PHARMACOKINETICS AND THE BIOEQUIVALENCE OF ORAL METHIMAZOLE IN HEALTHY THAI VOLUNTEERS, DETERMINED BY THE SENSITIVE ANALYTICAL METHOD, LC-MS/MS

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Abstract— The pharmacokinetic parameters and bioequivalence of test and reference methimazole products were determined. A randomized, open-label, single-dose, two-treatment, two-period, two-sequence, crossover design between the administration of 5 mg methimazole tablets with a one-week wash-out period was conducted on 22 healthy Thai volunteers. Each volunteer was assigned to receive a single dose of the test or reference product. At 0.0, 10, 20, 30 and 45 min, and then 1, 1.33, 1.67, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h after ingesting the pharmaceutical product, blood samples were collected. The plasma methimazole concentrations were analyzed by the validated LC-MS/MS. The Cmax, Tmax, T1/2, AUC0-48h, and AUC0-inf parameters were determined, and 90% confidence intervals were calculated based on the log-transformed data. The mean Cmax, AUC0-48h, AUC0-inf and T1/2 were 157.79 and 163.16 ng/ml, 1058.55 and 1040.52 ng.h/ml, 1160.16 and 1123.15 ng. h./ml and 6.29 and 6.01 h for the test and reference formulations, respectively. The mean ratios for the log-transformed data were -0.0261, 0.0234 and 0.0402 for Cmax, and AUC0-48h and AUC0-inf, respectively. In terms of the rate and extent of the absorption, the test and reference methimazole 5 mg tablets were bioequivalent.

Index Terms—Bioequivalence, LC-MS/MS, Methimazole, Pharmacokinetic,

I. INTRODUCTION

Methimazole (1-methyl-2-thioimidatozole) is an anti-thyroid agent which possesses inhibitory thyroid hormone synthesis activity. Its indicated use is in the treatment of hyperthyroidism and to improve hyperthyroidism in preparation for surgical treatment or radioactive therapy. Compared with propylthiouracil, another antithyroid medication, methimazole is at least 10 times more potent. The oral bioavailability of methimazole is 80-90%, it is rapidly absorbed from the gastrointestinal tract with peak plasma concentration within approximately 1 h after oral administration. It can pass through the placenta and pass into the milk in concentrations approximately equal to maternal blood. The protein binding is 0%. Methimazole has elimination half-life ranges from 5-13 h, approximately 12% is excreted in the urine within 24 h [1].

Although methimazole has been available on the market since the 1950s [2], this drug has been very important in the management of hyperthyroidism [3]. A range of generic methimazole products is worldwide available, although the study of bioequivalence is absent. As generic medical products play obvious and important roles in the Thai healthcare system, the study of bioequivalence could be an important tool to evaluate the similarity of efficacy and safety in two medical formulations.

The present study is the first bioequivalence of methimazole 5 mg tablets in healthy volunteers Thai in fasting conditions, and analyzed by the validated LC-MS/MS technique.

II. MATERIALS AND METHODS

A. Study design

This study was an open-label, randomized, single-dose, two-treatment, two-period, two-sequence, crossover design with a one-week wash-out period between the administration of the drugs in fasting conditions.

B. Clinical study

The clinical part of the present study was carried out in the Clinical Trial Unit, the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, and complied with the International Conference on Harmonisation's Good Clinical Practice Guidelines. The study protocol was accepted by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand on 22/01/2014.

Twenty-two healthy Thai male and female volunteers were equally enrolled, and calculated based on the intra-subject pharmacokinetics variations of approximately 20%. The drug administration sequences for each volunteer were randomly assigned by using a computer program from www.randomization.com. The inclusion criteria were BMI 18.5-25.0 kg/m^2, an age of between 18-40 years, healthy volunteers based on their medical history, a physical examination, the clinical laboratory test results being within the normal range or not clinically

significantly abnormal, being hepatitis B negative, being a non-smoker or a non-pregnant female, being unable to have children, being committed to using acceptable non-hormonal contraceptives in potentially childbearing female volunteers, and being willing to comply with the study's procedures and restriction. Volunteers with the following characteristics were excluded: those with a history of allergy to methimazole or the components of the investigation's medication, currently drinking or with a history of drinking alcohol, any substance abuse or smoking, taking any medications, especially enzyme modifying drugs within 14 days, or the consumption of oranges, pomelos or grapefruit within 7 days, or ingesting caffeine-containing beverages or food within 3 days before the study. Any volunteer who had a history of blood loss such as from surgery or blood donation within 3 months was also excluded. All the volunteers were briefed on the bioequivalence study's details, then the volunteers who met the above criteria were eligible for participation after voluntarily providing their written informed consent.

C. Study drug administration

All the volunteers were asked to fast and stay at the Clinical Trial Unit during the night before the date of the medical product administration for at least 10 h. On the administration day, each volunteer received a single oral dose of the test (TIMAZOL®, Sriprasit Pharma Co. Ltd., THAILAND) or the reference (TAPAZOLE®, OLIC(THAILAND) LTD) pharmaceutical product of one 5 mg methimazole tablet with 240 ml of water, according to the randomized plan. One hour before until one hour after administering medication the volunteers were not allowed to drink water. The volunteers were not allowed to lie down until 2 h after administering the medical product, after which they might resume normal activity. A standardized meal for lunch and dinner were served 4 and 10 h after the dose. For 12 h post-dose, the volunteers stayed in the medical ward of the Clinical Trial Unit. The study periods were separated by a 7-day wash-out period.

D. Sample collection and processing

Ten ml of blood was sampled at 0.0 (pre-dose), 10, 20, 30 and 45 min, and then 1, 1.33, 1.67, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h after medical ingestion, and transferred to a pre-labeled 10 ml tube coated with K2EDTA. Then it was transported to the Pharmacy Service Center, the Faculty of Pharmacy, Chiang Mai University at a controlled temperature, where the plasma samples were immediately separated and stored at -30 + 5 °C until being analyzed.

E. Analysis of the plasma methimazole concentration

The methimazole was extracted from the plasma by using the protein precipitation technique. Twenty-five µL of 132 µg/ml propylthiouracil, used as an internal standard, and 2.5 µL 20%v/v 2-propanol in dichloromethane were added to 225 µL of the plasma sample. The mixture was mixed, followed by centrifugation for 1 min at 4000 rpm. One hundred µL of the supernatant organic phase was transferred to another test tube and evaporated to a dry state at a reduced pressure. The dried residue was re-dissolved with 200 µL of the mobile phase. An aliquot of 5 µL was injected into the analytical column.

The LC-MS/MS analysis was conducted using a HPLC Agilent 1260 system consisting of a model 1260 Bind solvent delivery unit, a 1260 Hip on-line degasser, a 1260 Hip ALS auto injector and a 1260 TCC column oven in combination (Agilant, United States) with an API 3200 Mass spectrometer (AB Sciex, Singapore). A Hypurity C4 column (150 mm x 4.6 mm i. d., 5 µm) along with a Hypurity C4 guard column (10 mm x 4.0 mm i. d., 5 µm) was used for separation with the isocratic mobile phase, consisting of methanol and 2 mM of ammonium acetate (22:78 v/v) flowing at 0.8 ml/min. The column temperature was kept at 35 °C. The data were acquired and processed with Analyst software (Version 1.6.2).

The mass spectrometer was operated in multiple reaction monitoring mode and equipped with an electrospray ionization source. Quantification was achieved with MS/MS detection in a positive ion mode. The methimazole and internal standard were monitored at transition m/z 114.991→87.841 and 171.095→111.094, respectively. The ion spray voltage was set at 5000 V. The source parameters, i.e. the nebulizer gas, auxiliary gas, curtain gas and collision gas, were set at 60, 55, 30 and 8 psi, respectively. The compound parameter, i.e. the declustering potential, entrance potential, collision cell entrance potential, collision energy and collision cell exit potential, were 46, 8, 10, 23 and 4 V for methimazole, and 51, 8.5, 10, 29 and 4 V for prophythiouracil.

The analytical method for determining methimazole in human plasma by LC-MS/MS was validated according to the EMA (2011) [4] and US FDA (2001) [5] guidelines. The calibration curve shows a linearity in the range of 5-300 ng/ml with $r^2 >$ 0.99. The lower limit of quantification was 5 ng/ml, and there was accuracy and precision.

F. Pharmacokinetic parameters and statistical analysis

The pharmacokinetic parameters were analyzed including Cmax, Tmax, AUC0-inf, AUC0-48h [5]-[7], Ke and T1/2. Cmax and Tmax obtained from the actual data. AUC0-48h was calculated by the linear trapezoidal rule, AUC0-inf was estimated from AUC0-48h. 90% confidence intervals of Cmax, AUC0-inf and AUC0-48h were calculated based on the log-transformed data, and the pharmaceutical products were considered to be bioequivalent if both values were within 0.8000-1.2500. The

pharmacokinetic parameters, 90% confidence intervals, an ANOVA test for the sequence, period, subject (sequence) and treatment effects, the power of the test and intra-subject variation (%CV) were analyzed by using Phoenix™ Winnonlin® 6.3 computer software.

III. RESULTS

G. Demographic data

A total of 22 Thai volunteers with a mean age of 25.3 ± 3.8 years (range, 20-35 years); weight, 59.4 ± 9.0 kg (range, 45.0-79.7 kg); height, 165.5 ± 9.5 cm (range, 149-187 cm); and BMI 21.56 ± 1.80 kg/m^2 (range, 18.5-24.8 kg/m^2) were enrolled.

H. Pharmacokinetic characteristics

Table 1 shows a summary of the pharmacokinetics parameters of the methimazole, including Cmax, AUC0-48h, AUC0-inf, Tmax, T1/2 and Ke. The mean (range) Cmax values were 157.79 (83.14-234.60) ng/ml and 163.16 (88.69 - 259.44) ng/ml, and the mean AUC0-48h was 1058.55 (757.18-1546.54) ng. h/ml and 1040.52 (682.38-1454.39) ng. h/ml for the test and reference products, respectively. The mean AUC0-inf values for the test and reference products were 1160.16 (869.18-1604.51) ng. h/ml and 1123.15 (747.58-1518.69) ng.h/ml, respectively. The median (range) Tmax value for the test product was 0.50 (0.33-2.00) h, and from the reference product it was 0.50 (0.17-1.00) h, which was not significantly different between the two formulation ($p = 0.081$; Wilcoxon Signed Ranks Test), The average elimination plasma constant (Ke) value for the test (0.1133 h^{-1}) and reference (0.1193 h^{-1}) products were only slightly different. The average T1/2 values of both products were similar, 6.29 h for the test product and 6.01 h for the reference product. The concentration–time curve of the test and reference products is presented in Fig. 1.

The mean ratios for the log-transformed data of Cmax, AUC0-48h and AUC0-inf were close to zero: -0.0261, 0.0234 and 0.0402 for Cmax, AUC0-48h and AUC0-inf, respectively.

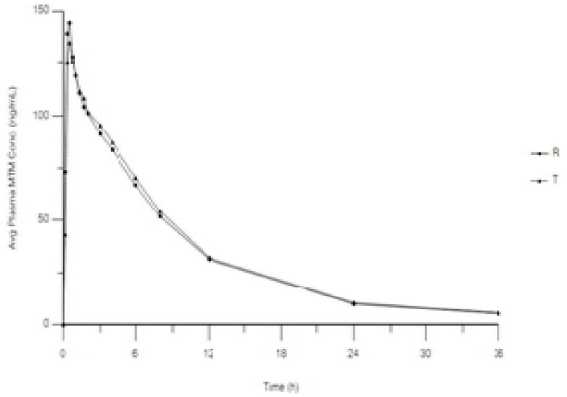


Fig. 1: Average plasma methimazole concentrations after the administration of a single oral dose of test (TIMAZOL®) 5 MG (-Δ-) (n = 22) and reference (TAPAZOLE®) 5 MG (-●-) (n = 22) tablets.

Table 1: Pharmacokinetic parameters of methimazole after the administration of the test and reference 5 mg tablets products to healthy Thai volunteers.

Parameter	Test product (n = 22)		Reference product (n = 22)	
	Arithmetic	Geometric	Arithmetic	Geometric
C_{max} (ng/mL)	157.79±43.48	151.61	163.16±50.59	155.62
AUC_{0-48h} (ng•h/mL)	1058.55±192.59	1042.24	1040.52±222.21	1018.15
AUC_{0-inf} (ng•h/mL)	1160.16±183.23	1146.64	1123.15±226.92	1101.47
$T_{1/2}$ (h)	6.29±1.44	6.12	6.01±1.52	5.81
T_{max} (h)	0.62±0.41	0.53	0.48±0.22	0.44
ke (h^{-1})	0.1169±0.0312	0.1133	0.1238±0.0371	0.1193

A significant difference in the subject nested within the sequence effect was found. The significant difference ($p < 0.05$) in the period effect was observed in both AUC0-48h and AUC0-inf. In addition, a significant effect for the treatment was found in AUC0-inf.

Adverse events were monitored during and until the end of 2 weeks after the medication dosing. One adverse event in one volunteer was found. This adverse event was mild in intensity, was not related to the study drug and was resolved spontaneously. No serious adverse events were reported.

IV. DISCUSSION

The mean pharmacokinetic parameters obtained from this study were within the similar ranges of those reported, including Cmax, AUC0-48h [2], Ke, T1/2 [8]-[10]. Nevertheless, the T1/2 observed in the present study was located in the wide range obtained from the literature, which was 5-13 h [1]. Okamura Y [2] and Skellern GG [9] reported 2.7 and 2.96 h of T1/2, respectively. This quite large difference in the value of T1/2 may have been due to the small size of these pharmacokinetic studies (ranging from 2-6 volunteers in each subgroup or study). However, in the present study, the sample size were 22 volunteers, which was calculated based on a previous study.

The significant difference ($p < 0.05$) in the subject nested within the sequence effect was found to be as usual, which might have been due to many factors such as genetic variation that influences the pharmacokinetics of the drug at different levels in each subject. A significant difference ($p < 0.05$) in the period effect was found for both AUC0-48h and AUC0-inf. The two main causes for the significant period effect are an equal carry-over effect and a true period effect. The former is obtained when the duration of the wash-out period is not sufficiently

long, and the drug levels could be detected in the pre-dose samples of the second period of the cross-over study for both formulations. A true period effect derives from a difference in the status of the subjects and/or changes in the environmental conditions that affect the bioavailability of the formulations. After all the chromatograms of the pre-dose samples in the second period had been carefully investigated, not even a single peak of the drug in the chromatogram was observed, indicating there was no carry-over effect found in the study. Thus, the significant period effect indicates that factors beyond the regular restriction in the bioequivalence study, as described in the protocol, such as the physiological status of the subjects or environmental conditions might have changed from period to period and affected the bioavailability of the formulations. Theoretically, because both the test and reference are affected in an equal way by the change in environmental/physiological conditions, their relationship (i.e. the possible differences between them) is not modified and the bioequivalence assessment is still valid. A significant effect for treatment was found in AUC0-inf. This can be present when the treatment mean square is small. In other words, the ANOVA procedure carried out is nothing, but the evaluation is identical to the power approach, so it can be said that a significant difference can occur at the moment the variability is low or the number of subjects is sufficiently high.

CONCLUSION

In the present study, 5 mg methimazole of two formulation, test and reference, met the regulatory criteria for bioequivalence based on the rate and extent of absorption. Both formulations were tolerated.

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