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Original article

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Abstract:

Pharmacokinetics of levoceterizine have been studied, however, obtained information was only from the Western people. In addition, the license of this drug formulation was expired few years ago. For approval of this new generic drug, the bioequivalence study is a registration requirement by the Thai Food and Drug Administration. Thus, the objectives of this study were to determine the pharmacokinetics of levocetirizine in healthy Thai volunteers and to compare the pharmacokinetics of a test (new generic; Avocet®) and reference (Xyzal®) formulations of levocetirizine 5 mg after single oral administration in healthy Thai volunteers. A single-dose, randomized-sequence, open-label, two-way crossover with a one-week washout period was conducted in 26 fasted volunteers. Levocetirizine was assayed by using a liquid-chromatography tandem mass spectrometry (LC-MS/MS) method. The C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, and $t_{1/2}$ evaluated from test drug administration were 226.50 ± 49.70 ng/mL, 1564.96 ± 336.29 ng/L/h, 1821.37 ± 434.38 ng/L/h, 1.00 ± 0.68 h, and 8.36 ± 3.28 h, respectively. The 90% CIs for the test/reference ratios of the log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 87.39 to 103.05%, 99.27 to 108.63%, and 95.21 to 111.42%, respectively. No serious adverse events were observed during conduct of this study and both formulations were well tolerated. In conclusion, a single 5-mg dose of the test formulation of levocetirizine met the regulatory criteria for bioequivalence to the reference formulation based on the rate and extent of absorption.

Keywords: Levocetirizine; Pharmacokinetics; Bioequivalence; Liquid chromatography tandem mass spectrometry; LC-MS/MS

Introduction

Levocetirizine, 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl] piperazine-1-yl]ethoxyl] acetic acid, is an active *R* enantiomer of cetirizine which was approved by the US Food and Drug Administration in 2008 for the relief of symptoms associated with seasonal allergic rhinitis and perennial allergic rhinitis and uncomplicated skin manifestations of chronic idiopathic urticaria in adults and children (age ≥ 6 years) [1-3]. It is a third-generation antihistamine developed from the second-generation antihistamine cetirizine. Its pharmacokinetic properties explain its good tolerability in patients, whereas the use of cetirizine is associated with a low incidence of anticholinergic effects such as dry mouth. Levocetirizine causes little sedation and has no cardiac adverse effects unlike highly lipophilic antihistamines [4, 5]. Moreover, it can be safely administered with virtually any other drug, as it has neither inducing nor inhibiting effects on the major drug-metabolizing enzymes [6]. The use of levocetirizine is therefore more effective with fewer side effects than cetirizine. Levocetirizine in the dosage of 5-10 mg was indicated to be sufficient to reach maximal antihistaminergic effects [5].

Although the pharmacokinetic characteristics of levocetirizine were reported previously by Strolin Benedetti *et al.* in 2001 [7], it was a study in only 4 subjects of healthy male volunteers. The obtained results of C_{max}, AUC and T_{max} were 0.27 ± 0.04 μg/mL, 2.31 ± 0.50 μg h/mL, and 0.75 ± 0.50 h, respectively. Levocetirizine is extensively absorbed from the gastrointestinal tract, reflecting in a total recovery of 98.3% of the dose as determined in the radiolabel study [8]. The plasma protein bound was high (91-92%), independent of plasma concentrations, the volume of distribution (Vd) was 0.3 l/kg, and the mean plasma $t_{1/2}$ was 7 h [7]. Levocetirizine is very poorly metabolized [7, 9]. *In vivo* in which a single oral dose of ^{14}C-labeled levocetirizine 5 mg was administered to those 4 healthy male subjects, 13 metabolites were detected in urine, representing only 2.4% of the dose at 48 h. Reported metabolic pathways included oxidation (hydroxylation, O-dealkylation, N-oxidation and N-dealkylation), glucuronidation, taurine conjugation and glutathione conjugation with formation of the mercapturic acids [1, 7]. The hepatic cytochrome P450 (CYP) isoenzyme responsible for dealkylation is CYP3A4; the enzymes responsible for aromatic oxidation have not fully been elucidated [1]. Levocetirizine is eliminated by renal excretion to a large extent. Metabolism is a minor route of elimination [7]. The elimination $t_{1/2}$ in healthy volunteers was approximately 8-9 h after administration of oral tablets or solution. Urinary excretion accounts for 85.4% of a dose and the feces for 12.9% [1]. Food had no effect on the extent of exposure (AUC) of the levocetirizine tablet, but T_{max} was delayed by about 1.25 h and C_{max} was decreased by about 36% after administration with a high fat meal [1, 7]. There was no evidence of chiral inversion of levocetirizine in humans [10]. The pharmacokinetics reported so far were all studied in Western people. There has not been any report about pharmacokinetics of levocetirizine in Asian people yet. Since ethnic contributes to difference in the pharmacokinetic characteristics especially in the metabolism of many drugs [11], the information of pharmacokinetic parameters of drug in specific subject is therefore very important in drug selection and optimization.

In Thailand, the only marketed levocetirizine 5 mg film-coated tablet is Xyzal®. This original levocetirizine is expensive, and unaffordable for long term treatment in many patients. The availability of a cheaper, generic formulation in the market would be benefit for the treatment of allergic rhinitis and chronic urticaria in patients who require levocetirizine treatment. However, to assure the quality of the generic product in compared with the original product, the bioequivalence study of those two products must be conducted. The objectives of this study were to determine the pharmacokinetics of levocetirizine in healthy Thai volunteers and to assess bioequivalence between original formulation (Xyzal®) and generic formulation namely Avocet® manufactured by Silom Medical Co., Ltd., Thailand in terms of extent and rate of absorption in healthy Thai volunteers under fasted condition.

Materials and Methods

Subjects

Twenty-six healthy Thai volunteers (both males and females) participated in this study were recruited through the Clinical unit, International Bio Service Co., Ltd. (Golden Jubilee Medical Center, Mahidol University) *via* advertisement. Volunteers were eligible based on the following criteria: age 18 to 45 years and body mass index between 18 and 25 kg/m^2. All volunteers had been determined healthy by medical history, physical examination, and laboratory analysis (complete blood count, blood urea nitrogen, serum creatinine, fasting blood sugar, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase) prior to the beginning of the study. Urine pregnancy tests were negative in all female volunteers and they must be willing to comply with applicable contraceptive requirements instead of using hormonal contraception within 14 days prior to study and throughout the entire study period and used a medically acceptable method of hormonal contraception or abstinence for 7 days after the study was completed. Volunteers did not have: history of allergy or sensitivity to levocetirizine or related substances; history of gastrointestinal; liver and kidney disorders or malabsorption; use of pharmacologic agents known to induce or inhibit drug metabolizing enzymes within 14 days before the first dose of study medication; drink of alcoholic beverages within 14 days before studying; usually consuming tobacco and unable to quit prior to and during the study periods; use of any medicine or food supplements, vitamins, mineral, herbal remedies within 14 days before first study drug administration and not willing to take them throughout participation period; and participation in other clinical studies within the past 30 days. The volunteers had been informed about the details including the risks and benefits of this study, and provided written informed consent before participating in the study. They were free to withdraw from the study at any time.

Study design

This study was a single-dose, randomized-sequence, open-label, two-way crossover bioequivalence study in 26 fasted subjects. The study protocol and informed-consent form were approved by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand prior to commencing and the study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guideline.

Subjects were admitted into the clinical unit and fasted 10 h before each study drug administration. Each subject was randomized and divided equally into two groups by the sequence of product taking, test and reference groups. The sequence of study product and unique subject number were assigned to each subject by order of arriving at the clinical unit on the first day of the first period. Volunteers were not allowed to drink water 1 h before dosing and administered the study medication along with 240 mL of water by nurse and under supervision of physician. During study period, standard meals were served at 4, 8 and 12 h after dosing.

Blood samples were collected for a total of 15 time points including prior drug administration (pre dose), at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h after drug administration. They were taken at these times because the plasma $t_{1/2}$ was known to be 7 h [7]. Blood samples (6 mL) were collected in 10-mL lithium heparin vacuum tube. These blood samples were cooled to 4°C, and centrifuged at 3000 rpm for 10 min at 4°C. All samples were centrifuged within 60 min of collection. After centrifugation, plasma was pipetted into duplicate polypropylene tubes and kept at -70°C until transferred for analysis.

The study procedures were similar between the two study periods with a wash out period of 7 days. Since the terminal elimination half-life of levocetirizine is about 7 h [7], the 7 days wash out period was considered sufficiently.

Adverse events

Subjects were confined to the research unit for a 24 h period of each dosing. Vital signs were measured and assessed as baseline prior to each study drug administration and blood pressure were repeated at every time point of blood collection up to 24 h of blood

sampling. In addition, vital signs were performed immediately if subjects experience any adverse events. Laboratory tests, in addition of vital signs, including urea nitrogen, blood sugar, serum creatinine, total bilirubin, AST, ALT, alkaline phosphatase and urine pregnancy test were performed at baseline and after completion of the study. All adverse events (AEs) occur were recorded in source document and evaluated for their seriousness, severity, and relationship to study medications.

Determination of plasma levocetirizine

Plasma concentrations of levocetirizine were determined by using a liquid-chromatography tandem mass spectrometry (LC-MS/MS) at the Bioanalytical Department of International Bio Service Co., Ltd., Thailand where certified Good Laboratory Practice (GLP) regarding the requirements for the study of bioequivalence. The analytical method was modified from the study of Morita *et al.* [12] The LC-MS/MS instrument used was a Varian model composing of Varian ProStar 410 Auto Sampler, Varian ProStar 210 Binary Solvent Delivery Module and Varian 1200L LC/MS equipment triple quadrupole (QqQ) mass spectrometer with Electrospray ionization (ESI) source (Varian Inc., CA, USA). The chromatographic and MS conditions were developed to obtain the most suitable for analysis. The column selected was Zorbax Eclipse XDB C18, 50 × 4.6 mm, 5 µm (Agilent Technologies, CA, USA). An isocratic mobile phase was used at 0.4 mL/min, and consisted of acetonitrile and 0.1% formic acid (80:20, v/v). The column temperature was set at 40°C. The ESI source operated in positive ionization mode. The following parameters were constant throughout the analysis: manifold temperature = 42°C, API drying gas (N_2) = 20 psi at 300°C, API nebulizing gas = 50 psi N_2, API housing temperature = 50°C, collision gas (Ar) = 2.0 mTorr, needle = 5500 V, shield = 600 V, scan time = 1.0 ms, detector = 1600 V. Multiple reaction monitoring transitions were at mass-to-charge ratios (*m/z*) of 389 → 201 and 383 → 337 for levocetirizine and loratadine (internal standard; IS), respectively.

The developed LC-MS/MS method was validated in terms of specificity, lower limit of quantification (LLOQ), linearity, accuracy, precision (intra-and interassay), recovery, matrix effect, and stability including: freeze-thaw, short-term, long-term, post-preparative and stock solution stability according to the Guidance for Industry, Bioanalytical Methods Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (US FDA, CDER) [13]. Briefly, six blank plasma samples from six individual subjects receiving no medication were used to validate the specificity of the method. The results should not present any chromatographic interference for levocetirizine and internal standard measurement. Five independent samples of spiked plasma with the lowest concentration of levocetirizine standard were determined for LLOQ. Linearity of levocetirizine at eight standard concentrations in range of 5-600 ng/mL was evaluated by using linear regression statistical analysis with 1/x weighting factor. Accuracy and precision were manipulated using a minimum of five determinations in each independent plasma sample having four concentrations of levocetirizine standard including LLOQ (5 ng/mL), low, medium and high quality control (QC) samples in the range of standard calibration curves (15, 250 and 500 ng/mL for low, medium and high QC samples, respectively). Recovery and matrix effect were performed by using a minimum of five determinations in each independent plasma sample having three concentrations of levocetirizine including low, medium and high QC concentrations.

For sample preparation, aliquots of 500 µL plasma were transferred in a 10-mL screw-capped glass test tube, 50 µL working solution of loratadine (IS) (6,000 ng/mL) was added and vortex-mixed for 3-5 seconds. Extraction solvent (3 mL), ethyl acetate, was added to each tube before capped and extracted by shaken at 300 rpm, room temperature for 5 min. The mixture was centrifuged at 4,000 rpm, 4°C for 5 min. The upper, organic layer was then transferred to a 10-ml glass test tube and evaporated under stream of nitrogen (15 psi) at 40°C to dryness. The resulting residue was reconstituted with acetonitrile:water (80:20, v/v). The obtained samples were transferred to microcentrifuge-tube and centrifuged at 13,000 rpm, room temperature for 5 min. Ten microlitres of clear obtained solution (supernatant) were analyzed.

The calibration standards, QC and all plasma samples were treated in the same manner as indicated.

Pharmacokinetic and statistical analysis

The individual plasma drug concentration-time curve was plotted and the pharmacokinetic parameters were calculated by noncompartmental methods using WinNonlin® software version 6.1 (Pharsight®, NC, USA). Accordingly, C_{max} and T_{max} were obtained directly from the concentration-time curves of levocetirizine. AUC_{0-t} was calculated according to the linear trapezoidal rule. $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t was the last measured concentration and λ_z was the slope of the linear regression of the log-transformed concentration-time curve. Levocetirizine plasma $t_{1/2}$ was calculated as $0.693/\lambda_z$ [14].

For the purpose of bioequivalence analysis C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were considered as the primary variables. Two-way analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for p-value equal to or less than 0.05.

The 90% confidence interval (90% CI) for the ratios of geometric mean test/reference for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were calculated based on least squares means from the ANOVA of log-transformed data. The 90% CI for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values of the test preparation over those of the reference product were estimated. If the 90% CI for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were within 80.00% to 125.00%, the 2 drug formulations would be considered bioequivalence [15].

Results

Demographic data

A total of 26 healthy Thai volunteers (14 males and 12 females) were enrolled and all completed the study. The demographic characteristics of the study population are summarized in Table 1. The result showed no difference between the two groups studied.

Adverse events

Four adverse events (AEs) were reported including somnolence, nausea, vomiting and hypotension. Somnolence is the most frequently AE occurred in both test and reference treatment groups (6 subjects [23.08%] for test and 3 subjects [11.54%] for reference product). Vomiting (1 subject [3.85%]), nausea (1 subject [3.85%]) and hypotension (2 subjects [7.69%]) occurred when received the test product. The mild vomiting occurred for only a minute at 5 h in one subject after received the test drug, considering no effect on pharmacokinetic evaluation. All AEs were transient and confirmed by the physician to be mild. No serious AEs were reported, and none of the subjects was withdrawn from the study due to AEs. The AEs found when administered the 2 formulations are summarized in Table 2. No clinically significant abnormalities were observed.

Analytical method validation

A sensitive and specific liquid chromatography-electrospray ionization mass spectrometric method was

Table 1 Baseline demographic and clinical characteristics of the study population

Characteristics		Group 1: TR* (n=13)	Group 2: RT* (n=13)
Gender	Male	6	8
	Female	7	5
Age (year ± SD)		24.46 ± 5.14	25.08 ± 4.42
Weight (kg ± SD)		59.72 ± 7.26	60.13 ± 8.33
Height (cm ± SD)		1.66 ± 0.08	1.65 ± 0.07
Body mass index (kg/m^2 ± SD)		21.66 ± 1.73	22.11 ± 2.48

*The sequence of product taken, TR = test-reference group and RT = reference-test group

Table 2 Adverse events (AEs) after administration of the test and reference formulation of levocetirizine 5-mg tablets in healthy Thai volunteers (n=26)

AE	Treatment groups		Relation to study drug	Severity	Action
	Test	Reference			
Body as a whole					
Somnolence	n = 6 (23.08%)	n = 3 (11.54%)	Probably related (test and reference)	Mild	Observation
Vomiting	n = 1 (3.85%)	-	Probably related (test)	Mild	Observation
Nausea	n = 1 (3.85%)	-	Probably related (test)	Mild	Observation
Cardiovascular					
Hypotension	n = 2 (7.69%)	-	Probably related (test)	Mild	Observation
No AE subjects	n = 16 (65.38%)	n = 23 (88.46%)			

Table 3 Pharmacokinetic parameters of levocetirizine after a single 5-mg oral dose of 2 formulations of levocetirizine tablets in healthy Thai volunteers (mean ± S.D., n=26)

Parameter	Treatment group		
	Test	Reference	Ratio (Test/Reference)
C_{max}, ng/mL	226.50 ± 49.70	241.41 ± 65.48	0.96 ± 0.24
T_{max}, h	1.00 ± 0.68	0.86 ± 0.43	1.50 ± 1.46
AUC_{0-t}, ng/L/h	1564.96 ± 336.29	1511.68 ± 344.29	1.04 ± 0.13
$AUC_{0-\infty}$, ng/L/h	1821.37 ± 434.38	1855.30 ± 972.80	1.02 ± 0.24
$t_{1/2}$, h	8.36 ± 3.28	8.99 ± 9.74	1.10 ± 0.46
k_{el}, h^{-1}	0.09 ± 0.04	0.10 ± 0.04	1.15 ± 1.14
% extrapolation	13.28 ± 7.68	13.04 ± 11.91	1.16 ± 0.54

developed and validated for the determination of levocetirizine in human plasma by using loratadine as an internal standard. The analytical method was linear in the range of 5-600 ng/mL levocetirizine ($R^2 > 0.99$). According to the FDA guidance on bioanalytic method validation, the mean value of quality control should be within 15% of the actual value except at LLOQ, where it should not deviate by > 20%. The LLOQ was established at 5 ng/mL with relative errors not exceed ± 20% (≤ 12.35%) and %CVs were ≤ 8.02% for intra-or interassay precision at 15, 250 and 500 ng/mL. The recovery values of levocetirizine and loratadine were 84.58 ± 9.30 and 109.22 ± 3.06%, respectively, indicating the high efficiency of the extraction procedure. The matrix effect for levocetirizine and loratadine were 104.32 ± 12.51 and 100.48 ± 9.71%, respectively, indicated that ion suppression or enhancement from the plasma matrix was consistent for this analytical method and would not interfere the measurement of the analytes. The analytical method met the criteria laid down in Guidance for Industry, Bioanalytical Methods Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (US FDA CDER, 2001, BP) [13]. Prior to study initiation, it was determined that both levoceterizine and loratadine were stable in human plasma under various conditions following 3 freeze-thaw cycles, for 6 h at room temperature, for up to 2 months following storage at -70°C, and for 48 h after being processed.

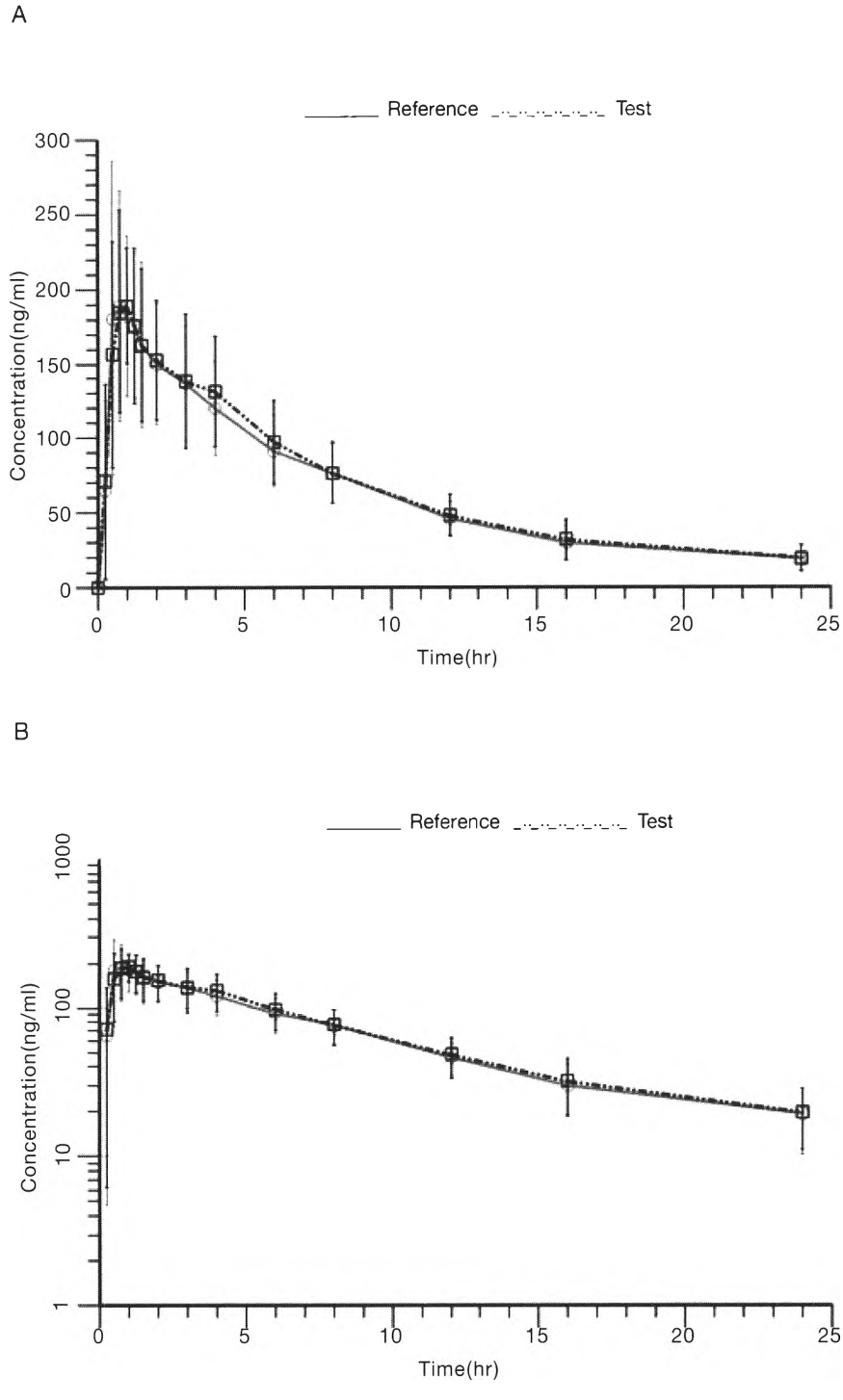

Figure 1 (A) Plasma concentration-time profiles of levocetirizine (Mean ± S.D., n=26) and (B) their semi-log scale plots

Pharmacokinetic analysis

The mean plasma levocetirizine concentrations over time and their Semi-log scale plot are illustrated in Fig. 1. The pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, k_{el}, and % extrapolation of AUC for both test and reference products are shown in Table 3. The mean C_{max} value of levocetirizine after administration of the test tablets was 226.50 ± 49.70 ng/mL and those after administration of the reference tablets was 241.41 ± 65.48 ng/mL. The mean T_{max} values were 1.00 ± 0.68 h for the test and 0.86 ± 0.43 h for the reference. Results for the extent of absorption, as determined from mean AUC_{0-t} and $AUC_{0-\infty}$ values, were 1564.96 ± 336.29 ng/L/h and 1321.37 ± 434.38 ng/L/h, respectively, after administration of the test and were 1511.68 ± 344.29 ng/L/h and 1355.30 ± 972.80 ng/L/h,

respectively, after administration of the reference tablets. The mean $t_{1/2}$ after administration of the test tablets (8.36 ± 3.28 h) and reference tablets (8.99 ± 9.74 h) are not significantly different. The mean ratio (test/reference) of all parameters were about 1, indicated the equal parameter values.

Bioequivalence analysis

Analysis of variance for the two way crossover test of C_{max}, AUC_{0-t} and $AUC0_{0-\infty}$ (Table 4) demonstrated no significant period, sequence and treatment effects for the log-transformed data. However, the significant subject nested in sequence effect was solely significant ($p < 0.05$) for all parameters which is usually seen in small sample size study.

The 90% CIs of the ratios (test/reference) for the log-transformed C_{max} (index of the rate of absorption) and for AUC_{0-t} (index of the extent of absorption) and the power of the test are listed in Table 5. The 90% CIs for the ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were

Table 4 Analysis of variance for two-way crossover of log-transformed pharmacokinetic parameters of levocetirizine after given a single dose (5 mg) of test and reference products in 26 healthy Thai volunteers

Source	**C_{max} (Ln transform data)**				
	D.F.	**SS**	**MS**	**F**	***p*-value**
Period	1	0.0437	0.0437	1.4488	0.2405
Subject (Sequence)	24	2.1215	0.0884	2.9321	0.0054*
Treatment (Formulation)	1	0.0356	0.0356	1.1823	0.2877
Sequence	1	0.0002	0.0002	0.0025	0.9604
Error	24	0.7235	0.0301		
Total	**51**	**2.9245**	**0.1980**		
Source	**AUC_{0-t} (Ln transform data)**				
Period	1	0.0009	0.0009	0.1033	0.7506
Subject (Sequence)	24	2.0368	0.0849	9.4262	0.0000*
Treatment (Formulation)	1	0.0185	0.0185	2.0527	0.1648
Sequence	1	0.2050	0.0250	2.4159	0.1332
Error	24	0.2161	0.0090		
Total	**51**	**2.4774**	**0.3183**		
Source	**$AUC_{0-\infty}$ (Ln transform data)**				
Period	1	0.0322	0.0322	1.1745	0.2893
Subject (Sequence)	24	3.5278	0.1470	5.3585	0.0001*
Treatment (Formulation)	1	0.0113	0.0113	0.4131	0.5265
Sequence	1	0.3582	0.3582	2.4368	0.1316
Error	24	0.6583	0.0274		
Total	**51**	**4.5878**	**0.5762**		

D.F. = Degree of freedom, SS = Sum of squares, MS = Mean square

*Significant; $p < 0.05$

Table 5 Comparison of 90% CIs for the log-transformed ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for levocetirizine following administration of 5 mg in 26 healthy Thai volunteers; and power

Parameter	**Ratio (Test/Reference)**	**90% CI**	**Power**
C_{max} (ng/mL)	0.96 ± 0.24	87.39-103.05	1.00
AUC_{0-t} (ng/L/h)	1.04 ± 0.13	99.27-108.63	1.00
$AUC_{0-\infty}$ (ng/L/h)	1.02 ± 0.24	95.21-111.42	1.00

87.39 to 103.05%, 99.27 to 108.63%, and 95.21-111.42%, respectively, with the powers of all parameters were higher than 80%.

Discussion

This study examined the pharmacokinetic properties and bioequivalence of 2 formulations of levocetirizine tablets in healthy Thai volunteers. The 90% CIs of levocetirizine were contained within the predefined bioequivalence criteria of 80% to 125% for both C_{max} and AUC.

There are few reports in the literature regarding the pharmacokinetics of levocetirizine and all are studied in healthy Western volunteers. Strolin Benedetti *et al.* [7] reported the pharmacokinetics of levocetirizine after administered a single dose 5-mg levocetirizine in healthy UK volunteers and C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, and $t_{1/2}$ obtained were 0.27 ± 0.04 μg/mL, 2.31 ± 0.23 μg/L/h, 2.96 ± 0.30 μg/L/h, 0.75 ± 0.50 h, and 10.66 ± 0.66 h, respectively. Another study was reported by Baltes *et al.* [10] that 10 mg levocetirizine were administered to healthy Belgium volunteers and the obtained C_{max}, AUC_{0-t}, T_{max}, and $t_{1/2}$ were 0.51 ± 0.11 μg/mL, 4.14 ± 0.74 μg/L/h, 0.73 ± 0.33 h, and 7.76 ± 1.59 h, respectively. The pharmacokinetic parameters in these two studies were comparable which the C_{max} and AUC in 10 mg dose increased two times compared to in 5 mg dose. In the present study which was studied in healthy Thai volunteers, the pharmacokinetic parameters obtained (from reference drug; Xyzal®) were 0.24 ± 0.06 μg/mL, 1.51 ± 0.34 μg/L/h, 1.85 ± 0.97 μg/L/h, 0.86 ± 0.43 h, and 8.99 ± 9.74 h for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, and $t_{1/2}$, respectively. As the result shown, the pharmacokinetic parameters were similar to previous studies except the AUC which appeared lower values. This may be related to the CYP450 genotype, which affects metabolism, because each race has a different ratio according to its genotype [16, 17]. Significant variability in the types and frequencies of CYP allelic variants has been found among ethnic groups [18], which may explain ethnicity-specific responses to drugs. Ethnic variations could also exist in terms of absorption and excretion of drugs, however, litter information currently exists to confirm such possibilities. In this study, C_{max} and T_{max}, which indicate the rate of absorption, of levocetirizine 5 mg (reference drug; Xyzal®) were not different between Thai (0.24 ± 0.06 μg/mL and 0.86 ± 0.43 h for C_{max} and T_{max}, respectively) and Western subjects (0.27 ± 0.04 μg/mL and 0.75 ± 0.50 h for C_{max} and T_{max}, respectively). Since levocetirizine is rapidly and extensively absorbed from the gastrointestinal tract to obtain a total recovery of 98.3% of the dose [8] within 1 h [7], there would no limited in absorption process. This can be explained by that levocetirizine exists almost exclusively as zwitterions in the pH region 3.5-7.5 [19]. Due to conformational flexibility and the formation of an internal ionic bond, the positive and the negative charges partly neutralize each other, rendering the molecule more lipophilic and hence better available for passive absorption [20]. The effects that C_{max} increased proportionally as dose increased (5 mg to 10 mg) (from previous studies) and T_{max} were not changed due to dose (0.75 ± 0.50 h and 0.73 ± 0.33 h for dose 5 mg and 10 mg, respectively) and also similar value of T_{max} was observed in this study (0.86 ± 0.43 h) could confirm the rapid and extensive absorption of levocetirizine. It exhibited passive absorption constituting non-saturable absorption kinetics of recommended dose administration (5-10 mg).

The pharmacokinetics may differ between children and adults. Five milligrams levocetirizine administered to Canadian children 6 to 11 years gave C_{max}, AUC_{0-t}, T_{max}, and $t_{1/2}$ = 0.45 ± 0.04 μg/mL, 3.55 ± 0.34 μg/L/h, 1.20 ± 0.20 h, and 5.70 ± 0.20 h, respectively [21], which were differed to those studied in adults. However, in Asian, there have not been reported in children yet. Further studies are needed to verify this hypothesis.

The results from ANOVA revealed a significant subject nested in sequence effect because of large variability between subjects which is usually seen in small sample size study. However, the sample size (26 subjects) used in this study is adequate to attain a power of 80% at a significance level of 0.05. Therefore, the subject nested in sequence would not expect to influence the comparison of the 2 formulations.

The pharmacokinetic parameters of levocetirizine 5 mg from the generic and the reference products in 26

healthy Thai volunteers were not significantly different. In addition, the 90% confidence interval of the ratio of C_{max} AUC_{0-t} and $AUC_{0-\infty}$ for levocetirizine of the generic and reference product were in the range of 80.00-125.00% as required by the US FDA [22] and the Thai FDA [15]. Therefore, bioequivalence was indicated between the generic Avocet® and original Xyzal® in terms of the rate and extent of drug entry into the systemic circulation. This generic product could be a candidate for a new registration product. The physician could use this generic product as an alternative to the original product for cheaper cost of treatment.

However, as with any clinical trial, the current study had several limitations that should be considered. This was an open-label study, so it might not address objectively the efficacy and safety profiles of the formulations tested. Because the data were only obtained from healthy volunteers who were administered a single dose in the fasted state, the therapeutic effect of long term use with multiple doses in patients should be considered for further evaluation.

Conclusion

This study contributes to pharmacokinetic and bioequivalence evaluation of the two formulations of levocetirizine 5 mg in healthy Thai volunteers. The extent of exposure (AUC) of levocetirizine in Thai volunteers was slightly different with the AUC reported previously in Western subjects. A single 5-mg dose of the test formulation of levocetirizine (Avocet® manufactured by Silom Medical Co., Ltd., Thailand) met the regulatory criteria for bioequivalence to the reference formulation (Xyzal®) based on the rate and extent of absorption. Both formulations were well tolerated.

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