Comparative Bioavailability of Two Brands of Ofloxacin in Healthy Human Volunteers

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Abstract

The relative bioavailability and pharmacokinetics of a locally manufactured (Oquin) and reference (Zanocin) formulations of ofloxacin were compared. Each volunteer received a single dose of 200 mg ofloxacin tablet, test or reference with a 7 days washout period. Multiple blood samples were collected and plasma concentrations of ofloxacin were analyzed by high performance liquid chromatography technique.

Following oral administration of both formulations, there was rapid and extensive oral absorption from gastrointestinal tract achieving maximum serum concentration within 2 hrs. The concentration-time profile generated by the two products was found to be superimposable. Peak plasma concentration (C_{max}), area under the serum concentration-time curve (AUC_{0-24}), area under the serum concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) and serum elimination half-life ($t_{1/2}$) were 1.98 ± 0.213 and 1.82 ± 0.194 µg/ml, 13.19 ± 1.45 and 12.56 ± 0.965 µg.hr/ml, 13.86 ± 1.49 and 13.14 ± 0.959µg.hr/ml, 5.55 ± 1.37 and 5.55 ± 0.715 hr for Oquin and Zanocin, respectively.

The result indicates that these two formulations have similar rate as well as extent of absorption. Hence these two products can be said to have comparable bioavailability.

Keywords: Ofloxacin; Pharmacokinetics; Bioavailability

Introduction

Ofloxacin [OFX] is a well known synthetic carboxyquinolone antimicrobial agent with a broad antimicrobial spectrum against gram positive and gram-negative bacteria and is considered safe. The bactericidal action of OFX results from interference with enzyme DNA gyrase that is needed for the synthesis of bacterial DNA (Okeri and Arhewoh, 2008). Its favorable pharmacokinetic feature includes good oral absorption and lack of metabolism resulting decrease in drug interaction. The presence of the methyl piperazine ring in ofloxacin probably leads to enhanced oral absorption and a long half-life. After oral administration it is excreted in the urine both unchanged ($\approx 70\%$ of the dose) and as three metabolites (Yuk et al., 1991; Lode et al., 1987).

OFX is made and marketed by several pharmaceutical manufacturer of Nepal. Availability of different formulations of the same drug substance, at the same strength, in the same dosage form and no in-vivo bioequivalence studies to prove their bioequivalence poses a special challenge to health care professionals of this country regarding the therapeutic equivalency of these products. If different formulations have different bioavailability, switching over to new brand may lead to inadequate therapeutic effect, toxicity or even cases of drug resistance can arise (FDA, 2001a; Midha et al., 1998).

This study was performed with an aim to observe the bioavailability of two brands of OFX, Oquin (manufactured by a local manufacturer) and Zanocin which is nationally authorized innovator brand of OFX in Nepal.

Subjects and Methods

Subjects

Eight healthy, nonsmoking, adult Nepalese male volunteers (mean age ± SD, 26.375 ± 5.449; range 22 -37 years) were selected for enrollment in the study. The mean body mass index (BMI) of the subjects was 21.395 ± 2.263 kg/m². All had normal renal and hepatic function. Subjects were included in the study after normal findings from physical examination, laboratory investigations (including hematological and biochemical tests, hepatitis and human immunodeficiency virus serological test).

Exclusion criteria were any history of a significant gastrointestinal condition that could potentially impair the absorption or disposition of the study medicine, use of prescribed medication (14 days prior to study) or OTC medication (48 hours prior to study), abuse of alcoholic beverages and history of allergy to the study drug. The volunteers were asked to abstain from taking any medication (including any prescription drugs) throughout the study period.

Prior to any screening procedures, written consent form was obtained from each subject participating in this study after adequate explanation of the aims, methods, objective, and potential hazards of the study.

The study was approved by the Institutional Review Committee of Kathmandu University School of medical Science/Dhulikhel Hospital (IRC-KUSMS). Protocol approval number is IRC-KUSMS 13/07.

Study drugs

Oquin tablet (OFX 200-mg, batch no. 62161) was used as test formulation. It was provided by Nepal Pharmaceuticals Laboratory, Birgunj, Nepal. Its pharmacokinetic parameters were compared with those of Zanocin (OFX 200-mg, batch no. 1760135), which is manufactured by Ranbaxy Private Limited, India.

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Study design

This was an open labeled, two periods, single dose study. On the first study day each volunteer was given one tablet of Oquin 200 mg and in the next study day they receive a tablet of Zanocin. There was 7 days washout period between study days. Each volunteer received the tablet with 240 ml of water. They received standard lunch after 4, snacks after 8 hrs and dinner after 12 hrs of drug administration. The volunteers were ambulatory throughout the study but were prohibited from strenuous physical activity, smoking, alcohol and stimulating beverages containing xanthine derivatives (tea, coffee, and soft drinks containing caffeine) were also prohibited. Volunteers were monitored constantly throughout the study period by a medical doctor.

Blood sampling

Blood samples of 5 ml volume were collected in vacutainer tubes (without an anticoagulant) before drug administration and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 16, 24 hrs after dosing via an intravenous cannula place in the volunteers' forearm. The blood samples were allowed to clot at room temperature for 20 minutes. Then they were centrifuged at 3000 rpm for 15 minutes. Serum was transferred into a separate Eppendorf tubes and was promptly frozen at –80°C until assay. Each tube was properly labeled stating patient identity and time of sampling done after drug administration.

Analysis of ofloxacin concentration in human plasma

The plasma samples were analyzed using a reversed-phase high performance liquid chromatographic (RP HPLC) method. The isocratic HPLC system (Shimadzu) comprised of a pump (LC - 20AD), autosampler (SIL- 20AD), photo-diode array detector (SPD - M20A) and a column oven (CTO - 10 AS VP). The separation was performed on CAPCELL PAK C18 column (250mm × 4.6mm ID × 5µm) and the wavelength of detector was set at 294nm.

Pharmacokinetic analysis

The various pharmacokinetic parameters were calculated using WinNonlin software. The maximum serum concentration (C_{max}) and the time to reach Cmax (T_{max}) were determined by visual inspection of the individual serum-concentration time profiles. Terminal elimination rate constant (Kel) was calculated from a semilog plot of the serum concentration versus time curve. The terminal half-life was calculated by the formula 0.693/kel. The area under the concentration-time curve (AUC) calculated by the linear trapezoidal method.

Results

Single oral dose of Oquin (NPL) or Zanocin (Ranbaxy) were given to eight Nepalese, healthy, male volunteers (mean age ± SD, 26.375 ± 5.449; range 22 -37 years) on each study days.

Drug concentrations in serum were assayed by HPLC. The column used was C18 Capcell 25 mm, internal diameter 4.6 mm. The system was operated at 294nm. Temperature of column oven was set at 40°C.

Mobile phase consisted of acetonitrile and 0.0625% triethylamine in water (12.5: 87.5, pH adjusted to 2.5 with orthophosphoric acid). The mobile phase was prepared daily and delivered at the flow rate of 1.2 ml/min. The retention time for OFX was 10.7± 0.8 minutes and that for the internal standard (ciprofloxacin) was 13 ± 0.76 minutes. The typical assay time is 15 minutes. The software used for data acquisition was LC solution. This method was validated by following international guidelines (Zendelovska and Stafilov, 2005; Shah et al., 2000). The standard curves were linear over the concentration ranges of 0.1 to 5µg/ml. with a correlation coefficient of 0.9999. The lower limit of quantification of OFX was found to be 0.05µg/ml. The plasma samples of concentration 0.1, 0.5, 1, 2.5 and 5 µg/ml were prepared in triplicate on each three separate days and analyzed by the method developed. The intra-day and inter-day degree of precision and accuracy of the method is expressed as coefficient of variation and relative error respectively (Table 1). The intraday and interday coefficients of variations were less than 14.42% for all the selected concentrations. The relative errors at all the studied concentrations were less than 6.2%. These data indicate the considerable degree of precision and reproducibility of the method, both during the analytical run and between different runs. And it is obvious from the relative error that the method is remarkably accurate which ensures that reliable results are obtained.

A single stage liquid-liquid extraction method using methanol (3 times the volume of plasma) was used in this study. This method gave a good recovery (Table 2) with minimal time for extraction.

Pharmacokinetic parameters were calculated using WinNonlin software. The pharmacokinetic analysis after oral dosing of Oquin and Zanocin was based on an open two-compartment model (Lode et al., 1987). The various pharmacokinetic parameters after the administration of OFX 200 mg immediate release formulations manufactured by above mentioned pharmaceutical companies are shown in Table 3.

The mean serum concentration time profile of two formulations as shown in the graph was similar and super imposable (Figure 1). The mean of C_{max} was 1.82 µg/ml ± 0.19 (%CV 10.70) for reference product and 1.98 ± 0.21 µg/ml (%CV 10.80) for test product. For t_{max} the mean values were found to be similar for both the reference and test product and it was 2.00 ± 0.92 (%CV 48.80) for reference and

Concentration added (mcg/ml)	Calculated concentration (mcg/ml)		Standard deviation		% Coefficient of variation		Relative error %	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.1	0.096	0.104	0.013	0.015	13.541	14.42	-4.000	4
0.5	0.531	0.522	0.011	0.024	2.071	4.597	6.2	4.4
1	1.015	1.015	0.062	0.044	6.104	4.334	1.5	1.5
2.5	2.499	2.536	0.014	0.116	0.56	4.574	-0.04	1.4
5	4.994	5.165	0.302	0.259	6.047	5.014	-0.12	3.3

Table 1: Intra-day and inter-day accuracy and precision data.

Added concentration (µg/ml)	Absolute recovery (%)		
	Mean ± SD (n = 3)	Range (min-max)	CV (%)
1	93.85 ± 11.907	80.1 – 100.75	12.688
2.5	93.696 ± 3.822	89.80 – 97.44	4.079
5	98.269 ± 5.939	91.88 – 103.623	6.043

Table 2: Recovery data of OFX at three concentrations.

Reference formulation, 200-mg Tablet			
Pharmacokinetic parameters (no of subjects=8)	Geometric Mean	SD	CV (%)
C_{max} (mcg/ml)	1.82	0.19	10.70
T_{max} (hr)	2.00	0.92	48.80
AUC_{0-24} (mcg.hr/ml)	12.56	0.96	7.70
$AUC_{0-\infty}$ (mcg.hr/ml)	13.14	0.959	7.30
$T_{1/2}$ (hr)	5.55	0.715	12.90
K_{el} (1/hr)	0.13	0.0174	13.40
Test formulation, Oquin 200-mg Tablet			
Pharmacokinetic parameters (no of subjects=8)	Geometric Mean	SD	CV (%)
C_{max} (mcg /ml)	1.98	0.21	10.80
T_{max} (hr)	2.00	0.53	26.50
AUC_{0-24} (mcg.hr/ml)	13.19	1.45	11.00
$AUC_{0-\infty}$ (mcg.hr/ml)	13.86	1.49	10.80
$T_{1/2}$ (hr)	5.55	1.37	24.80
K_{el} (1/hr)	0.13	0.02	19.90

C_{max} (mcg /ml) =peak plasma concentration; T_{max} (hr) =time to reach peak serum concentration; AUC_{0-24} (mcg.hr/ml) =the area under the serum concentration-time curve; $AU_{C0-\infty}$ (mcg.hr/ml) =the area under the serum concentration-time curve extrapolated to infinity; $T_{1/2}$ (hr) =serum elimination half life; K_{el} (1/hr) =elimination rate constant; CV =coefficient of variation; SD =standard deviation

Table 3: Mean pharmacokinetic parameters of OFX 200-mg tablets

2.00 ± 0.53 (%CV 26.50) for test product. For AUC 0-24, the values obtained were 12.56 ± 0.96 (%CV 7.70) µg.hr/ml and 13.19 ± 1.45 (%CV 11.0) µg.hr/ml for reference and test product respectively.

AUC is important in determining the BA and BE of a drug product. The values of AUC_{0-24} for all volunteers were found to be greater than 80% of $AUC_{0-\infty}$. The mean $AUC_{0-\infty}$ values were found to be 13.14 ± 0.959 (%CV 7.30) µg.hr/ml for reference and 13.86 ± 1.49 (%CV 10.80) µg.hr/ml for test product. Half-life of OFX was found to be 5.55 hr (5.55 ± 1.37 hr for test product, 5.55 ± 0.715 for reference), Kel was 0.13 ± 0.0174 (%CV13.40) for reference and 0.13 ± 0.02 (%CV 19.90) for test.

The table 4 shows the 90% confidence intervals of ratio of test and reference (T/R) between the 2 formulations regarding AUC_{0-24}, $AUC_{0-\infty}$ and C_{max}. Ratio of Least square Means (T/R) percent was found to be 105.159 for AUC_{0-24}, 105.506 for $AUC_{0-\infty}$ and 111.394 for C_{max}. The study revealed that at a 90% confidence interval, AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} were found to be within the range of 97.81% and 113.07%, 98.59% and 112.93%, and 102.14 and 121.47% respectively. All of these values are within the bioequivalence accepted range of 80% - 125%; however, the sample size in this study is not sufficient to provide significant

Test /Reference Log transformed data			
Parameters	Mean Ratio (Test/Reference)	90%CI	
AUC_{0-24} (mcg.hr/ml)	105.159	97.81	113.07
$AUC_{0-\infty}$ (mcg.hr/ml)	105.506	98.58	112.93
Cmax (mcg /ml)	111.394	102.14	121.47

Table 4: 90% Confidence interval for different pharmacokinetic parameters from log data for assessment of bioequivalence.

power for concluding these two products bioequivalent. But it is apparent that pharmacokinetic parameters including bioavailability for both of these products are comparable and these products can be proven bioequivalent in a study with sufficient number of volunteers.

Discussions

BA and BE studies provide important information which ensure safety and effectiveness of medicines to patients and practitioners. Providing two drug products bioequivalent entails a similarity in rate and extent to which a drug in a dosage form becomes available for biological absorption. Area under the curve is accepted as a good indicator of the extent of absorption, whereas C_{max} and T_{max} are considered estimators of ratio of absorption (FDA, 2001a; GIBB, 2001; FDA-OGD, 1992; FDA, 2001b).

The aim of this study was to compare the bioavailability of two formulations of OFX 200-mg tablets, Zanocin (reference formulation) and Oquin (test formulation). The study revealed that pharmacokinetic parameters of these two products are almost similar and OFX concentration-time profiles generated by the two products were essentially super-imposable.

The PK parameters obtained were in good agreement with those reported in literature. Study done by Lode et al. (1987) shows similar type of result (C_{max}=1.19±0.43, t_{max} 76.8±39.2 mins, $AUC_{0-\infty}$ 14.6±2.7) (Lode et al., 1987). Other two crossover study of comparison of two formulations of 200 mg tablet of OFX studies have reported were slightly different but comparable with that of the present study (Oliveira et al., 1999; Flor et al., 1991).

It can be concluded that PK values of OFX obtained from test (Oquin) and reference were comparable in the extent of bioavailability ($AUC_{0-\infty}$ and C_{max}) and in the rate of absorption (C_{max} and T_{max}). They can be proven bioequivalent in a crossover bioequivalence study.
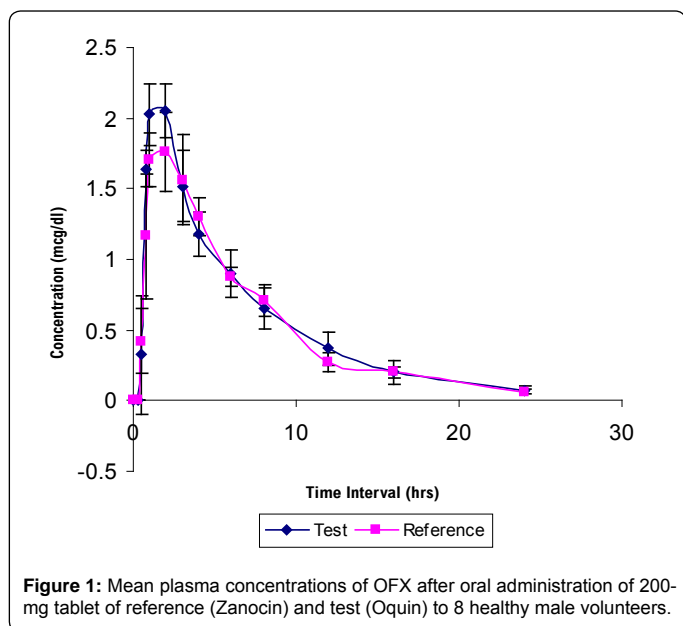

Figure 1: Mean plasma concentrations of OFX after oral administration of 200-mg tablet of reference (Zanocin) and test (Oquin) to 8 healthy male volunteers.

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