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Average Bioequivalence of Clarithromycin Immediate Released Tablet Formulations in Healthy Male Volunteers

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ABSTRACT

The objective of this study was to assess average bioequivalence of two immediate released tablet formulations of 500-mg clarithromycin tablets in 24 healthy Thai male volunteers. In a randomized, single dose, fasting state, two-period, crossover study design with a 1-week washout period, each subject received a 500-mg clarithromycin tablet. Plasma samples were collected over a 24-hour period after oral administration and were analyzed by using a validated method using high performance liquid chromatography with electrochemical detection. Pharmacokinetic parameters were determined by using noncompartmental analysis. The time to reach the maximal concentration (t_{max}, h), the peak concentration (C_{max}, ng/mL), and the area under the curve ($AUC_{0-\infty}$, ng.h/mL) of the Reference and Test formulations were 2.0 ± 0.8 vs. 2.2 ± 0.9, 2793 ± 1338 vs. 2642 ± 1344, and 17912 ± 7360 vs. 17660 ± 7992, respectively. Relative bioavailability was 0.99. The 90% confidence interval of C_{max} and $AUC_{0-\infty}$ were 82.6–112.1% and 84.7–112.0%. Bioequivalence between the Test and Reference formulation can be concluded.

Key Words: Bioequivalence; Pharmacokinetics; Clarithromycin; Electrochemical detection; Dissolution profile.

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INTRODUCTION

Clarithromycin is a broad spectrum macrolide antibacterial agent that is effective both in vitro and in vivo against major pathogens responsible for respiratory tract infections such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella spp.*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*.[1–4] Its chemical structure is derived from erythromycin A by methylation at position 6 of the lactone ring, preventing the hydrolysis of the lactone ring at low pH and formation of the inactive 6,9–9,12 spiroketal derivative. These improve antimicrobial activity and pharmacokinetic properties in comparison with erythromycin, including increased oral bioavailability, increased plasma concentrations, longer half-life to allow twice daily administration, and extensive diffusion into saliva, sputum, lung tissue, epithelial lining fluid, alveolar macrophages, neutrophils, tonsils, and nasal mucosa in middle ear fluid.[4–5] Moreover, its properties also allow a decrease in incidence of gastrointestinal effects.[3]

Clarithromycin is well absorbed from the gastrointestinal tract, but its systemic bioavailability (55%) is relatively low due to first-pass metabolism. It undergoes rapid biodegradation to produce the microbiologically active 14-hydroxy-metabolite. The dose-dependent maximal plasma concentrations (C_{max}) of clarithromycin and its 14-hydroxy metabolite are achieved within 3 hours following a single oral dose.[6] The presence of food appears to have no clinically significant effect on clarithromycin pharmacokinetics.[1,6] The maximal plasma concentrations of clarithromycin after single doses of 250 and 500 mg administration were 0.78 ± 0.25 mg/L and 2.12 ± 0.83 mg/L, respectively. The time to peak concentration was 1 to 3 hours.[7,8]

Clarithromycin is well distributed throughout the body and achieves higher concentrations in tissues than in the blood as well as the 14-hydroxy clarithromycin, its major and active metabolite.[1,6] The primary metabolic pathways are oxidation, N-demethylation, and hydroxylation by cytochrome P450 (CYP) 3A isozymes.[5–6] Both parent and its primary metabolite are mainly excreted in the urine.[6] Mean values of total body clearance and renal clearance in adults range from 29.2 to 58.1 L/hr and 6.7 to 12.8 L/hr, respectively.[5]

A reduction in urinary clearance in the elderly and in patients with renal impairment accompanied with increases in area under the plasma concentration-time curve and peak plasma concentrations as well as prolonged elimination half-life have been reported.[5] A dosage adjustment for clarithromycin should be considered in the patients with a creatinine clearance less than 1.8 L/hr.[5] Mild hepatic impairment does not significantly alter clarithromycin pharmacokinetics.[6] The recommended dose for treatment of community-acquired upper and lower respiratory tract infections in hospital and community settings is 500 to 1000 mg/day for 5 to 14 days.[6] Drug interactions related to the cytochrome P450 system may occur when clarithromycin is used.[1]

Objectives of the Study

The objectives of this study were to compare in vitro dissolution profiles and in vivo bioequivalence of two formulations of 500-mg clarithromycin tablets: Crixan® from Ranbaxy, India (test formulation) and Klacid® from Abbott, USA (reference formulation) in 24 healthy Thai male volunteers. The clinical protocol was reviewed and approved by the Ethics Committee of the Ministry of Public Health, Thailand.

EXPERIMENTAL

Clarithromycin Preparations

Reference preparation: Klacid® 100 tablets (Abbott, USA) containing 500 mg clarithromycin per tablet (Lot no. 51215VA, Mfd. 03/1999).

Test preparation: Crixan® 100 tablets (Ranbaxy, India) containing 500 mg clarithromycin per tablet (Lot no.1088686, Mfd. 03/2000).

In Vitro Studies: Protocol and Data Analysis

The in vitro dissolution study was designed to theoretically investigate the influence of drug release properties, dissolution of solid dosage form, and dissolution of drug particles in immediate release dosage products.

Dissolution testing of twelve tablets of the test product vs. twelve tablets of reference product was conducted Dissolution testing by following (USP) 24 dissolution method.[9] Dissolution studies were performed using USP apparatus 2 (Vankel VK7000, Cary, NC) in 900 mL of 0.1 M sodium acetate

buffer (pH 5.0) at rotation speed of 50 rpm, with a constant temperature at $37 \pm 0.5^{\circ}$C. Five-milliliter samples were withdrawn at 5, 10, 15, 20, 30, and 45 minutes and replenished with 5 mL of fresh dissolution medium. The dissolution samples were filtered with 0.45 μm nylon filter prior to analysis.

The filtered portion of the solutions were analyzed with high pressure liquid chromatography (HPLC) using isocratic elution over a normal phase column with UV detector (Model SPD-10AVP, Shimadsu Corporation, Japan) for their clarithromycin contents at 210 nm. The percentage of drug dissolved was calculated based on the concentrations of drugs. The dissolution profile comparison, when appropriate, was carried out using f_2 similarity factor.[10] The similarity factor is a logarithmic reciprocal square-root transformation of the sum of squared error and is a measurement of the similarity in the percentage of dissolution between the two curves.

$$f_2 = 50 \times \log\left\{\left[1 + (1/n)\sum(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

Two dissolution profiles are considered similar when the f_2 value is greater than or equal to 50. Note that both test and reference products dissolve 80% or more within 30 minutes to comply with USP 24 monograph. Moreover, the guidelines for dissolution testing indicated that if more than 85% of clarithromycin is released from the tablets at 15 minutes, the profile comparison with an f_2 is unnecessary.

In Vivo Studies: Study Design and Clinical Protocol

Twenty-four healthy Thai male volunteers with the ages of 20.6 ± 3.6 years and body mass index of 20.3 ± 0.9 were included in a randomized, single dose, fasting, two-period, two-sequence, crossover study with a 1-week washout period. All healthy volunteers provided written informed consent before enrollment. The volunteers were nonsmoking, nonalcoholic, and free from cardiac, hepatic, renal, gastrointestinal, and hematological diseases, as assessed by physical examination and the following laboratory tests: complete blood count, total bilirubin, plasma creatinine, blood urea nitrogen, (AST), (ALT), alkaline phosphatase, and hepatitis B surface antigen.

During each period, the volunteers were admitted to the Bioequivalence Test Center, at Naresuan University at 6:00 p.m. and had an evening meal before 9:00 p.m. After an overnight fast, they received a single 500-mg clarithromycin dose of each of the formulations at 7:00 a.m. along with 240 mL of water. They were then in the seated position at least 30 minutes and then fasted for 2 hours. A standard lunch and an evening meal were provided at 4 and 9 hours after dosing. No other food was permitted during the study period. Liquid consumption was allowed ad libitum after lunch but xanthine containing and acidic beverages were prohibited. After each period of the study, the volunteers were reexamined by a physician.

Clarithromycin Chemical Analysis

Seven milliliters of each blood sample was collected into a lithium-heparinized containing tube by catheterized venupuncture at forearms before dosing and at 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, and 24 hours after the administration of each clarithromycin formulation. The blood samples were centrifuged (2000 g, 10 minutes) and the plasma samples were separated within 10 minutes after collecting blood. All samples then were stored at -80°C until analysis.

The plasma samples were assayed by a validated high pressure liquid chromatographic method with electrochemical detection modified from the methods of Chu et al., using roxithromycin as an internal standard.[7,8] In brief, 0.5 mL of plasma sample was extracted by liquid-liquid extraction. The evaporated sample was reconstituted with 200 μL of mobile phase, and 100–150 μL of the eluate was injected. The HPLC systems consisted of Constametric 3200, an isocratic pump (Thermo Separation Products, San Jose, CA), series 200 autosampler (Perkin Elmer, Wellesley) and Coulochem II electrochemical detector (Environmental Sciences Associates, Chelmsford, USA) with +850 mV (guard cell), +500 mV (E1), and +780 mV (E2). The Alltech Rocket C-8 analytical column (Alltech, Deerfield, IL) 3 μ, 53 × 7.0 mm was employed. The mobile phase was composed of acetonitrile:methanol:acetate buffer (50:10:40) with pH 7.5. The flow rate was 1.3 mL/min. The calibration curve of clarithromycin ranged from 90 to 3600 ng/mL in which the peak area ratio of the analyte to the internal standard was fitted to a quadratic regression equation with linear $(1/x)$ weighting and the correlation coefficient (r) equaled to 0.9995. The limit of quantitation was 18 ng/mL. The extraction efficiency and its coefficient of variation (% C.V.) in spiked plasma samples with clarithromycin concentrations of 90, 360, and 1800 ng/mL were 95.0, 90.5, and 98.2% and 8.3, 5.6, and 3.3% respectively.

The extraction efficiency and its % C.V. for the internal standard in spiked plasma samples were 89.9% and 7.6% respectively.

Pharmacokinetic and Statistical Analysis

A noncompartmental pharmacokinetic method was employed to determine the pharmacokinetic parameters of clarithromycin. The time to peak plasma concentration (T_{max}) and the peak concentration (C_{max}) were obtained directly from the plasma clarithromycin concentrations. The area under the concentration-time curve ($AUC_{0-\infty}$) and terminal half-life ($t_{1/2}$) were determined by using WinNonlin Standard (version 3.0).

An analysis of variance (ANOVA) was performed on the pharmacokinetic parameters C_{max} and $AUC_{0-\infty}$, using general linear models (GLM) procedures, in which sources of variation were sequence, subjects within sequence, period, and preparation. Then the 90% confidence intervals of the Test/Reference ratios for C_{max} and $AUC_{0-\infty}$ (log transformed) were determined. Bioequivalence between two formulations can be concluded when the 90% confidence intervals for these pharmacokinetic parameters of two products are found within the acceptable range of 80–125%.[11]

RESULTS AND DISCUSSION

In Vitro Studies

The labeled amounts of both test and reference products are 97.65% and 94.45%, respectively, which are within the range requested by USP 24. The cumulative amounts (%) of clarithromycin dissolved in vitro from both reference and test product are plotted in Fig. 1. The dissolution testing data from the test and reference products consistently indicate more than 80% of the labeled amount of clarithromycin is dissolved in 30 minutes as required in USP 24.

The overall profiles up to 30 minutes generate an f_2 value of 38.17, whereas a single point calculation at 15 minutes result in an f_2 value of 64.52. Moreover, the guidance for dissolution testing indicated that more than 85% if of clarithromycin is released from the tablets at 15-minutes, the profile comparison with an f_2 is unnecessary. In this case, the test and reference products release $82.80 \pm 5.44\%$ and $84.61 \pm 3.79\%$, respectively, at the 15-minute time point, and these values represent the value at the plateau level. Therefore, both products' release profiles should not have a significant effect on in vivo bioavailability.

In Vivo Studies

Clarithromycin was well tolerated. No volunteer was withdrawn and no serious adverse event was found during the study. However, mild gastric discomforts were observed in two subjects and the symptoms appeared to disappear spontaneously.

Pharmacokinetics of Clarithromycin

Average concentration-time courses of clarithromycin after single 500-mg clarithromycin tablet administrations of both preparations are shown in

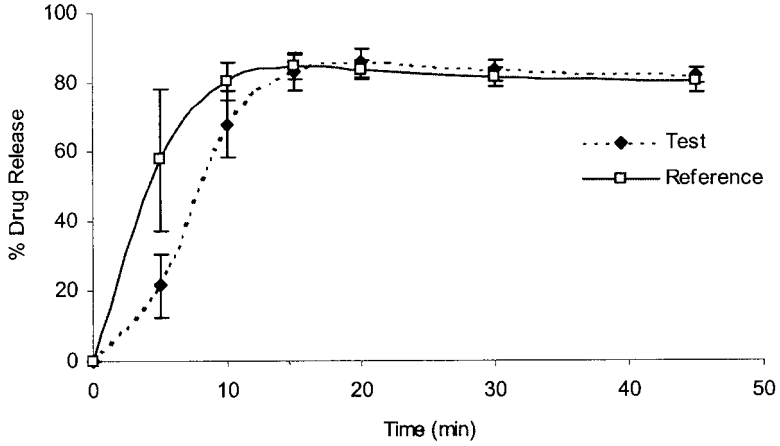

Figure 1. Dissolution profiles of clarithromycin tablets.

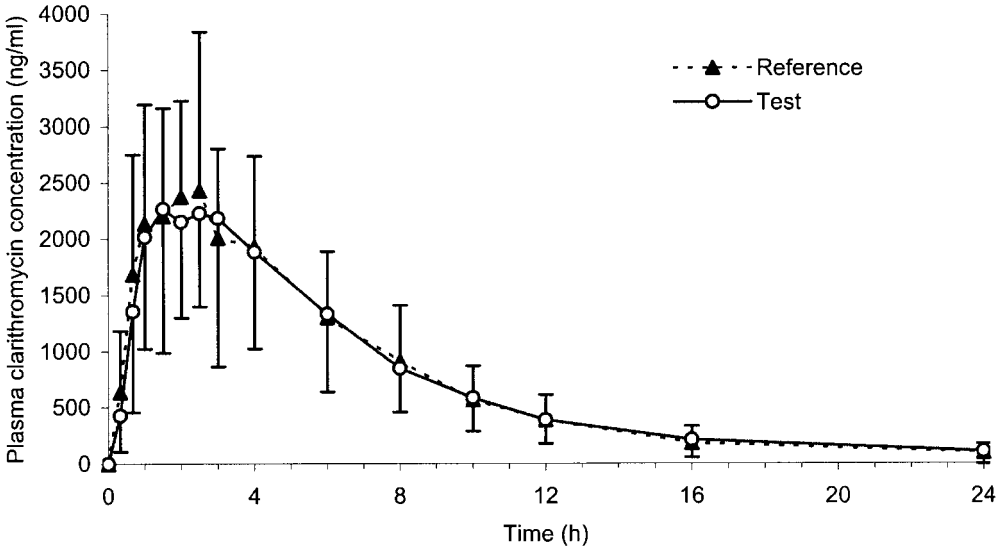

Figure 2. Average plasma concentration-time curve of clarithromycin after 500-mg single dose administration of reference and test formulations in 24 healthy Thai male volunteers.

Table 1. Pharmacokinetic parameters (mean ± SD) of clarithromycin after 500-mg single dose administration of reference and test formulations in 24 healthy Thai male volunteers.

	Pharmacokinetic parameters			
Formulations	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng.h/mL)	Terminal $t_{1/2}$ (h)
Reference	2.0 ± 0.8	2793 ± 1338	17,912 ± 7360	3.6 ± 1.8
Test	2.2 ± 0.9	2642 ± 1344	17,660 ± 7992	3.9 +1.9

Fig. 2. Pharmacokinetic parameters of clarithromycin are summarized in Table 1. Maximal clarithromycin levels were observed after 2.2 ± 0.9 hours (Test) and 2.0 ± 0.8 hours (Reference). The average peak concentration (C_{max}) and area under the concentration-time curve ($AUC_{0-\infty}$) of Test and Reference were 2642 ± 1344 vs. 2793 ± 1338 (ng/mL) and 17,660 ± 7992 vs. 17,912 ± 7360 (ng.h/mL). The mean terminal half-lives were 3.9 ± 1.9 hours for Test and 3.6 ± 1.8 hours for Reference. The relative bioavailability between Test and Reference Formulation was 0.99.

Bioequivalence Analysis of Clarithromycin

The Test/Reference ratio for C_{max} and the 90% confidence interval were 0.95 and 82.6–112.1%. The Test/Reference ratio for $AUC_{0-\infty}$ and the 90% confidence interval were 0.99 and 84.7–112.0% (Tables 2 and 3).

However, the dissolution profiles of both formulations are slightly different before 15 minutes and appear to be similar after 15 minutes (Fig. 1). However the in vivo bioequivalence is not different, which may be owing to, at 15 minutes, the location of clarithromycin in the gastrointestinal tract, which is not the primary site of clarithromycin absorption and has relatively small adsorptive surface area compared to the small intestine. If the major site of drug absorption is the stomach, the early time point difference in the dissolution profile will affect the in vivo bioavialability and its bioequivalence.

CONCLUSION

The average bioequivalence of 500-mg clarithromycin tablets of the test formulation compared with the reference formulation was studied in 24

Table 2. ANOVA table of C_{max} and $AUC_{0-\infty}$ (logarithmically transformed) of clarithromycin after 500-mg single administration of reference and test formulations in 24 healthy Thai male volunteers.

Source of variation	DF	SS	MS	Computed F	p-value
C_{max}					
Total	47	9.745			
Subject	1	0.121	0.121	0.355	0.557
Subject (sequence)	22	7.515	0.342	3.597	0.002
Formulation	1	0.018	0.018	0.191	0.666
Period	1	0.002	0.002	0.018	0.896
Error	22	2.089	0.095		
$AUC_{0-\infty}$					
Total	47	7.645			
Subject	1	0.030	0.030	0.112	0.741
Subject (sequence)	22	5.850	0.266	3.332	0.003
Formulation	1	0.008	0.008	0.105	0.748
Period	1	0.001	0.001	0.012	0.913
Error	22	1.756	0.080		

Table 3. Ninety percent confidence interval of C_{max} and $AUC_{0-\infty}$ (logarithmically transformed) of clarithromycin after 500-mg single administration of reference and test formulations in 24 healthy Thai male volunteers.

	90% confidence interval	
	C_{max}	$AUC_{0-\infty}$
Test/reference	82.6–112.1	84.7–112.0

healthy Thai male volunteers. The in vitro dissolution study indicated that the percentage of label amount and percentage of clarithromycin dissolved from both the test and reference products complied with USP 24 monograph and the guidelines for dissolution testing from U.S. Food and Drug Administration (FDA). Regarding the pharmacokinetic study, both formulations were equivalent in terms of rate and extent of absorption. Based on the U.S. (FDA) bioequivalence guidelines, 90% confidence intervals of C_{max} and $AUC_{0-\infty}$ ratios of clarithromycin of these two preparations (Test/Reference) were in the acceptable range of 80–125%. Consequently, the test product containing 500 mg of clarithromycin per tablet was equivalent to the reference product containing 500 mg of clarithromycin per tablet and can be used interchangeably in clinical practice.

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