Indonesian J. Pharm. Vol. 23 No. 3 : 183 – 187
ISSN-p : 0126-1037

Research Article

A BIOAVAILABILITY STUDY OF INDONESIAN GENERIC TABLET OF CAPTOPRIL IN HEALTHY VOLUNTEERS

Agung Endro Nugroho*, Arief Rahman Hakim, Purwantiningsih and Lukman Hakim

*Laboratory of Pharmacology and Toxicology, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Sekip Utara Jogjakarta, Indonesia, 55281.

Submitted: 05-06-2012
Revised: 17-06-2012
Accepted: 28-06-2012

*Coresponding Authors
Agung Endro Nugroho

Email: agungendronugroho@yahoo.com

ABSTRACT

Captopril is a selective inhibitor of angiotensin-converting enzyme (ACE) and is formulated by several pharmaceutical companies in Indonesia. This study was conducted to compare the bioavailability of a captopril tablet with reference products in healthy volunteers. The relative bioavailability of captopril was determined in single dose, randomized, crossover, and two-phase studies. The relative bioavailability of the test product (a generic captopril 50 mg tablet) with respect to the reference product was determined. Twelve healthy volunteers in two groups took part in these studies and took either the test or reference tablets in the first phase and received the other tablet in the second phase of each study. The bioavailability parameters include the peak concentration of captopril in serum (Cmax); the time to achieve the peak concentration (Tmax); and the area under the curve of captopril in serum versus time. Non-compartmental analysis on observed concentration versus time data has resulted in the mean value of Cmax of 545.26 ± 22.90 ng/mL (test product) and 548.91 ± 25.07 ng/mL (reference product) and mean Tmax of 1.13 ± 0.08 hours (test product) and 1.08 ± 0.08 hours (reference product), mean of AUC_{0-7} value of 1820.51 ± 75.31 ng. hour/mL (test product) and 1822.09 ± 99.29 ng. hour/mL (reference product), and mean of AUC_{0-inf} value of 1967.83 ± 95.65 ng. hour/mL (test product) and 1996.94 ± 124.52 ng. hour/mL (reference product). Based on the data, it can be concluded that there is no significant difference ($p>0.05$) in bioavailability between both captopril Tablet (test and reference product).

Key words: Bioequivalence, Captopril, HPLC, Human serum, Generic

INTRODUCTION

Captopril is the first orally active angiotensin-converting enzyme (ACE) inhibitor which is widely used antihypertensive drug. Angiotensin-converting enzyme is a peptidyl carboxypeptidase that catalyzes the conversion of angiotensin I to the active octapeptide angiotensin II in blood and other tissues, and may also reduce the degradation of bradykinin (Dollery, 1991; Jackson and Garrison, 1996). The drug is also used in the management of hypertension, in cardiac failure, following myocardial infarction (especially when there is ventricular dysfunction, even when this is mild), progressive renal insufficiency and in diabetic nephropathy (Parfitt and Martindale, 1999; Rang *et al.*, 1999). Captopril also has antithrombotic effect by reducing venous thrombus weight in rats. The mechanism of antithrombotic action of captopril is dependent on the suppression of coagulation cascade and the enhancement of the fibrinolytic processes (Chabielska *et al.*, 2005).

In the body, approximately 68 to 76% of an oral dose is absorbed, and bioavailability of unchanged captopril is 62 – 65 %. Captopril is covalently bound to plasma protein. Peak blood levels are reached about an hour after an oral dose. The elimination half-life has been reported to be 1 to 2 hours, and elimination predominantly by renal excretion. Captopril is rapidly and extensively metabolized interaction involving its sulfhydryl group. Main pathway involves the formation of disulfide dimer of this drug as well as mixed conjugates with indogenous thiol-containing compounds and plasma proteins (Dollery, 1991).

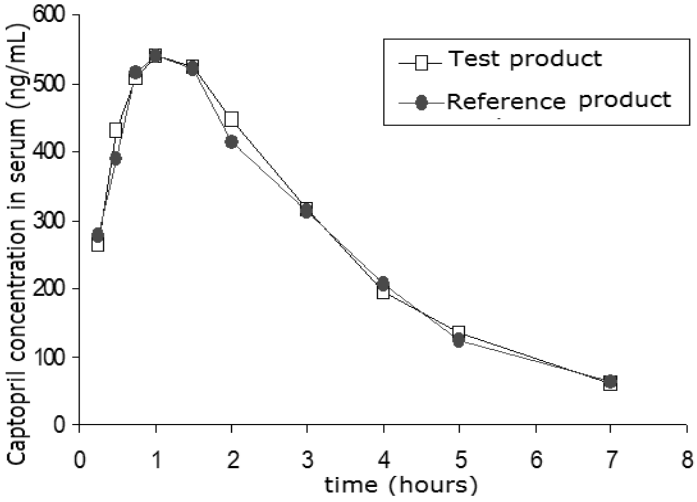

Figure 1. Mean plasma captopril concentrations (n = 12) following single-dose administration of 1 x 50 mg reference product and Generic Captopril (test product) tablets.

There are several clinical side effects of captopril including hypotension occurring in patients receiving it after the first dose, skin rash, cough and taste disturbance (dysgeusia). Captopril is contraindicated for patient with pregnancy, renal failure, the elderly and children. Therefore it is not recommended for these patients (Dollery, 1991; Jackson and Garrison, 1996). However, considering its side effects, administration of captopril must be monitored based on the benefit-risk ratio. Therefore, its pharmacokinetic data are important to ensure safety and product quality.

The objective of this research was to evaluate the bioavailability of a 50 mg Indonesian Generic tablet of Captopril (test product) compared to that of a reference product. The pharmacokinetic parameters were used to describe the bioavailability of captopril in the body include the peak concentration of captopril in serum (Cmax); the time to achieve the peak concentration (Tmax); and the area under the curve of captopril in serum versus time. Other parameters such as mean residence time (MRT), half-time ($t_{1/2}$), volume distribution at steady state (Vdss) and total clearence (Cl) of captopril are reported as complementary.

METHODOLOGY

Study protocol

The research had been carried out in 12 healthy volunteers of both sexes, each 6 males and 6 females, aged between 20-40 years, weight of 40-60 kg, height around 150-170 cm. The volunteer's body weight is about 70-150 % from ideal body weight versus body height (Suyono, 1996). The volunteers health were delineated from clinical and laboratory examination results, including a routine laboratory examination on the blood and urine, hepatic and renal function. These results were used to fulfill inclusion criteria of the study. An individual who was found to have renal failure, hepatic disease history, allergy to captopril and related compounds, or has smoking habit, drinking alcohol, was excluded from this research (exclusion criteria). At least within two weeks before this pharmacokinetic study has been commenced and during the research in progress, the volunteers were not allowed to consume any drugs.

Before the research commenced, the volunteers were given explanation about the research (informed consent) and given opportunity to ask about it as much as possible. Moreover, each volunteer was asked to sign a statement letter of no objection to follow the research.

During the research process, any complaint which was an indication the appearance of side effect obtained much attention and measures as good as possible by the medical doctor. The research protocol has obtained an ethical clearance from the Ethical Committee of Human Biomedical Research of Gadjah Mada University, Yogyakarta, Indonesia.

Blood sampling

Before the study was performed, each volunteer was asked to fast overnight. Drinking of plane water without sugar was allowed ad libitum. At 07.00 a.m. a blood sample (7.0 mL) was taken for a blank, and then was followed by drug administration orally with 50 mg dose of the test product (Generic Captopril 50 mg tablet) and the reference product (Capoten® 50 mg tablet, Bristol-Myers Squibb). The blood samples were taken serially at 0.25; 0.5; 0,75; 1; 1,5; 2; 3, 4; 5 and 7 hours using vacuntainer tubes. The serum was separated and stored in a freezer (-20°C) in glass tubes at the upright position and were packed in aluminum foil (to avoid contact with rubber cover and photo decomposition) pending for the drug concentration analysis. Breakfast was given 4 hours after the drug administration and the next meals were arranged after at the tenth hour taking sample. During the process of the research, each complaint on side effect obtained serious handling by the medical advisor.

Assay

Analysis was performed using high-performance liquid chromatographic (HPLC) with UV detector (Jankowski *et al.*, 1995). To 1 mL serum in was added 30 µL *p*-bromophenacyl bromide 1 mg/mL in acetonitrile and also added 50 µL NaOH 100 mM. The mixture was left for 30 min. After adding 75 µL HCl 1M, 150 µL buffer acetate 200 mM pH 4.0, extracted with 4 mL benzene. The mixture centrifuged 3000 rpm for 20 menit. Its organic phase is moved into clean tube, and evaporated to dryness under nitrogen. The residue was reconstituted in 200 µL mobile phase and 20 µL was injected onto the HPLC column (LiChroCART® 125-4 LiChrospher® 100 RP-18; 5 µm). The mobile phase is mixture between acetonitril and 1 % acetic acid (6 : 4), with flow rate of 1.5 mL/min. Detection was at 245 nm. The analytical method was fully validated in terms of linearity, accuracy and precision according to USP guidelines.

Pharmacokinetic variables

Some pharmacokinetic variables were calculated for each volunteer and product, using actual blood sampling times to compare the rate and extent of absorption of captopril in this study. The pharmacokinetic analysis from captopril serum concentrations versus time was performed using non-compartmental analysis (Benet and Galeazzi, 1979). The values of Cmax, Tmax were taken from the observed value of the drug concentration curve versus time of each volunteer. The areas under the plasma concentration curves (AUC_{0-t}) were calculated using the linear trapezoidal rule. The value of MRT, $t_{1/2}$, Vdss, and Cl were calculated using PK (Pharmacokinetic) Function software (Under Microsoft Excel). The pharmacokinetic parameter values of both captopril products were compared statistically using a paired t-test with a confidence level of 95 % (SPSS program, 10.00 version).

RESULT AND DISCUSSION

Validation of the analytical method

The method was linear over a range of 20 – 800 ng/mL of drug in plasma. The method accuracy for concentrations ranging between 40 – 400 ng/mL and 500 ng/mL was 80 – 90%, and the value of random analytical error was 5 – 7 %. The last value is parameter of inprecision of an analytical method. The limit of quantification was found to be 20 ng/mL.

Pharmacokinetic results

Figure 1 shows an average concentration of captopril in serum versus time of 12 volunteers, after the administration of a single dose of 50 mg Generic Captopril tablet (test product) and 50 mg reference tablets. From this figure it appears that the rates of absorption for both products were relatively similar after the drug administration. After reaching the peak, the curve decreased with rapid elimination.

Table I. The mean values of Tmax, Cmax and AUC after an oral single dose administration of 50 mg Generic Captopril and reference tablets in 12 healthy volunteers

No.	Parameter	Unit	Product	
			Generic Captopril Mean ± SEM	Reference product Mean ± SEM
1	Tmax	hours	1.13±0.08(*)	1.08±0.08(*)
2	Cmax	ng/mL	545.26±22.90(*)	548.91±25.07(*)
3	AUC_{0-7}	ng.hours/mL	1820.51±75.31(*)	1822.09±99.29(*)
4.	AUC_{0-inf}	ng.hours/mL	1967.83±95.65(*)	1996.94±124.52(*)

(*) Paired t-test results for all values of both products indicate not significantly different (P>0.05)

Table II. The mean values of MRT, $t_{1/2}$, Vdss and total clearance after an oral single dose administration of Generic Captopril and reference tablets in 12 healthy volunteers

No.	Parameter	Unit	Product	
			Generic Captopril Mean ± SEM	Reference product Mean ± SEM
1	MRT	hours	2.98±0.10 (*)	3.10±0.10 (*)
2	$t_{1/2}$	hours	1.68±0.07 (*)	1.78±0.06 (*)
3	Vdss	ML	26.01±1.14 (*)	25.87±1.25 (*)
4	Cl	mL/hours	76.75±2.81 (*)	79.50±3.72 (*)

(*) Paired t-test results for all values of both products indicate not significantly different (P>0.05)

The parameter values of captopril pharmacokinetics obtained after the administration of the two products are presented in tables II. The parameters used to evaluate the bioavailability of both products are captopril peak concentrations in serum (Cmax), the time required to achieve the peak concentrations (Tmax), and area under the curve of captopril concentration in serum versus time (AUC).

From the observed captopril concentration in serum versus time and the pharmacokinetic values in 12 healthy volunteers after the administration (50 mg; oral) of Generic Captopril and reference product, the following results indicated that the mean AUC_{0-7} value of Generic Captopril tablet is 1820.51 ± 75.31 ng.hours/mL (1461.52 to 2334.98 ng.hours/mL) and that of reference product is 1822.09 ± 99.29 ng.hours/mL (1448.28 to 2728.47 ng.hours/ mL); the mean AUC_{0-inf} value of Generic Captopril tablet is 1967.83 ± 95.65 ng.hours/mL (1529.13 to 2756.40 ng.hours/mL) and that of reference product is 1996.94 ± 124.52 ng.hours/mL (1550.27 to 3213.94 ng.hours/ mL); the mean Cmax value for Generic Captopril tablet is found to be 545.26 ± 22.90 ng/mL (ranging from 421,86 to 678,73 ng/mL) and that for reference product tablet is 548,91 ± 25,07 ng/mL (ranging from 404,29 to 690,62 ng/mL); and the mean Tmax value for Captopril tablet is 1.13 ± 0.08 hours (0.75 to 1.5 hours) while that for reference product tablet is 1.08 ± 0.08 hours (0.75 to 1.5 hours). Statistically, these pharmacokinetic parameter values for both Generic Captopril and reference product are not significantly different (P>0.05).

The values of mean (± SEM) MRT, $t_{1/2}$, Vdss and Cl of captopril in from all subjects may be seen in the following table II. Mean residence time (MRT), half-life time ($t_{1/2}$), volume of distribution at steady state (Vdss) and total clearance (Cl) were presented as a

complement. The pharmacokinetic parameter values for both Generic Captopril and reference tablets are not significantly different (P>0.05).

According to these result, the values of C_{max}, AUC_{0-7}, AUC_{0-inf} and T_{max} (as measures of the rate and extent of absorption of captopril, respectively) for Generic Captopril and reference tablets are not significantly different (P>0.05). It indicated that the test products (Captopril) are bioequivalent to the reference product with respect to the rate and the extent of absorption of captopril for 50 mg tablets.

CONCLUSION

Based on the data, it can be concluded that captopril tablets manufactured in Indonesia are comparable to foreign brands and can produce acceptable plasma concentrations.

ACKNOWLEDGMENTS

The authors would like to thank to PT. Hexpharm Jaya for financial support, and the nursing and technical staff of State Hospital of Sleman for their assistance throughout this study.

REFERENCES

Benet, L. Z. and Galeazzi, R. L., 1979, Noncompartmental determination of the steady-state volume of distribution, *J.Pharm.Sci.*, 68: 1071-1074.

Chabielska, E., Mogielnicki, A., Kramkowski, K. and Buczko, W., 2005, Antithrombotic effect of captopril and enalapril in old rats, *Pharmacol Rep.*, 57(1):135-137.

Dollery, S. C., 1991, Captopril, *Therapeuctic Drugs*, Vol. 2, Churchill Livingstone, *Edinburgh*, pp. C44 – C48.

Jackson, E. K. & Garrison, J.C., 1996, Renin and Angiotensin, IN : GOODMAN GILMAN (Ed.). *Phamacological basic of Therapeutics*, pp. 733-758 (New York : McGraw-Hill Health Professions Division, 9th Ed.).

Jankowski, A., Skorek, A., Krzysko, K., Zarzyxki, P.K., Ochocka, and R.J., Lamparczyk, H., 1995, Captopril : determination in blood and pharmacokinetics after single oral dose, *J. Pharm. Biomed. Anal.*, 13 : 655 – 660.

Parfitt, K. and Martindale, W., 1999, *Martindale: the Complete Drug Reference*, pp. 836 – 837 (London : UK Pharmaceutical Press, 32th Ed.).

Rang, H.P., Dale, M.M., and Ritter, J.M., 1999, *Pharmacology*, p. 1-44, 94-156 (Melbourne : Churchill Livingstone, 4th Ed.)

Suyono, S., 1996, Diet Pada Diabetes, Dalam Noer, Hm S. (ed.), *Buku Ajar Ilmu Penyakit Dalam* (Jakarta : Penerbit FKUI, Ed. 3).