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A Randomized, Single Dose Bioequivalence Study of Two Tablet Formulations of Balofloxacin In Healthy Human Subjects Under Fasting Conditions

Prabhavathi K[1*], JC Shobha[1], Radhika S[1]

1.Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences (NIMS), Hyderabad

ABSTRACT

To compare the bioavailability of two tablet formulations of Balofloxacin 100 mg in adult, male, healthy human subjects under fasting conditions.The study was conducted as an open label, balanced, randomized, two-period, two-sequence, two treatment, single dose cross over study to determine the bioequivalence of two tablet formulations of Balofloxacin 100 mg in 24 healthy, adult, male, human subjects under fasting conditions. Serial blood samples were collected at 0, 0.5, 1.0, 1.50, 2.0, 2.50, 2.75, 3.0, 3.25, 3.50, 3.75, 4.0, 4.50, 4.75, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0h during each study period. A washout period of 7 days was given between two study periods. 90% confidence interval(CI) for the ratio of logarithmic transformed pharmacokinetic parameters Cmax, Tmax, AUC0-t and AUC 0- ∞ were used to determine bioequivalence. The data of 23 subjects was analyzed in the study. Tmax (hrs), Cmax (µg/ml), AUC 0-t and AUC0-∞ (µg.h/ml) for test formulation were 1.174±0.535 , 852.431±361.274, 4682.785±1616.552 and 5375.882±1727.286 and that of reference formulation were 1.033±0.428, 850.238±312.422, 4447.628±1240.125 and 5125.723±1304.619 respectively. The 90% CI for the T/R ratios of log transformed Cmax, AUC0-t and AUC 0-∞ were 89.58%-108.19%, 98.86%-109.88% and 97.70%-110.01% respectively. The two tablet formulations of Balofloxacin 100mg (both test and reference) met the requisite bioequivalence criteria (80-125%) .

Keywords: Bioequivalence, Balofloxacin, Urinary Tract Infection

*Corresponding Author Email: prabhas_world@yahoo.co.in

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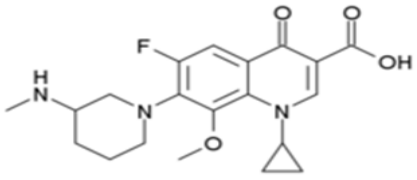
INTRODUCTION

Balofloxacin (Q-35), an orally active new 3rd generation fluoroquinolone antibiotic of molecular mass 389.42 g/mol with chemical formula C20H24FN3O4 has been developed by Choongwae pharmaceuticals, Korea and approved by the Korean FDA in December 2001 for UTI. [1,2] In India, it was approved for the treatment of uncomplicated UTI in 2009. [3] Very limited clinical studies are available for Balofloxacin (Q-35), to understand its existence and importance as an anti-infective agent.[1]

Balofloxacin (Q-35) has broad spectrum of activity against various respiratory pathogens, including gram-positive and gram negative organisms[4] i.e. methicillin-resistant staph aureus,[5] L.interrogans,[6] Mycoplasma, E. coli, and P.aeruginosa, Enterobacter aerogenes. Addition of piperidine group at C7 position [Figure1] gives enhanced activity against Gram positive bacteria. The introduction of a methoxy group into the 8 position [Figure1] of quinolones contributes to markedly decreased phototoxicity and increased activity against anaerobic bacteria and quinolone resistant staphylococci.[7] The antimycobacterial activity of balofloxacin was comparable or slightly inferior to that of levofloxacin (LVFX) for tuberculosis and other various atypical mycobacterial species. Balofloxacin tablet is as effective and safe as levofloxacin tablet in the treatment of uncomplicated urinary tract infection.[8]



Balofloxacin (Q-35)

Systematic (IUPAC) name: 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylaminopiperidin-1-yl)-4-oxoquinoline-3-carboxylic acid[1]

Figure 1: Structure of Balofloxacin (Q-35)

HPLC is the most sensitive, rapid and accurate method for determination of Balofloxacin in human plasma and study its pharmacokinetics and relative bioavailability.[9] Pharmacokinetic data revealed that Balofloxacin is well-absorbed after oral doses.[10] It has time to peak concentration of 1 hour. [10,11] In healthy subjects, mean peak plasma levels of 2.2 µg/mL were achieved 1 hour after single dose of 200 mg. Steady-state volume of distribution in healthy subjects after 200mg doses is 38L. Optimal doses for oral route have not been clearly established while analysis of data from pharmacokinetic studies suggests that twice daily dosing may be required in many patients. [10]

As pharmacokinetic data revealed that most of an oral dose is excreted unchanged in the urine, [10] dose reduction may be required in patients with renal impairment. Urinary excretion data suggest minimal hepatic metabolism. Elimination half-life is 7 to 8 hours. Delayed and reduced urinary recovery and a prolonged half-life of balofloxacin have been reported in elderly subjects (66 to 79 years of age), total/renal clearance was also reduced and renal clearance correlated significantly with creatinine clearance (CLcr). The absorption of the drug was also delayed in the elderly, most likely related to reduced gastrointestinal motility.[10] Dose adjustments for balofloxacin in elderly patients should be based on renal function.

In RTI, UTI (uncomplicated or complicated) and obstetric or gynaecological infections, oral doses of 100 to 400 mg once or twice daily have been administered. Duration of therapy was 3 to 14 days in RTI/UTI and 3 to 9 days in obstetric/gynaecological infections.[12,13,14] Nausea, heartburn, dizziness, fever, indigestion, urticaria are the common adverse effects.

Very limited clinical studies are available for Balofloxacin (Q-35), to understand its existence as an anti-infective agent and it is not yet licensed in many other countries including US.[1] But however as it is available in India under various brand names for the treatment of uncomplicated UTI such as cystitis and urethritis [3] and as the same formulations of the same drug by different manufacturers may differ with respect to bioavailability, this study has been taken up with the objective to compare the bioavailability of two tablet formulations (test and reference) of balofloxacin 100mg in healthy, adult, male, human subjects under fasting conditions.

MATERIALS AND METHODS:

Ethical considerations:

The study was conducted according to a protocol approved by the Institutional ethics committee. This research was carried out in accordance with the Good Clinical Practice guidelines and the principles enunciated in the Declaration of Helsinki. All the subjects provided written informed consent before entering the study.

Study Design:

The study was conducted as an open label, balanced, randomized, two period, two sequence, two treatment, two way cross over bioequivalence study in 24 healthy adult, male, human subjects, under fasting conditions to compare the bioavailability of the test product (T) with the reference formulation (R).

Study subjects:

The study included male human subjects who voluntarily have given their written informed consent to participate in the study, aged 18-45yrs with a body weight and height within normal

range and healthy as evidenced by medical histories, complete physical examination, routine laboratory tests and ECG performed within 21days prior to commencement of the study. Subjects were excluded if they have received any medication during the two weeks period prior to the start of the study. They were not permitted to take any prescription and OTC medications, grape fruit juice subsequently until the completion of the study. Subjects had to abstain from any xanthine-containing food or beverages or alcoholic products for 48 h prior to dosing and throughout the sampling schedule during each period. Smokers and alcoholics were also excluded from the study.

Study Methodology:

Eligible subjects were admitted and housed at the study site, 12 h before dose. Each subject received single oral dose of assigned formulation after an overnight fast of at least 10hrs based on randomization along with 240ml of drinking water in each period. Sitting /upright posture was maintained for 2hrs post dose. A total of 23 blood samples (5 ml each) were collected pre-dose and at 0.50, 1.0, 1.50, 2.0, 2.50, 2.75, 3.0, 3.25, 3.50, 3.75, 4.0, 4.25, 4.50, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0 and 48.0h post dose in potassium EDTA containing tubes through an indwelling cannula placed in a forearm vein during each period. After collection, the blood samples were centrifuged at 3800rpm, for 10 minutes, at 10±2ºc to separate the plasma. All plasma samples were stored in suitably labeled duplicate polypropylene tubes at -20ºC until analysis. Subjects were discharged 24 h after dose during each period. A washout period of 7 days was given between two periods. The same procedures were followed in the second period of the study.

Bioanalytical method:

A validated LC-MS-MS method with appropriate quality control samples was used for the analysis of Balofloxacin plasma samples.

Pharmacokinetic analysis:

The following pharmacokinetic parameters for both Test and Reference formulations of balofloxacin were calculated which include,

$AUC_{0\text{-}72hrs}$: The area under the plasma concentration versus time curve from 0 to 72 hrs , as calculated by the linear trapezoidal method.

$AUC_{0\text{-}\infty}$: The area under the plasma concentration versus time curve, from 0 to infinity was calculated as the sum of the $AUC_{0\text{-}72}$ plus $AUC_{72\text{-}\infty,}$ calculated by the linear trapezoidal method.

C_{max}: Maximum drug concentration achieved in systemic circulation following drug administration.

T_{max}: The time required to achieve maximum drug concentration in systemic circulation.

K(el): Apparent first order elimination rate constant calculated from a semi log plot of the plasma concentration versus time curve. The parameter was calculated by linear square regression analysis using last three (or more) non zero plasma concentrations.

T**1/2**: The apparent terminal elimination half-life was calculated as 0.693/kel

The preparations were considered to be bioequivalent when the limits of the 90% confidence interval (CI) for the ratios of the means of C_{max} and AUC of test and reference drug falls within 80%-125%.[15]

Statistical analysis:

The following analysis was conducted using WinNonlin soft ware (Pharsight Version 5.0.1 or higher). The AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values were log converted before analysis. Ratio of mean (in percentage) was calculated using the least square mean (LSM) for log transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values. Ratios of mean were expressed as percentage of the LSM for the respective treatment comparisons. The 90% CI of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for the ratio of test and reference was determined to assess the bioequivalence between different products using the equivalence interval of 0.80 and 1.25. Analysis of variance (ANOVA) was also performed and a statistically significant difference was considered at $P<0.05$.

RESULTS AND DISCUSSION:

Urinary Tract Infection caused by Gram negative and Gram positive bacteria is one of most common bacterial infections. Achievement of rapid cure is desirable to prevent the complications of urinary tract infections. [16]

Fluoroquinolones are the mainstay for treatment of urinary tract infections. They have very good urinary concentration, tissue penetration, apart from having good coverage on uropathogens with high clinical success rate. Balofloxacin, a 3rd generation quinolone, has very good activity against Gram negative and Gram positive organisms causing urinary tract infections. Unlike other fluoroquinolones, Balofloxacin has good activity against Gram positive bacteria. Also the incidence of adverse effects seen with Balofloxacin is negligible compared to other fluoroquinolones due to structural modifications. [16]

Bioavailability and/or bioequivalence studies play a key role in the drug development of generic products. Pharmacokinetic bioequivalence is used to demonstrate that generic and innovator drugs are essentially similar in terms of efficacy and tolerability. Pharmacokinetic bioequivalence studies employ healthy volunteers and crossover designs to minimize the magnitude of interindividual variability in a single-dose pharmacokinetic bioequivalence study.

Based on these considerations, we performed the present bioequivalence study to compare the bioavailability of two formulations (test and reference) of balofloxacin 100mg tablets. In the present study, 24 healthy volunteers were randomized, which satisfies the sample size requirement for a bioequivalence study.[15] The randomization of study groups was sufficiently balanced to avoid bias of sequence allocation. Although 26 eligible healthy male subjects were recruited in the study, only 24 subjects were randomized and two were standby. The analysis included data from 23 subjects out of 24 subjects who participated in the study. One subject data was not included in analysis as he did not come for the second period of the study due to unknown reason. The mean age ± S.D and weight of the subjects was 22.6±3.97yrs, 58.8kg respectively.

Table1: Mean ± SD and log transformed pharmacokinetics parameters of two formulations of balofloxacin in 23 healthy human subjects

Formulation type	Cmax (µg/ml)	AUC0-t (µg.h/ml)	AUC0-∞ (µg.h/ml)	Tmax (hr)	T1/2 (h)	Kel (h-1)
Reference(R)						
AM	850.238±312.422	4447.628±1240.125	5125.723±1304.619	1.033±0.428	6.172±1.123	0.116±0.019
GM	798.542	4311.508	4991.304	0.972	6.08	0.114
CV(%)	36.7	27.9	25.5	41.5	18.2	16.8
Test (T)						
AM	852.431±361.274	4682.785±1616.552	5375.882±1727.286	1.174±0.535	6.311±1.594	0.116±0.028
GM	801.387	4486.518	5169.443	1.057	6.132	0.113
CV(%)	42.4	34.5	32.1	45.6	25.3	23.8
Ratio of LSM T/R(%)	98.44	104.22	103.67			
90% CI T/R(%)	89.58-108.19	98.86-109.88	97.70-110.01			

AM-Arithmetic mean, GM - Geometric mean, LSM - Least square mean, CI - Confidence interval, n=23

The following pharmacokinetic parameters; Cmax, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $T_{1/2}$ and Kel values of all the 23 subjects who completed the study is shown in [Table1] as both arithmetic and geometric means. The T/R (Test/Reference) ratios for the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and Cmax were 98.44%, 104.22%, and 103.67% respectively. The 90% confidence interval(CI) for the ratios between the means of C_{max} and AUC of test and reference drug were within the limits of bioequivalence (80-125%). The mean plasma concentration vs time profiles of all 23 subjects, for test and reference products of balofloxacin 100 mg are shown in Figure 2. There was no period, sequence and formulation effect as indicated by the P values in both the groups as shown in [Table2]. Statistical analysis in our study demonstrated that sequence and

period effects were not statistically significant. The T/R (Test/Reference) ratios for the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were 98.44%, 104.22%, and 103.67% respectively. 90% CI for the T/R ratios of log-transformed pharmacokinetic parameters for both Test and Reference products were within the limits of bioequivalence.

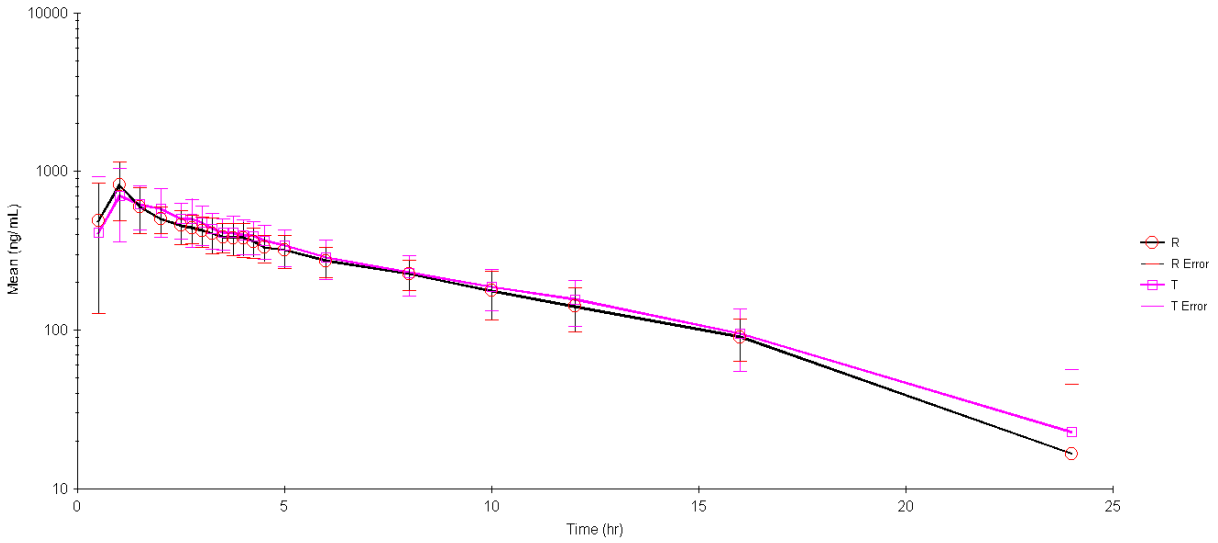


Figure 2 Mean concentration–time profiles in plasma following the administration of Balofloxacin 100 mg manufactured by different pharmaceutical companies to healthy volunteers. Each point represents the geometric mean and standard error obtained from 23 subjects

Table 2: Period, Sequence and Formulation effect as indicated by the P values

Extract from ANOVA analysis P-value	Cmax	AUC0-t	AUC0-∞
Formulation	0.7776	0.1921	0.3075
Period	0.0137	0.6972	0.8255
Sequence	0.5716	0.7766	0.7128

In our study, the geometric means of Tmax and T1/2 for both reference and test products are almost similar (0.972 and 6.08 vs 1.057 and 6.132 in hrs). In a pharmacokinetic bioequivalence study of two formulations of balofloxacin 200mg conducted in China by SUI Yin et al,[9] the half-lives for test and reference formulations were 9.10±1.70 and 8.44±1.13 hrs respectively. In the study done by SUI Yin et al, [9] Tmax values [for test, 0.88±0.64 and for reference, 0.96±0.48 hrs] were in agreement with the values that were obtained in our study [Test, 1.174±0.535 hrs and reference 1.033±0.428 hrs].

In our study T/R ratio of AUC0-∞ was 103.67% which was in agreement with the T/R ratio of AUC0-∞ (105.1%) in the study done by SUI Yin et al.[9] The two formulations (test and

reference) analyzed in the present study satisfied the criteria for pharmacokinetic bioequivalence which is 80-125%. There were no serious adverse events occurred throughout the study.

CONCLUSION:

The results obtained from 23 subjects in this study confirm that both the test and reference formulations containing balofloxacin 100mg met the bioequivalence criteria after single dose administration in adult, male, healthy human subjects under fasting conditions.

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