Bioequivalence Study of 30 mg Pioglitazone Tablets in Thai Healthy Volunteers

Somruedee Chatsiricharoenkul MD*, Piyapat Pongnarin MSc*,
Korbtham Sathirakul PhD**, Supornchai Kongpattanakul MD*

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

Objective: *To compare the bioequivalent parameters of 30 mg pioglitazone tablets manufactured locally (Glista®) and originally (Actos®).*
Material and Method: *A randomized, single dose, two-treatment, two-period, two-sequence crossover study was conducted. Twenty-four healthy volunteers were recruited at Siriraj Clinical Research Unit. Each subject received a 30 mg pioglitazone tablet of both formulations with at least a week washout period. Blood samples were collected over 48 h after the oral administration. The plasma fractions were analyzed for pioglitazone using a liquid chomatography-mass spectrometry (LC-MS/MS).*
Results: *Twenty-four volunteers enrolled in the present study. Pharmacokinetic parameters were determined using the non-compartment model. The 90 percent confidence intervals of the mean ratios (test/reference) of* C_{max} *(86.2687-113.7313%),* $AUC_{0 \rightarrow t}$ *(85.7139-114.2861%) and* $AUC_{0 \rightarrow \infty}$ *(81.7820-118.2180%) fell within the acceptable range (80-125%) for bioequivalent eligibility. Both preparations were well tolerated and had a few non-serious adverse events.*
Conclusion: *The 2-tablet preparations of pioglitazone were bioequivalent in Thai healthy volunteers.*

Keywords: *Pioglitazone, Bioequivalence*

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Pioglitazone is a thiazolidinedione antidiabetic agent used in the treatment of type 2 diabetes. Thiazolidinediones act mainly by sensitizing the liver and peripheral tissues to the effects of insulin, which results in improving insulin-mediated glucose disposal. The thiazolidinediones can bind with high affinity to and activate the nuclear peroxisome proliferator activated receptor-γ (PPAR-γ). PPAR-γ activation by thiazolidinediones results in the expression of specific genes involved in the regulation of carbohydrate and lipid metabolism, as well as promoting adipocyte differentiation. Pioglitazone stimulates the uptake of glucose and fatty acids into cells by promoting the synthesis and expression of cellular glucose and fatty acid transporters[1]. Many studies of pioglitazone demonstrated the improvement of glycemic control, HbA1c, fasting plasma glucose levels, and serum lipid profiles[2,3].

Correspondence to : Chatsiricharoenkul S, Department of Pharmacology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-7571, Mobile: 081-636-1717, Fax: 0-2411-5026

The structural formulation of pioglitazone hydrochloride is (±) -5 - [p - [2 - (5-ethyl - 2-pyridyl) ethoxy] - 2, 4-thiazolidinedione hydrochloride. The empirical formula is $C_{19}H_{20}N_2O_3S$•HCl. The molecular weight is 392.90. At steady state, maximum plasma drug concentrations (C_{max}) were 0.7 and 1.2 mg/L, and times to C_{max} (t_{max}) were 4.8 and 3.7 h, for pioglitazone at 15 and 30 mg/d, respectively. Pioglitazone undergoes extensive hepatic metabolism, predominantly via by the cytochrome P450 (CYP) 2C8 system. Its elimination half-life was 3-7 h. Pioglitazone and its metabolites were excreted via urine (15-30%). The remainders were excreted into bile and feces[4-6]. The recommended dose for the treatment of type 2 diabetes was 15-30 mg/d. Pioglitazone could be given as monotherapy or in combination with another antidiabetic agent or insulin[7,8].

Bioequivalence study examines whether two different formulations of the same drug behave simi-

larly in terms of their exposure[9,10]. It is an attractive method that assures clinicians that two formulations of the same drug will result in similar toxicity and efficacy. Clinicians can confidentially prescribe less expensive generic drugs as alternatives for the original drugs.

The objective of the present study was to assess the bioequivalence of two formulations of 30-mg pioglitazone tablets (Glista® from Berlin Pharmaceutical Industry Co.,Ltd. Thailand as the test formulation; and Actos® from Takeda Pharmaceutical Company Ltd., Osaka, Japan as the reference formulation) in healthy Thai volunteers. The protocol has been approved by the Ethical Committee of the Faculty of Medicine, Siriraj Hospital, Mahidol University (No.189/2548) on August 29, 2005.

Material and Method

Piogltazone Preparations

Reference preparation: Actos® (Takeda Pharmaceutical Company Ltd., Osaka, Japan) containing 30 mg pioglitazone per tablet (Lot no. 0079, Mfg. date 27 August 2004, Exp. date 27 Aug 2007).

Test preparation: Glista® (Berlin Pharmaceutical Industry Co., Ltd. Thailand) containing 30 mg pioglitazone per tablet (Lot no. 05116, Mfg. date 15 August 2005, Exp. date 15 Aug 2007).

Volunteers

Twenty-four healthy Thai volunteers aged between 18-45 years with a body mass index between 18-24 kg/m^2 were recruited at Siriraj Clinical Research Center, Siriraj Hospital. After explaining the details and the purposes of the present study, all healthy volunteers provided written informed consents. They 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 investigations: complete blood count, BUN, creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, fasting blood sugar, and hepatitis B surface antigen. Urine pregnancy tests were negative in all female volunteers. Volunteers did not have a history of allergy to pioglitazone and/or its constituents and did not receive other medicines within 14 days before the first study drug administration.

Study design

Randomized, single dose, fasting, two-period, two-sequence, crossover study with at least one week washout period was conducted. Volunteers were allocated into two equal groups. Each volunteer was assigned to a particular study group using a pre-printed randomization table generated by Microsoft Excel.

During each period, the volunteers were admitted to the Siriraj Clinical Research Center, Siriraj Hospital. After overnight fasting for at least 8 hours, they received a 30-mg pioglitazone tablet along with 240 mL of drinking water. Volunteers continued fasting for 4 and 2 h for food and water respectively after drug administration. Before and after each period of the present study, the volunteers were examined by a physician. For the safety of volunteers, blood sugar was monitored at 12, 24, and 48 h after drug administration.

Sample collection and pioglitazone analysis

Five mL of each blood sample was collected by catheterized venupuncture at forearms from each subject. Fifteen samples were collected: 0 (before the dosing), 0.25, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, and 48 h after the oral administration. The blood samples were centrifuged and the plasma fractions were collected and kept at -70°C until analysis.

Plasma pioglitazone was measured by a validated liquid chomatography-mass spectrometry (LC-MS/MS) method[11-14]. Rosiglitazone was used as an internal standard. Pioglitazone was extracted by liquid-liquid extraction technique, using methyl-t-butyl ether and 1-chlorobutane. All of organic phase was evaporated to dryness under nitrogen gas. The residual was redissolved and injected to HPLC. The mobile phase consisted of acetonitrile and ammonium acetate. The analytical equipment used included an HPLC device coupled with a mass selective detector. The selected reaction monitoring (SRM) used the range from *m/z* 356.98 to 119.08 for pioglitazone and from *m/z* 358.00 to 135.06 for internal standard. Validation of this method was performed as recommended by the USFDA. Calibration curve was linearity with $r^2 = 0.998843$ and the lower limit of quantification for the validated assay was 0.5 ng/ml. The mean inter-assay accuracy and precision (CV (%)) for pioglitazone ranged from 97.54 to 104.00 and 7.24 to 14.03%, respectively. Pioglitazone level was calculated using MassLynx version 4.0.

Pharmacokinetic and statistical analysis

A non-compartmental pharmacokinetic model was used to determine the pharmacokinetic parameters of pioglitazone. The pharmacokinetic parameters, i.e., $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, C_{max}, t_{max}, $t_{1/2}$ were determined using WinNonlin edition version 3.1. Statistical comparisons

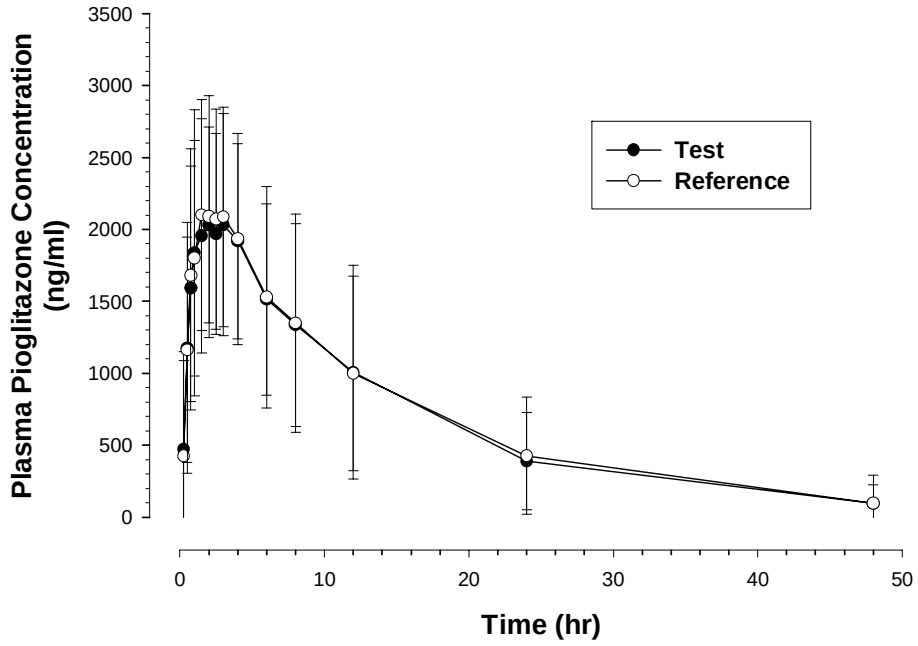

Fig. 1 The average pioglitazone plasma concentrations at different time points over 48-hours period (R = Reference, T = Test)

between pharmacokinetic parameters of the two products were analyzed using two-way ANOVA with $p < 0.05$ for statistical significance to assess the effect of formulation, periods, sequence, subjects within sequence. The 90 percent confidence intervals of the test/reference ratio of C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ using log transformed data were determined. The bioequivalence between the two formulations would be accepted if the 90 percent confidence intervals (CI) of the log transformed C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ of test fell within 80-125% of the original product[9,10].

Results

Twenty-four volunteers (15 male and 8 female) enrolled in the present study. A volunteer violated the protocol because a mistake of sequencing between test and reference therefore corresponding data was removed accordingly. Pioglitazone was well tolerated. There were only three adverse events (common cold, dyspepsia, and acute diarrhea) and nobody had hypoglycemic symptom. No serious adverse event was found throughout the present study. No significant difference was observed in any of the analyzed pharma-

Table 1. Pharmacokinetic parameters of Reference (Actos®) and Test (Glista®) with 90% confidence interval (CI) of the mean ratios (generic/original) of log transformed values

Pharmacokinetic parameters	Product (Mean ± SD)		90% confidence interval (CI) of the mean ratios (generic/original) of log transformed values
	Test (Glista®)	Reference (Actos®)	
t_{max} (hr)	1.954	2.014	-
$t_{1/2}$ (hr)	9.818	9.130	-
C_{max}(ng/ml)	2,265.551±864.919	2,187.328±852.731	86.27-113.73
$AUC_{0\rightarrow t}$(ng·hr/ml)	26,128.863±17,023.690	26,704.665±17,723.773	85.71-114.29
$AUC_{0\rightarrow\infty}$(ng·hr/ml)	29,446.032±24,970.133	28,122.425±23,793.962	81.78-118.22

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

cokinetic parameters (Table 1). The generic formulation had a C_{max} at 2,265.551 ng/mL, a t_{max} at 1.954 h while the original formulation had a C_{max} at 2,187.328 ng/mL, a t_{max} at 2.014 h (Fig. 1).

Ninety percent CI of the mean ratios (generic/original) of the log transformed of the C_{max}, $AUC_{0 \rightarrow t}$ and $AUC_{0 \rightarrow \infty}$ were 86.27-113.73%, 85.71-114.29 and 81.78-118.22% respectively. Since the 90% CI for C_{max}, $AUC_{0 \rightarrow t}$ and $AUC_{0 \rightarrow \infty}$ fell within the predefined bioequivalence acceptance limits (80-125% of the originator); the generic and original formulations were considered bioequivalent. Hence, Glista® could be concluded as having comparable pharmacokinetic profiles with Actos®.

Discussion

Pioglitazone is an antidiabetic agent that has a good result for glycemic control and improves serum lipid profile. The analytical method (LC-MS/MS) utilized to determine the concentrations of pioglitazone in human plasma demonstrated good precision and accuracy. The present study employed a randomized, single dose, two treatments, two periods, two sequences crossover design to study the bioequivalence in 24 healthy volunteers. The study design and sample size are considered most appropriate and standard for this type of study.

The overall pharmacokinetic parameters from both formulations were similar but were not similar to data previously published[4-6]. The present result has shown that metabolism of Thai people is probably different from foreign people. However, the sample size was too small to conclude. With no serious clinical adverse events, it was concluded that a single dose of test, compared to reference formulation, was well tolerated.

Conclusion

From the present study, the bioequivalence of both formulations of 30-mg pioglitazone tablets was based on the equivalences in C_{max}, $AUC_{0 \rightarrow t}$ and $AUC_{0 \rightarrow \infty}$.

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การศึกษาชีวสมมูลของยาเม็ดไพโอกลิตาโซนขนาด 30 มิลลิกรัมในอาสาสมัครไทยสุขภาพ แข็งแรง

สมฤดี ฉัตรสิริเจริญกุล, ปิยภัทร พงศ์นรินทร์, กอบธัม สถิรกุล, สุพรชัย กองพัฒนากูล

วัตถุประสงค์: เพื่อศึกษาชีวสมมูลของยาเม็ดไพโอกลิตาโซน ขนาด 30 มิลลิกรัม ระหว่างผลิตภัณฑ์ยาสามัญ Glista® กับผลิตภัณฑ์ยาต้นแบบ Actos®

วัสดุและวิธีการ: อาสาสมัครสุขภาพดีจำนวน 24 คน ได้รับการคัดเลือกให้มาเข้าร่วมการศึกษาที่ศูนย์วิจัยคลินิกศิริราช รูปแบบการศึกษาที่ใช้คือ randomized, single dose, two treatments, two periods, two sequences crossover study อาสาสมัครแต่ละคนได้รับประทานยาเม็ดไพโอกลิตาโซนขนาด 30 มิลลิกรัมทั้งสองตำรับ โดยมีระยะเวลา washout period นานอย่างน้อย 1 สัปดาห์ มีการเก็บตัวอย่างเลือดในช่วงเวลา 48 ชั่วโมง ตัวอย่างเลือดจะได้รับการวิเคราะห์โดยวิธี liquid chromatography-mass spectrometry ที่ได้รับการตรวจสอบความถูกต้องแล้ว

ผลการศึกษา: อาสาสมัครจำนวน 24 คนได้เข้ามาร่วมในการศึกษา แต่เนื่องจากมีการเบี่ยงเบนจากโครงร่างงานวิจัย 1 ราย จึงไม่นำข้อมูลจากอาสาสมัครรายนั้นมาใช้ในการวิเคราะห์ ผลการศึกษาค่าทางเภสัชจลนศาสตร์โดยใช้การวิเคราะห์แบบ non-compartmental analysis โดยมีค่า 90% ความเชื่อมั่นของค่า log ของค่าเฉลี่ยของยา Glista® ต่อ Actos® ดังนี้ ค่า Cmax เท่ากับ 86.2687-113.7313% ค่า $AUC_{0\rightarrow t}$ เท่ากับ 85.7139-114.2861% และค่า $AUC_{0\rightarrow\infty}$ เท่ากับ 81.7820-118.2180% ซึ่งทุกค่าอยู่ในเกณฑ์ของการยอมรับความเท่าเทียมกันในการศึกษาชีวสมมูล (80-125%) ยาทั้ง 2 รูปแบบมีความปลอดภัยและพบเหตุการณ์ไม่พึงประสงค์ที่ไม่รุนแรงเพียงเล็กน้อย

สรุป: ยาเม็ดไพโอกลิตาโซนทั้ง 2 ตำรับมีชีวสมมูลซึ่งกันและกัน เมื่อศึกษาในอาสาสมัครไทยสุขภาพดี