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Influence of *Escherichia coli* lipopolysaccharide induced fever on the plasma kinetics of pazufloxacin in buffalo calves

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Pazufloxacin has potent broad spectrum activity against gram-positive and gram-negative bacteria including variety of resistant strains and anaerobic bacteria (Zhanel *et al.* 2006). Fever, one of the most common manifestations of many infectious diseases, is reported to change the pharmacokinetics of drugs (Kumar *et al.* 2013). So, the study on, influence of fever on the pharmacokinetics of antibiotics is essential. The purpose of this study was to determine the pharmacokinetic variables of pazufloxacin in febrile buffalo calves following intravenous administration. From the pharmacokinetic data, recommendations were made for optimal dosage regimen of pazufloxacin in febrile buffalo calves.

The experiments were performed in 6 healthy male buffalo calves of 6–12 months age and weighing between 80 and 150 kg. The animals were acclimatized in the animal shed of the department under uniform conditions for 3 weeks before start of experiment. During this period animals were subjected to regular clinical examination. Animals were maintained on green fodder, wheat straw and water *ad lib*. Before the start of experiment, permission for experiments on these animals was obtained from the Institutional Animal Ethics Committee.

Pazufloxacin mesilate purchased commercially was injected into the jugular vein of each of these 6 healthy animals @ 5 mg/kg body weight. After 1 month the study was repeated after inducing a febrile state in the same animals. Fever was induced by single/repeated intravenous injection of *E. coli* lipopolysaccharide @ 1 µg/kg body weight (Dardi *et al.* 2005). Fever was monitored by measuring the rectal temperature at every 30 min intervals. Once fever was induced pazufloxacin was injected intravenously to these 6 animals @ 5 mg/kg body weight. Blood samples (4–5 ml each) were withdrawn from the contra lateral jugular vein into heparinized glass test-tube before administration and at 2.5, 5, 10, 15, 30, 45 and 60 min and 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 16 h after administration of drug. Plasma was separated after centrifugation at 2,000 rpm for 15 min at room temperature and stored at –20°C till analysis usually the next day.

Plasma concentrations of pazufloxacin were determined using high performance liquid chromatography (Phapale *et al*. 2010). HPLC system consists of a single pump and autosampler injector with 200 microlitre loop, dual wavelength UV detector, flourescent detector and Total Chrome software for analysis. A reverse phase c18 column (particle size 5µ, 4.6 × 250 mn) was used as a stationary phase. The mobile phase consists of buffer (20 mM citric acid and 5 mM of 1-octane sulphonic acid, pH 5 was adjusted with sodium hydroxide) and acetonitrile was mixed in the ratio of 81:19 v/v. The flow rate of mobile phase was 1ml/min. The detection for plasma sample was performed with flourescent detector at 330 nm (excitation) and 394 nm (emission). Samples were analyzed for 28min. Plasma standards/samples (500µl) were vortex mixed for 10s with 100 µl internal standard solution (10 $\mu g.ml^{-1}$). After addition of 400µl acetonitrile, the mixture was vortex mixed for 5 min, left at room temperature for 30 min, and centrifuged at 10,000 rpm for 10 min. The supernatant (200µl) was vortex mixed with 800µl buffer for 5 min, and 1µl of the solution obtained was injected for LC analysis. Buffer used in the mobile phase and for sample preparation was prepared by mixing aqueous 20 mM citric acid (2.1g) and 5mM 1-octane sulphonic acid (0.55 g) salt in 750 ml of HPLC water. Volume of the buffer was raised to 800 ml with HPLC water. An 81:19 (v/v) mixture of buffer and acetonitrile was used as isocratic mobile phase. The mobile phase was delivered at a flow rate of 1.0 ml/min. Before use, the mobile phase was filtered through a 0.22µm millipore filter.

The calibration curve for plasma samples was constructed in the range of 0.02-2.5 µg/ml. The limit of detection was 0.02 $\mu g\ ml^{-1}$ in plasma. The value of regression coefficient (R^2) was 0.99. Mean recovery from plasma at concentration

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0.02 to 2 µg/ml ranged from 89–100%. For plasma sample overall RSD was less than 10%. The kinetic parameters were calculated from the formulae derived for a 2-compartment open model (Gibaldi and Perrier 1982). The statistical analysis was performed using SPSS 12.0 software package. The dosage regimen (D) of pazufloxacin was also determined based on kinetic data by using following formulae:

$$D = C_p(min).\ Vd(e^{\beta\tau})$$

where C_p(min) is the minimum therapeutic concentration of pazufloxacin, t is the dosage interval and other parameters are defined in Table 1.

The administration of *E. coli* lipopolysaccharide induced fever within 2–2.5 h, which persisted for 16 h. At least 2°F increase of temperature from the normal temperature was taken as the time of pazufloxacin administration. The mean plasma concentrations of pazufloxacin as a function of time following intravenous administration in healthy and febrile animals are shown in Fig. 1.

Table 1. Pharmacokinetic parameters of pazufloxacin following its single IV dose of 5 mg.kg^{-1} body weight in healthy and febrile buffalo calves

Parameter	Unit	Healthy	Febrile
Cp^o	µg.ml^{-1}	19.4±1.0	17.9±0.49
A	µg.ml^{-1}	18.5±0.96	17.1±0.56
α	h^{-1}	7.5±0.85	4.37±0.18**
$t_{½\alpha}$	h	0.09±0.01	0.16±0.01**
B	µg.ml^{-1}	0.87±0.13	0.77±0.11
b	h^{-1}	0.26±0.01	0.25±0.01
$t_{1/2\beta}$	h	2.68±0.10	2.74±0.10
k_{12}	h^{-1}	6.68±0.82	3.81±0.19**
k_{21}	h^{-1}	0.58±0.06	0.43±0.03
k_{12}/k_{21}	Ratio	11.8±1.47	9.09±0.92
k_{10}	h^{-1}	0.52±0.01	0.38±0.01**
AUC	µg.ml^{-1}.h	9.38±0.45	9.84±0.37
AUMC	µg.ml^{-1}.h^2	17.8±0.95	25.8±0.72**
$Vd_{(SS)}$	L.kg ^{1}	1.03±0.06	1.35±0.09*
$Vd_{(area)}$	L.kg ^{1}	2.09±0.16	2.01±0.08
Cl_B	ml.kg^{-1}.h^{-1}	539.7±28.3	510.6±18.2
MRT	h	1.91±0.04	2.63±0.09**
AUC/MIC	Ratio	93.8±4.54	98.4±3.67

Values given are mean±SE of the results obtained from 6 animals. Fever was experimentally induced with intravenous injection of *E. coli* lipopolysaccharide (1 µg.kg^{-1}). *significantly ($p<0.05$) different as compared to corresponding values of healthy animals. **significantly ($P<0.01$) different as compared to corresponding values of healthy animals. C_p^o, plasma drug concentration immediately following intravenous injection of single dose; A, B, zero-time plasma drug concentration intercepts of regression lines of distribution and elimination phases respectively; α and β, distribution and elimination rate constants, respectively; $t_{1/2\alpha}$, distribution half life; $t_{1/2\beta}$, elimination half life; K_{12}, K_{21}, rate of transfer of drug from central (blood) to peripheral (tissues) compartment and vice-versa; k_{10}, elimination of drug from central compartment; AUC, total area under plasma drug concentration-time curve; AUMC, area under the first moment of the plasma drug concentration-time curve; Vd(ss), apparent volume of distribution, based on steady state plasma levels; $V_{d(area)}$, apparent volume of distribution, based on area under curve; Cl_B, total plasma clearance; MRT, mean residence time; MIC, minimum inhibitory concentratrion.

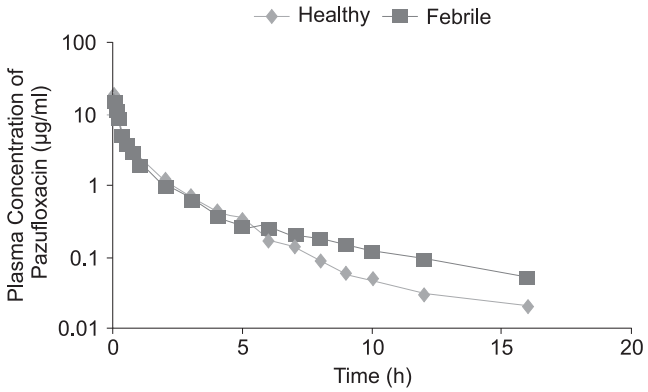

Fig. 1. A semi logarithmic plot of plasma levels time profile of pazufloxacin after a single intravenous dose of 5 mg kg^{-1} body weight in healthy and febrile buffalo calves. Values given are mean±SE of 6 animals.

Evaluation of the results on plasma pazufloxacin levels against time indicated that pharmacokinetics of pazufloxacin after its intravenous administration in healthy and febrile buffalo calves follow 2- compartment open model. The plasma concentration time data were adequately described by the equation:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

where, Cp is pazufloxacin concentration at time t, A and B are zero time intercepts of the distribution and elimination phase of the plasma concentration time curve, α and β are the distribution and elimination rate constants, respectively, and 'e' represents the base of natural logarithm. At 2.5 min, the peak plasma level of pazufloxacin in febrile buffalo calves was almost similar to healthy buffalo calves. At most of the times during distribution phase the plasma concentration in febrile buffalo calves was lower than in healthy animals but it was higher during the elimination phase. Lower concentration of cefuroxime (Chaudhary *et al.* 1999), ceftriaxone (Dardi *et al.* 2005) and moxifloxacin (Pathania and Sharma 2010) in buffalo calves were reported.

The various pharmacokinetic parameters are presented in Table 1. The low values of the distribution rate constant in febrile animals indicated that pazufloxacin is slowly distributed into various body fluids and tissue compartment than healthy animals. The volume of distribution at steady state ($Vd_{(ss)}$) is the constant that expresses the amount of drug in the body at steady state as a proportion of the corresponding C_{ss} (the expected plasma concentration at steady-state) (Toutain and Bousquet-Melou 2004). Pazufloxacin exhibited a relatively high volume of distribution at steady state ($Vd_{(ss)}$) in healthy and febrile

buffalo calves which shows that there is a relatively quick and wide distribution of pazufloxacin after intravenous administration.

The volume of distribution at steady state $Vd_{(ss)}$ suggested a wide penetration through biological membrane and good tissue distribution. But here it is necessary to mention that volume of distribution is a theoretical measurement and the possibility that it may exceed the volume of total body water emphasizes this fact. Drugs have different affinities for different body tissues and observation of a large Vd does not indicate the location of the relevant drug in the body. Changes in the permeability of biological membrane barrier and/or tissue and plasma pH during fever may alter the distribution pattern of drugs. These changes may be more important in patient suffering from infectious encephalitis, prostatitis, arthritis and mastitis.

The total body clearance (healthy=539.7±28.3 ml/kg/h; febrile=510.6±18.2 ml/kg/h) suggested the medium elimination of pazufloxacin in buffalo calves. The Cl_B value of danofloxacin (Sappal *et al.* 2009), moxifloxacin (Pathania and Sharma 2010) and gatifloxacin (Raipuria *et al.* 2007) in buffalo calves were 0.71±0.10, 0.37±0.11, and 0.24±0.08 L/kg/h, respectively. Toutain and Bousquet-Melou (2004) indicated breakpoint clearance values to classify clearance as high, medium, or low as 28, 12 and 5.8 ml/kg/min, based on extraction ratios of 0.35. 0.15 and 0.05, respectively. In this study, the pazufloxacin clearance, 9 ml/kg/min (healthy) and 8.5 ml/kg/min (febrile), may be classified as medium.

The elimination half-life of pazufloxacin was not affected in febrile conditions. The value of elimination half-life ($t_{1/2\alpha}$) of buffalo calves is longer than the corresponding values reported in rabbits, rats and mice, but it is shorter than the values reported in dogs (Nakata *et al.* 1999). The value of elimination half-life ($t_{1/2\beta}$) in rabbits, rats, mice and dogs has been reported at 1.0, 0.88, 0.23, and 4.5 h, respectively. The difference in elimination half life could be due to difference in age, sex and breed of the animal.

The main objective of present study was to determine a satisfactory dosage regimen of pazufloxacin in febrile calves. It is not axiomatic to compute the dosage regimen of pazufloxacin to be used effectively in clinical practice for the treatment of mild to severe bacterial infections, without having first conducted a detailed pharmacokinetic study. With the minimum therapeutic plasma concentration of pazufloxacin as 0.1 µg/ml which was found to be the most effective against the majority of sensitive gram-positive and gram-negative pathogens, the convenient and suitable dosage regimen of cefepime in healthy and febrile animals was 4.7 mg/kg and 4.0 mg/kg, respectively, repeated at 12 h intervals.

On the basis of present study, lipopolysaccharide induced fever markedly altered the dosage regimen of pazufloxacin in buffalo calves, hence, the dosage obtained in healthy animal needs to be modified. By doing so, the therapeutic efficacy of pazufloxacin may be increased and the adverse effects related to drug as well as the cost factor of treatment may decrease.

SUMMARY

The pharmacokinetics of pazufloxacin was studied in healthy and febrile buffalo calves (5 mg/kg, IV). The fever was induced with *Escherichia coli* lipopolysaccharide. The drug concentration in plasma was detected by HPLC method. At 2.5 min the concentration of pazufloxacin in healthy and febrile animals were 17.8 0.6 µg/ml and 15.4±0.5 mg/ml, respectively and drug was detected up to 16 h. The distribution half-life of pazufloxacin increased from 0.09±0.01 h in healthy animals to 0.16±0.01 h in febrile animals. Drug distribution was altered by fever as febrile animals showed higher volume of distribution (1.35 0.09 L/kg) higher than normal animal (1.03±0.06 L/kg). Total body clearances in healthy and febrile animals were 539.7±28.3 and 510.6±18.2 ml/kg/h, respectively. To maintain minimum therapeutic concentration of 0.1 µg/ml, a satisfactory dosage regimen of pazufloxacin in healthy and febrile buffalo calves would be 4.7 mg/kg and 4 mg/kg body weight, respectively, to be repeated at 12 h intervals.

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