Bioequivalence Study of Sildenafil 20 mg Tablets in Healthy Thai Male Volunteers

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Abstract

The present study aims to evaluate the bioequivalence of the generic formulation, sildenafil GPO 20 mg tablets to the originator, Revatio® 20 mg tablets. The study was a randomized, single dose, two-way, cross-over, open-label study which included 32 healthy Thai male volunteers under fasting conditions with a 7-day washout period. After drug administration, blood samples have been collected at the predefined time points and the plasma concentrations of sildenafil and its metabolite, piperazine N-desmethylsildenafil, were analyzed using validated LC-MS/MS method. The pharmacokinetic parameters assessed were area under the plasma concentration time curve from time 0 hour to 24 hours (AUC_{0-24}), AUC from time zero to infinity ($AUC_{0-\infty}$), the peak plasma concentration (C_{max}), and time to achieve the C_{max} (t_{max}). The geometric mean (90% CI) values for the ratio of AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} of the test product over those of the reference product were 101.0% (92.0-110.9%), 100.8% (92.1-110.4%) and 100.9% (90.0-113.1%) for sildenafil, respectively. The geometric mean ratios (90% CI) for piperazine N-desmethylsildenafil were 100.6% (95.1-106.4%), 99.8% (95.2-104.6%), and 100.8% (92.0-110.3%) for AUC_{0-24}, $AUC_{0-\infty}$ C_{max}, respectively. The differences between the test and reference product in t_{max} values were not statistically significant (P>0.05). The 90% confidence intervals of the test/reference AUC ratio and C_{max} ratio of sildenafil and piperazine N-desmethylsildenafil were within the acceptance range for bioequivalence. There was no adverse event observed during this bioequivalence study. Both formulations were equivalent in terms of extent and rate of absorption.

Keyword: Pulmonary arterial hypertension, Phosphodiesterase-5 inhibitor, Bioequivalence, Sildenafil, Pharmacokinetics

INTRODUCTION

Pulmonary arterial hypertension (PAH) is one type of pulmonary hypertension (PH) which is characterized by elevation in pulmonary arterial pressure. Patients will be diagnosed as PAH if they have mean pulmonary arterial pressure ≥ 25 mmHg and pulmonary wedge pressure ≤ 15 mmHg at rest measured by cardiac catheterization[1]. The causes of PAH may be idiopathic or associated with other abnormalities such as collagen vascular disease, HIV infection, drugs and toxins, etc[1-2]. Patients with this condition may suffer from many symptoms e.g. dyspnea, fatigue, edema, exertion intolerance[3].

Sildenafil, an orally active potent phosphodiesterase-5 (PDE5) inhibitor with its chemical structure described in Figure 1, has been used for treatment of PAH. Inhibition of PDE5 increases intracellular cGMP smooth muscle that results in smooth muscle relaxation. This can lead to pulmonary vascular dilatation in PAH patients[4].

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Multicenter, randomized, double-blind, placebo-controlled trial has shown improvement in exercise capability, WHO functional classification of pulmonary arterial hypertension, and hemodynamics in symptomatic PAH patients as resulting from the effect of the drug[5]. In addition, the study revealed mild to moderate adverse effects related to vasodilation effect of the drug[5]. In the US and Europe, the approval dosage is 20 mg three times a day and may be taken with or without food[4].

Sildenafil is absorbed rapidly after oral administration due to its desirable physicochemical properties (e.g. $pK_a = 6.5$ and $\log D_{7.4} = 2.7$)[6]. Its maximal plasma concentration (C_{max}) is reached (or T_{max}) within approximately 1 hour in the fasting state[4]. The mean absolute oral bioavailability is about 41% because of first-pass metabolism. After orally taken, AUC and Cmax increase proportionally with the dose range 20-40 mg. When taken with food, its time to achieve C_{max} (or T_{max}) and C_{max} is delayed by 1 hour and reduced by 29%, respectively. Sildenafil is extensively cleared through CYP3A4 microsomal enzyme. It has one active metabolite, piperazine N-desmethylsildenafil, which also has the same phosphodiesterase (PDE) selectivity profile as its parent drug with activity about 50% of sildenafil. In PAH patients, plasma levels of piperazine N-desmethylsildenafil metabolite are approximately 72% of sildenafil after 20 mg three times daily dosing with unknown subsequent effect on efficacy[7].

A generic product of sildenafil has been developed for clinical use with a lower cost compared with an original brand. Although containing the same active pharmaceutical ingredient, both products may differ from each other by formulation, or manufacturing process which may consequently affect some pharmacokinetic properties of the drug especially rate and extent of absorption. Therefore, the bioequivalence study is conducted to demonstrate the similarity and to confirm the interchangeability between the two preparations in human. The purpose of this study was to evaluate the bioequivalence of two oral preparations of sildenafil 20 mg tablets in healthy Thai male volunteers.

MATERIALS AND METHODS

Study drugs

The reference product of sildenafil 20 mg tablets was Revatio® manufactured by Pfizer PGM, Poce Sur Cisse, France (batch no. A062504, Mfd Jul 2011, Exp Jun 2014). The test product was sildenafil GPO 20 mg tablets manufactured by The Government Pharmaceutical Organization, Thailand (batch no. S540386, Mfd 07 Nov 2011, Exp 07 Nov 2013).

Subjects

Thirty two Thai male volunteers aged between 18 and 55 years old were enrolled for the study. Subjects were healthy on the basis of physical, laboratory, and medical history examinations. The participants who were contraindicated or hypersensitive to sildenafil or any excipient in the formulation were excluded. Those with underlying diseases, positive hepatitis B test, cigarette smoking, alcohol dependence, heavy consumption of xanthine containing products, or consumption of any medication prior to the study for 14 days are not included. Volunteers who donated blood or participated in any clinical trial prior the starting date of this study for 1 month were also excluded. The study protocol was approved by the Institute for the Development of Human Research Protections (IHRP).

Study design and method of drug administration

A randomized, open label, two treatment, two period, two sequence, single dose, crossover, bioequivalence study was carried out at Clinical Research Center, Department of Medical Sciences, Ministry of Public Health, Thailand. Each subject randomly received a single dose of the assigned formulation, administered with

240 ml of water after an overnight fasting of at least 10.0 h. Washout period was at least 7 days between treatments. Each participant received an identical food and fluid intake in both periods of the study. Blood samples were collected into K2EDTA vacutainers by the indwelling catheter for twenty sampling times (0.00, 0.17, 0.33, 0.50, 0.67, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 10.00, 12.000, 16.00 and 24.00 hours). After collection, the blood samples were centrifuged at 3000 ± 100 rcf for 5 minutes at below 10°C, and plasma in the supernatant portion was separated. All plasma samples were divided into two aliquots and transferred to suitably labeled polypropylene tubes. Then, plasma samples were stored at -65±10°C until analysis.

Determination of sildenafil and piperazine N-desmethylsildenafil concentration in plasma.

The concentrations of sildenafil and piperazine N-desmethylsildenafil were analyzed by a liquid chromatography with tandem mass spectrometer detector (LC-MS/MS). Sildenafil-d8 and piperazine N-desmethylsildenafil-d8 were used as internal standards (IS) for sildenafil and piperazine N-desmethylsildenafil, respectively. MRM transitions (m/z) for each molecule were: 475.210 283.160 for sildenafil, 461.190 283.200 for piperazine N-desmethylsildenafil, 483.260 283.200 for sildenafil-d8, and 469.240 283.180 for piperazine N-desmethylsildenafil-d8. The polarity mode for molecular fragmentation was positive mode. The chromatographic system consisted of ACE 5 CN, 150 x 4.6 mm i.d. column. The mobile phase was a mixture of 1 mM ammonium formate (pH 6.0) and methanol (10:90 v/v). The system was binary flow with flow rate of 1.0 ml/min and set the temperature at 40°C. The analytes and internal standards were extracted from plasma using liquid-liquid extraction method before transferred into appropriate autosampler vials for analysis.

Statistical methods and data analysis

Both primary and secondary pharmacokinetic parameters of sildenafil and piperazine N-desmethylsildenafil were calculated. The areas under the plasma concentration versus time curve from time zero to the last measurable concentration (AUC_{0-t}) were calculated by linear trapezoidal method. The areas under the plasma concentration versus time curve from time zero to infinity ($AUC_{0-\infty}$) were computed by using following equation: $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$ where C_t was the last measurable concentration and λ_z was the terminal elimination rate constant. Maximal plasma concentration (C_{max}) and time to achieve maximal plasma concentration (T_{max}) were obtained directly from each subject's plasma concentration versus time profile. The terminal elimination rate constant was estimated by linear least squares regression analysis using at least last three or more non-zero plasma concentration values. The terminal elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/\lambda_z$. The residual area in percentage (AUC_%Extrap_obs) was determined by the formula, $[(AUC_{0-\infty}-AUC_{0-t})/AUC_{0-\infty}]$ x 100. Ratio of AUC_{0-t} and $AUC_{0-\infty}$ was also computed. The pharmacokinetic parameters were calculated by non-compartmental model using Phoenix WinNonlin Software version 6.3. The primary pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) were transformed to natural logarithm (ln) scale before carrying out the statistical analysis. The comparison of the pharmacokinetic parameters was carried out using PROC GLM of SAS® version 9.3. Analysis of variance was carried out by employing PROC GLM of SAS® version 9.3 for un-transformed and in-transformed pharmacokinetic parameters.

RESULTS AND DISCUSSION

All of the subjects were healthy Thai having normal values of all clinical and laboratory parameters with demographic data shown in Table 1. Out of 32 subjects, 31 subjects completed the study of which

Table 1. Demographic data for sildenafil bioequivalence study in 32 subjects

	Mean (SD)	Range
Ages (year)	30.9 (8.1)	18-49
Weight (kg)	65.3 (6.3)	54.9-79.2
Height (cm)	173.6 (6.1)	165-187
BMI (kg/m^2)	21.7 (1.7)	18.3-24.8

the data were used for pharmacokinetic evaluation of sildenafil and piperazine N-desmethylsildenafil.

The analytical method was developed and validated. The retention times of sildenalfil, sildenafil-d8, piperazine N-desmethylsildenafil, and piperazine N-desmethylsildenafil-d8 were 2.3, 2.3, 3.8, and 4.1 min, respectively. The lower limit of quantifications (LLOQ) of the method were 1.003 and 0.500 ng/ml, whereas the linearity ranges were 1.003 to 515.047 and 0.500 to 76.453 ng/ml with a coefficient of determination greater than 0.98 for sildenafil and piperazine N-desmethylsildenafil, respectively.

The mean plasma concentration time profile of sildenafil and piperazine N-desmethylsildenafil after single oral administration of the sildenafil 20 mg tablets (both the test and the reference drug) are showed in Figure 2 and Figure 3, respectively. The geometric mean ratios (90% CI) of the test/reference drug for sildenafil were 100.9% (90.0-113.1%) for C_{max}; 101.0% (92.0-110.9%) for AUC_{0-24}; and 100.8% (92.1-110.4%) for $AUC_{0-\infty}$. The geometric mean ratios (90% CI) of the test/reference drug for piperazine N-desmethylsildenafil were 100.8% (92.0-110.3%) for C_{max}; 100.6% (95.1-106.4%) for AUC_{0-24}; and 99.8% (95.2-104.6%) for $AUC_{0-\infty}$. The 90% CIs of the test/reference C_{max} and AUC ratio and ratio of sildenafil and piperazine N-desmethylsildenafil were within the acceptance range for bioequivalence. The differences of t_{max} values between the test and reference drug products for both sildenafil and piperazine N-desmethylsildenafil were not found to be statistically significant (P>0.05). The values of the pharmacokinetic parameters and the geometric mean ratios (90% CI) of sildenafil and piperazine N-desmethylsildenafil for both formulations are presented in Table 2 and Table 3.

Table 2. Pharmacokinetic parameters and statistical comparison of sildenafil after single-dose oral administration of sildenafil 20 mg tablets of the test and the reference drug

Parameters	Test produc mean (SD)	Reference product mean (SD)	Geometric mean ratio of test/reference drug (90% CI)
AUC_{0-24} (ng.h/mL)	233.55(71.56)	233.46(77.11)	101.0% (92.0-110.9%)
$AUC_{0-\infty}$ (ng.h/mL)	238.81(71.92)	239.02(77.26)	100.8% (92.1-110.4%)
C_{max} (ng/mL)	105.16(35.44)	104.72(39.13)	100.9% (90.0-113.1%)
$T_{1/2}$ (h)	2.29(0.56)	2.35(0.45)	-
t_{max} (h)*	0.67(0.33-1.75)	0.67(0.33-2.50)	-

* The values are reported as median (minimum-maximum)

Table 3. Pharmacokinetic parameters and statistical comparison of piperazine N-desmethyl-sildenafil after single-dose oral administration of sildenafil 20 mg tablets of the test and the reference drug

Parameters	Test produc mean (SD)	Reference product mean (SD)	Geometric mean ratio of test/reference drug (90% CI)
AUC_{0-24} (ng.h/mL)	58.55 (25.18)	59.70 (31.07)	100.6% (95.1-106.4%)
$AUC_{0-\infty}$ (ng.h/mL)	62.71 (25.57)	64.07 (31.49)	99.8% (95.2-104.6%)
C_{max} (ng/mL)	18.90 (6.87)	18.64 (6.82)	100.8% (92.0-110.3%)
$T_{1/2}$ (h)	3.70 (0.78)	3.92 (0.97)	-
t_{max} (h)*	0.67 (0.33-1.50)	0.67 (0.67-2.00)	-

* The values are reported as median (minimum-maximum)

The $AUC_{0-24}/AUC_{0-\infty}$ ratio of sildenafil in each subject was more than 80% (94.4%–99.1% for the test drug and 93.8%–99.3% for the reference drug). The ratio for piperazine N-desmethylsildenafil was also more than 80% (86.5-97.3% for the test drug and 81.6-96.8% for the reference drug). These demonstrate the adequacy of sampling time point to capture the absorption phase. The power for all pharmacokinetics parameters were also greater than 90% indicating that the number of subjects was enough to confirm the statistical conclusions.

There was no adverse event developed during this bioequivalence study.

CONCLUSION

Based on the pharmacokinetics and the results of this study, it can be concluded that Sildenafil GPO 20 mg tablets and Revatio 20 mg tablets were bioequivalent with regard to extent and rate of absorption.

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