Comparative Fasting Bioavailability of 2 Cilostazol Formulations in Healthy Thai Volunteers: An Open-Label, Single-Dose, Randomized, 2-Way Crossover Study

Pinpilai Jutasompakorn MD*,
Supornchai Kongpatanakul MD*, Piyapat Pongnarin MSc*,
Korbtham Sathirakul PhD**, Somruedee Chatsiricharoenkul MD*

* Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
** Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

Objective: To evaluate the bioequivalence of 50 mg cilostazol tablets manufactured locally (Citazol®) and originally (Pletaal®) in healthy Thai volunteers.

Material and Method: An open-label, single dose, randomized, two-period, two-sequence, crossover study in 30 healthy volunteers. Each volunteer received a 50 mg cilostazol tablet of both formulations with a washout period of at least 14 days. Blood samples were obtained at pre-dose and over 48 hours after dosing. Cilostazol plasma concentrations were quantified by using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Results: The 30 volunteers completed the entire study. The geometric mean ratios (GMR) (test/reference) between the two formulations of cilostazol were 112.38% (101.70%-124.19%) for C_{max}; 103.66% (96.06%-111.86%) for AUC_{0-48}; and 95.14% (86.12%-105.12%) for $AUC_{0-\infty}$. There was no statistical difference of the T_{max} between the two formulations (p>0.05). No serious adverse events related to the studied drugs were found.

Conclusion: No significant difference in the analyzed pharmacokinetic parameters was found between the two formulations of 50 mg cilostazol tablets. Therefore, it can be concluded that these two cilostazol tablet formulations were considered bioequivalent.

Keywords: Cilostazol, Bioequivalence

Cilostazol is a cyclic nucleotide phosphodiesterase III inhibitor that has been approved by the Food and Drug Administration (FDA) for the treatment of intermittent claudication in patients with peripheral artery disease[1,2]. Its main physiological effects are vasodilatation and inhibition of platelet aggregation[3]. Recent trials have shown the effects of cilostazol in preventing secondary stroke in Asian population and preventing coronary artery restenosis post-endovascular treatments[4-6]. Cilostazol is rapidly absorbed after oral administration, peak plasma concentration (C_{max}) occurs three hours after drug administration and decline biexponentially with concentration detectable in the plasma for at least 36 hours[7]. A high fat meal increases absorption, with a 90% increase in C_{max} and a 25% increase in AUC[8]. It is metabolized extensively by cytochrome P-450 (CYP) enzymes, mainly CYP3A4 and, to some degree, CYP2C19, to active metabolites. The primary route of elimination is the urine (74%) and the remainder excrete in the feces (20%) with apparent elimination half-life of 11 to 13 hours[7,9].

Cilostazol 50 mg tablet has currently marketed in Thailand, specifically-Pletaal®, which is an original formulation, is costly. An introduction of generic drugs would reduce drug costs and benefit consumers dramatically. Hence, a pharmacokinetic comparison between original and generic drug is needed before such an introduction. An in vivo bioequivalence study is required for product registration to be marketed in the country[10]. The present study was designed to determine the bioequivalence of the generic formulation of 50 mg cilostazol (Citazol®, Standard Chem. & Pharm. Co., Ltd., Taiwan) in comparison to the original formulation (Pletaal®, Otsuka Pharmaceutical Co., Ltd., Korea).

Correspondence to:
Chatsiricharoenkul S, Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Phone: +66-2-4197571, Fax: +66-2-4115026
E-mail: somruedee.cha@mahidol.ac.th

Material and Method

Study drugs

Citazol® manufactured by Standard Chem. & Pharm Co., Ltd., Taiwan (Lot No. 004, Mfg. date 9 February 2009) and Pletaal® manufactured by Korea Otsuka Pharmaceutical Co., Ltd., Korea (Lot No. PM 809027, Mfg. date 2 September 2008), were used as test and reference formulations, respectively. Both formulations were prepared as tablets containing 50 mg cilostazol.

Volunteers

Sample size was calculated to yield a power of 80% and using an alpha level of 0.05. Assuming the percentage intra-subject coefficient of variation (CV) for C_{max} and area under curve (AUC) was 23%[7,11,12], the 90% CI indicated that 26 subjects would be sufficient for the study. Four subjects were added to the calculated subject number to compensate for a possible predicted dropout rate of 15%.

Thirty healthy Thai volunteers aged between 18 and 45 years with a body mass index between 18 and 25 kg/m², were informed of the details and purposes of the present study, and provided written informed consent before participation. Volunteers were assessed to be in good physical condition by clinical screening included history taking, physical examination and the following laboratory tests, complete blood count, blood urea nitrogen, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, fasting blood sugar, urinalysis, and hepatitis B surface antigen. Eligible volunteers must not smoke for at least 30 days before participating in the study. Pregnant or lactating women or positive pregnancy test were ineligible for enrollment. Exclusion criteria included allergy to either cilostazol or related structure. Volunteers who used any drugs, food supplements, vitamins, mineral, herbal remedies, and contraceptives hormones within 14 days before participated in the study were excluded.

Study design

The present study was designed as an open-label, single dose, fasting, two-treatment, two-period, two-sequence randomized crossover with at least 14 days washout period. The volunteers were randomized and assigned equally into two groups, by the sequence of product taking those are Test-Reference (TR) and Reference-Test (RT) group. The volunteers are randomly allocated to groups by 'subject number' based on their order of arriving at the clinic on admission day. Odd and even numbers were used to indicate TR group and RT group respectively. Both the investigators and the volunteers knew the formulation being given. However, to avoid bias in analysis, the randomization code was blinded to bio-analytical staff. All volunteers were admitted on the night before administration day and were hospitalized at research ward for 24 hours after dosing. A single dose of 50 mg cilostazol, either reference or test formulations, was administered with 220 mL water under fasting condition for at least 10 hours. In each period, 18 blood samples were obtained, sampling time points were 0 hour (pre-dose sample) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 18, 24, 36, and 48 hours after dosing. The clinical part was conducted at Siriraj Clinical Research Center and the bioanalytical part was done at Siriraj Bioequivalent Center, Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University. The protocol had been approved by the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (No.077/2552) on September 4, 2009.

Plasma cilostazol analysis

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was developed by our scientists for the determination of cilostazol in human plasma. Ketoprofen was used as an internal standard. Cilostazol was extracted by liquid-liquid extraction technique (LLE), using methyl-t-butyl ether and dichlorobutane. Chromatographic separation was carried out on LC-MS/MS with C18 column. A mobile phase consisting of acetonitrile and 2 mM ammonium acetate (50:50% v/v) was delivered with a flow rate of 0.2 mL/min[13,14]. Mass spectra were obtained using a Quattro Micro mass spectrometer (Micromass Technologies, UK) equipped with electrospray ionization (ESI) source. The mass spectrometer was operated in the multiple reaction monitoring (MRM) modes. Sample introduction and ionization was electrospray ionization in the positive ion mode. The mass transition ion-pair was selected as m/z 370.347 to 288.198 for cilostazol and m/z 255.114 to 209.207 for ketoprofen. The data acquisition was ascertained by Masslynx 4.0 software. Validation of this method was performed as recommended by the USFDA guidelines[15]. The relative standard deviation (RSD) of quality control should be within 15% of the actual value, except at lower limit of quantitation, where RSD should be within 20%. The assay was linear

over a range of concentrations of 0.02 to 1,000 ng/mL ($r^2 = 0.999401$), with a lower limit of quantitation of 0.02 ng/mL. No interfering peaks were observed in the validation process. The recovery ranged from 85.92 to 100.61% for cilostazol. From these quality control samples, interday accuracy ranged from 91.87 to 98.36% and interday precision, expressed as percent coefficient of variation (%CV), ranged from 6.19 to 8.75%. In addition, intraday accuracy and precision (%CV) ranged from 86.98 to 104.95% and from 1.19 to 12.76%, respectively.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were calculated by non-compartmental methods using WinNonlin® software version 3.1 (Scientific Consulting Inc., Apex, North Carolina). Cilostazol bioequivalence between the two treatments were compared with respect to $AUC_{0-\infty}$, AUC_{0-48}, C_{max}, T_{max}, $T_{1/2}$, and λ_z. For the purpose of bioequivalence analysis, $AUC_{0-\infty}$, AUC_{0-48}, and C_{max} were considered as the primary variables. C_{max} and T_{max} of cilostazol were taken directly from the concentration-time data. The AUC_{0-48} was calculated using the log-linear trapezoidal approach. The $AUC_{0-\infty}$ was calculated by the formulation $AUC_{0-\infty} = AUC_{0-48} + (C_{t\,last} / \lambda_z)$, where $C_{t\,last}$ was the last detectable concentration. The λ_z was the elimination rate constant calculated from the log (ln) transformation of concentration-time curves. The plasma concentration half-life ($T_{1/2}$) was calculated by using the formulation $T_{1/2} = 0.693/ \lambda_z$.

Two-way analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences, and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for p-value equal to or less than 0.05. The 90% confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for AUC_{0-48}, $AUC_{0-\infty}$, and C_{max} was calculated based on least squares means from the ANOVA of log-transformed data. A non-parametric statistical analysis, Friedman's test using Kinetics 2000 software was performed on T_{max} and considered significant difference between test and reference formulations when $p<0.05$. The bioequivalence between the two formulations would be accepted if the 90% confidence interval (CI) of the log transformed AUC_{0-48}, $AUC_{0-\infty}$, and C_{max} of test fell within 80 to 125% of the original product.

Results
Demographic data

Demographic characteristics of volunteers between the two groups were not significantly different. The mean age of volunteers in group TR (seven males and eight females) was 31.4 years and the mean body mass index (BMI) was 22.4 kg/m². For RT group (eight males and seven females), the mean age was 31.3 years, and the mean BMI was 21.9 kg/m². The 30 enrolled volunteers completed the entire study. Demographic data of volunteers are summarized in Table 1.

Table 1. Demographic data and baseline characteristics of volunteers

| Characteristics	Mean ± SD	
	TR Group (n = 15)	RT Group (n = 15)
Gender		
Male	7	8
Female	8	7
Age (years)	31.4±5.3	31.3±4.5
Weight (kg)	62.2±9.3	58.5±9.3
Height (cm)	166.4±8.7	163.1±7.6
Body mass index (kg/m²)	22.4±2.0	21.9±2.0
Vital signs		
Temperature (°C)	36.4±0.4	36.2±0.2
Pulse (beats/minute)	76.5±13.3	72.8±8.6
Respiratory rate (time/minute)	19.9±0.5	20.0±0.0
Systolic blood pressure (mmHg)	108.3±10.8	109.3±9.9
Diastolic blood pressure (mmHg)	70.2±7.5	69.5±8.6

TR = Test-Reference; RT = Reference-Test
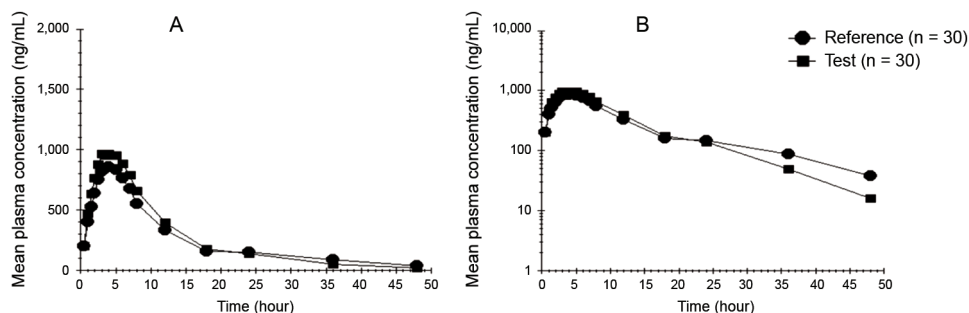
Fig. 1 Geometric mean of plasma concentration-time profile of a test (Citazol®) and reference (Pletaal®) formulation of 50 mg cilostazol after a single administration in 30 healthy Thai volunteers. Lower limit of quantitation = 0.02 ng/mL; normal plot (A) and semilogarithmic plot (B).

Pharmacokinetic parameters

The geometric means of C_{max}, AUC_{0-48}, $AUC_{0-\infty}$, T_{max}, and $T_{1/2}$ were summarized in Table 2. The geometric means of plasma concentration-time profile of cilostazol were also presented in Fig. 1.

Bioequivalence analysis

ANOVA of the log-transformed data of C_{max}, AUC_{0-48}, and $AUC_{0-\infty}$ obtained from the test and reference formulations demonstrated no significant period, sequence and treatment effects ($p>0.05$).

Table 2. Summary of the pharmacokinetics parameters

Pharmacokinetic parameters	Geometric means (%CV)	
	Reference product (Pletaal®)	Test product (Citazol®)
Cmax (ng/mL)	1,010 (28.7)	1,130 (31.9)
AUC_{0-48} (ng.h/mL)	12,400 (33.4)	12,900 (29.8)
$AUC_{0-\infty}$ (ng.h/mL)	14,200 (43.2)	13,500 (30.2)
T_{max} (h)	3.41 (43.5)	2.7 (56.7)
λ_z (h^{-1})	0.0632 (67.8)	0.0852 (58.0)
$T_{1/2}$ (h)	11.0 (67.8)	8.13 (58.0)

AUC = area under plasma concentration-time curve; C_{max} = maximal plasma concentration; T_{max} = time for the maximal plasma concentration; $T_{1/2}$ = half-life; λ_z = elimination rate constant

However, the significant subject nested in sequence effect was solely significant ($p<0.05$) for all parameter which was normally seen in small sample size bioequivalence studies (data not shown). The statistical analysis of cilostazol obtained from the present study (n = 30) showed that the point estimate (90% confidence interval) of the geometric mean ratio (test/reference) of the log transformed of C_{max}, AUC_{0-48}, and $AUC_{0-\infty}$ were within the equivalence criteria (80.00-125.00%) which was 112.38% (101.70-124.19%) for C_{max} ratios and 103.66% (96.06-111.86%) for AUC_{0-48} ratios and for $AUC_{0-\infty}$ was 95.14% (86.12-105.12%) with the power more than 80% (Table 2.). There was no statistical difference of median T_{max} between the test and reference formulations ($p>0.05$).

Tolerability

Adverse events were monitored and recorded in case report forms based on volunteer interview and physical examination. Treatments were generally well tolerated. Twenty-five post-dose adverse events were reported in 15 volunteers (nine events from the reference product and 16 from the test product). The most frequently reported adverse event was headache (13), which had frequently occurred in previous studies[8,16]. Additionally, other adverse events including dizziness (3), conjunctivitis (1), fatigue (1),

Table 3. Statistical summary of the comparative bioavailability data of cilostazol (n = 30)

Dependent	Geometric mean ratio (T/R)	90% confidence interval (CI)	Power	Intra-subject coefficient of variation (%CV)
Ln (C_{max})	112.38	101.70-124.19	0.9775	23.04
Ln (AUC_{0-48})	103.66	96.06-111.86	0.9986	17.46
Ln ($AUC_{0-\infty(obs)}$)	95.14	86.12-105.12	0.9780	22.98

T = test product (Citazol®); R = reference product (Pletaal®)

muscle strain (1), tingling of fingers and toes (1), diarrhea (1), abdominal discomfort (1), nausea (1), dyspepsia (1), and rash (1) were also reported. No serious adverse events were observed throughout the study.

Discussion

The bioequivalence study of 50 mg cilostazol tablets formulations was conducted in 30 healthy Thai volunteers between a generic product (Citazol®) and the reference product (Pletaal®). The analytical method (LC-MS/MS) utilized to determine the concentrations of cilostazol in plasma demonstrated good precision and accuracy. The present study design and sample size were considered most appropriate and standard for this type of study. The results showed that both formulations were well tolerated. The 90% confidence intervals for log-transformed geometric mean test/reference formulation ratios of primary parameters including C_{max}, AUC_{0-48}, and $AUC_{0-\infty}$ were entirely within 80% and 125%[17]. There was no statistically significant difference of T_{max} between the reference and the test products ($p>0.05$).

Conclusion

From the present study, both reference and test products of 50-mg cilostazol tablets were bioequivalent as rate and amounts of drug absorption.

What is already known on this topic?

Cilostazol has been approved by the Food and Drug Administration (FDA) for the treatment of intermittent claudication in patients with peripheral artery disease.

What this study adds?

A generic product (Citazol®) could be a substitute of innovator product (Pletaal®) based on this present bioequivalence study. This can reduce drug costs for consumers, patient care organizations, and governments.

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การศึกษาชีวสมมูลของยาเม็ด cilostazol ขนาด 50 มิลลิกรัม ในอาสาสมัครไทยที่มีสุขภาพแข็งแรง

พิณพิไล จูทะสมพากร, สุพรชัย กองพัฒนากูล, ปิยาภัทร พงศ์นรินทร์, กอบธัม สถิรกุล, สมฤดี ฉัตรสิริเจริญกุล

วัตถุประสงค์: เพื่อศึกษาชีวสมมูลของยาเม็ด cilostazol ขนาด 50 มิลลิกรัม ระหว่างผลิตภัณฑ์ยาสามัญ citazol® ของบริษัท ยูเนียน แลบบอราทอรี่ ประเทศไทย กับผลิตภัณฑ์ยาต้นแบบ Pletaal® ของบริษัท โอตสึกะ ฟาร์มาซูติคอล

วัสดุและวิธีการ: การศึกษานี้เป็นแบบ open-label, single dose, 2-treatment, 2-period, 2-sequence randomized crossover design ในขณะท้องว่าง มีระยะเวลา washout period อย่างน้อย 14 วัน ทำการศึกษาในอาสาสมัครไทย สุขภาพดี ทั้งเพศหญิงและชาย จำนวน 30 ราย ทำการวัดระดับ cilostazol ในตัวอย่างเลือดของอาสาสมัคร ตั้งแต่ก่อนรับประทานยาและตรวจวัดเป็นระยะจนถึง 48 ชั่วโมง การวัดระดับยานี้ใช้เทคนิค liquid chromatography with tandem mass spectrometry (LC-MS/MS) และนำค่าที่วัดได้มาหาค่าทาง เภสัชจลนศาสตร์โดยใช้ non-compartment model

ผลการศึกษา: ที่ระดับความเชื่อมั่น 90% เมื่อเปรียบเทียบสัดส่วนของค่าเฉลี่ยระหว่างผลิตภัณฑ์ยาสามัญกับผลิตภัณฑ์ยาต้นแบบ ของ cilostazol จะได้ค่าของ C_{max}, AUC_{0-48} และ $AUC_{0-\infty}$ เท่ากับ 112.38% (101.70%-124.19%), 103.66% (96.06%-111.86%) และ 95.14% (86.12%-105.12%) ตามลำดับ

สรุป: ที่ระดับความเชื่อมั่น 90% เมื่อเปรียบเทียบอัตราส่วนของค่าเฉลี่ยเรขาคณิตระหว่างผลิตภัณฑ์ยาสามัญกับผลิตภัณฑ์ยาต้นแบบ ของค่าสำคัญทางเภสัชจลนศาสตร์อยู่ในช่วงที่ได้รับการยอมรับคือ 80.00%-125.00% ดังนั้นจึงสรุปได้ว่าผลิตภัณฑ์ยาสามัญมีชีวสมมูลกับผลิตภัณฑ์ยาต้นแบบ