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PHARMACOKINETIC VARIATIONS OF OFLOXACIN IN NORMAL AND FEBRILE RABBITS

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ABSTRACT

The influence of experimentally *Escherichia coli*-induced fever (EEIF) on the pharmacokinetics of ofloxacin was evaluated. Ofloxacin was administered @ 20 $mg.kg^{-1}$ body weight intravenously to a group of eight healthy rabbits and compared these results to values in same eight rabbits with EEIF. Pharmacokinetic parameters of ofloxacin in normal and febrile rabbits were determined by using two compartment open kinetic model. Peak plasma level (C_{max}) and area under the plasma concentration-time curve ($AUC_{0-\alpha}$) in normal and febrile rabbits did not differ ($P>0.05$). However, area under first moment of plasma concentration-time curve ($AUMC_{0-\alpha}$) in febrile rabbits was significantly ($P<0.05$) higher than that in normal rabbits. Mean values for elimination rate constant (K_e), elimination half life ($t_{1/2\beta}$) and apparent volume of distribution (V_d) were significantly ($P<0.05$) lower in febrile rabbits compared to normal rabbits, while mean residence time (MRT) and total body clearance (Cl) of ofloxacin did not show any significant difference in the normal and febrile rabbits. Clinical significance of the above results can be related to the changes in the volume of distribution and elimination half life that illustrates an altered steady state in febrile condition; hence, the need for an adjustment of dosage regimen in EEIF is required.

Key words: Ofloxacin, *Escherichia coli*-induced fever, pharmacokinetics.

INTRODUCTION

Ofloxacin, a fluorinated carboxyquinolone, is the racemate, (±)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1, 4-benzoxazine-6-carboxylic acid (Mitsuhashi, 1986). Ofloxacin and the other newer quinolones are well absorbed orally with the exception of norfloxacin. Ofloxacin is rapidly absorbed in a dose-dependent manner. Bioavailability of ofloxacin after oral administration has been reported to be 80 to 90% and it is widely distributed into body tissues. Its volume of distribution (V_d) varies between 1.0 and 1.5 $L.kg^{-1}$ after oral or intravenous administration.

The fluoroquinolones are a series of synthetic antibacterial agents which are used for the treatment of a variety of bacterial infections. All the fluoroqui-nolones exhibit such distributional and antimicrobial properties that make them potentially useful in veterinary medicine (Iqbal *et al*., 2007). Low protein binding is common to all flouroqui-nolones. Mean ofloxacin serum protein binding is 25% and is not affected by drug concentration. Ofloxacin has a pyridobenzoxazine ring that appears to decrease the extent of parent compound metabolism. In individuals with normal renal function, less than 5% of ofloxacin is excreted in the urine as metabolites (Lode *et al*., 1987). This drug is almost completely eliminated in an unchanged form by the kidneys, with about 80% being excreted in 24 hours. After a 100 mg dose, the concentration in urine peaked at 115 $\mu g.mL^{-1}$ at 2 to 4 hours and declined to 36 $\mu g.mL^{-1}$ at 12 to 24 hours after administration (Goodman and Gillman, 1992).

Ofloxacin was found to have a broad antibacterial spectrum which includes both gram-positive and gram-negative aerobic and anaerobic species. Its activity is generally higher than that of pipemidic acid and nalidixic acid. It has proved to be more active than norfloxacin against staphylococci and streptococci. In the case of enterobactriaceae and pseudomonadacae, ofloxacin and norfloxacin possess comparable activities. Ofloxacin was the most active compound against a number of aerobic isolates which are resistant to cefotaxime and ceftriaxone (Brown, 1996).

Ofloxacin has been well studied in patients with diminished renal function. Since ofloxacin is primarily eliminated renally, decreased glomerular filtration rate (GFR) would be expected to have profound effects on its deposition. Values of $t_{1/2}$ are linearly related to creatinine clearance (CLCR) until CLCR falls below 20 $ml.min^{-1}$ (1.2 $L.h^{-1}$), when renal $t_{1/2}$ rises sharply. The t_{max} of ofloxacin may be delayed in patients with GFR less than 30 $mL.min^{-1}$ (1.8 $L.h^{-1}$). V_d does not appear to be influenced in the presence of renal dysfunction (Fillastre *et al*., 1987).

Ofloxacin can penetrate into the intra-alveolar fluid of patients treated for drug-resistant tuberculosis. The lung epithelial lining fluid concentrations were consistently higher than the minimal inhibitory concentrations of *Mycobacterium tuberculosis,* as determined in vitro (Chierakul *et al*., 2001).

Oral ofloxacin bioavailability was significantly altered in rats with hepatic fibrosis. AUC and C_{max} were reduced, while the absorption $t_{1/2}$, t_{max} and elimination $t_{1/2}$ were significantly prolonged, indicating that both the extent and rate of ofloxacin absorption were decreased. Up-regulated P-glycoprotein expression and increased cytochrome P-450 isoenzymatic activities of small intestines in rats with hepatic fibrosis may contribute to the decreased bioavailability and increased elimination of ofloxacin after oral elimination (Hui *et al*., 2006).

One of our major aims of conducting pharmacokinetic studies of antimicrobial agents is to formulate optimal dosage regimens that would be useful in the treatment of diseases caused by susceptible pathogens in animals. Most of these studies are, however, conducted on healthy animals and the pharmacokinetic data generated is extrapolated for use in diseased human beings and animals. Such extrapolation of pharmacokinetic data does not seem scientifically valid because several bacterial and blood protozoan infections and related diseases in which these drugs are used to induce pathophysiological changes cause rise in body temperature and alterations in basal metabolic rate in the animal body (Kume and Garg, 1986). Studies have shown that diseases, particularly, those that are associated with fever, markedly alter the pharmacokinetics of drugs in animals (Van-Miert, 1990). These alterations in pharmacokinetic parameters during disease states may influence the efficacy of antibacterial therapy. To obtain the optimal efficacy of a drug, it is necessary to modify its dosage regimens on the basis of pharmacokinetic data of the drug obtained during the actual disease state (Kumar and Malik, 1999).

The pharmacokinetics of ofloxacin has been well characterized in healthy volunteers, with very few investigations in patients. Differences in study design, pharmacokinetic model and patient populations give rise to an inherent variability in pharmacokinetic parameters obtained. Therefore, the objective of the present study was to elaborate pharmacokinetic variations of ofloxacin in normal and febrile rabbits.

MATERIALS AND METHODS

Animals

For the determination of pharmacokinetic variations of ofloxacin in normal and febrile condition, mixed breed rabbits were used. The study was conducted on clinically healthy male rabbits (n = 8), weighing 1-1.5 kg. They were kept for two weeks before commencement of the experiment. During the experimental period, the animals were maintained on fresh green fodder thrice a day and water was provided *ad-libitum*. All animals were kept under the same experimental conditions with natural day and night cycle.

Study conditions

These normal rabbits were further used for similar studies following the experimentally *Escherichia coli*-induced fever (EEIF). The use of same rabbits was proceeded to minimize any individual to individual variations. For the induction of EEIF, *Eschrichia coli* in sterilized saline water were injected in the marginal ear vein of each rabbit at a dosage rate of 0.01 $mL.kg^{-1}$ body weight. The suspension was prepared by inoculating the pure cultures of *Eschrichia coli* in nutrient broth and a 10 fold dilution of the suspended broth was prepared to have a 130×10^7 $count.mL^{-1}$. Counting of the organisms was done on the Neuobaur scale after serial dilution of the suspension. Rectal temperature was recorded to ascertain febrile condition (Riffat *et al*., 1982). The study was approved by the Board of Advance Studies and Research, The Islamia University of Bahawalpur and was carried out according to the ethical principles.

Sampling procedure

Ofloxacin was administrated as a single intravenous dose @ 20 $mg.kg^{-1}$ body weight (Tarivid infusion, 200 mg/100 mL of distilled water, Aventis Pharmaceuticals, Pakistan) at the climax of fever. Blood samples (1.5 mL) were collected in heparinized test tubes by jugular vein puncture prior to and after administration of the drug at 0, 0.084, 0.167, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 and 6.0 hour in normal rabbits and after the production of diseased condition in same rabbits after a washout period of seven days. Plasma was harvested by centrifugation at 3000 rpm for 15 min and stored at -20ºC until analysis.

Sample analysis

Stock solution of 1 $mg.mL^{-1}$ of ofloxacin was prepared in mobile phase. Sonication of solution enhanced dissolution. Stock solution was prepared on daily basis.

For extraction of protein in rabbit plasma after administration of drug, a previously used extraction method was followed with some modification (Samanidou *et al*., 2003). A 50 µL of rabbit plasma was taken and treated with 400 µL of acetonitrile in order to precipitate proteins. After vortex mixing for two minutes, the sample was centrifuged at 3500 rpm for 15 min and supernatant was evaporated at 45ºC under a nitrogen stream. The residue was reconstituted to volume (50 µL) with mobile phase. Extraction recovery was done by comparing the peak area of an extracted spiked sample with peak area of direct injection of the mobile phase containing same concentration of pure drug.

Standard curve, in a range of 0.1, 0.2, 0.3, 0.5, 1.0, 2.0 and 3.0 $ng.mL^{-1}$, was constructed to encompass the anticipated range of plasma ofloxacin concentration found in healthy rabbits. A 20 µL extracted sample was injected and spectra of each concentration were taken.

Chromatographic conditions involved HPLC system (Perkin-Elmer), Hypersil ODS C18 (4.6 × 250 mm, 5 µm) and UV detector operated at 290 nm (λ_{max}) at a flow rate of 1.5 mL.min^{-1}. Mobile phase consisted of 0.1M phosphoric acid (adjusted to pH 2.5 with a solution of 45% potassium hydroxide) and acetonitrile mixed in a ratio of 75:25 (v/v).

Pharmacokinetic analysis was determined by using software, Kinetica and relevant mathematical calculations were made using MS Excel 2007 Windows professional XP. Two compartment open kinetic model and paired t-test were applied using SPSS 12.0.

RESULTS

Standard curves were prepared on regular basis to confirm the validity of method of analysis by examining the percentage recovery (more than 90%) with fairly logical precision. The data for standard curve is presented in Fig. 1. The mean plasma ofloxacin concentration versus time profiles after single intravenous doses of ofloxacin @ 20 mg.kg^{-1} body weight are presented in Fig. 2. After intravenous dosing to normal and febrile rabbits, mean ofloxacin concentrations at 5 min were 66.79 ± 0.28 and 78.99 ± 0.42 µg/ml respectively. After this time-point, a rapid decrease in mean plasma concentrations (24.57 ± 0.51 and 35.19 ± 0.68 µg/ml in normal and febrile states, respectively) was observed at 15 min, followed by a slow fall till one hour (9.79 ± 0.28 and 12.33 ± 0.21 µg/ml in normal and febrile states).

The pharmacokinetic parameters after an intravenous dose of 20 mg.kg^{-1} in normal and febrile rabbits are presented in Table 1 and Fig. 2. Average normal body temperature of all healthy rabbits was 99.2°C. After the administration of *Escherichia coli*, mean body temperature of all rabbits was found to be 105.1°C in one hour.

DISCUSSION

Escherichia coli-induced fever (EEIF) was produced within 30 minutes after injection of bacterial suspension and persisted during the experiment.

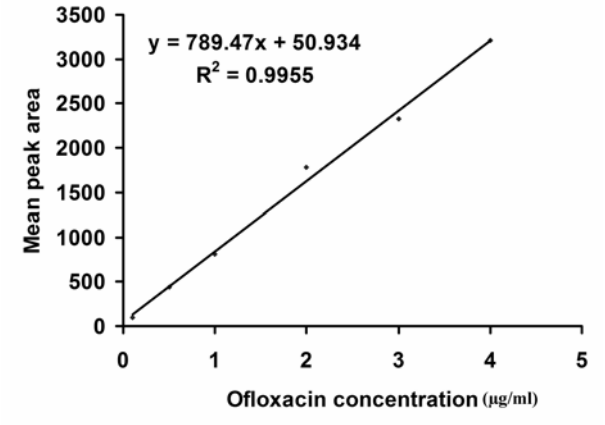

Fig. 1: Calibration curve of ofloxacin in healthy rabbits

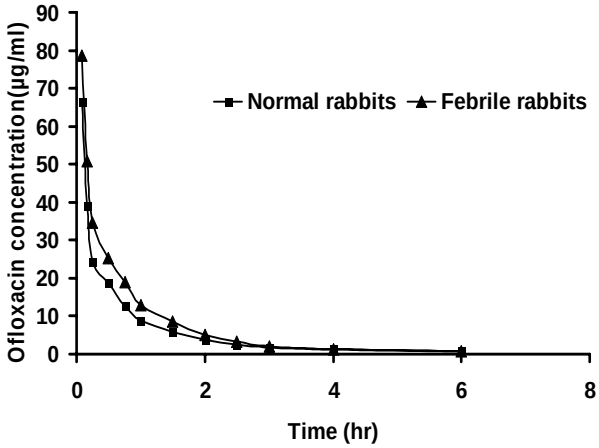

Fig. 2: Pharmacokinetic profiles of ofloxacin in healthy and febrile rabbits.

EEIF induced alterations in the pharmacokinetics of ofloxacin are evidenced by lower values of $V_{d,}$ $t_{1/2\alpha,}$ K_e and Cl, while higher values of $C_{max,}$ MRT and AUMC obtained from febrile rabbits compared to values obtained from the same animals before induction of fever.

In the present study, area under first moment of plasma concentration-time curve ($AUMC_{0-\alpha}$) of ofloxacin in normal and febrile rabbits was observed to be 25.20 ± 1.85 and 49.76 ± 5.71 µg.h^2.mL^{-1} respectively, reflecting a significantly ($P<0.05$) higher value in febrile rabbits than normal animals and indicating more absorption of drug in febrile condition. Increase in $AUMC_{0-\alpha}$ in febrile condition was also observed in cefepime (Ayman *et al*., 2006).

$AUC_{0-\alpha}$ is the integral of drug blood levels over time from zero to infinity and is a measure of drug actually absorbed in the body. As a consequence of reduction of body clearance in febrile rabbits, the area under the plasma concentration vs time curve increased non-significantly ($P>0.05$) with the induction of EEIF ($AUC_{0-\alpha}$ = 11.46 ± 0.45 and 16.16 ± 0.68 µg.h.mL^{-1} in healthy and febrile rabbits, respectively). Increased $AUC_{0-\alpha}$ in febrile condition than normal was also described in marbofloxacin treated goats (Waxman *et al*., 2001).

In the present study, plasma concentrations of ofloxacin at all sampling time points were higher in febrile rabbits compared to normal rabbits. The higher plasma concentrations in febrile condition than normal indicates more absorption of drug in febrile condition. Similar observations have been recorded for enorfloxacin in goats (Rao *et al*., 2000) and cefepime in rabbits (Waxman *et al*., 2001).

Table 1: Comparison of mean (± SEM; n = 8) bioavailability and disposition kinetics of ofloxacin in normal and febrile rabbits after an intravenous dose of 20 mg/kg

Serial No.	Pharmacokinetic parameters	Normal	Febrile
1	Plasma concentration (C_p , µg/ml)	6.17 ± 0.10	6.05 ± 0.20 NS
2	Maximum plasma concentration (C_{max}, µg/ml)	66.48 ± 2.07	78.64 ± 0.66 NS
3	Elimination rate constant (K_{el}, h^{-1})	0.55 ± 0.03	0.38 ± 0.02 NS
4	Absorption half life ($t_{1/2\alpha}$, h)	0.70 ± 006	2.48 ± 0.19 **
5	Elimination half life ($t_{1/2\beta}$, h)	1.77 ± 0.06	1.22 ± 0.05 NS
6	Apparent volume of distribution (V_d, L/kg)	4.47 ± 0.11	2.19 ± 0.09 *
7	Volume of distribution at steady state (V_{SS}, L/kg)	3.81 ± 0.07	3.74 ± 0.20 NS
8	Area under the plasma concentration-time curve ($AUC_{0-\alpha}$, (µg.h/ml)	11.46 ± 0.45	16.16 ± 0.68 NS
9	Under first moment of plasma concentration-time curve ($AUMC_{0-\alpha}$, (µg.h^2 / ml)	25.20 ± 1.85	49.76 ± 5.71 *
10	Mean residence time (MRT, h)	2.18 ± 0.80	3.03 ± 0.23 NS
11	Total body clearance (TCR, $Lh^{-1}kg^{-1}$)	0.18 ± 0.01	0.13 ± 0.01 NS

***=**Significant difference (P<0.05); **=Highly significant difference (P<0.01); NS=Not significant difference.

The maximum plasma concentration (C_{max}) of ofloxacin in febrile and normal rabbits was observed to be 78.64 ± 0.66 and 66.48 ± 2.07 µg.mL^{-1}, respectively, which reveals a non-significant difference. The higher value of C_{max} has been observed for other drugs after intravenous administration of *E. coli* endotoxin including marbofloxacin (Waxman *et al*., 2001). The higher value of C_{max} in febrile rabbits appears to be due to faster absorption of drug from its site of administration. It is probable that increased cardiac output and blood flow to the muscles prompted the drug molecules to move faster from their site of deposition to the blood circulation, resulting in more absorption of ofloxacin during fever (Kume and Garg, 1986).

The mean volume of distribution (V_d) of ofloxacin in the present study was found to be 4.47 ± 0.11 L.kg^{-1} in normal rabbits, indicating wide tissue distributions. These findings reflect marked capacity of drug to penetrate into biological membranes and are consistent with low degree of binding of drug to plasma protein. The higher volume of distribution of quinolones has been seem in humans (Goodman and Gillman, 1992). In the present study, the volume of distribution at steady state decreased non-significantly (P>0.05) in febrile conditions (V_{ss} =3.82 ± 0.073 L.kg^{-1} in normal and 3.12 ± 0.2 L.kg^{-1} in febrile rabbits).

The distribution half life ($t_{1/2\alpha}$) was significantly (P<0.05) higher in febrile rabbits than normal animals ($t_{1/2\alpha}$ = 0.70 ± 0.06 h in normal and 2.48 ± 0.20 h in febrile rabbits). This indicates faster absorption of the drug in febrile condition. The elimination half life ($t_{1/2\beta}$) was non-significantly (P>0.05) lower in febrile rabbits than normal animals, ($t_{1/2\ \beta}$ = 1.22 ± 0.05 h in febrile and 1.77 ± 0.06 h in normal rabbits). Lower $t_{1/2\beta}$ of ofloxacin noted in febrile rabbits is probably related to increase in blood flow through kidneys. The elevation of central body temperature by endotoxin or by extra-corporeal heating blood produces significant increase in hepatic as well as renal blood flows. Typhoid vaccine-induced fever in man was associated with marked increase in hepatic renal blood flows, which largely accounted for the augmented cardiac output associated with fever. The elevation of body temperature by heating the blood in the pump in cardio-pulmonary bypass in rabbits and dogs resulted in an increase in renal blood flow and in glomerular filtration rates. The shortened half life of salicylamide was also observed in pyrogens-induced febrile volunteers than the controls (Gillenwater *et al*., 1964; Satinsky and Konecke, 1970).

After the induction of EEIF, the elimination of the drug was non-significantly (p<0.05) slower in febrile rabbits (0.13 ± 0.01 L.h^{-1}.kg^{-1}) than healthy animals (0.18 ± 0.01 L.h^{-1}.kg^{-1}). It was observed that elimination of ofloxacin was altered by EEIF. It is probable that the increase in tubular re-absorption and a decrease in GFR and tubular secretion induced by EEIF play an important role in the decrease of body clearance of drugs, including ofloxacin which are widely eliminated by the renal route. The slow elimination of drugs in febrile condition is also noted for sulphadiazine in rabbits (Ahmad and Nawaz, 1995; Waxman *et al*., 2001).

MRT provides a quantitative estimation of persistence time of drug in the body. In the present study, MRT of ofloxacin in normal and febrile rabbits was observed to be 2.18 ± 0.80 and 3.03 ± 0.23 h, respectively, reflecting a non-significantly (P>0.05) higher value in febrile rabbits. Similar findings were made earlier (Ahmad and Nawaz, 1995; Waxman *et al*., 2001).

The present study elaborates differences between the pharmacokinetics of ofloxacin in healthy and febrile rabbits. Plasma concentrations of ofloxacin were higher at all sampling points and $AUC_{0-\alpha}$ was higher in case of EEIF, which could be explained by the slow elimination of drug in febrile condition. As a consequence of the reduction of body clearance in febrile rabbits, K_{el} is significantly decreased in febrile

rabbits. The higher AUC, AUMC and MRT values in febrile rabbits observed in this study show that the drug remains in the body for a comparatively longer duration in the febrile condition (also supported by the increased values of $t_{1/2\alpha}$). On the basis of these results, it is concluded that fever has a profound effect on the pharmacokinetics of drugs. Therefore, dosage adjustment is necessary when the drugs are given in hyperthermia or in any other disease.

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