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A crossover randomized bioequivalence study of two formulations of clarithromycin in healthy male volunteers

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Abstract

A crossover-randomized bioequivalence study of two oral formulations of 500 mg clarithromycin tablet, an antibiotic, was carried out in 15 healthy male Bangladeshi volunteers. The test and reference formulations were CLARIN™ (Drug International Ltd, Bangladesh) and KLACID™ (Abbott Laboratories, USA), respectively. Each tablet was administered with 150 mL of water to subjects after overnight fasting on 2 treatment days separated by 1 week washout period. After dosing, serial blood samples were collected for a period of 24 hours. The plasma concentration of clarithromycin was estimated using a validated HPLC method. The pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and K_{el} were determined. The C_{max} for test and reference drugs were 3.12 ± 0.88 and 3.37 ± 1.2 µg/mL (90% CI: 84.86-115.13%) respectively. The T_{max} for test and reference drugs were 1.50 ± 0.32 and 1.40 ± 0.28 hours (90% CI: 90.66-109.33%) respectively. The mean $AUC_{0\rightarrow 24h}$ for test and reference drugs were 13.78 ± 5.02 and 16.40 ± 4.56 µg.hr/mL (90% CI: 88.17-111.82%) respectively. The relative bioavailability of text/reference drug was 84.02%. The mean $t_{1/2}$ and k_{el} for test drug were 2.96 hours and 0.2562 respectively whereas the values for reference drug were 3.37 hours and 0.2698 respectively. The 90% CI for the test and reference drugs were found within the acceptance range of 80-125%.

Keywords: Bioequivalence, Clarithromycin, Pharmacokinetics, Liquid chromatography

Introduction

Bioequivalence is a term in pharmacokinetics used to assess the expected in vivo biological equivalence of two proprietary preparations of a drug. Two products are said bioequivalent means they are expected to show all

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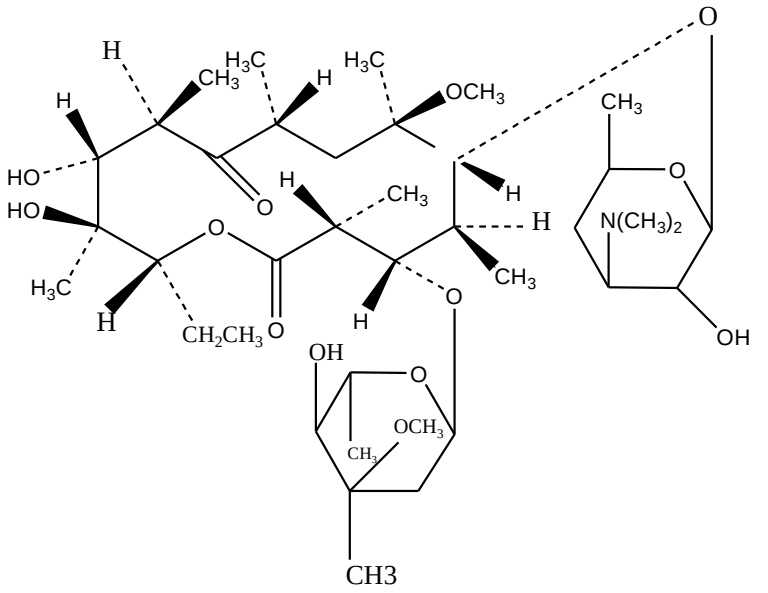
intents and purposes similar. Bioavailability is the rate and extent to which an active drug or metabolite enters the general circulation, thereby permitting access to the site of action. Bioavailability is determined either by measuring the concentration of the drug in the body fluids or by the magnitude of the pharmacologic response [1]. Clarithromycin (6-0-methylerythromycin) is a newer macrolide that is similar to erythromycin in its mechanism of action [2]. Macrolide antibiotics contain a many-membered lactone ring (14- membered ring for clarithromycin) to which are attached one or more deoxy sugars [3]. Clarithromycin is semi synthetic derivative of erythromycin. Clarithromycin is derived from erythromycin by addition of a methyl group and has improved acid stability and oral absorption compared with erythromycin [4].

Clarithromycin is absorbed rapidly from the gastrointestinal tract after oral administration, but first-pass metabolism reduces its bioavailability to 50% to 55%. Peak concentrations occur approximately 2 hours after drug administration. Clarithromycin may be given with or without food, but the extended release form, typically given once daily as a 1 g dose, should be administered with food to improve bioavailability. Steady state peak concentrations in plasma of 2-3µg/ml are achieved after 2 hours with a regimen of 500 mg every 12 hours, or after 2 to 4 hours with two 500 mg extended release tablets given once daily [3]. A 500 mg dose of clarithromycin produces serum concentration of 2-3 µg/mL. The longer half life of clarithromycin (6 hours) compared with erythromycin permits twice daily dosing. Clarithromycin penetrates most tissue well, with concentrations equal to or exceeding serum concentrations. The action of clarithromycin is better by its widespread distribution into tissues and by the development of primary microbiologically active metabolite, 14(R) hydroxyclarithromycin [5]. The newer macrolides, azithromycin and clarithromycin, should also demonstrate effective although there is very little existing data on their use in or facial infections. They include the advantages over erythromycin such as less GI toxicity, high tissue concentration, better gram- negative range, and once or twice daily dosing for patient compliance.



$C_{38}H_{69}NO_{13}$ (Erythromycin,6-O-methyl-6-O-Methylerythromycin)

The study was conducted to assess the bioequivalence of a test product CLARIN™ (clarithromycin 500 mg per tablet; Drug International Ltd, Bangladesh) with a reference product KLACID™ (clarithromycin 500 mg per tablet; Abbott Laboratories, USA) by measurement of plasma concentrations of clarithromycin by HPLC and calculation of bioequivalence parameters.

Materials and Methods

Protocol/design: Randomized, open-label, two-way crossover bioequivalence study was carried out with a washout period of 7 days. There was no major deviation made from the approved protocol.

Study subjects: Volunteers were collected from volunteer bank which was made by counseling with every individual. Numbers of subjects were fifteen and average age of the volunteers was 26.17 ± 2.68 years, average height was 165.13 ± 3.11 cm; average weight at screening examination was 55.81 ± 4.15 kg. Blood sample were collected after drug administration by two phases.

Study medication: CLARIN^{TM} (batch no.: 0210, manufacturing date: 02/2010, expiry date: 01/2012) was obtained from Drug International Ltd, Bangladesh as test formulation and KLACID^{TM} (batch no.: 6004967, manufacturing date: 08/2009, expiry date: 31/8/2013) was collected from Abbott Laboratories, USA as reference formulation.

Dosage regimen: Each healthy volunteer received each of the treatments as a single dose in accordance with a randomization scheme with a washout period of 7 days.

Institutional review board: The protocol and the ethical aspect of this study were approved by the seven membered Institutional review board of Khwaja Yunus Ali Medical College Hospital, Sirajgonj, Bangladesh. The protocol was approved with minor modifications on 12/2/2010.

This study was conducted in accordance with International Conference of Harmonization (ICH) and Good Clinical Practice (CGP) guidelines adopted by the European Agency for the evaluation of Medicinal products (EMEA).

Informed consent: The purpose of the study was explained to each volunteer in local language (Bengali) before starting the study by medical officer. Written informed consent was taken from a volunteer only when he agreed to participate in the study. Any question raised by the volunteer was discussed with the medical officer in detail. A copy of written informed consent was attached in the protocol.

Hospital admission: After screening, volunteers were admitted into the hospital ward (12 bedded especially designed for bioequivalence study) one day before starting the study. In this study, at a time 8 volunteers were admitted into the ward. The blood samples (2 mL in each time) were taken immediately before and at 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 hours after administering clarithromycin.

Analytical procedure: A validated liquid chromatography method (HPLC) was used for determination of clarithromycin in human plasma with a UV-Visible detector. Column type used was Cromasil C_{18} (150 mm × 4.6 mm, i.d.) 5 µ and the mobile phase was phosphate buffer: Acetonitrile (80:20). Procedures of validation and acceptance criteria were based on “FDA Bio-

analytical Method validation guidelines [6]. The retention time of clarithromycin was 15.1 min. The limit of detection (LOD) was 3.1 µg/mL whereas limit of quantification (LOD) was 12.5 µg/mL.

The extent of absorption was determined by $AUC_{0\rightarrow 24h}$ of clarithromycin. The rate of absorption was determined by C_{max} and t_{max}. The half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of clarithromycin were used to further characterize the pharmacokinetic outcome of this study.

Results

Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were taken directly from observed concentration ***vs*** time data. The elimination rate constant (K_{el}) was estimated by a non-linear least square regression analysis of the individual concentrations observed as a function of time during the elimination phase. The apparent elimination half life ($t_{½}$) was obtained by dividing 0.693 by K_{el}. The area under the curve (AUC) of clarithromycin in plasma from time zero to last quantifiable time point (t), $AUC_{(0\text{-}t),}$ was calculated using the linear trapezoidal rule. The AUC from time zero to infinity, $AUC_{(0\text{-}\infty)}$, was calculated from the sum of $AUC_{(0\text{-}t)}$ and C_{last}/K_{el}, where C_{last} was the last measurable concentration of clarithromycin in plasma [7].

The mean (± SD) $AUC_{0\rightarrow 24h}$ for CLARIN™ for 15 volunteers was 13.78 ± 5.02 3.1 µg.hr/mL whereas it was 16.40 ± 4.56 µg.hr/mL for KLACID™ (90% CI- 88.17 to 111.82%) (Table 1). The relative bioavailability (CLARIN™ / KLACID™ ratio) was 84.02%.

The mean C_{max} for CLARIN™ was 3.12 ± 0.88 µg/mL whereas it was 3.37 ± 1.2 µg/mL for KLACID™ (90% CI: 84.86-115.13%). The T_{max} for CLARIN™ and KLACID™ were 1.50 ± 0.32 and 1.40 ± 0.28 hours (90% CI: 90.66-109.33%) respectively. The mean $t_{1/2}$ and k_{el} for test drug were 2.96 hours and 0.2562 respectively whereas the values for reference drug were 3.37 hours and 0.2698 respectively (Table 1).

Table 1: Pharmacokinetic parameters following oral administration of CLARIN™ (test) and KLARICID™ (reference)

Parameters	CLARIN™		KLACID™	
	Mean ± SD	90% CI	Mean ± SD	90% CI
C_{max}	3.12 ± 0.88 µg/mL	88.14%-111.85%	3.37 ± 1.2 µg/mL	84.86% - 115.13%
T_{max}	1.50 ± 0.32 hours	90.66%-109.33%	1.40 ± 0.28 hours	91.42% - 108.57%
$AUC_{0\rightarrow24h}$	13.78 ± 5.02 µg.hr/mL	84.54%-115.45%	16.40 ± 4.56 µg.hr/mL	88.17%-111.82%
$t_{1/2}$	2.96 ± 13.79 hour	85.47%-114.52%	3.37 ± 11.71 hour	84.86%-115.13%
K_{el}	0.2562 ± 0.0430	93.67%-109.28%	0.2698 ± 0.005	81.54%-118.60%

$AUC_{0\rightarrow24h}$ = Area under the plasma concentration–time curve from zero hours to 24 hours; C_{max} = maximal plasma concentration; t_{max} = time for the maximal plasma concentration; $t_{1/2}$ = half-life; K_{el} = elimination rate constant.

The 90% confidence intervals for the CLARIN™ (test) and KLACID™ (reference) were found within the acceptance range of 80–125% (Table 1).

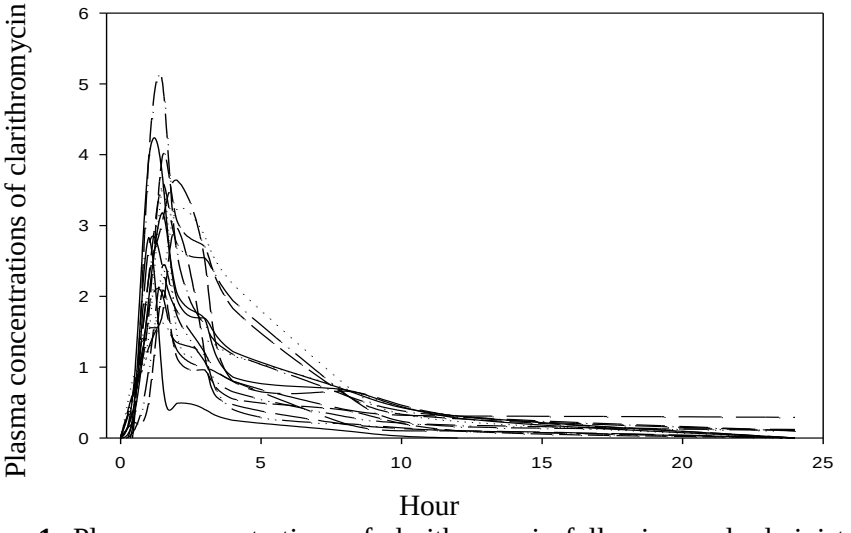


Figure 1: Plasma concentrations of clarithromycin following oral administration of single dose (500 mg) of CLARIN™ (test) in 15 healthy male human volunteers

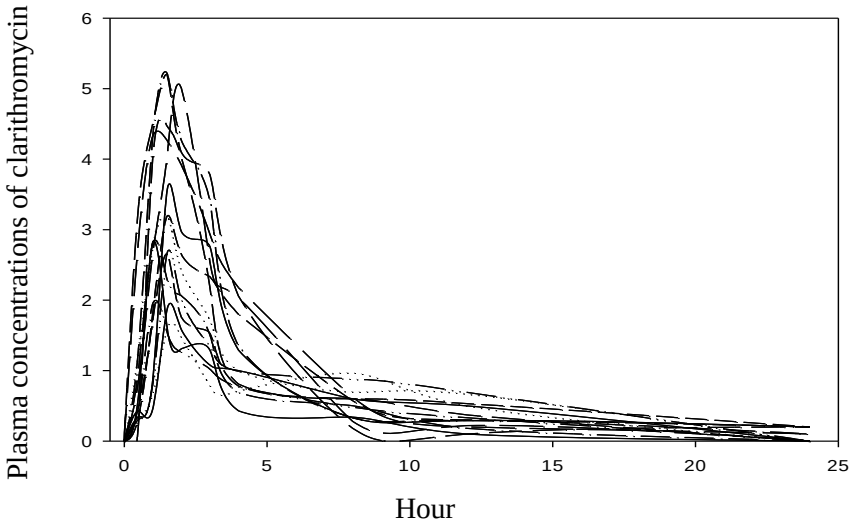


Figure 2: Plasma concentrations of clarithromycin following oral administration of single dose (500 mg) of KLACID™ (reference) in 15 healthy male human volunteers

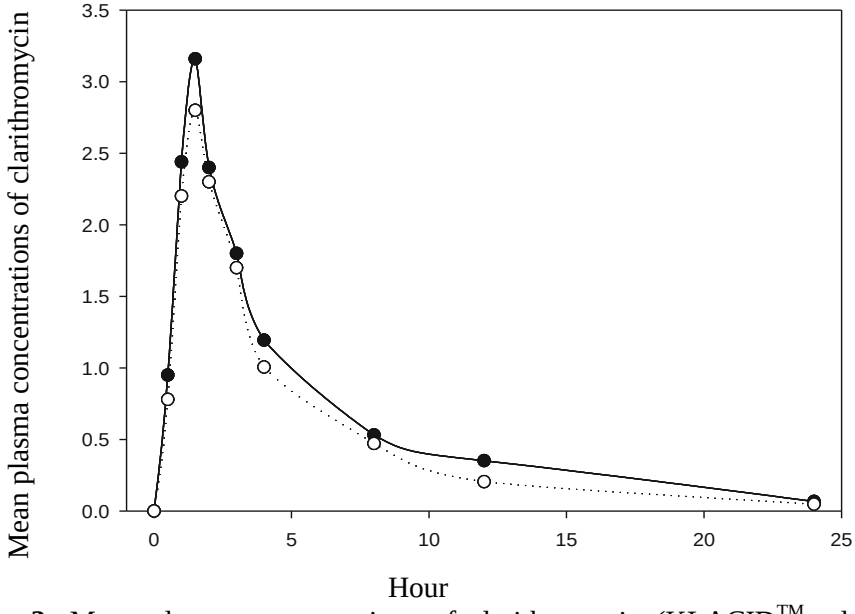


Figure 3: Mean plasma concentrations of clarithromycin (KLACID™- closed circle; CLARIN™- open circle) in 15 human volunteers

Tolerance: Single dose of 500 mg of both clarithromycin products were well tolerated by the volunteers.

Discussion

It is necessary to be bioequivalent for any local product to reference product to exclude any clinically important difference in the rate and extent at which the active entity of the drug becomes available at the site of action. The study was limited by inclusion of healthy male volunteers and each volunteer was administered a single dose in the fasted state. The current study had some limitations that should be considered. Our study examined the pharmacokinetic properties and bioequivalence of two formulations of clarithromycin capsules in healthy Bangladeshi male volunteers. The pharmacokinetic parameters calculated for both the test and reference formulations were not significantly different, which reflects the comparable pharmacokinetic characteristics of two formulations [6].

Mean half-life of CLARIN^{TM} (test) was found 2.96 hours, higher than that of KLACID^{TM} (reference) which was 3.37 hours (Figure 3). Since clarithromycin inhibits liver enzyme CYP3A, clinicians have to be aware that plasma levels of drugs that are metabolized by this enzyme may increase. This enzyme inhibition may be less in Asian people than Western, so increased enzyme activity may cause rapid inactivation of drug as well as lower half life [8]. The study population might be fast acetylator than the Western people, so the half life was found lesser in CLARIN^{TM} (test). Plasma protein level would be higher in Western population than the study population, which may be responsible for increased half-life of KLACID^{TM} (reference). An internationally published pharmacokinetic study of lafutidine performed on healthy Japanese male volunteers showed a C_{max} of 133.90 ng/mL and t_{max} of 1.84 h [9]. A similar study performed on healthy Chinese volunteers showed a C_{max} of 151.55 ± 54.49 ng/mL and t_{max} of 1.60 ± 0.40 h [10]. This particular study performed on an Indian subpopulation showed a C_{max} of 265.15 ± 49.84 ng/mL and t_{max} of 0.95 ± 0.24 h [11]. The estimated pharmacokinetic parameters of the test and the reference formulations in this study have higher levels than Japanese and Chinese clinical studies probably due to racial and genetic differences in the population studied.

Conclusion

CLARIN™ (test) is bioequivalent to KLACID™ (reference) based on the rate and extent of absorption and can be used interchangeably in clinical setting. The method described for the quantification of clarithromycin and its main metabolite is accurate and sensitive. Since there were no significant differences in the bioequivalence determined using the pharmacokinetic parameters of either clarithromycin or 14-hydroxyclarithromycin, we suggest that future bioequivalence trials of this drug may be performed by quantifying clarithromycin only.

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