Original Article

PHARMACOKINETICS AND BIOEQUIVALENCE STUDIES OF WARFARIN SODIUM 5 MILLIGRAMS TABLET IN HEALTY THAI SUBJECTS

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ABSTRACT

Objective: The present study aimed to evaluate the bioequivalence between the generic warfarin sodium tablet and a reference product when gave as equal labeled doses in healthy Thai subjects under fasting condition.

Methods: A randomized, open-label, single dose, two treatments, two periods, two sequences, crossover design between 5 mg of warfarin administration under fasting condition was conducted in 22 male and female healthy Thai subjects. Each subject was assigned randomly to receive a single oral dose of the test formulation or the reference formulation of 5 mg warfarin tablets. Study periods were separated by a 14-day washout period. Blood samples were collected at 0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 12.0, 24.0, 36.0, 48.0 and 72.0 h after drug administration. A simple, sensitive and specific HPLC method was used for quantification of warfarin in plasma. Pharmacokinetic parameters were analyzed including C_{max}, T_{max}, t1/2 and AUC_{0-72h} .

Results: Twenty subjects, selected randomly from healthy adult Thai subjects were enrolled, age of 22.5 + 3.1 years, weight, 59 + 6 kg. Twenty-one subjects completed both periods of the study. The mean C_{max} values were 759.63 and 778.20 ng/ml and the mean AUC_{0-72h} were 20010.89 and 20418.55 ng. h./ml for test and reference formulations, respectively. The mean ratios for log-transformed data were 0.9955 and 0.9971 for C_{max}, and AUC_{0-72h}, respectively. The 90% confidence intervals of the ratios of C_{max} and AUC_{0-72h} between test and reference tablets were 88.23% – 105.70% and 94.40% – 99.61%.

Conclusion: It can be concluded that test and reference warfarin 5 mg products were bioequivalent in terms of rate and extent of absorption.

Keywords: Pharmacokinetic, Bioequivalence, Warfarin, HPLC, Validation, Human plasma.

INTRODUCTION

Warfarin sodium is a synthetic derivative of 4-hydroxycoumarin. It is an indirect-acting anticoagulant by interfering with the action of reduced vitamin K, which is necessary for the γ-carboxylation of several glutamic acid residues in the precursor protein of these coagulation factors resulting in the synthesis alteration of blood coagulation factor II (prothrombin), factor VII (proconvertin), factor IX and factor X in the liver. Warfarin is used for prophylaxis and treatment of venous thrombosis, pulmonary embolism, thromboembolic complications associated with atrial fibrillation and/or cardiac valve replacement, and as an adjunct in the treatment of coronary occlusion. The drug is also used to reduce the risk of death, re-infarction, and thromboembolic events such as stroke or systemic embolization following myocardial infarction [1-2].

The present study aimed to evaluate the bioequivalence between the generic warfarin sodium tablet and a reference product when gave as equal labeled doses in healthy Thai subjects under fasting condition.

MATERIALS AND METHODS

Study design

Randomized, open-label, single dose, two treatments, two periods, two sequences, crossover design was used with 2 weeks washout period between drug administration under fasting condition.

Clinical study

The clinical study was carried out in accordance with the International Conference on Harmonisation's Good Clinical Practice Guideline and was conducted at the Clinical Trial Unit, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The study protocol and the informed consent was approved by the Ethical Review Committee, Faculty of Pharmacy, Chiang Mai University, Thailand on September 18th 2012.

Twenty two Thai healthy subjects, consisting of males and females equally, calculated based on the intra-subject pharmacokinetics variations of 15% [3] were enrolled. They were included after passing clinical screening procedures including a physical examination and clinical laboratory tests. Subjects with the following conditions were excluded: allergic history to warfarin or the components of the investigations medication, history of alcohol drink or substance abuse or smoking, positive results to HIV and/or hepatitis clinical laboratory tests, taken any medications especially enzyme modifying drugs within 14 days before study and history of blood loss such as from surgery. Subjects who met the above criteria were eligible for participation in the study after voluntarily providing written informed consent.

Study drug administration

All subjects were admitted to the medical ward, Clinical Trial Unit, before 9.00 pm the night before the date of study and fasted overnight for at least 10 h. About 8.00 am of the study day, each subject was assigned randomly to receive a single oral dose of the test formulation or the reference formulation of warfarin tablet 5 mg with 240 mL of water. Subjects were not allowed to recline until 2 h after ingestion of the drug product and might resume normal activity thereafter. The strenuous activity was prohibited during the confinement periods. A standardized meal was served at 4 and 10 h after dosing. During 12 h post dose, subjects were confined in a medical ward of Clinical Trial Unit. Study periods were separated by a 14-day washout period.

Sample collection and processing

Blood samples were collected by direct venipuncture or from an indwelling cannula, which was placed in an arm vein of the subject. Eight mL of blood samples were taken at 0.0 (pre-dose), 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 12.0, 24.0, 36.0, 48.0 and 72.0 h after drug administration and transferred to pre-labeled 10

ml Vacutainer® containing heparin sodium as anticoagulant. They were immediately transferred to the analytical facility at Pharmacy Service Center, Faculty of Pharmacy, Chiang Mai University under controlled temperature of 0+ 5 °C, where plasma samples were promptly separated and stored at -30 + 5 °C until analyzed. Analysis of plasma warfarin concentration.

Twenty five µL of 30.0 µg/ml ofloxacin (internal standard) solution and 1.0 mL of acetonitrile were added to the plasma sample. The mixture was mixed at 2000 rpm for 5 minutes followed by centrifugation for 10 minutes at 10900 rpm. Three hundred µL of supernatant liquid were transferred into a vial and then 700 µl of the mobile phase were added. An aliquot of 80 µl was injected into the HPLC column. The HPLC system (Shimadzu, Kyoto, Japan) consisted of a model LC–10ATvp pump, a model DGU-14A degasser, a model SIL–10ADvp autoinjector, a model CTO-10AS vp column oven and a model RF-10AXL Spectrofluorometric detector. Data were acquired and processed with Shimadzu software (Class-VP version 6.14). Separation was achieved on Mightysil RP-18 GP column (150 mm x 4.6 mm i. d., 5 µm) along with a Mightysil RP-18 GP guard column (5 mm x 4.6 mm i. d., 5 µm). A mobile phase consisting of methanol and 50 mM phosphate buffer pH 7.0 (40:60 v/v) was filtered through 0.2 µm cellulose acetate membrane filter and sonicated before use. Flow rate was 1.1 ml/min and injection volume was 80 µl. The column temperature was controlled at 30 °C. The analyte and internal standard were detected and quantitated with a fluorometer. The excitation and emission wavelengths of fluorescence detector were set at 313 and 400 nm, respectively. The analytical method for determination of warfarin in human plasma by HPLC was validated according to EMA and US FDA guidances.[4-8]

Pharmacokinetic parameters and statistical analysis

Pharmacokinetic parameters were analyzed including C_{max}, T_{max}, AUC_{0-72h} (Truncated AUC)[9-10], k_e and $t_{1/2}$. C_{max} and T_{max} were obtained from the actual data. AUC_{0-72h} was calculated by the linear trapezoidal rule. 90% confidence intervals of C_{max}, and AUC_{0-72h} were calculated based on log-transformed data and the drug products were consider bioequivalence if both values were within 0.8000 – 1.2500. The pharmacokinetic parameters, 90% confidence intervals, ANOVA test for sequence, period, subject (sequence), and treatment effects, power of test and intra-subject variation (%CV) were analysed by using Phoenix™ Winnonlin® 6.3 computer software.

RESULTS

Validation of HPLC assay

A simple, sensitive and specific high-performance liquid chromatography (HPLC) method was developed and validated for quantification of warfarin in human plasma. Sufficient separation of warfarin peak and ofloxacin peak from other interferences was observed. The calibration curve for the analyte was linear in the range of 42 – 1263 ng/ml with coefficient of determination ≥ 0.99. The lower limit of quantification (LLOQ) for warfarin was 42 ng/ml. The within-run and between -run accuracy and precision were within the acceptable ranges. Warfarin was stable in plasma at least 5 hours at room temperature, and at least 60 days at -30 ± 5 °C. The hemolysed plasma and hyperlipidemic plasma had no significant effect on the accuracy and precision of the analysis. Results obtained from dilution integrity and partial volume analysis demonstrated that the analytical method was reliable and reproducible. Slight variations in mobile phase composition and switching columns did not significantly affect the analysis results. The method has been found to be highly precise, accurate, robust and suitable for the determination of warfarin in plasma samples from the bioequivalence study of warfarin tablets.

Demographic data

Twenty-two subjects, selected randomly from healthy adult Thai subjects were enrolled as planned number of subjects in approved protocol with mean age of 22.5 + 3.1 years (range, 19–30 years); weight, 59 + 6 kg (range, 43–70 kg); and height, 169 + 7 cm (range, 154–185 cm). Twenty-one subjects completed both periods of the study. One female subject withdrew for personal reason without having any adverse event. There were 5 adverse events reported in 5 subjects including upper respiratory tract infection, rhinorrhea and sore throat. All of them were not serious adverse events. The adverse events were not related to the study drug products.

Pharmacokinetic characteristics

Average plasma warfarin concentration–time curve of test and reference formulations is presented as fig. 1. Parameters include C_{max}, AUC_{0-72h}, AUC_{0-inf}, T_{max}, $T_{1/2}$ and K_e are collated in table 1. The mean (range) C_{max} values was 759.63 (496.59 -1127.27) ng/ml and 778.20 (561.58-978.29) ng/ml and the mean AUC_{0-72h} were 20,010.89 (16,210.96-27,052.50) ng. h./ml and 20,418.55 (16,185.31-25,002.45) ng. h./ml for test and reference formulations, respectively. The mean AUC_{0-inf} values for test and reference formulations were 29,378.94 (21,509.31 - 44,153.52) ng. h./ml and 29,343.61 (20,751.01-41,811.93) ng. h./ml, respectively. The median (range) T_{max} value for the test formulation was 0.50 (0.25-4.00) h and from the reference formulation was 0.75 (0.25-3.00) hour, it was not significantly difference between two formulation (p = 0.981; Wilcoxon Signed Ranks Test), The average elimination plasma constant (K_e) value for the test (0.0164 h^{-1}) and reference (0.0173 h^{-1}) products were only slightly different, and as a result, the average $T_{1/2}$ values of both products were similar (44.00 h for test product and 41.67 h for reference product).

The mean ratios for log-transformed data were 0.9955, 0.9971 and 0.9994 for C_{max} and AUC_{0-72h}, respectively. Values close to 1 indicate the similarity of both products in terms of pharmacokinetic parameters.
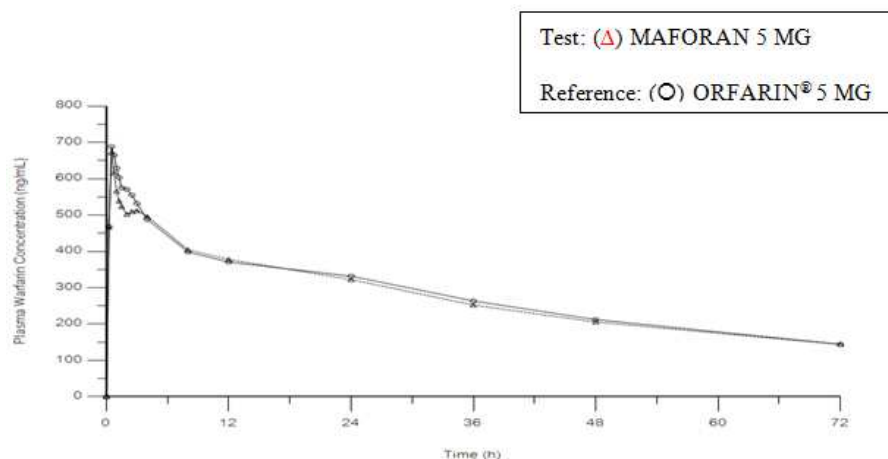

Fig. 1: Average plasma warfarin concentrations at various sampling times of all subjects (n=21) after taking a single oral dose of test (Δ) and reference (O) tablets

Table 1: Pharmacokinetic parameters of warfarin after administration of test and reference formulations to healthy Thai subjects

Pharmacokinetic parameter	Test formulation		Reference formulation	
	Arithmetic	Geometric	Arithmetic	Geometric
$AUC_{0 \to t}$ (ng·h/mL)	20010.89 + 3093.65	19801.34	20418.55 + 2547.41	20267.96
$AUC_{0 \to inf}$ (ng·h/mL)	29378.94 + 6050.31	28825.07	29342.61 + 5402.13	28872.88
C_{max} (ng/mL)	759.63 + 170.00	742.19	778.20 + 119.48	769.39
T_{max} (h)	1.05 + 1.19		0.95 + 0.70	
$T_{1/2}$ (h)	44.00 + 8.76		41.67 + 8.06	
k_e (h^{-1})	0.0164 + 0.0037		0.0173 + 0.0034	

Table 2: Ratio of log-transformed least square mean and 90% confidence interval of pharmacokinetic parameters used in bioequivalence evaluation (n =21)

Parameter	Ratio of least square mean (%)	90% confidence interval
C_{max} * (ng/ml)	96.57	88.23 - 105.70
AUC_{0-72h} * (ng. h.r./ml)	96.97	94.40 - 99.61

* Ln data transformation

Bioequivalence analysis

The geometric means of C_{max} were 742.19 ng/ml and 769.39 ng/ml for test and reference formulations. The geometric means of AUC_{0-72h} were 19,801.34 and 20,267.96 ng. h./ml and of AUC_{0-inf} were 28825.07 and 28872.88 ng. h./ml test and reference tablets, respectively. The 90% confidence intervals of the ratios of C_{max} and AUC_{0-72h} between test and reference tablets were 88.23% – 105.70% and 94.40% – 99.61%, as shown in table 2. The _post-hoc_ power of test for C_{max} and AUC_{0-72h} between two formulations were 99.01%, and 100.00%. The values above 80% indicated a sufficient number of subjects were included into the study. The intra-subject coefficients of variation of C_{max} and AUC_{0-72h} were 17.03% and 5.03%.

The sequence, inter-individual, period and formulation effects between test and reference tablets assessed by ANOVA were not significantly different for C_{max} at a significance level of 5% ($p < 0.05$). The sequence and formulation effects between test and reference tablets were also not significantly different for AUC_{0-72h}. However, the inter-individual variability effects (subject effect) and period effect were statistically significant different at a significance level of 5% ($p < 0.05$) for AUC_{0-72h}.

DISCUSSION

The mean pharmacokinetic parameters obtained from this study were in the similar ranges of those reported by Yacobi and coworkers (C_{max}: 607 ng/ml, AUC_{0-inf}: 26300 ng. h./ml and T_{max}: 0.67 hour for reference formulation). The $T_{1/2}$ obtained from this study was located in the range obtained from the literature which was 20-60 hours.[1,1][1-1]2[])The data showed very low intra-subject variation in the extent of warfarin absorption which was similar to those reported by Yacobi and colleagues [3].

The significant difference in subject effect might be due to many factors such as genetic variation [11-13] that influences to pharmacokinetic of the drug at difference levels in each subject. The significant period effect indicates that the study conditions might have changed from period to period and the analysis does adjust for this in estimating the treatment effects. The exact factors that influence to the significant difference in period effect in this study could not be clearly identified.

CONCLUSION

The pharmacokinetic parameters of both test and reference warfarin 5 MG tablets in Thai healthy subjects were determined. 90% confidence intervals of the log of the ratio of C_{max} (88.23% – 105.70%) and AUC_{0-72h} (94.40% – 99.61%) between test and reference tablets were within the equivalence criteria of 80.00% - 125.00%. The powers of test for C_{max} (99.01%) and AUC_{0-72h} (100.00%) were found to be greater than 80%. Therefore, it can be concluded that they are bioequivalent in terms of rate and extent of absorption [14].

ABBREVIATION

AUC_{0-72h} = Area under the plasma concentration versus time curve up to the last sampling time at 72 h, AUC_{0-inf} = Area under the plasma concentration versus time curve with the concentration at 72 h extrapolated based on the elimination rate constant (Ke), C_{last} = Last plasma concentration, C_{max} = Maximum observed plasma concentration, %CV = Intra-subject variation, HPLC = High performance liquid chromatography, IS = Internal standard, LLOQ = Lower limit of quantification, Ke = Elimination rate constant, T_{max} = Time to C_{max}, $T_{1/2}$ = Elimination half life.

CONFLICT OF INTERESTS

Declared None

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