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Research Article

Bioequivalence Study of Two Ticagrelor 90 mg Tablets in Healthy Thai Volunteers under Fasting Conditions

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ABSTRACT

Patients with acute coronary syndrome require antiplatelet therapy to reduce risk of thrombotic cardiovascular events. Ticagrelor is a potent oral antiplatelet agent that reversibly binds to P2Y12 Adenosine Diphosphate (ADP) receptor thereby preventing platelet aggregation. Since the use of ticagrelor is manageable in outpatient setting, the Government Pharmaceutical Organization (GPO), Thailand had developed a generic product of ticagrelor 90 mg tablets to reduce cost of treatment and improve accessibility to medicines for Thai patients. An open label, randomized, two-way crossover, single dose bioequivalence study was conducted under fasting conditions. The ANOVA showed no significant effects of sequence, formulation and period on the ln-transformed primary pharmacokinetic parameters. The 90% confidence intervals for the ratio of geometric least squares mean were 97.95%-108.87%, 98.21%-109.10% and 89.71%-107.78% for $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$ and Cmax, respectively. Both treatments were well tolerated by the study subjects. It could be inferred that two tablet formulations were bioequivalent and could be used interchangeably regarding the similarity on the pharmacokinetics and tolerability.

Keywords: Ticagrelor; Bioequivalence; Pharmacokinetics; Antiplatelet

INTRODUCTION

Acute Coronary Syndrome (ACS) is a clinical syndrome caused mainly by thrombus formation due to coronary plaque instability and rupture, leading to myocardial ischemia, hypoxia and even myocardial necrosis [1]. Patients with ACS require antiplatelet therapy to reduce risk of thrombotic cardiovascular events [2-4]. Ticagrelor is a potent oral antiplatelet agent that was first approved by U.S. FDA in 2011. Due to cyclopentyl triazolopyrimidine structure (Figure 1), ticagrelor reversibly binds to P2Y12 Adenosine Diphosphate (ADP) receptor thereby preventing platelet aggregation [5]. In comparison with antiplatelet agents in thienopyridine group, e.g. clopidogrel and prasugrel, ticagrelor also inhibits P2Y12 receptor on vascular smooth muscles, thus reducing vasoconstriction and increasing myocardial perfusion [5,6]. In addition, ticagrelor and its active metabolite demonstrate antiplatelet activity whereas thienopyridine agents are prodrugs which require metabolic activation [7].

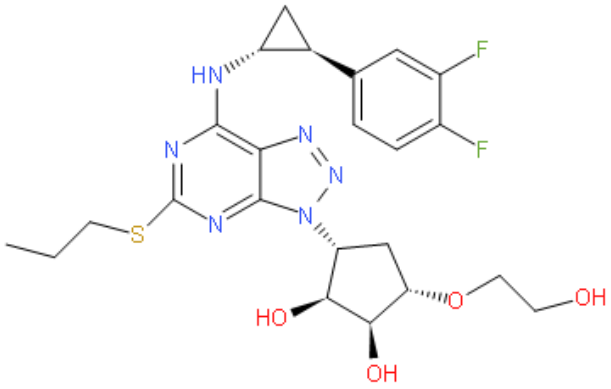

Figure 1: Structure of ticagrelor.

The recommended dose of ticagrelor includes an oral loading dose

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of 180 mg followed by 90 mg twice daily. It can be co-administered with the maintenance dose of 75-100 mg of aspirin [4]. Ticagrelor is rapidly absorbed following oral administration. High fat meal slightly increases the extent of absorption while delay the time to achieve maximum concentration. However, administration with food minimally affects the pharmacokinetics of ticagrelor in terms of clinical significance [8]. It is mainly metabolized to active metabolite by cytochrome P450 3A4. Ticagrelor and its active metabolite exhibit linear pharmacokinetics over the therapeutic range [9]. No dose adjustment for is required for severe renal impairment or mild hepatic impairment [10,11]. Therefore, the use of ticagrelor is manageable in outpatient setting.

The Government Pharmaceutical Organization (GPO), Thailand had developed a generic product of ticagrelor 90 mg tablets to reduce cost of treatment and improve accessibility to medicines for Thai patients. To ensure that the generic product, TAGRELOR maintains the same quality and safety as the reference product, BRILINTA bioequivalence study was conducted in Thai adult volunteers under fasting conditions.

MATERIALS AND METHODS

Study products

Ticagrelor 90 mg tablets were compared between the test product (TAGRELOR, Lot No. S645076) manufactured by the Government Pharmaceutical Organization, Thailand and the reference product (BRILINTA, Lot No. 60040519) manufactured by AstraZeneca AB, Sweden.

Study subjects

The sample size was computed with the following assumptions: T/R ratio between 95.0% and 105.0%, approximately 18% intra-subject variability and 95% of power with 5% significant level which yielded the sample size of 25 subjects for establishing bioequivalence with adequate power. However, 34 subjects were enrolled in the study accounting for 25% dropouts or withdrawals [12].

Both male and female subjects between 18 and 55 years of age with the Body Mass Index (BMI) between 18.0 and 30.0 kg/m^2 were enrolled in the study. The inclusion criteria also included normal medical history, 12 lead EKG and chest X-ray. In case of female subjects, they were not pregnant or breastfeeding. They also agreed to use acceptable birth control method during the study. Subjects with allergic history to ticagrelor or any excipients in the formulations, positive results for HBsAg, Anti-HCV, Anti-HIV and COVID-19 RT-PCR were excluded. Before start dosing, the subjects were instructed to abstain from alcohol, tobacco, and xanthine products. The subjects were informed about risks and benefits of the studies and gave written informed consent before study participation.

Study design

An open label, randomized, two-way crossover, single oral dose bioequivalence study under fasting conditions was conducted [13]. After an overnight fasting for at least 10 hours, one tablet of either the test or reference product was orally administered to each subject in sitting position with 240 mL of water as per the randomization schedule, followed by mouth and hand check to assess the dosing compliance. At least 7-day washout period was applied between two study periods. Any adverse events were monitored throughout study. The study protocol was reviewed and approved by Institute for the Development of Human Research Protections (IHRP), Department of Medical Sciences, Ministry of Public Health, Thailand (COA No. IHRP2022017). This study conducted following to the ICH GCP, Declaration of Helsinki and the standard operation procedures of International Bio Service Co., Ltd., Golden Jubilee Medical Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.

Blood sampling

Blood samples were collected through an indwelling intravenous cannula placed in a forearm vein of the subjects and transferred to tubes containing K_2EDTA as an anticoagulant. Total 22 blood samples were collected at pre-dose (0 hour) and at 0.5, 1, 1.33, 1.67, 2, 2.33, 2.7, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 34, 48 and 72 hours post-dose from each subject in each period. Each sample was centrifuged to separate plasma and divided into 2 aliquots for respective sample analysis. The plasma samples were stored at -65 ± 10°C until completion of analysis.

Study sample analysis and incurred sample reanalysis

Ticagrelor and ticagrelor-d7 (internal standard) were extracted from plasma samples using liquid-liquid extraction method. Briefly, 200 µL of plasma sample was aliquoted and 50 µL of internal standard solution was added. Then, 3 mL of extraction solution (Methyl-tert-butyl-ether: Hexane (80:20, v/v)) was added. The samples were centrifuged at 4000 ± 100 rcf at 10°C for 5 minutes. The plasma layer was flash frozen and the organic layer was transferred to pre-labeled tube. The content was evaporated at 40°C to dryness and reconstituted with mobile phase (acetonitrile: 0.2% formic acid solution (v/v) (70:30, v/v)). The samples were analyzed using a validated liquid chromatography tandem mass spectrometer (LC-MS/MS) method over the concentration range of 2.057-1495.974 ng/mL following EMA and U.S. FDA guideline on bioanalytical method validation [14,15]. Nexera™ (Shimadzu Corporation, Japan) coupled with TSQ Quantum Ultra; (Thermo Fisher Scientific, USA) was used. The mobile phase was pumped at a flow rate of 0.7 mL/minute through ACE 5 C18 100 × 4.6 mm column. Mass spectrometer was operated in direct flow mode. The transition of precursor to product ion was monitored in positive mode at m/z 523.16 to 133.06 for ticagrelor and m/z 530.20 to 153.07 for ticagrelor-d7. Software Xcalibur™ version 4.0.27.42 and LCquan™ version 3.0.26.0 were used for data acquisition and evaluation of chromatographic data. The samples were analyzed following in-house SOPs and European Medicines Agency guideline on the investigation of bioequivalence [16]. Incurred Sample Reanalysis (ISR) was performed to confirm the reliability of the concentration data. As per EMA guideline on method validation, 130 were selected for reanalysis but the results were not use for pharmacokinetic parameter calculation [14].

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated using non-compartmental model of Phoenix WinNonlin software version 6.4 (Pharsight Corporation, USA), The maximum measured plasma concentration (C_{max}), area under the plasma concentration versus time curve from time zero to the last sampling time point ($AUC_{0\text{-}tlast}$) and area under the concentration versus time curve from time zero to time infinity ($AUC_{0\text{-}\infty}$) were considered as the primary pharmacokinetic parameters, while the time to achieve

C_{max} (t_{max}), elimination rate constant (λ_z) and half-life ($t_{1/2}$) were considered as the secondary parameters.

The statistical analysis was carried out using PROC GLM SAS® version 9.4 (SAS Institute Inc., USA). The analysis of Variance (ANOVA) was used to determine the effects of the formulation, period, and sequence on ln-transformed primary parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max}). ANOVA model included sequence, formulation, and period as fix effects and subject (sequence) as a random effect. Sequence effect was tested using subject (sequence) as an error term. The 90% Confidence Intervals (CIs) for the ratio of geometric least squares mean (test/reference) of ln-transformed primary parameters were calculated. Bioequivalence between two formulations was to be concluded if the 90% confidence interval was within the acceptance range of 80.00%-125.00% for ln-transformed primary pharmacokinetic parameters. Wilcoxon signed-rank test was used to compare median t_{max} of the test and reference products. All statistical calculations were performed at a significance level of 5% (α=0.05).

RESULTS

Demographic characteristics of study subjects

Thirty-four healthy Thai male and female subjects were enrolled and randomly divided into TR and RT group equally. The mean ± SD of age, height, weight and BMI of enrolled study subjects were 37.53 ± 10.94 years, 1.65 ± 0.08 m, 63.14 ± 11.20 kg and 23.22 ± 3.46 kg/m^2, respectively. There were 3 subjects withdrawn due to having high risk of coronavirus infection prior to check-in of period-II. Therefore, there were 31 subjects who completed the study.

Study sample analysis and incurred sample reanalysis

Total 1430 Samples were collected and analyzed in 20 analytical runs. The correlation coefficient of each analytical run constructed from 8 calibration standards was more than 0.99. Four levels of quality control samples were used to demonstrate the precision and accuracy in each analytical run. The inter-run precision and accuracy of quality control samples ranged from 3.7% to 5.6% of the Coefficient of Variation (CV) and 95.7% to 104.1% of the nominal concentrations, respectively. Three samples were reanalyzed due to significant variations in response of internal standard accounting for 0.2% of total analyzed samples. For ISR, 130 samples were selected for reanalysis. There were 129 samples (99.2%) having percent difference between the original and reanalyzed concentrations less than ± 20%. The results indicated that the analytical method was reliable and reproducible.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of healthy Thai subject who completed the study after receiving the test and reference products are summarized in Table 1. The mean plasma concentration-time profiles of ticagrelor are shown in Figure 2. After oral administration, ticagrelor was rapidly absorbed and reached the maximum plasma concentration around 700 ng/mL within the median t_{max} at 2 hours for both test and reference formulations. The terminal half-life of ticagrelor was approximately 9 hours. The ANOVA showed no significant effects of sequence, formulation and period on the ln-transformed primary pharmacokinetic parameters. The 90% confidence intervals for the ratio of geometric least squares mean of primary parameters were within the acceptance range of 80.00%-125.00% (Table 2). Wilcoxon signed-rank test showed no significant difference in median t_{max} between the test and reference products (p-value=0.6741).

Table 1: Pharmacokinetic parameters of ticagrelor for the test and reference products.

Parameter (Unit)	(Mean ± SD, N=31)	
	Test	Reference
$AUC_{0\text{-}tlast}$ (ng.hr/mL)	4632.137 ± 434.625	4497.844 ± 470.623
$AUC_{0\text{-}\infty}$ (ng.hr/mL)	4698.446 ± 447.062	4553.560 ± 484.774
C_{max} (ng/mL)	701.387 ± 211.198	706.747 ± 222.186
Median t_{max} (hr) (Min , Max)	1.67 (1,4.5)	2 (1,3.5)
λ_z (1/hr)	0.085 ± 0.028	0.084 ± 0.022
$t_{1/2}$ (hr)	9.179 ± 3.601	8.980 ± 3.004
Extrapolated AUC (%)	1.473 ± 0.672	1.242 ± 0.461

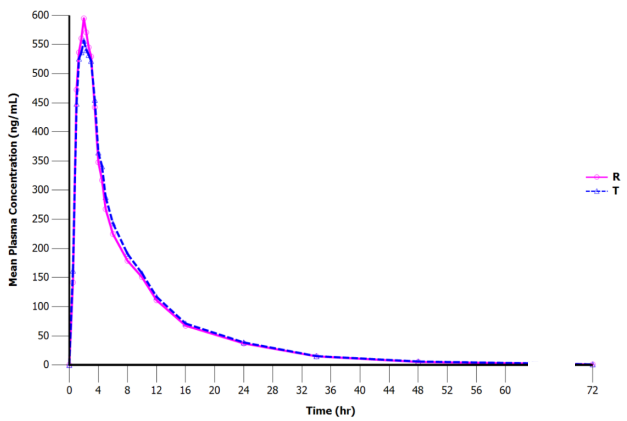

Figure 2: The mean ticagrelor plasma concentration-time profiles of the test and reference products (N=31).

Table 2: Statistical comparison of primary parameters between test and reference products (N=31).

Parameters	Ratio of Geometric least squares mean (90% CI)	Power	Intra-subject CV (%)	ANOVA (p-value)		
				Sequence	Formulation	Period
ln ($AUC_{0\text{-}tlast}$)	103.3 (97.95-108.87)	100.00%	12.3	0.7327	0.3102	0.6755
ln ($AUC_{0\text{-}\infty}$)	103.5 (98.21-109.10)	100.00%	12.2	0.7576	0.2738	0.6957
ln (C_{max})	98.3 (89.71-107.78)	98.90%	21.5	0.9434	0.7577	0.8656

Tolerability

The adverse events were closely monitored. Total 21 adverse events were reported in 16 subjects after receiving a single dose

of ticagrelor (Table 3). There were 11 adverse events reported in 9 subjects who received the test product whereas 10 adverse events were reported in 7 subjects who received the reference product. All reported adverse events were possibly related to the study drug, but the intensity was mild.

Table 3: List of adverse events.

Adverse event	Incidence (N)	
	Test	Reference
Increased alanine transaminase	1	1
Decreased hemoglobin and hematocrit	4	2
Increased lymphocyte	1	1
Increased neutrophil	0	1
Increased platelet count	0	1
Decreased red blood cell count	1	0
Increased serum albumin	0	1
Increased serum creatinine	1	0
Increased white blood cell count	1	0
Increased uric acid	2	3
Total	11	10

DISCUSSION

The study was designed to evaluate the bioequivalence between two ticagrelor 90 mg tablets in healthy Thai volunteers under fasting conditions as no significant food effect has been observed on the pharmacokinetics of ticagrelor. In addition, fasting condition is considered as the most sensitive condition to detect the formulation differences [13,16]. Both male and female subjects were enrolled in this study because the developed product is intended to be used in both genders. The pharmacokinetic parameters of ticagrelor characterized in this study were similar with the results of the study in healthy Chinese subjects. The mean C_{max} ± SD of the reference formulation was 525.82 ± 157.60 ng/mL in Chinese subjects, while that of the reference formulation was 706.747 ± 222.186 ng/mL in Thai volunteers. The C_{max} was attained with in the same range for both populations. However, the $AUC_{0-\infty}$ was slightly higher in Thai population as compared with Chinese population.

With evaluable data from 31 subjects, the bioequivalence was successfully established with the power greater than 90%. The intra-subject variability on the C_{max} observed in this study was comparable with the values used for sample size calculation. Considering the results of ANOVA, no significant effects of sequence, formulation and period on the ln-transformed primary pharmacokinetic parameters. Moreover, the 90% CIs for the ratio of geometric least squares means of $AUC_{0-tlast}$, $AUC_{0-\infty}$ and C_{max} were within the acceptance criteria of 80.00%-125.00%. The hematologic changes observed in this study was not clinically relevant with no further follow-up required. There were no dropouts due to drug intolerance and any serious adverse events reported in this study. Ticagrelor has been reported to be associated with bleeding events and dyspnea which were not observed in this study [4,7]. However, the adverse events upon continuous use should be monitored carefully.

CONCLUSION

The bioequivalence study in healthy Thai volunteers demonstrated that pharmacokinetics describing rate and extent of absorption of TAGRELOR (test formulation) were statistically comparable to BRILINTA (reference formulation). Both treatments were well tolerated by the study subjects. It could be inferred that two tablet formulations were bioequivalent and could be used interchangeably regarding the similarity on the pharmacokinetics and tolerability.

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