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Comparative Bioavailability Study of Two Ramipril Tablet Formulations in Indonesian Healthy Volunteers

Yahdiana Harahap[1], Lucy Sasongko[2], Budi Prasaja[3]*, Windy Lusthom[3], Evy C. Setiawan[3], Raria S. Meliala[3], Lipin[3]

[1]Department of Pharmacy, Faculty of Mathematic and Science, University of Indonesia, Depok (Indonesia)
[2]School of Pharmacy, Bandung Institute of Technology, Bandung (Indonesia)
[3]Clinisindo Laboratories, Jakarta (Indonesia)

Abstract

Aim

To compare the bioavailability of two ramipril tablet formulations – 10 mg Prohytens® tablets as test formulation and 10 mg Triatec® tablets as reference formulation.

Methods

A single-dosed, open-label, randomized two-way crossover design under fasting period with two weeks wash out period was evaluated in 24 subjects. For the analysis of pharmacokinetic properties, the blood samples were drawn taken up to 72 hours after dosing. Plasma concentration of ramipril and ramiprilat were determined using liquid chromatography – tandem mass spectrometry method with TurboIonSpray mode. Pharmacokinetic parameters $AUC_{0-t,}$ $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence after log-transformation of data and ratios of t_{max} were evaluated non-parametrically.

Results

The point estimates and 90% confidence intervals (CI) for $AUC_{0-t,}$ $AUC_{0-\infty}$ and C_{max} for ramipril were 93.21% (85.67-101.41%), 93.45% (85.88-101.69%), 94.02% (80.09-110.38%) and for ramiprilat were 92.26% (87.76-96.99%), 94.59% (89.71-99.73%) and 91.55% (84.88-98.74%).

Conclusion

These results indicated that the two formulations of ramipril were bioequivalent and thus may be prescribed interchangeably.

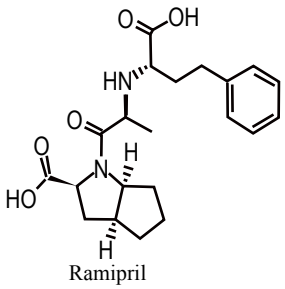
Keywords: Angiotensin-coverting enzyme inhibitor (ACEi); Bioequivalence and bioavailability; Ramipril; Ramiprilat

Introduction

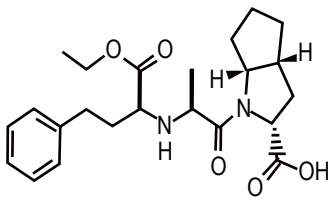
Ramipril, 2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo (3-3-0) octane-3-carboxylic acid (Figure 1), is a potent angiotensin-converting enzyme inhibitor (ACEi) that is used in the treatment of hypertension, heart failure, in patients after myocardial infarction (MI), in the prevention of diabetic and non-diabetic nephropathy, and in the prevention of MI in high-risk patients. In general, ramipril exerts its effects through actions in renin-angiotensin-aldosterone system (RAS). It inhibits the conversion of angiotensin I to angiotensin II through actions of ACE, thus decreasing the vasoconstriction, sympathetic activation, and trophic changes in the heart and blood vessels that angiotensin produces (Smith and Ball, 2000; Vuong and Annis, 2003).

Ramipril acts as a prodrug of the diacid ramiprilat, its active metabolite. After oral doses at least 44 to 66% is absorbed. Absorption is not influenced by silmutaneously intake of food. Ramipril is metabolized in the liver to ramiprilat; other metabolites are inactive. Peak plasma levels of ramipril are reached within one hour of administration with peak levels of ramiprilat are achieved 2 to 4 hours after an oral dose of ramipril (van Griensven et al., 1995). The C_{max} for ramipril is about 12 ng/mL and the AUC is about 15 ng.h/mL after the single oral administration of 5 mg ramipril (Mendes et al., 2006). Ramiprilat is about 56% bound to plasma proteins. After oral administration, 60% of the dose is found in the urine and 40% is found in the feces. The effective half-life for accumulation of ramiprilat is 13 to 17 hours after daily doses of ramipril 5 to 10 mg (Smith and Ball, 2000; Vuong and Annis, 2003).

The present study was conducted to investigate the pharmacokinetics and bioavailability of two ramipril tablet formulations in order to prove bioequivalence between both formulations.



Ramipril
CAS #87269-97-4; MW 388.46



Ramiprilat
CAS #87333-19-5; MW 416.5

Figure 1: Chemical structure of Ramipril and Ramiprilat.

***Corresponding author:** Budi Prasaja, S.Si., Apt, MM, Clinisindo Laboratories, Jl. Ulujami Raya 12, Jakarta 12250 (Indonesia), Tel: +6221 73889918; Fax: +6221 73885453; E-mail: budi.prasaja@clinisindo.com

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Subjects, Materials and Methods

Ethics consideration

The protocol study was reviewed by the Committee of The Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by the National Agency of Drug and Food Control (Jakarta, Indonesia).

This study was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human volunteers and Good Clinical Practice (GCP). All participants signed a written informed consent after they had been informed of the nature and details of the study in accordance with Indonesian Guidelines for Bioequivalence Studies.

Study design

The study was based on single-dose, open-label, randomized two-way crossover design under fasting period with two weeks wash out period. Subjects were randomized to one of the two sequences to receive the formulations according to randomization scheme. The test preparation was 10 mg of Prohytens® tablets, manufactured by PT. Novell Pharmaceutical Laboratories, Indonesia (Batch no. 10M035) and the reference formulation was 10 mg Triatec® tablets, manufactured by PT. Aventis Pharma, Indonesia (Batch no. 065U014).

Based on previous study, the sample size n = 24 subjects was sufficient to ensure power of 80% for correctly concluding bioequivalence under the following assumption: $\alpha = 0.05$, $0.95 < \mu_T / \mu_R < 1.05$ and an intra-subject variability of 20% (Diletti et al., 1991; Mendes et al., 2006).

Subjects

A total of 26 volunteers were selected among Indonesia residents and participated in this study. First twenty-four volunteers (19 males and 5 females) were available for pharmacokinetic evaluation. If volunteers could not complete the entire study (dropout/withdrawn), volunteers were replaced with two reversed volunteers based on the same randomization code. The demographic data of twenty-four volunteers are shown in Table 1. Volunteers were selected after passing a clinical screening procedure including a physical examination, ECG and clinical laboratory tests (hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, total cholesterol, creatinine, urine analysis, pregnancy test (for female subjects) and negative results of HBsAg, anti HBC and anti HIV. Volunteers were excluded if they had a history of peptic ulcer, any illness of the hepatic, renal and cardiovascular system, taken alcohol or other medications for a long period of time, had hypersensitivity to ramipril or related ACEi, had received any investigation drug within four weeks (or suitable longer period for slowly eliminated drugs) of enrollment and donation or loss more than 450 ml of blood within 3 months prior to the screening of the study.

	Mean (± SD) Value	Range
Age (years)	29.0 ± 5.8	20 - 38
Weight (kg)	59.7 ± 7.4	48 - 73 kg
Height (m)	164.9 ± 5.9	152 - 175
Body mass index (kg m^{-2})	21.9 ± 2.2	119 - 25

Table 1: Demographic data for ramipril bioequivalence study in 24 volunteers.

Drug administration and sampling

All volunteers avoided using other drugs for at least two weeks prior to the study and until after its completion. They also refrain from ingesting alcohol, caffeine, chocolate, tea or coke-containing beverages at least 48 hours before each dosing and until the collection of the last blood sample. The grapejuice was not prohibited (Bailey and Dresser, 2004).

Volunteers were confined to clinical unit of Clinisindo Laboratories one night before study to assure the fasting condition (10 hours before drug administration). On the study day, subjects were given one tablet of either product with 240 ml of water. No food was allowed until 4 hours after dose administration. Water intake was allowed 2 hours after the dose. Standard meals were served at 6 hours (±1008 calories) and 12 hours (±836 calories), snacks were served at 4 hours (±165 calories) and 8 hours (±160 calories) after drug administration. Total calories were calculated by nutritionist.

Subjects were remained upright (sitting or standing) for the first 4 hours. Subjects were confined at clinical unit of Clinisindo Laboratories for 24 hours after dosing and and did not permitted to take strenuous exercise during the sampling days. Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling.

5 ml of the venous blood were collected at pre dose, 10, 20, 40, 60, 80, 100, 120 minutes, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 36, 48 and 72 hours after drug administration in the heparinized tubes. After blood separation, plasma was frozen at -20ºC until analysis (Mendes et al., 2006; van Griensven, 1993).

After two weeks wash out period, subjects returned to Clinisindo Laboratories and the blood sample analysis was repeated in the second period in the same manner to complete the crossover design.

Analytical method

The concentration of ramipril and ramiprilat in plasma was determined using LC-MS/MS method with TurboIonSpray mode. Enalapril (CAS# 75847-73-3, MW 376.4) was used as the internal standard (Bo Yuan et al., 2000). The method has already been validated in terms of selectivity, sensitivity, linearity, accuracy and precision, recovery and also has been verified just before being used in study. The limit of quantification for ramipril and ramiprilat was 0.1 ng/mL and 0.2 ng/mL, respectively. The standard calibration curves for ramipril and ramiprilat were ranged from 0.1-100 ng/mL and 0.2-100 ng/mL, respectively. The best linear fit and least-squares residual for the calibration curve were achieved with $1/x^2$ weighing factor. The recoveries of ramipril and ramiprilat were 80.90%, 84.09%, 79.13% and 36.99%, 37.49%, 38.42% at low medium and high QC samples. The analytical separation was performed on a Synergi 4µ POLAR-RP-80A, 50 x 2.00 mm, 4 µm (Phenomenex®, USA) and protected by guard column AQ C18, 4 x 2.0 mm (Phenomenex®, USA). The mobile phase was used gradient of 0.1 % formic acid in acetonitrile and 0.1 % formic acid in water, pumped 0.6 mL/min for 4.0 min run time. The column temperature was maintained at 40°C. Briefly, a 500 µL of human plasma in polypro-

pylene tube was added with internal standard, 100 µL of 1 M phosphoric acid and 100 µL of methanol:water (50:50). After mixing, 3 mL of ethyl acetate was added and vortex mixed for 2 min. The mixture was centrifuged at 3000 rpm for 10 min. The organic phase was removed and evaporated to dryness under vacuum at 60°C for 15 min. The residue was reconstituted with methanol:water (1:1). A volume of 10 µL aliquot was injected into the LC MS/MS system. The retention time for ramipril and ramiprilat was 0.41 min and 0.34 min, respectively.

Safety evaluation

Analysis of safety-related data was considered using the more common adverse events which occurred after initiation of study treatment and supported by the following more detailed tabulations and analysis.

Pharmacokinetic and statistical analysis

The bioequivalence was determined using the primary parameters, $AUC_{0-t,}$ $AUC_{0-\infty,}$ $C_{max.}$ The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly by inspection of the individual drug plasma concentration time data, and were used as measures of rate of absorption. The area under the plasma concentration time curve up to the last time (t) showing a measurable concentration (C_t) of the analyte (AUC_{0-t}) was calculated using the trapezoidal rule. The elimination rate constant (K_{el}) was calculated by the technique of least-squares regression from the data of the last 3-8 points of each plasma concentration data curve. The $AUC_{0-\infty}$ values were determined by adding the quotient of C_t and the appropriate K_{el} to the corresponding $AUC_{0-t,}$ that is:

$$AUC_{0-\infty} = AUC_{0-t} + C_t / K_{el} \quad (1)$$

where C is the estimated last plasma concentration. The apparent elimination half-life ($t_{½}$) of ramipril and ramiprilat in plasma was calculated by using the following equation:

$$t_{½} = (\ln 2) / K_{el} \quad (2)$$

For the parameters of $AUC_{0-t,}$ $AUC_{0-\infty}$ and C_{max} a multiplicative model was assumed, and analysis of variance (ANOVA) was applied using the respective ln-transformed data. For estimation of bioequivalence the 90% CI of the geometric mean ratio test/reference (T/R) for $AUC_{0-t,}$ $AUC_{0-\infty}$ and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was 80-125%. All statistical analyses were performed using EquivTest version 2.0 software (Statistical Solution, Cork, Ireland).

Causal Relation to Study drug	Events	Total
Related	Dizziness	22
	Hypotension	13
	Headache	6
	Fatigue	20
	Dry Cough	4
	Nausea	3
	Vomiting	1
	Sleep Disturbance	2
Probable	-	0
Possible	Sleepy	10
Unrelated	Cold Sweating	2
		83

Table 2: Disposition of adverse events.

Results

Clinical observation

Both ramipril formulations were well-tolerated at the administered dose and no significant adverse clinical events were observed. Twenty three out of 26 volunteers experienced 83 adverse events during the study. All adverse events were of mild intensity and recovered without concomitant medication. There were no serious adverse events. However, all events resolved completely. The disposition of adverse events is shown in Table 2.

Pharmacokinetic evaluation

A total of 26 volunteers were invited to participate in this study. First twenty-four volunteers were available for pharmacokinetic evaluation. The mean ramipril and ramiprilat concentration versus time profiles for both formulations are shown in Figure 2 and Figure 3. The pharmacokinetic parameters used to assess the bioequivalence of the test formulation versus the reference were $AUC_{0-t,}$ $AUC_{0-\infty}$ for the extent of the absorption and C_{max} and t_{max} for the rate of absorption. Descriptive statistics of the pharmacokinetic parameter for ramipril and ramiprilat test and reference are summarized in Table 3 where the geometric mean values and the range for the $AUC_{0-t,}$ $AUC_{0-\infty,}$ C_{max} and $t_{½}$ values obtained for each formulation are shown. The pharmacokinetic

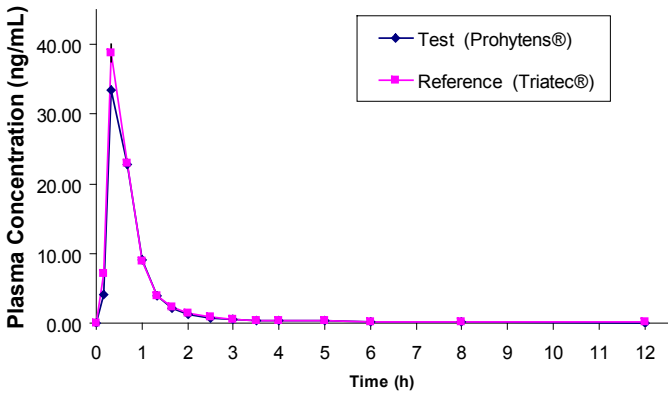

Figure 2: Arithmetic mean plasma concentration-time profiles of ramipril after a single dose of two 10 mg ramipril tablets of two different formulations.

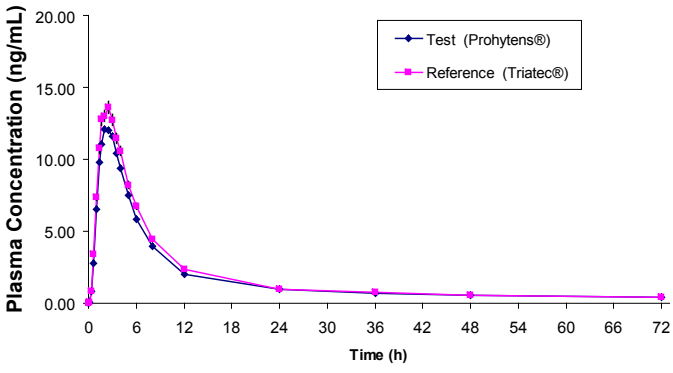

Figure 3: Arithmetic mean plasma concentration-time profiles of ramiprilat after a single dose of two 10 mg ramipril tablets of two different formulations.

Ramipril		
Parameters	**Test Formulation**	**Reference Formulation**
C_{max} (ng/mL) Geometric mean Range	35.06 13.77-78.07	37.29 16.91-96.22
AUC_{0-t} (ng.h/mL) Geometric mean Range	21.62 11.53-65.48	23.19 11.90-54.23
$AUC_{0-\infty}$ (ng.h/mL) Geometric mean Range	21.87 11.60-66.51	23.40 12.06-54.90
$t_{1/2}$ (h) Geometric mean Range	0.93 0.36-2.74	0.96 0.38-5.07
t_{max} (h) Median Range	0.33 0.33-0.67	0.33 0.33-0.67
Ramiprilat		
Parameters	**Test Formulation**	**Reference Formulation**
C_{max} (ng/mL) Geometric mean Range	11.17 4.45-41.34	12.21 4.03-37.43
AUC_{0-t} (ng.h/mL) Geometric mean Range	109.99 53.43-310.00	119.15 65.04-325.18
$AUC_{0-\infty}$ (ng.h/mL) Geometric mean Range	134.48 77.07-329.41	141.63 69.03-342.80
$t_{1/2}$ (h) Geometric mean Range	40.27 22.42-57.59	35.64 12.15-59.07
t_{max} (h) Median Range	2.5 1.67-4	2.75 1.67-4

Table 3: Mean pharmacokinetic parameters for ramipril and ramiprilat after administration of the two formulations to 24 volunteers.

RAMIPRIL		
Parametric Analysis	**T/R Point Estimate**	**Confidence Limits**
C max	94.02	80.09 - 110.38
AUC_{0-t}	93.21	85.67 - 101.41
$AUC_{0-\infty}$	93.45	85.88 - 101.69
$t_{1/2}$	97.45	81.40 - 116.68
RAMIPRILAT		
Parametric Analysis	**T/R Point Estimate**	**Confidence Limits**
C max	91.55	84.88 - 98.74
AUC_{0-t}	92.26	87.76 - 96.99
$AUC_{0-\infty}$	94.59	89.71 - 99.73
$t_{1/2}$	110.45	97.94 - 124.55

Table 4: Statistical evaluation of AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and $t_{½}$ for ramipril and ramiprilat of two formulations from 24 volunteers.

characteristic t_{max} was presented as mean values. For ramipril, the mean obtained values for test and reference products were 35.06 and 37.29 ng/mL for C_{max}; 21.62 and 23.19 ng.h/mL for AUC_{0-t}; 21.87 and 23.40 ng.h/mL for $AUC_{0-\infty}$. The median t_{max} for both formulations was 0.33 h. For ramiprilat, the mean obtained values for test and reference products were 11.17 and 12.21 ng/mL for C_{max}; 109.99 and 119.15 ng.h/mL for AUC_{0-t}; 134.48 and 141.63 ng.h/mL for $AUC_{0-\infty}$. The median t_{max} was 2.5 h (test) and 2.75 h (reference).

Statistical evaluation

The results of the bioequivalence analysis for ramipril and ramiprilat are given in Table 4. The intra-subject variability of ramipril in the AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $t_{½}$ estimates from the coefficient of variables as determined by ANOVA were 17.00%, 17.03%, 32.36%, and 36.30%, respectively. The intra-subject variability of ramiprilat in the AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $t_{½}$ estimates from the coefficient of variables as determined by ANOVA were 10.05%, 10.63%, 15.23%, and 24.23%, respectively.

Discussion

The aim of the randomized, single blind, two-period, cross over study with a washout period of 2 weeks was to evaluate the bioavailability of the test and the reference ramipril tablet administered as 10 mg single oral dose each.

From the result of the safety evaluation, it was concluded that both of test and reference preparations were well-tolerated. A clinically relevant difference to the adverse events stated in the literature was not detected.

In the study the last sampling time for ramipril was 12 hours. Ramipril was rapidly absorbed and the elimination was fast and 5 hours after administration only 9 subjects showed detectable ramipril concentration in two period. The formation of ramiprilat was fast and the elimination was slower than ramipril.

In literature, ramiprilat displays triphasic elimination kinetics with half-lives of 2 to 4 hours, 9 to 18 hours, and greater than 50 hours. This triphasic elimination is due to extensive distribution to all tissues (initial half-life), clearance of free ramiprilat from plasma (intermediate half-life), and dissociation of ramiprilat from tissue ACE (terminal half-life) (Brunton et al., 2007). In this study the half-lives were 40.27 and 35.64 hours for test and reference and also the reported study by Mendes et al. Thus, the calculated half-lives may be biased because of their dependence on the duration of blood sampling (Ruf et al., 1994).

Theoritically, the duration of the study was more than 3 time of half-life. But for the BE study, a sampling period longer than 72 hours is not considered necessary (CHMP, 2008).

According to FDA guidance on ramipril, the analytes that should be measured were ramipril and ramiprilat. The bioequivalence data is concluded based on ramipril data while the metabolite data is submitted as supportive evidence of comparable therapeutic outcome. (CDER Guidance on Ramipril, 2008).

The intra subject variability of AUC_{0-t} for ramipril and ramiprilat was 17.00% and 10.05%, respectively. Considering this result the sample size of 24 subjects was sufficient in order to conclude bioequivalence with the power of 80% at the 5% nominal level (Diletti et al., 1991).

As shown in Table 3, the results of the statistical evaluation for 90% confidence interval of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $t_{½}$ for ramipril and ramiprilat were entirely included within the bioequivalence acceptance limit of 80 – 125% (CPMP, 2001).

In conclusion, the two ramipril formulations were equivalent with respect to the rate and extent of absorption and it can be assumed to be therapeutically equivalent and exchangeable in clinical practice.

Acknowledgement

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