Research Article

Bioequivalence study of two oral formulations of clarithomycin following a single dose administration to healthy male subjects under fasting conditions

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1. Abstract

Background: Clarithromycin is a semi-synthetic macrolide antibiotic used in a wide range of bacterial infections as a single or combination therapy. Several generic formulations of clarithromycin are marketed in Iraq, but their bioavailability and hence therapeutic equivalence is not available. Therefore, demonstration of bioequivalence is warranted to determine the bioequivalence between medicinal products in Iraqi individuals. **Objectives:** The aim of this study was to assess bioequivalence between a single dose from two products: Rithro Tablets (500 mg clarithromycin), produced by Avenzor, as a test product, in comparison with Klacid ® Tablets (500 mg clarithromycin), produced by Abbott, as a reference product in healthy male Iraqi subjects under fasting conditions. **Methods:** This study was an open label, randomized, fasting, single dose, two-treatment, two-sequence, two-period, crossover, laboratory-blind study in 26 healthy subjects conducted under fasting conditions. Blood samples were collected over a 24-hour period after dosing and plasma samples were analyzed for clarithromycin by high performance liquid chromatography with electrochemical detection. The products were compared using pharmacokinetic parameters derived from the plasma concentration-time profile of clarithromycin: T_{max}, $T_{1/2}$, $K_{elimination}$ $(\lambda)_z$, first order elimination rate constant, C_{max}, AUC_{0-t}, & AUC_{0-} . The average bioequivalence of the two products were concluded if the two-sided 90 % confidence interval (CI) for the test to reference ratio of the population means is within 80.00 – 125.00 % for each of intransformed data of C_{max}, AUC_{0-t} & AUC_{0-} . Tolerability was assessed based on changes in vital signs and laboratory tests, and questioning subjects about adverse events. **Results:** There were no statistically significant differencesin the plasma concentration-time profile of the test and reference compound. The 90% confidence intervals for C_{max} were 91.36 - 119.16%, for AUC_{0-t} 87.34 - 105.45% and for AUC_{0-} 85.93 - 103.66%. The bioequivalence parameters for the extent of absorption (AUC_{0-} and AUC_{0-t}) and the rate of absorption (C_{max} and T_{max}) were all within the acceptance range for 90% CI. There were few adverse events and subjects have tolerated both treatments during both periods of the study. **Conclusions:** The results obtained in this study showed that the two tablet preparations of clarithromycin Rithro/Avenzorand Klacid®/ Abbott were bioequivalent following an oral dose of 500 mg administration under fasting condition to healthy adult males.

Keywords: Bioequivalence, Clarithomycin, Pharmakokinetic parameters.

2. Introduction

Clarithromycin is a semi-synthetic macrolide antibiotic that reversibly binds to P site of 50S ribosomal subunit of susceptible organisms and may inhibit RNA-dependent protein synthesisthere by inhibiting bacterial growth[1]. Clarithromycin and its 14-hydroxy metabolite are active against Gram-positive and -negative pathogens. Clarithromycin is used in the treatment of a wide range of bacterial infections including upper and lower respiratory infection, skin and soft tissue infection, acute maxillary sinusitis, immunomodulator in sepsis and in peptic ulcer disease as part of 2-3 drug combination for eradication of *H. pylori*[2, 3].

Clarithromycin is rapidly and well absorbed from the gastro-intestinal tract after oral administration. Following oral administration, Clarithromycin bioavailability is about 50% and its peak in plasma is 2-3 hours (immediate release) and 8 hours (extended release). Clarithromycin has half-life 3-7 hours, about 50% plasma protein binding and is widely distributed in most body tissues except the central nervous system[4, 5].

Several generic formulations are available and have been used extensively in many developed and developing countries to reduce drug expenses and thereby national medical expenditure[6, 7, 8].According to the European Medicines Agency[9], a generic drug is a medicinal product having the same qualitative and quantitative composition in active substances and the same pharmaceutical form as the reference medicinal product and whose bioequivalence with the reference medicinal product has been demonstrated to be within acceptable predefined limits. Such limits are represented by the AUC and AUC values ranging from less than 20% to more than 25% of the brand name drug values (90%CI 0.8–1.25).

A post-marketing study by Nightingale [10] looked at quality of 65 generic clarithromycin products manufactured in 18 countries with that of the innovator product manufactured by Abbott Laboratories, showing that generic tablets are sometimes not comparable in vitro with the originator product or do not reach bioequivalence. These findings suggest that results achieved with branded clarithromycin (Abbott Laboratories) should not be extrapolated to generic products. Since generic products do not consistently meet the specifications of the Abbott product, bioequivalence studies with the generic products are warranted to determine its PK parameters. In Europe [8] and USA [11], the classical way to generate equivalence between different formulations is bioequivalence study based on in vivo bioavailability.

In this context, the aim of this study was to assess bioequivalence between a single dose from two products: Rithro Tablets (500 mg clarithromycin), produced by Avenzor, as a test product, in comparison with Klacid ® Tablets (500 mg clarithromycin), produced by Abbott, as a reference product in healthy male subjects under fasting conditions.

3. Subjects and Methods

3.1 Drug Description

Clarithromycin is a semi-synthetic macrolide antibiotic. Chemically, it is 6-O-methylerythromycin. The molecular formula is $C_{38}H_{69}NO_{13}$, and the molecular weight is 747.96.
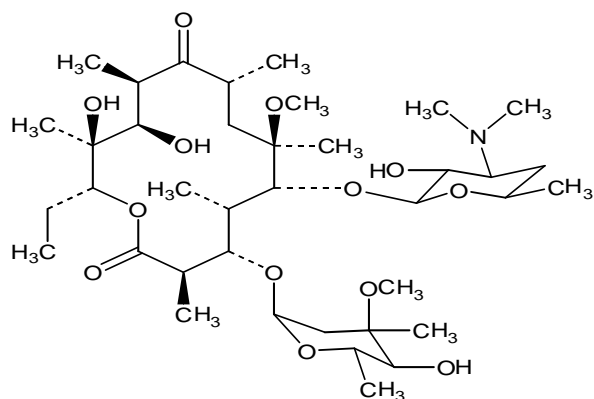
The structural formula is:

Figure 1: Clarithromycin

3.2. Subjects

Twenty-six adult males participated in the study. The mean age ± SD of subjects were 25.32 ± 5.66 (range, 19 to 43 years), and body mass index was 24.54 ± 2.97 kg/m^2 (range, 20.3 to 30.1 kg/m^2). All subjects were judged to be healthy on the basis of the results of medical history, clinical examination, blood pressure, ECG recordings and hematological analyses. Subjects included in this study had no history of or a contraindication/ allergy to study drug, had not taken any drugs for at least 7 days prior to and during the study and avoided alcohol or xanthine-containing foods and beverages for 48 hours prior to and during the course of the study.

3.3. Study Design

This study was conducted as an open label, randomized, fasting, single dose, two-treatment, two-sequence, balanced, and two-period crossover design with a washout interval of one week between dosing. The test preparation used was Rithro Tablets (500 mg clarithromycin), produced by Avenzor, as a test product, and Klacid ® Tablets (500 mg clarithromycin), produced by Abbott, as a reference product.

The study took into consideration the design for bioequivalence studies described in the US FDA guidance on bioavailability and bioequivalence studies[11]and the EMEA guidelines[9]. The subjects were randomly divided into two groups using simple randomization (table of random numbers). Subjects were equally distributed to the two possible sequences (TR & RT, T for test & R for reference) using the table of random numbers.

After an overnight fasting of at least 10 hours, each subject received a single 500 mg tablet of either the test or the reference product with 240 ml of bottled tepid water. Standardized lunch was served at 4 hours after drug administration. All subjects were instructed to avoid xanthine-containing products and grape fruit juice during the study, as clarithromycin and grape fruit are major inhibitor of cytochrome P450 3A4 isozyme[12]. The study took into consideration the principles of the Declaration of Helsinki for medical research involving human subjects [13].

3.4. Sample collection and analysis

Blood samples (7 ml) were collected from all subjects into pre-labeled heparinized tubes through an indwelling catheter in the subject forearm according to the following schedule:

Before dosing (zero time) and at 20, 40, 60, 80 and 100 minutes and 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16 and 24 hours post dosing.

Collected blood samples were immediately centrifuges at 4°C, 4000 rpm for 5 minutes. Supernatant plasma was transferred and divided into two pre-labeled Eppendorf tubes. Samples were stored at− 70 $\pm$ 15°C until analysis.

Concentration of clarithromycin in plasma was determined using a validated high liquid chromatographic assay with electrochemical detection.

The methods were developed in agreement with the US FDA "Bioanalytical Method Validation" guideline[14]. The method validation included Specificity, Linearity, Precision, Accuracy, Recovery and Stability.

This assay employed samples that were cleaned online via a column-switching technique; roxithromycin was used as the internal standard. The lower limit of quantitation (LLOQ) of clarithromycin in plasma was 50 ng/mL. A standard curve including blank matrix was generated for each analytic run and was used to determine clarithromycin concentrations in the unknown authentic sample. No determinations were done by extrapolation below the LLOQ or above the upper limit of quantitation of the standard calibration curve.

Calibration curves standards pass with a deviation of less than 15 % from nominal value except for the LLOQ which should not deviate more than 20 %. 75 % or a minimum of six standards should pass including the LLOQ and upper limit of quantification.

At least 67 % of analyzed quality control samples should be within 15 % of their respective nominal value.

3.5. Pharmacokinetic and statistical evaluation

Pharmacokinetic parameters for clarithromycin for test and reference product were calculated from the plasma concentration-time profile of Clarithromycin: T_{max}, T_{half}, $K_{elimination}$ (λ_z), C_{max}, AUC_{0-t}, & AUC_{0-} .
Non-compartmental analysis was followed in calculating pharmacokinetic parameters using commercially available computer software program (Kinetica 2000 version 4.0, InnaPhase Corporation, Philadelphia, Pennsylvania)as follows:

The values C_{max} and T_{max} were obtained directly from the concentration versus time curve of each subject.
The terminal elimination rate constant ($K_{elimination}$ or λ_z) was estimated for each subject and for each treatment via linear regression of the last points (at least three points were used) at the terminal phase of the log-concentration versus time curve of each subject.

T_{half} was calculated from $0.693/\lambda_z$

AUC_{0-t} was calculated by Trapezoidal rule.

AUC_{0-} was calculated from the sum of AUC_{0-t} and AUC_{t-} (Extrapolated Area ($AUC_{Extrapolated}$)) which is

the Area under the plasma concentration-time curve from t_{last} to infinity, calculated as C_{last}/λ_z. It is also called residual Area ($AUC_{Residual}$) or tail Area (AUC_{tail}))

The % extrapolated AUC was calculated as ($AUC_{t-\infty}/AUC_{0-\infty}$) $\times$ 100

T_{half}, $K_{elimination}$, $AUC_{0-\infty}$ were not reported for subjects who did not exhibit obvious terminal elimination phase. Data from subjects who had experienced emesis within 2 times median T_{max} was excluded from the analysis.

Statistical analysis included descriptive statistics: Arithmetic Means, Geometric Means, Ratio of Means, Maximum Values, Minimum Values, Standard Deviation (SD), Coefficient of Variation (CV), Number of data points (N).

One-way-analysis of variance (ANOVA) was used for the calculation of the effect of the following factors: Period, sequence, subjects nested in sequence and treatment (Formulation or Product). ANOVA was calculated for the untransformed values of T_{max}, T_{half}, $K_{elimination}$ (λ_z), C_{max}, AUC_{0-t}, & $AUC_{0-\infty}$. ANOVA were also calculated for the log-transformed values of C_{max}, AUC_{0-t}, & $AUC_{0-\infty}$.

The average bioequivalence of the products was concluded if the two-sided 90 % confidence interval for the test to reference ratio of the population means was within 80 – 125 % for each of the in transformed data C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

For all parameters, the difference between formulations was considered statistically significant at $P < 0.05$. The two formulations were considered bioequivalent if the 90% CI for the test to reference ratio of intransformed C_{max} and AUC fell between 0.80 and 1.25. The FDA guidance for statistical analysis [15] has been considered in the analysis of the study data.

Results

The plasma clarithromycin concentration-time curves for the test and reference preparations are illustrated in Figure 1. Clarithromycin was rapidly absorbed and was measurable 20 minutes after oral administration. Mean peak concentration (C_{max}) was reached 1.53 and 1.84 hour after oral dosing of test and reference compounds, respectively (Table 1). The mean terminal half-life (T_{half}) was 4.68 ± 0.94 hours and 4.68 ± 0.94 hours for the test and reference formulation, respectively.
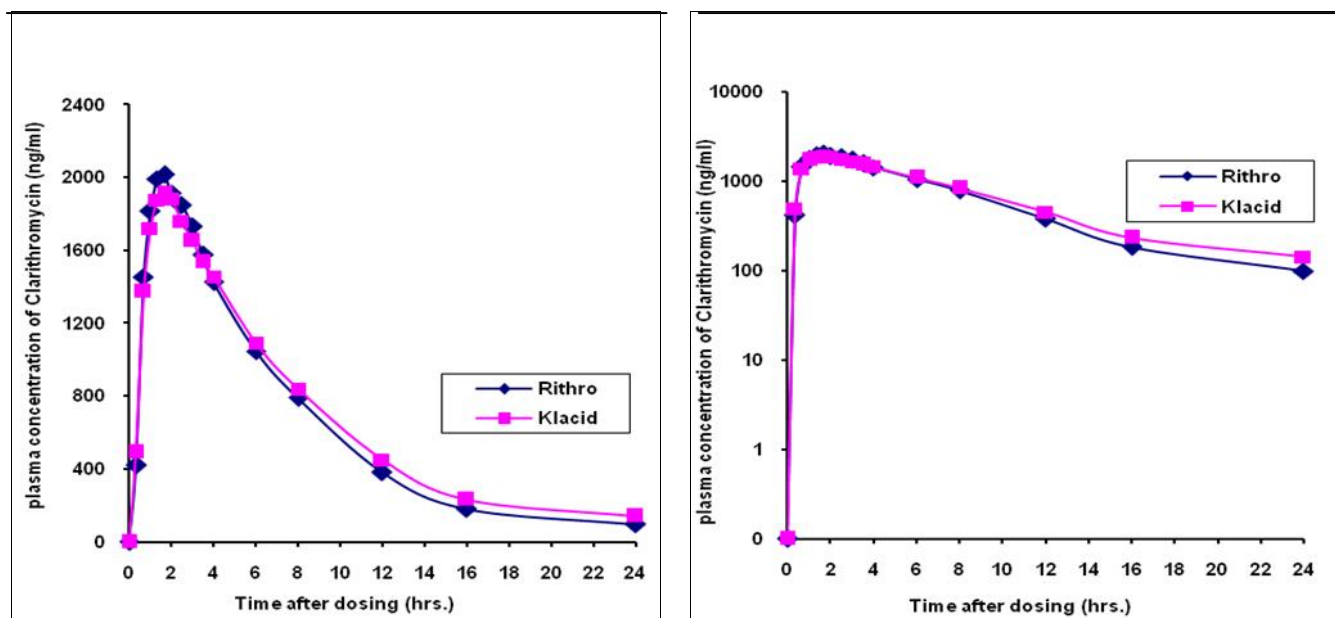

Figure 1. Mean Plasma clarithromycin concentration-time curve (a) and Log curve (b) following a single oral dose of 500 mg clarithromycin in reference (Klacid®, Abbott) and test (Rithro, Avenzor) formulations to healthy subjects (n=26).

The peak plasma concentration (C_{max}) for the test and reference compounds (mean ± SD) were 2175.3 ± 686.4 and 2089.2 ± 632.6 ng/ml, respectively (Table 1). The area under the plasma concentration-time curve (from time 0 to time t (AUC_{0-t}) were 15221.4 ± 4669.0 and 16070.5 ± 5457.2 ng·h/mL, respectively. There were no statistically significant differences ($p > 0.05$) in the plasma concentration-time profile of the test and reference compound (Table 1).

Table 1. Pharmacokinetic results: Mean ± SD of Pharmacokinetic Parameters calculated for the test product Rithro Tablet (500 mg clarithromycin)**,** and reference product Klacid® Tablets (500 mg clarithromycin).

Parameter	Test Product (Mean ± SD)	Reference Product (Mean ± SD)	Ratio T/R (Mean ± SD)	P value: ANOVA
C_{max}(ng/ml)	2175.3 ± 686	2089.2 ± 633	1.14 ± 0.60	0.346*
AUC_{0-t} (ng Î hr/ml)	15221 ± 4669	16071 ± 5457	1.0 ± 0.28	0.812*
AUC_{0-} (ngÎ hr/ml)	15761 ± 4885	17080 ± 6577	0.98 ± 0.26	0.538*
T_{max}(hr)	1.53 ± 0.45	1.84 ± 1.17	1.05 ± 0.47	0.643*
T_{half} (hr)	4.68 ± 0.94	4.90 ± 1.68	1.01 ± 0.22	0.170*
$K_{elimination}$(hr^{-1})	0.153 ± 0.028	0.156 ± 0.049	1.04.± 0.30	0.070*

C_{max} = peak plasma concentratin; AUC_{0-t} = area under the plasma concentration curve for time zero to time t; AUC_{0-} = AUC for time zero to infinity; T_{max}= time to reach C_{max}; **T_{half}** = elimination half -life; $K_{elimination}$ =terminal elimination constant; %AUC_{extra} = % AUC extrapolated.
ANOVA: Analysis Of Variance Test.
* No Significant difference (P > 0.05).

The results obtained showed that the relative bioavailability between the reference and test compound for C_{max} (91.36 - 119.16%), AUC_{0-t} (87.34 - 105.45%) and AUC_{0-} (85.93 - 103.66%) were all within the bioequivalence acceptance criteria of 80-125 % (Table 2).

Table 2. Results of the two-sided 90 % confidence interval (90% CI) and Geomean for test to reference ratio of formulations of clarithromycin 500-mg tablets (Acceptance criterion for bioequivalence: 90% CI, 80–125.%)

Parameter	Geomean Ratio	Lower Limit	Upper Limit
C_{max}	104.34	91.36	119.16
AUC_{0-t}	95.97	87.34	105.45
AUC_{0-}	94.38	85.93	103.66

Both preparations were well tolerated by the participants. One subject had epigastric pain 1.2 hour post dosing of reference compound. This was mild and transient and did not result in any withdrawal from the study.

Discussion

The use of generic preparation of a therapeutically well-established active drug has to be justified by a well-designed, conducted and analyzed bioequivalence study. Ramirez et al [16] evaluated 124 bioequivalence studies with 80 active substances, showing that the plasma concentration-time profile is highly affected by the performance of the dosage formulation as bioequivalence of generic compound may differ from brand compound despite the similarity provided by usually employed test dissolution methodology. This study was carried in healthy male Iraqi subjects to assess bioequivalence of Rithro**,** a generic product produced by Avenzor from Syria with Klacid® Tablets (500 mg clarithromycin), produced by Abbott, as a reference product.

The results of this study demonstrated that the plasma concentration-time profile of generic and reference compound had comparable pharmacokinetic parameters.

Clarithromycin undergoes significant first-pass metabolism to its active 14-hydroxy metabolite[17]. In this study, evaluation of bioequivalence of clarithromycin was based upon the measurement of the parent compound. This approach is supported by Lerner et al. [18] who suggested measurement of clarithromycin in bioequivalence studies as there is no significant differences in the bioequivalence of either the parent drug or the its active metabolite.

Comparison of the therapeutic performance of two medicinal products containing the same molar dose and under similar experimental conditions in terms of the rate & extent of absorption is critical for assessing the possibility of supplanting an innovator with any essentially similar medicinal product; pharmaceutical alternative [19]. The results of this study showed that the pharmacokinetic parameters for the Rithro and Klacid® compounds in regards to extent of absorption ($AUC_{0-\infty}$ and AUC_{0-t}) and the rate of absorption (C_{max} and T_{max}) were not significantly different. In addition, the average bioequivalence of the products was to be concluded if the two-sided 90 % confidence interval for the test to reference ratio of the population means is within 80– 125 % for each of the ln-transformed data C_{max}, AUC_{0-t} & $AUC_{0-\infty}$. The results obtained for the two formulations showed that the 90% CIs for the ratios of mean C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 1.04, 0.96 and 0.94, respectively, thus meeting FDA bioequivalence acceptance criteria of 0.8 to 1.25.

Taken together, the pharmacokinetic and bioequivalent values obtained with the test and reference formulations were not significantly different which reflects the similar characteristics of the two formulations and can interchangeably be used in clinical practice.

In this study, the elimination half-life of the reference compound in plasma was 1.84 h after a single dose of 500 mg. In comparison to the results of the present study, earlier bioequivalence studies have shown that the mean C_{max} values were lower in Greek male volunteers 1884 ng/ml [20] and $T_{1/2}$ values were longer in healthy male Greek (2.38 h) and Thai (3.1 h) volunteers[20, 21]. These differences may indicate that race is an influential factor on the pharmacokinetic profile of clarithromycin.

The study drugs were well tolerated by all subjects with minor and transient adverse effects.

Conclusion

The results obtained showed that the test product Rithro Tablets (500 mg clarithromycin), produced by Avenzor were essentially bioequivalent to the reference product Klacid® Tablets (500 mg clarithromycin), produced by Abbott, and it can thus be assumed that they are therapeutically equivalent and exchangeable in clinical practice. There were negligible adverse events and subjects have tolerated both treatments during this study.

Acknowledgments

Conflict of interest

The author has declared no conflict of interest.

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