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Comparative Bioequivalence Studies of Pantoprazole 40 mg Delayed-Release Tablet Formulations in Healthy Thai Volunteers

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Abstract

Pantoprazole is a H^+,K^+-ATPase enzyme inhibitor for the treatment of acid-related gastrointestinal diseases. The Government Pharmaceutical Organization (GPO), Thailand had developed Pantoprazole GPO® (pantoprazole 40 mg delayed-release tablets) as a generic substitute for the corresponding innovator product, CONTROLOC® 40 mg. Two separate single-dose studies were conducted under fasting and fed conditions to demonstrate bioequivalence for the delayed-release dosage forms as per the regulatory requirements. A randomized-sequence, open-label, 2-period crossover design was used for the single-dose fasting study while a randomized-sequence, open-label, 4-period crossover fully replicate design was used for the single-dose fed study. In both studies, plasma samples were collected over a period of 36 hours and analyzed using a validated liquid chromatography tandem mass spectrometry method. The studies demonstrated the effect of food in the delay of pantoprazole absorption. However, bioequivalence was successfully established under both fasting and fed conditions. The 90% confidence intervals of geometric least squares mean ratio (test/reference) for log-transformed AUC_{0-36h}, $AUC_{0-\infty}$ and C_{max} observed in the fasting study were 95.68-106.21%, 95.52-105.93% and 97.97-122.28%, respectively. Similarly, the values observed in the fed study were also within 80.00-125.00% of bioequivalence criteria (92.35-104.44% for AUC_{0-36h}, 91.89-100.76% for $AUC_{0-\infty}$, and 85.00-105.87% for C_{max}). Both treatments were well tolerated, and no serious adverse events were reported. It was concluded that two pantoprazole 40 mg tablet formulations were bioequivalent based on insignificant difference in terms of rate and extent of absorption describing by peak drug concentration (C_{max}) and both area under concentration-time curves (AUC_{0-36h} and $AUC_{0-\infty}$).

Keywords: Pantoprazole; Bioequivalence; Pharmacokinetics; LC-MS/MS

Introduction

Pantoprazole is a benzimidazole derivative inhibiting acid secretion in the stomach. It is activated in the acidic environment of the parietal cells, and the active sulfenamide metabolite exerts antisecretory activity by inhibiting H^+,K^+-ATPase enzyme [1]. Oral 40-80 mg of pantoprazole daily is recommended for the treatment of moderate and severe reflux oesophagitis, gastric ulcer, duodenal ulcer, Zollinger-Ellison-Syndrome and other pathological hyper secretory conditions. In addition, it is also used in a combination with antibiotics to eradicate *Helicobacter pylori* infection [2].

Since pantoprazole is acid-labile, the delivery system should be gastro-resistant to prevent pantoprazole from degradation in the stomach [3]. Pantoprazole exhibits linear pharmacokinetics over the dose range of 10-80 mg. After oral administration at 40 mg, maximum concentration (C_{max}) of pantoprazole was attained at 2-3 hours with 77% absolute bioavailability [4]. Administration of pantoprazole with food could delay time to achieve C_{max} (t_{max}) by 3-4 hours and decrease the extent of systemic exposure [5]. Pantoprazole is highly bound to serum proteins (98%) while having low apparent volume of distribution of 0.15 L/kg. A total clearance of pantoprazole is about 0.15 L/kg with a terminal half-life ($t_{1/2}$) of 1 hour [4]. Mainly, pantoprazole is metabolized in the liver and the metabolite is subsequently excreted in urine. Pharmacokinetics of pantoprazole is altered in severe liver cirrhosis patients whereas it is comparable between healthy subjects and renally impaired patients [6,7].

The Government Pharmaceutical Organization (GPO), Thailand had developed Pantoprazole GPO® (pantoprazole 40 mg delayed-release tablets) as a generic substitute for the corresponding innovator product, CONTROLOC® 40 mg. Two separate single-dose

studies were conducted under fasting and fed conditions to demonstrate bioequivalence for the delayed release dosage forms as per the regulatory requirements [8,9]. The purposes of these studies were to compare pharmacokinetic parameters describing the rate and extent of absorption of the test and reference formulations, and to evaluate the tolerability of the formulations in healthy Thai subjects.

Materials and Methods

Study products

Pantoprazole GPO®, pantoprazole 40 mg delayed-release tablets (Lot No. S620041) manufactured by GPO, Thailand were used as the test product and CONTROLOC®, pantoprazole 40 mg gastro-resistant tablets (Lot No. 443226) manufactured by Takeda GmbH, Germany were used as the reference product.

Methods

Sample size calculation was based on probability of greater than 90% for concluding bioequivalence within the acceptance bioequivalence limits of 80.00-125.00% at a significant level of 5% [10]. In-house data on the maximum intra-subject variability for the primary pharmacokinetic parameter, C_{max} of pantoprazole was found to be around 24% and the expected T/R ratio was 95% for the single-dose fasting study which yielded a sample size of 34 subjects. However, 42 healthy Thai subjects were enrolled in the fasting study considering 20% dropout and withdrawal rate. Compared with the single-dose fasting study, higher intra-subject variability was anticipated (approximately 40%), thus a replicate design has been suggested for the single-dose fed study [11]. A fully replicate crossover design was selected since fewer number of subjects are required compared to 2-period crossover design or 3-period partial replicate design. In addition, this design allows estimation of intra-subject variability for each formulation and the individual response can be estimated more precisely [12,13]. The sample size for establishing bioequivalence at T/R ratio of 110%, significant level 5% and power ≥ 90% was 39 subjects [12]. With regards to higher expected dropout rate (approximately 30%) due to multiple periods of the study, 52 healthy Thai subjects were enrolled in the single-dose fed study.

Subjects

The age and body mass index of subjects were within the range of 18-55 years and 18-30 kg/m^2, respectively. All subjects had acceptable medical history, physical examination results and clinical laboratory measurements prior to study initiation. Female subjects were not pregnant or breastfeeding throughout the study. The subjects had no history of hypersensitivity to pantoprazole or any excipients, allergy to other medications, alcohol dependence, drug abuse, recent blood donation, and recent clinical drug research participation. They were instructed to abstain from smoking and taking any medications prior to dosing and during the entire study. Consumption of any grapefruit, pomelo or orange-based products, and xanthine containing products were restricted at least 24-48 hours prior to dosing and throughout the study. All subjects provided the written informed consent before study participation at International Bio Service Co., Ltd., Golden Jubilee Medical Center, Mahidol University, Thailand.

Study design

The bioequivalence studies were conducted as per the protocol, ICH 'Guidance on Good Clinical Practice', Declaration of Helsinki, and the standard operation procedures (SOPs) of International Bio Service Co., Ltd., Golden Jubilee Medical Center, Mahidol University, Thailand. The clinical study protocols were approved by the Institute for the Development of Human Research Protection (IHRP), Department of Medical Sciences, Ministry of Public Health, Thailand. A randomized-sequence, open-label, 2-period crossover design was used for the single-dose fasting study while a randomized-sequence, open-label, 4-period crossover fully replicate design was used for the single-dose fed study. All enrolled subjects were admitted to the clinical facility one day prior to study initiation. The test or reference product was given as per the randomization schedule.

In the single-dose fasting study, 42 subjects were enrolled and randomly divided into two groups, test-reference (TR) and reference-test (RT) (Table 1). The investigational product was administered after at least 10-hour fasting in each period. In contrast, 52 subjects were enrolled and randomly divided into TRTR and RTRT groups in the single-dose fed study. The subjects in TRTR group received the test product in period I and III, and switched to the reference product in period II and IV. The dosing sequence in RTRT group was done in a reverse order (Table 2). Each subject had a high fat and high calorie breakfast within 30 minutes before dosing in each period. The activities of each subject were standardized in both studies including administration of drug with 240-mL water in sitting posture, food restriction for 4 hours post-dose, and water intake restriction for an hour pre- and post-dose. The washout period between the study periods was 7 days for both studies. Physical and clinical laboratory examinations were performed periodically to evaluate tolerability and to ensure welfare of study subjects. The subjects were monitored for any adverse events or complaints throughout the study.

Group	Period I	Period II
1	Test (T)	Reference (R)
2	Reference (R)	Test (T)

Table 1: Dosing sequences of the single-dose fasting study

Group	Period I	Period II	Period III	Period IV
1	Test (T)	Reference (R)	Test (T)	Reference (R)
2	Reference (R)	Test (T)	Reference (R)	Test (T)

Table 2: Dosing sequences of the single-dose fed study

Blood sampling

Approximate 3 mL of each blood sample was drawn into heparinized tube using syringe through an indwelling intravenous cannula placed in the forearm vein of the subjects. Total 23 blood samples were collected from each subject at pre-dose (0 hour), 0.5, 1, 1.33, 1.67, 2, 2.23, 2.67, 3, 3.33, 3.67, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, 24 and 36 hours post-dose in each period of the fasting study. The sampling time points were adjusted to capture the delayed t_{max} in the fed study. Therefore, total 26 blood samples were collected from each subject at pre-dose (0 hour), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 20, 24, 30 and 36 hours post-dose in each period of the fed study. After sample collection, the blood samples were centrifuged at 3,000±100 relative centrifugal force (rcf) for 5 minutes at below 10 °C to obtain plasma for pantoprazole assay. Each plasma sample was separated into two aliquots, and subsequently stored upright in a freezer maintained below -50 °C until analysis.

Chemicals and reagents

Pantoprazole sodium, reference standard bearing Lot No. R022R0, 93.8% purity was procured from USP (Rockville, MD). Pantoprazole D6, internal standard bearing Lot No. CS-PO-389, 97.8% purity was procured from Clearsynth Labs Ltd. (Mumbai, India). All solvents used for sample analysis were HPLC grade. Only ultrapure water (in-house) was used in all experiments. The reagents used for sample preparation were analytical grade.

Sample analysis and incurred sample reanalysis (ISR)

The plasma samples were analyzed at GPO, Thailand as per in-house SOPs complying with the international guidelines [14,15]. The samples from the same subject were analyzed in the same analytical run along with 10 calibration standards (2.031 to 6068.514 ng/mL) and 16 quality control samples at 4 different levels. Pantoprazole and the internal standard were extracted from 50 µL of plasma using 0.1% ammonia solution (v/v) and methanol. Then the samples were centrifuged to separate the precipitates. The supernatants were transferred into appropriate vials for analysis.

The plasma concentrations of pantoprazole were determined using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method: Nexera™ (Shimadzu Corporation, Japan) coupled with TSQ Quantum Ultra (Thermo Fisher Scientific, USA). Each sample was injected at 5 µL onto ACE 5 C18 150×4.6 mm column. The isocratic mobile phase consisting of methanol and 0.1% formic acid (80:20, v/v) was pumped at a flow rate of 1 mL/min. The autosampler and column oven temperatures were set at 4 °C and 40 °C, respectively. The transition of precursor to product ion was monitored in positive mode at m/z 384.04 to 199.99 for pantoprazole, and m/z 390.07 to 206.02 for pantoprazole D6. Data acquisition and evaluation of chromatographic data were performed using Xcalibur™ version 34.0.27.42 and LCquan™ version 3.0.26.0.

The study samples having concentrations close to maximum concentration and in the elimination phase of each subject in each period were chosen for incurred sample reanalysis (ISR) according to EMA guideline on bioanalytical method validation [14]. However, the concentrations from ISR were not used for pharmacokinetic calculation.

Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed by non-compartmental analysis (Phoenix WinNonlin Software Version 6.4, Pharsight Corporation, USA). The C_{max} and t_{max} of pantoprazole were directly obtained from the pharmacokinetic profiles. The elimination rate constant (λ_Z) was determined from the slope of terminal log-linear portion of the pharmacokinetic profiles. The apparent $t_{1/2}$ was calculated as $0.693/\lambda_Z$. The area under the curve from time zero to last measurable time point (AUC_{0-36h}) of pharmacokinetic profiles was calculated by the trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was determined as $AUC_{0-t} + C_t/\lambda_Z$, where C_t is last measurable concentration. The AUC_{0-36h}, $AUC_{0-\infty}$ and C_{max} were reported as primary pharmacokinetic parameters, whereas the t_{max}, λ_Z, $t_{1/2}$ and the time prior to first measurable concentration (t_{lag}) were reported as secondary pharmacokinetic parameters.

The statistical analysis was carried out using PROC GLM (SAS® Version 9.4, SAS Institute Inc., USA). Analysis of variance (ANOVA) was performed for log-transformed pharmacokinetic parameters: AUC_{0-36h}, $AUC_{0-\infty}$ and C_{max}. Effects of period, treatment, and sequence on primary pharmacokinetic parameters were included in ANOVA mixed-effect model. The significance of these effects

was determined using F-test. The 90% confidence intervals (CIs) for the ratio of geometric least squares mean (test/reference) were calculated for the log-transformed primary pharmacokinetic parameters. Bioequivalence was to be concluded when the 90% CIs were within the acceptable range of 80.00-125.00%. Wilcoxon signed-rank test was performed to compare t_{max} of the test and reference products. All statistical calculations were performed at a significance level of 5% ($\alpha = 0.05$).

Results

Demographic characteristics of subjects

In the single-dose fasting study, 42 subjects were enrolled and there were 2 withdrawn subjects due to adverse events. An additional subject dropped out due to personal reason before check-in to period II (Figure 1). In the single-dose fed study, 52 subjects were enrolled and there were total 8 dropout and withdrawn subjects (Figure 2). Out of 8, there were 3 subjects had abnormal clinical laboratory findings and were withdrawn by principle investigator before check-in to period IV. Total 7 subjects completed 3 study periods, and 5 of them received one reference and two test formulations. Another dropout subject completed 2 study periods by receiving one test and one reference formulation. The demographic characteristics of enrolled subjects in both studies are summarized in Table 3.

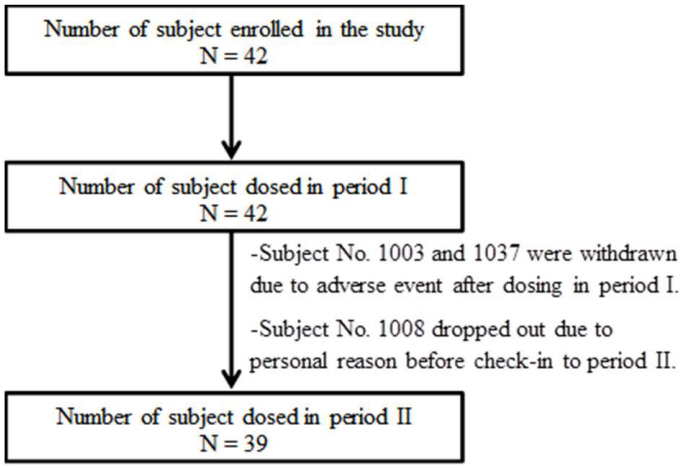

Figure 1: Flow chart of the single-dose fasting study

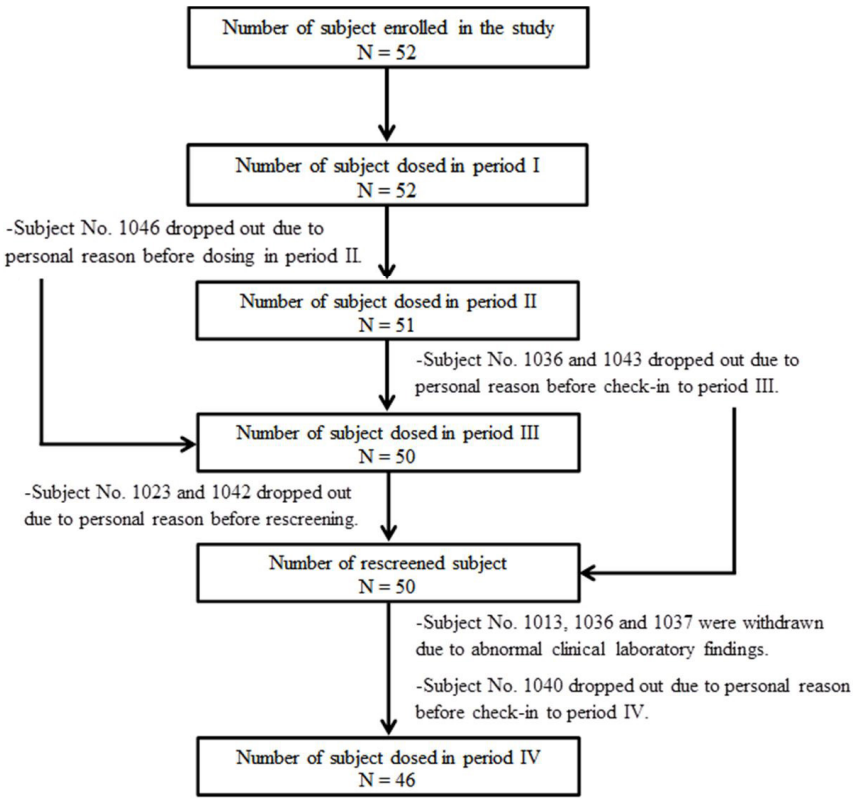

Figure 2: Flow chart of the single-dose fed study

Demographic characteristics	Single-dose fasting study (N = 42)	Single-dose fed study (N = 52)
Age (year)	35.67 ± 9.04	33.54 ± 9.87
Weight (kg)	64.98 ± 11.18	62.85 ± 13.22
Height (m)	1.64 ± 0.09	1.62 ± 0.09
BMI (kg/m^2)	24.02 ± 2.76	23.83 ± 3.34

Table 3: Demographic characteristics of enrolled subjects (Mean ± SD)

Sample analysis

A total of 1,847 samples from the single-dose fasting study were successfully analyzed in 20 analytical runs. A total of six samples accounted for 0.3% of total samples were reanalyzed due to concentration above the highest calibration curve standard. In the single-dose fed study, total 5,174 samples were collected in four study periods. The samples were analyzed in 52 analytical runs. There were 2 samples (0.04%) subject to repeat analysis due to processing error. The correlation coefficient calculated from 10 calibration standards was more than 0.99 for all analytical runs. The analysis details of study samples from both studies are presented in Table 4.

Sample analysis details	Single-dose fasting study	Single-dose fed study
Number of samples	1847 samples	5174 samples
Number of analytical runs	20 analytical runs	52 analytical runs
Between-run precision of the calibration curve standards	0.7% to 1.9% of the CV	1.3% to 4.4% of the CV
Between-run accuracy of the calibration curve standards	97.4% to 101.6% of the nominal values	97.1% to 101.6% of the nominal values
Between-run precision of the quality control samples	1.5% to 3.3% of the CV	1.9% to 4.9% of the CV
Between-run accuracy of the quality control samples	100.5% to 105.2% of the nominal values	91.8% to 93.9% of the nominal values

Table 4: The analysis details of study samples

ISR was carried out in two separate analytical runs for 162 samples selected from the single-dose fasting study. The difference between original and ISR concentrations of all incurred samples was less than 20%. Total 398 samples were selected from the single-dose fed study for ISR and 396 accounted for 99.5% had percent difference between original and ISR concentrations less than 20%. The ISR results of the samples obtained from both studies met the acceptance criteria as per EMA guideline on bioanalytical method validation [14]. The reanalysis using incurred samples confirmed reproducibility of the validated bioanalytical method for the study samples.

Pharmacokinetic and statistical analysis

The data from 39 subjects participating in the single-dose fasting study were used for pharmacokinetic and statistical analysis. Although there were total 8 dropout and/or withdrawn subjects in the single-dose fed study, all 52 subjects received both test and reference products in the study and their plasma concentration data were eligible for pharmacokinetic and statistical analysis. The mean plasma concentration-time profiles of pantoprazole after administration of the test and reference products under fasting and fed conditions are illustrated in Figure 3. Pharmacokinetic parameters of pantoprazole for the test and reference products are summarized in Table 5.

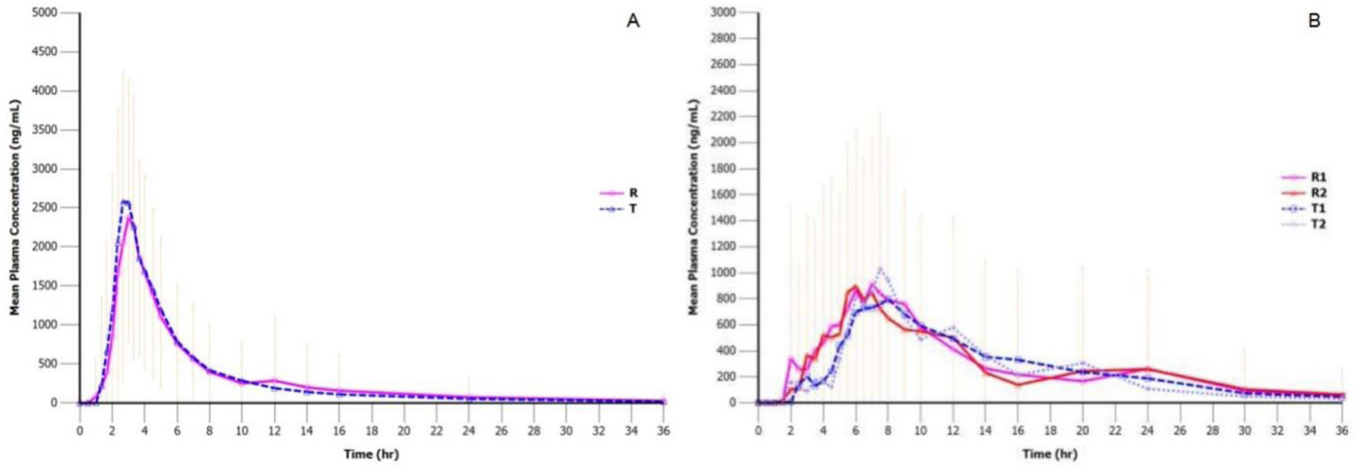

Figure 3: Mean plasma concentration-time profiles of pantoprazole after administration of test product-T and reference product-R in healthy Thai volunteers under fasting conditions **(A)** and fed conditions **(B)**

Parameter (Unit)	Un-transformed data (Mean ± SD)			
	Fasting		Fed	
	Test (N = 39)	Reference (N = 39)	Test (N = 101)	Reference (N = 98)
$AUC_{0\text{-}36h}$ (µg.hr/mL)	11.3 ± 11.1	11.3 ± 10.9	9.52 ± 9.15	10.3 ± 10.2
$AUC_{0\text{-}\infty}$ (µg.hr/mL)	11.6 ± 11.9	11.6 ± 12.0	10.3 ± 10.9	10.7 ± 11.5
C_{max} (µg/mL)	3.85 ± 1.26	3.68 ± 1.48	2.77 ± 1.13	2.92 ± 1.17
t_{max} (hr)*	2.67 (1.33,5.00)	2.67 (1.33,12.0)	7.50 (2.00,24.0)	6.28 (2.00,24.0)
λ_z (1/hr)	0.35 ± 0.16	0.33 ± 0.14	0.38 ± 0.19	0.39 ± 0.18
$t_{1/2}$ (hr)	2.81 ± 2.38	2.92 ± 2.28	2.71 ± 2.43	2.78 ± 2.72
t_{lag} (hr)*	1.67 (0.50,4.00)	1.67 (0.50,10.0)	6.50 (1.00,16.0)	5.50 (1.00,20.0)
Extrapolated AUC (%)	0.78 ± 2.02	0.97 ± 2.76	1.51 ± 3.83	1.47 ± 3.70

*t_{max} and t_{lag} were reported in Median (Min, Max).

Table 5: Pharmacokinetic parameters of pantoprazole for test and reference formulations in healthy Thai volunteers

On the ANOVA of log-transformed $AUC_{0\text{-}36h}$, $AUC_{0\text{-}\infty}$, and C_{max}, no significant effects of sequence, formulation or period were observed in the single-dose fasting study (Table 6). However, sequence effect was observed on the log-transformed $AUC_{0\text{-}36h}$, $AUC_{0\text{-}\infty}$ and C_{max} in the single-dose fed study ($p < 0.05$, Table 7). In both studies, the 90% CIs of the geometric least squares mean ratio between the formulations of log-transformed $AUC_{0\text{-}36h}$, $AUC_{0\text{-}\infty}$ and C_{max} were within the acceptance range for bioequivalence. Wilcoxon signed-rank test did not detect the significant difference in the median t_{max} between the test and reference products given under fasting and fed conditions ($p > 0.05$).

Parameter	Geometric least squares mean ratio (90% CI)	Power	Intra subject CV (%)	ANOVA (p-value)		
				Sequence	Formulation	Period
ln ($AUC_{0\text{-}36h}$)	100.8 (95.68-106.21)	100.0	13.7	0.2207	0.7964	0.4373
ln ($AUC_{0\text{-}\infty}$)	100.6 (95.52-105.93)	100.0	13.6	0.2308	0.8486	0.4135
ln (C_{max})	109.4 (97.97-122.28)	95.2	29.6	0.1442	0.1775	0.7957

Table 6: Statistical comparison of primary pharmacokinetic parameters between test and reference formulations in the single-dose fasting study (N = 39)

Parameters	Geometric least squares mean ratio (90% CI)	Power	Intra subject CV (%)		ANOVA (p-value)		
			Test	Reference	Sequence	Formulation	Period
ln ($AUC_{0\text{-}36h}$)	98.2 (92.35-104.44)	100.0	24.9	29.2	<0.0001	0.6281	0.1900
ln ($AUC_{0\text{-}\infty}$)	96.2 (91.89-100.76)	100.0	18.6	15.0	<0.0001	0.1691	0.3985
ln (C_{max})	94.9 (85.00-105.87)	95.5	47.5	48.1	0.0059	0.4278	0.5392

Table 7: Statistical comparison of primary pharmacokinetic parameters between test and reference formulations in the single-dose fed study (N = 52)

Safety

Study	Adverse event	Incidence (N)	
		Test	Reference
Fasting study	Nausea and vomiting	0	1
	Urticaria	1	0
	Total	1	1
Fed study	Nausea and vomiting	0	1
	Faintness	1	0
	Fever	1	1
	Increased ALT and AST	1	0
	Decreased hemoglobin	1	0
	Decreased platelet	1	0
	Dizziness	3	0
	Headache	0	1
	In utero exposure	0	1
	Asymptomatic hypertension	1	1
	Total	9	5

Table 8: List of adverse events

Both test and reference products were well tolerated by the study subjects. Two post-dose adverse events were reported in 2 subjects in the single-dose fasting study. Urticaria was reported in the subject after receiving the test product whereas nausea and vomiting was reported in the subject after receiving the reference product. Total fourteen adverse events were reported in the single-dose fed study. The most frequently reported adverse event in this study was dizziness. Nine adverse events were reported in 8 subjects after receiving the test product whereas five adverse events were reported in 2 subjects after receiving the reference product (Table 8). In general, all adverse events found in both studies were mild in the intensity and could resolve without any medical treatment.

Discussion

In our studies, the pharmacokinetics of pantoprazole was characterized in Thai population under fasting and fed conditions. The pharmacokinetics of pantoprazole was comparable between the test and reference formulations. It was observed that food slightly decreased the C_{max} and AUC of pantoprazole for both test and reference formulations. However, food effect was more pronounced when considering the increased t_{max} and t_{lag}, which can be explained by the delayed gastric emptying [5]. These findings were in accordance with the data from other bioequivalence studies [16,17]. Although reported half-life values of pantoprazole was approximately 1-2 hours, a possibility of no measurable concentration up to 24 hours was demonstrated under fed conditions [16]. Therefore, the last sampling time point in this study was assigned at 36 hours. In addition, more number of sampling time points were designed for the fed study since board range of t_{max} had been reported in different studies [16-18]. Washout period between the administrations of two formulations was 7 days to ensure complete drug elimination given that at least 5 half-lives are required [9]. Even though pantoprazole must be converted to its active form to exert the activity, the conversion occurs in the parietal cells, not in the systemic circulation. Furthermore, the rate and extent of absorption derived from parent compound is more relevant to drug release from the formulation [19]. Therefore, only pantoprazole concentration was measured according to FDA published product-specific guidance for generic drug development [20]. Apart from ISR, the precision and accuracy determined by calibration standards and quality control samples in the analytical runs also suggested that the analysis was reliable and reproducible.

The fasting and fed bioequivalence studies were conducted using different study designs. A fully replicate design was selected for fed study since it is recommended for highly variable drug (intra-subject variability ≥ 30%). With this design, fewer number of subjects are required and the intra-subject variability can be determined for each of the test and reference product [11-13]. The data from subjects who completed either two or three study periods with an intervention of only one reference formulation were not used for the calculation of within-subject standard deviation of reference product (S_{WR}). Based on the calculated S_{WR} of 0.4564 for C_{max}, bioequivalence limit for C_{max} can be widen up to 70.69-141.46% considering scaled-average approach [11]. However, the 90% CI of the geometric least squares mean ratio between the formulations of log-transformed C_{max} met the standard bioequivalence criteria regardless of expansion of bioequivalence limits.

Both studies could demonstrate bioequivalence between the test and reference formulations with the power greater than 90%. However, the sample size for both studies was calculated by overestimating the dropout rate, especially for the fed study which included 4-study period participation. In addition, higher number of subjects for a fully replicate design is required by EMA compared with regulatory requirements of FDA [12]. With utilization of this design, the recruitment of 52 subjects in the fed study was more than necessary and led to statistical overpower. ANOVA did not show any significant effects of period and treatment (formulation) whereas the sequence effect was observed on the primary pharmacokinetic parameters in the single-dose fed study. It is interesting that this effect was not observed in the fasting study, in which the study conduct was standardized in the same manner. The case record forms were thoroughly inspected and only eligible subjects were enrolled in each study period. The analytical method used for study sample analysis was successfully validated in compliance with the regulatory guidance [14,15]. No significant amounts of drug were found in any pre-dose samples indicating sufficient washout of drug between study periods. Moreover, sampling period was appropriately designed since the elimination phase was well captured and the extrapolation of AUC was less than 2% suggesting that $AUC_{0-\infty}$ was reliably estimated. The samples from the same subjects were analyzed altogether in the same analytical run and the randomization sequence was not accessible for all analysts during the analytical phase. It was ensured that all samples were treated in the same manner. However, it is important to note that the sequence effect was tested using subject nested within sequence as an error term. Considering high intra-subject variability under fed conditions, it potentially produced the difference over the study periods. Although the sequence effect existed, it did not affect the results of bioequivalence as the intra-subject variability values observed for both products were comparable and the ANOVA adjusted the product effect for the sequence effect [21].

Conclusion

The statistical comparison of AUC_{0-36h}, $AUC_{0-\infty}$, and C_{max} of the test and reference formulations administered under both fasting and fed conditions indicated that there was no significant difference between two formulations. The pharmacokinetics of pantoprazole was affected by food intake, especially the t_{max} and t_{lag}. Nevertheless, the studies successfully established bioequivalence between Pantoprazole GPO® and CONTROLOC® 40 mg under both fasting and fed conditions. The test and reference formulations were well tolerated and no subjects developed serious adverse events. As a result, the bioequivalence between these two formulations can be concluded based on insignificant difference in terms of rate and extent of absorption describing by C_{max} and both AUCs.

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