวสมมูล ดังนั้นยาเม็ดอีนาลาพริลทั้งสองตำรับจึงมีชีวสมมูลซึ่งกันและกัน

คำสำคัญ : ชีวสมมูล, อีนาลาพริล, อีนาลาพริแลต

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