

BIOEQUIVALENCE STUDY OF TWO ORAL FORMULATIONS OF CLARITHROMYCIN IN HUMAN MALE SUBJECTS

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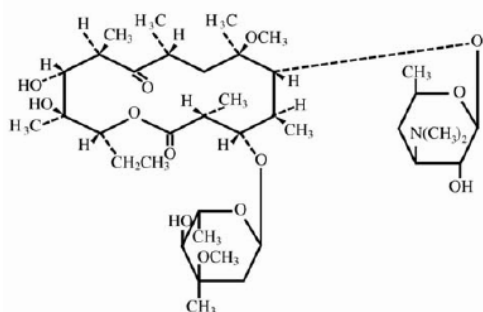
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ABSTRACT

To assess the bioequivalence of tablets formulations of Clarithromycin 500mg each of test and reference products. A single post oral dose of each formulation was given to 14 male healthy volunteers. The study was conducted phase 1, open-label, randomized, complete two-way crossover designed with 7 days wash out period. The plasma concentration of Clarithromycin was quantified by validated microbiological assay method. The precision of the method was evaluated using calibrated 14-hydroxyClarithromycin concentration was detected semi quantitatively as equivalent of Clarithromycin /ml. The peak plasma concentrations of $(3.63 \pm 0.80 \text{ ug/ml})$ and $(3.31 \pm 0.35 \text{ ug/ml})$ was attained in about 1.42 hours and 1.49 hours for both test and reference Clarithromycin tablets respectively. The mean $\pm$ SD values for total area under the curve (AUC) were 22.07 ± 4.90 and $20.16 \pm 2.35 \text{ h.mg/L}$ for both test and reference tablets respectively. This study indicated that the differences in all the bioequivalence parameters for test and reference Clarithromycin formulations are statistically non-significant; hence both formulations are considered bioequivalent.

Keywords: Clarithromycin, area under the curve, peak plasma level, bioequivalence studies.

INTRODUCTION



$\text{C}_{38}\text{H}_{69}\text{NO}_{13}$ (Erythromycin, 6-O-methyl-6-O-methylethylerythromycin)

The macrolides stand out as antibiotics for various infections mostly those concerning intracellular and/or respiratory pathogens. Erythromycin is a successful drug for a lot of acute or facial infections. 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 enhanced patient compliance. Macrolide concentration in inflammatory cells and transport to the site of infection is a diverse advantage over other antibiotics. Clarithromycin is a macrolide antibiotic with wide range of action in vitro against clinically vital gram positive aerobes and

anaerobes. The action of Clarithromycin is better by its widespread distribution into tissues and by the development of primary microbiologically active metabolite, 14(R) hydroxyClarithromycin (1988). Clarithromycin has been accepted as a drug for managing of upper and lower respiratory tract infections and has shown to have an elevated rate of clinical efficacy for respiratory tract and skin infections and excellent patient tolerance. The action of Clarithromycin is improved by its widespread distribution into tissues and the formation of a therapeutically active metabolite, 14(R) hydroxyl-Clarithromycin. Numerous investigators have studied the pharmacokinetics, drug-drug interactions, and clinical safety of this macrolide. Clarithromycin has the major advantages above other macrolides, like Erythromycin, is its improved range of action which include gram negative, H. Influenzae. Clarithromycin's activity against H. influenzae is more or less exclusively due to that of 14(R) hydroxyl Clarithromycin (14OHC) and MICs 90% of isolates are inhibited, against 1mg/liter, respectively. Approximately 25% of the bio-available Clarithromycin is metabolized to 14(R) hydroxyl Clarithromycin (14HOC) and as result its peak concentrations that near the minimum inhibitory concentration (MIC) at which 90% of isolates are inhibited for H. influenzae. As result, anything that decreases the concentration of 14(R) hydroxyl Clarithromycin may negatively affect the Clarithromycin's activity against H. influenzae. In the past this occurs in nature due to the normal inter-individual inconsistency of any metabolic process. This metabolic inconsistency has translated into H.influenzae

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eradication changeability in clinical trials (range 20 to 100% eradication).

MATERIALS AND METHODS

The study was conduct by applying the norms of Good Clinical Practice guidelines. Persons who volunteered were registered for the advance study. The age was between twenty to twenty four years, male and healthy non-smoker volunteers with homogenous body weight.

Drug information

Test drug: Clarithromycin 500 mg Tablets

B. No. Test

Mfg.date November 2008.

Exp.date Use within two years.

Only for Experimental purpose.

Reference drug: *Clarithromycin* 500 mg Tablets (market image)

An overnight fasting, volunteers received one dose of Clarithromycin 500-mg each of reference or test tablets with 240 ml of water in randomized designed by dividing into two groups of fourteen volunteers in each group. The bioequivalence studies with two formulations a replicated-crossover design was use. A washout period of seven day between dosing of test and reference tablets employed.

Sample collection and treatment

A control venous blood sample was taken from every volunteer as blank before drug administration. After post oral drug dosing, successive blood samples of volume 5ml were drawn at time interval 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, and 12 hours in heparinized centrifuge tubes particularly arranged for this study. Prior to freezing these tubes were centrifuged under refrigeration for 15

minutes at about 2000 rpm. The plasma was collected by separation technique and store at -20°C until on the day of analysis.

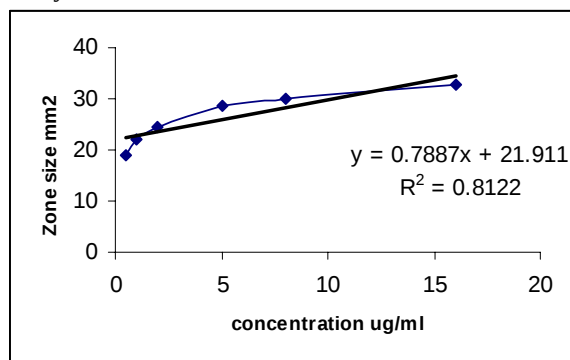


Fig. 1: Standard curve of Clarithromycin in plasma.

Demographic and clinical data

Demographic and estimated clinical data were recorded for all the volunteers are depicted in fig. 2.

Microbiological agar diffusion method

Clarithromycin content in the plasma sample was estimated by using Microbiological assay technique using *Streptococcus faecalis* as investigation organism employed by Arrct *et al.* 1971. This procedure has the advantage to estimate the microbiologically active moieties of drug in biological samples, hence, considered a legitimate method for analysis of most of antibiotics. For estimation of Clarithromycin in biological fluid (duplicate) the Disc Agar Diffusion technique was employed. The concentrations of Clarithromycin in the samples were estimated by zones of inhibitions by using regression equation standard curve fig.1. The standards were run with each analysis. The regression coefficient value was ($R^2 = 0.8122$).

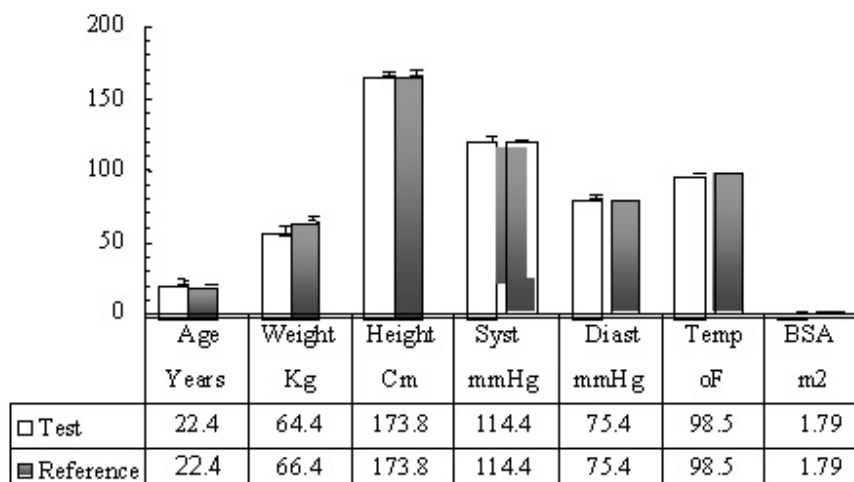


Fig. 2: Showing mean values for age, body weight, height, blood pressure, body temperature and body surface area of all healthy male volunteers used for the study of bioequivalence of Clarithromycin.

Conc.ug/ml	Average (n=5) Zone size (mm)
0.5	19.1
1	22.2
2	24.4
5	28.6
8	30.0
16	32.8

Bioequivalence/Bioavailability parameters

The plasma content of Clarithromycin from every volunteer will be plotted on a semi logarithmic scale against time. This data was used to calculate pharmacokinetics and bioavailability parameters with the help of a PC-Computer Program, APO, MWPHARM version 3.02 a MED1WARE product Holland. Area under curve (AUC) from time t to ∞ (infinity) was calculated with poly-exponential and trapezoidal methods. Bioavailability parameters such as C_{max} , T_{max} and AUC were determined and bioequivalence comparisons were performed by Student t-test: paired two samples for means.

RESULTS

The demographic data of volunteers participate in the present research work on pharmacokinetic/bioequivalence of Clarithromycin test and reference samples are represented in fig. 2. This is apparent from the result that healthy male subjects in both groups are homogenous in terms of mean $\pm$ SD age (22.4 & 22.4 years), weight (64.4 & 64.4 kg), height (173.8 & 173.8 cm), and body surface area (1.79 & 1.79 m^2). The plasma mean drug

concentration-versus-time profiles are presented in fig. 3 collected from study subjects following oral administration of Clarithromycin. The plasma mean concentration (ug/ml) of Clarithromycin test and reference in fourteen volunteers are presented in table 1. The maximum plasma concentrations of Clarithromycin (3.63 ± 0.80 ug/mL) and (3.31 ± 0.35 ug/mL) was attained in about 1.42 ± 0.28 hour and 1.49 ± 0.16 for both test and reference respectively. Total area under the curve (AUC) mean $\pm$ SD values were 22.07 ± 4.90 , and 20.16 ± 2.35 hmg/1 for both test and reference tablets. Pharmacokinetic mean value for both Clarithromycin tablets are shown in table 2. The clearance mean $\pm$ SD values were 23.83 ± 5.70 and 25.08 ± 3.78 l/h for both formulations respectively. The half-life ($t_{1/2}$) expressed in hrs are 3.17 ± 0.71 and 3.09 ± 0.62 respectively. It is apparent from the results presented in table 2 that all the pharmacokinetic parameters for both Clarithromycin tablets are statistically non-significant.

The assessment of mean $\pm$ SD "bioequivalence" and pharmacokinetics parameters of Clarithromycin Test and Reference formulations are presented in table 2.

DISCUSSION

Statistical evaluation of the bioequivalence among the two tablet formulations did not disclose any major differences which have been summarized in table 2. The accessibility of this significant drug in a variety of brands in Pakistan raises the need to carry out pharmacokinetic and bioequivalence studies for a variety of tablet

Table 1: Plasma concentration (μ g/mL) of Clarithromycin test tablet and 500 mg reference tablet in 14 healthy Male subjects.

Time Hours	0.5	1	1.5	2	2.5	3	4	5	6	8	12
Test	0.88 $\pm$ 0.46	1.61 $\pm$ 0.71	2.71 $\pm$ 0.91	2.84 $\pm$ 1.02	2.48 $\pm$ 0.84	2.12 $\pm$ 0.69	1.70 $\pm$ 0.61	1.48 $\pm$ 0.57	1.26 $\pm$ 0.54	0.83 $\pm$ 0.32	0.00 $\pm$ 0.00
Reference	1.58 $\pm$ 0.69	2.69 $\pm$ 0.85	2.64 $\pm$ 0.69	2.36 $\pm$ 0.66	2.08 $\pm$ 0.63	2.08 $\pm$ 0.63	1.39 $\pm$ 0.41	1.11 $\pm$ 0.29	0.80 $\pm$ 0.29	0.80 $\pm$ 0.29	0.00 $\pm$ 0.00
Ratio T/R	1.11	1.13	1.04	1.05	1.00	1.04	1.03	1.06	1.05	1.00	1.00

Table 2: Mean $\pm$ SE Bioequivalence parameters of Clarithromycin test and reference tablet in 14 healthy male subjects.

Bioequivalence parameters	Units	Test Mean $\pm$ SD	Reference Mean $\pm$ SD	% Difference	t- test two tail	P >0.01
AUC	[h.mg/l]	22.07 $\pm$ 4.90	20.16 $\pm$ 2.35	9.494	0.2193	NS
Ln(AUC)	[h.mg/l]	1.3332 $\pm$ 0.1010	1.301 $\pm$ 0.0562	2.450	0.3334	NS
Tmax	[h]	1.42 $\pm$ 0.28	1.49 $\pm$ 0.165	4.619	0.4112	NS
Cmax	[mg/L]	3.63 $\pm$ 0.80	3.31 $\pm$ 0.35	9.746	0.2010	NS
Ln(Cmax)	[mg/L]	0.5497 $\pm$ 0.0988	0.517 $\pm$ 0.0496	6.308	0.3035	NS
Cl	[l/h]	23.83 $\pm$ 5.70	25.08 $\pm$ 3.78	4.990	0.5218	NS
T $\frac{1}{2}$ β	[h]	3.17 $\pm$ 0.71	3.09 $\pm$ 0.62	2.567	0.7638	NS
MRT	[h]	5.36 $\pm$ 0.60	5.26 $\pm$ 0.42	1.899	0.6348	NS

formulations in objective population. The current scheme was based, to examine the disposition and bioequivalence of two post oral (500mg) administration of Clarithromycin tablets. The systemic absorption of post oral drug in a solid dose is comprises of three different steps that can notably affect the pharmacokinetic and bioequivalence parameters:

1. Disintegration of the drug product.
2. Dissolution of the drug in the fluids at the absorption site.
3. Transfer of drug molecules across the membrane lining the gastrointestinal tract into the systemic circulation.

Bioequivalence is an evaluation of the bioavailability of two drug formulations or more and these contain the similar drug molecules are bioequivalent if their rates and amounts of absorption are the same. For bioequivalence studies, maximum plasma concentration (C_{max}), maximum time (T_{max}) and AUC are frequently use parameters (table 2). Giving post oral of 500mg Clarithromycin, (C_{max}) of (3.63 ± 0.80 ug/mL) and (3.31 ± 0.35 ug/mL) were achieved in about 1.42 ± 0.28 hour and 1.49 ± 0.16 (T_{max}) for both test and reference Clarithromycin (fig. 3). These values are comparable to the literature values of 2.34 ug/ml & 2.27 ug/ml with T_{max} 1.94 h & 1.92 (TsigHua 1998), 3.09 ± 0.29 ug/mL & 2.98 ± 0.37 ug/mL with T_{max} 1.37 ± 0.35 h & 1.56 ± 0.42 h (TsigHua 2000) and 2.47 ± 0.30 ug/ml & 2.26 ± 0.25 ug/ml with T_{max} 2.06 ± 0.2 h & 2.11 ± 0.42 h (TsigHua 2003), respectively with 500 mg P.O dose. In current study the mean $\pm$ SD values for total AUC were 22.07 ± 4.90 , and 20.16 ± 2.35 mg h/1 for both test and reference tablets. This parameter are comparable to the reported values of 21.7 ± 2.71 mg.h/ml & 22.42 ± 3.5 , 22.6 ± 3.6 & 23.0 ± 3.9 and (21.50 ± 3.53) 19.10 ± 2.39 & 19.92 ± 2.63 (TsigHua 1998, 2000 & 2003) . The half-life ($t_{1/2\beta}$) expressed in hours showed the 3.17 ± 0.71 and 3.09 ± 0.62 respectively and comparable to 4.59 ± 0.99 & 4.72 ± 0.85 /h and 4.07 ± 0.92 and 4.09 ± 0.71 /h (TsigHua 1998, 2000).

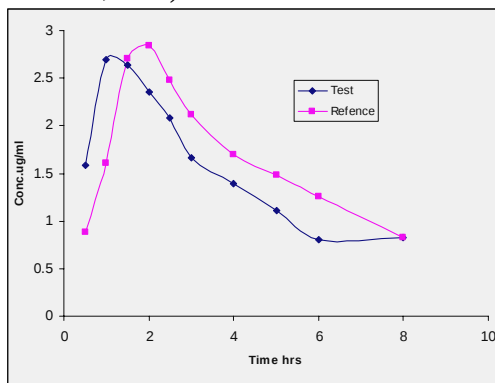


Fig. 3: Showing mean $\pm$ SD values of the plasma concentration of Clirithromycin after 500 mg oral dose of Test and Reference Tablets given to 14 volunteers on both ordinary graph scale.

In current study the critical bioequivalence parameters i.e. AUC, T_{max} and C_{max} of both test and reference Clarithromycin tablets are in the range of 80 to 125 % (109.5%). So this work demonstrates that the bioequivalence metrics among the bioavailability parameters of Clarithromycin tablet did not illustrate major differences, therefore both test and tablets are bioequivalent.

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