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Pharmacokinetics of moxifloxacin in *Escherichia coli* lipopolysaccharide-induced febrile buffalo calves

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ABSTRACT

Pharmacokinetics and urinary excretion of moxifloxacin was studied in *Escherichia coli* lipopolyssacharide (1 mg/kg b.wt.,iv)-induced fever in buffalo calves (n=6). A single intravenous dose of 5 mg/kg body weight was administered in each of the 6 buffalo calves once body temperature was elevated by 1–2°C. Pharmacokinetic analysis of disposition data were best described by 2 compartment open model. The concentration of moxifloxacin in plasma of febrile buffalo calves was detected up to 12 h. The elimination half life and volume of distribution of moxifloxacin after intravenous administration were 2.99±0.10 h and 1.79±0.14 L/kg respectively. The distribution half-life and area under curve (AUC) were 0.09±0.01 h and 12.3±0.76 µg/ml h respectively. The total body clearance (Cl_B) and tissue:plasma (T:C) ratio were 414.3 ± 24.9 ml/kg/h and 0.22 ± 0.05 respectively. At the end of 24 h, the urinary excretion was approximately 10% of total dose. Using the surrogate marker of AUC/MIC, it was determined that a dosage of 5 mg/kg of moxifloxacin administered by the iv route in febrile buffalo calves is likely to be effective against bacterial isolates with MIC < 0.1 µg/ml.

Key words: Buffalo, Calves, Fever, Moxifloxacin, Pharmcokinetics

Moxifloxacin is a novel fourth-generation fluoroquinolone with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria. It is also effective against anaerobic and atypical organisms such as *Mycoplasma* and *Chlamydia* species. It has the highest potency in its class against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Kowalski *et al.* 2003). Fever is one of the important clinical signs of bacterial infections in animals. Febrile conditions in different species alter the pharmacokinetic properties of various chemotherapeutic agents (Kumar and Malik 2003, Pawar and Sharma 2008). Pharmacokinetics of antimicrobials are markedly altered in disease conditions (Lesar and Zaske 1984, Dardi *et al.* 2005, Sharma *et al.* 2005), hence the dosage regimen obtained in healthy subjects cannot be extrapolated in clinical cases to treat diseased animals. For a judicious therapy, it is essential to study the pharmacokinetics and formulate the dosage regimen of a drug in the disease condition in which it is to be used clinically. Therefore in the present study, the pharmacokinetic variables and urinary excretion of moxifloxacin following intravenous administration were determined in febrile buffalo calves.

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MATERIALS AND METHODS

Healthy male buffalo calves (6), 6–12 months old and weighing between 80 and 150 kg, were acclimatized in the animal shed of the department under uniform conditions for 3 weeks before the start of experiment. During this period the animals were subjected to regular clinical examination. Animals were maintained on green fodder, wheat straw and water *ad lib*.

Before the start of experiment, the animals were kept in metabolic stalls of standard size. The stalls were prepared in such a way that all the urine voided by animals could be collected over the chosen time intervals without any contamination or spillage. Fever was induced by intravenous injection of *E. coli* lipopolysaccharide @ 1 mg/kg body weight (Pawar and Sharma 2008). Fever was monitored by measuring the rectal temperature at every 30 min intervals. Once fever was induced, moxifloxacin hydrochloride was injected into the jugular vein of each of 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 1, 2.5, 5, 10, 15, 30, 45 and 60 min and 2, 3, 4, 5, 6, 8, 10, 12, 16 and 24 h after administration of drug. Plasma was separated after centrifugation at 2000 rpm for 15 min at room temperature and stored at –20°C till analysis, usually the next day. The urine samples were collected at 4, 8, 12, 20 and 24h after the drug administration. The volume of urine was measured and approximately 8–10 ml was frozen for analysis. The concentration of moxifloxacin was determined in plasma and urine by microbiological assay technique (Arret *et al.* 1971) using *E. coli* (MTCC 739) as the test organism. The limit of detection of method was 0.1 mg/ml. The standard curve of moxifloxacin in buffalo calf plasma was linear between 0.125 and 1 mg/ml. The intra-day and inter-day coefficients of variance were less than 5 and 10% respectively. The kinetic parameters were calculated from the formulae derived for a 2-compartment open model (Notari 1980, Gibaldi and Perrier 1982).

RESULTS AND DISCUSSION

No diurnal variations in basal temperature of buffalo calves were observed, as values recorded for a week did not show significant differences. The iv administration of *E. coli* lipopolysaccharide-induced fever within 1 h which persisted for 24 h. Some animals also showed sign of restlessness, anorexia, coughing, lacrimation and dry muzzle. At least 2°F increase of temperature from the normal temperature was taken as the time of moxifloxacin administration. The mean plasma concentrations of moxifloxacin as a function of time following intravenous administration in febrile animals are shown in Fig. 1.

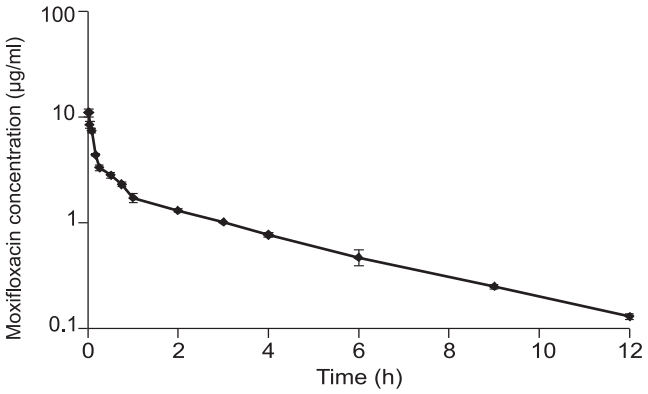

Fig 1. Semilogarthmic plot of plasma levels time profile of moxifloxacin in febrile buffalo calves following a single intravenous dose.

The evaluation of the results on plasma moxifloxacin levels against time indicated that pharmacokinetic of moxifloxacin after its intravenous administration in febrile buffalo calves follow two-compartment open model. The plasma concentration time data were adequately described by biexponential equation:

$$Cp = Ae^{-at} + Be^{-bt}$$

where Cp is the concentration of drug in the plasma at time t; A is the intercept of the distribution phase with the concentration axis expressed as µg/ml; B is the intercept of the elimination phase with the concentration axis expressed as µg/ml; α is the distribution rate constant expressed in units of reciprocal time (per hour); and e is the natural logarithm base. Moxifloxacin after intravenous administration has also been reported to follow two-compartment open model in rabbits (Fernandez-Varon *et al.* 2005, Carceles *et al.* 2006), lactating goats (Fernandez-Varon *et al.* 2006) and ewes (Goudah 2008). A comparison of plasma levels of moxifloxacin in febrile animals with healthy animals (Pathania 2008), indicated that the plasma concentrations in

Table 1. Pharmacokinetics of moxifloxacin in febrile buffalo calves (n=6) following its single IV injection (5 mg/kg body weight)

Parameter[a]	Unit	Mean±SE
$Cp°$	µg/ml	12.1 ± 0.43
A	µg/ml	10.0± 0.41
α	h	7.99 ± 0.44
$t_{½\alpha}$	h	0.09 ± 0.01
B	µg/ml	2.06 ± 0.17
β/h	0.23 ± 0.01	
$t_{1/2\beta}$	h	2.99 ± 0.10
K_{12}	h	6.40 ± 0.42
K_{21}	h	1.54 ± 0.10
K_{12}:K_{21} ratio		4.24±0.41
K_{el}	h	0.28 ± 0.01
$t_{½}K_{el}$	h	2.47 ± 0.12
AUC	µg/ml h	12.3 ± 0.76
AUMC	µg/ml h^2	44.1 ± 4.21
$Vd_{(B)}$	L/kg	2.51± 0.19
$Vd_{(SS)}$	L/kg	2.17 ± 0.16
$Vd_{(area)}$	L/kg	1.79 ± 0.14
Cl_B	ml/kg/h	414.3 ± 24.9
T:P	Ratio	0.22 ± 0.05
MRT	h	3.57 ± 0.17

[a] Kinetic parameters as described by Gibaldi and Perrier (1982)

C^o_p =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; AUC=total area under plasma drug concentration-time curve; AUMC= Area under the first moment of the plasma drug concentration-time curve; $V_{d(B)}$ = apparent volume of distribution, based on zero time plasma drug concentration intercept of elimination phase Vd(ss)= apparent volume of distribution, based on steady state plasma levels; $V_{d(area)}$ =apparent volume of distribution; Cl_B= total plasma clearance; T/P= tissue/plasma ratio of drug concentration; MRT= Mean residence time.

febrile animals were lower than healthy animals from 1 min to 6 h. Accordingly, lower concentration of gentamicin in febrile goats (Ahmad *et al.* 1994), cefazolin in febrile goats (Roy *et al.* 1992); and cefuroxime (Chaudhary *et al.* 1999), ceftriaxone (Dardi *et al.* 2005) and cefepime (Pawar and Sharma 2008) in crossbred calves were reported.

The various pharmacokinetic parameters are presented in Table 1. The high value of the distribution rate constant (7.99±0.44/ h) in febrile animals indicated that moxifloxacin is rapidly distributed into various body fluids and tissue compartment. The higher value of $Vd_{(area)}$, $Vd_{(ss)}$, $Vd_{(B)}$ revealed that the drug is extensively distributed to various body fluids and tissues. Volume of distribution is a theoretical value, and it may exceed the volume of total body water at times. Drug has different affinities for different body tissues but a large V_d does not indicate the exact location of the relevant drug in the body. But high tissue distribution of moxifloxacin is further corroborated by higher values of K_{12}:K_{21} ratio and lower value of K_{el}.

The elimination half life and elimination rate constant in buffalo calves were 2.99±0.10 h and 0.23±0.01/h respectively. From the half life obtained in our study, moxifloxacin does not seem to offer advantages over other fluoroquinolones. The value of elimination half life of buffalo calves is longer than the corresponding value reported in rabbits and lactating goats (Fernandez-Varon *et al.* 2005,2006) and ewes (Goudah 2008), but it is shorter than the value reported in camels (Abd El-Aty *et al.* 2007). The elimination half life in rabbits, lactating goats, ewes and camels was reported as 1.84± 0.12, 1.94±0.41, 1.77±0.23 and 12.26±5.83 h respectively.

While comparing the total body clearance in febrile animals with that of healthy animals (Pathania 2008), it was found that the value of Cl_B in febrile animals (414.3±24.9 ml/kg/h) is not significantly different as compared to the healthy animals (371.211.2 ml/kg/h).

In the present study, a significant increase was found in the value of MRT in febrile buffalo calves in comparison with healthy animals (Pathania 2008). The MRT increased from 2.79±0.19 h in healthy animals to 3.57±0.17 h in febrile animals. The mean urine concentration of moxifloxacin along with cumulative per cent of total drug excreted in urine at different time intervals are shown in Fig. 2. The peak concentration of moxifloxacin (35.4±11.0 mg/ml) was determined in urine sample collected at 4–8 h. Total urinary recovery of drug was 9.33±0.37% in 24 h, which was similar to that of healthy buffalo calves (Pathania 2008).

For a concentration-dependent drug, such as moxifloxacin, successful treatment usually correlates with AUC_{24}/MIC and C_{max}/MIC, and high ratios of the latter were found associated with a lower incidence of the development of resistance (Drusano *et al.* 1993, Lode *et al.* 1998). Animal models with different quinolones showed that an AUC_{24}:MIC ratio of about 100 h or a C_{max}:MIC ratio of 10 should be achieved to give

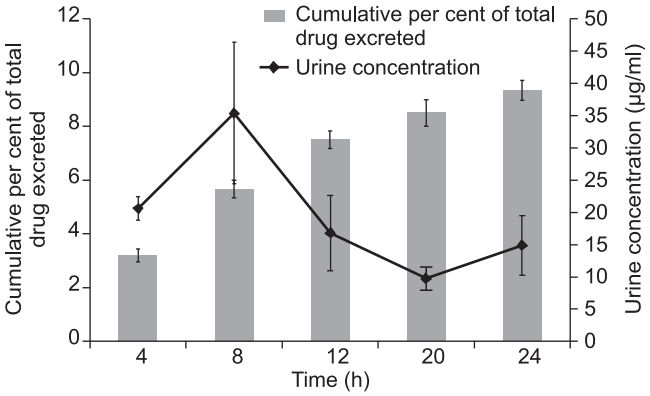

Fig 2. Urinary concentration and cumulative % of total moxifloxacin excreted in urine of febrile buffalo calves.

maximum clinical and microbial efficacy (Turnidge 1999). However, it should be noted that for concentration- dependent antibacterial the numerical values of AUC_{24}:MIC, C_{max}:MIC while as for time-dependent antibacterial T > MIC, parameters are used as a surrogate markers to predict optimal dosage, which were generated in experimental infections in laboratory animals (Toutain and Lees 2004). However, these numerical values may or may not be applicable in infections of buffalo calves. Little is known about MICs of moxifloxacin against the most specific and important pathogens affecting buffalo calves. But MICs against sensitive strains of different micro-organisms range from 0.01 to 0.5 µg/ml (Woodcock *et al.* 1997). Using the surrogate marker of AUC_{24}:MIC, it was determined that moxifloxacin is likely to be effective against bacterial isolates with MIC < 0.1 µg/ml for 12 h in febrile buffalo calves when drug is administered @ 5 mg/kg body weight by iv route. Therefore, moxifloxacin @5 mg/kg body weight needs to be repeated after every 12 h to combat infections amicable to this antibacterial drug. A cumulative 10% of this drug was eliminated in 24 h through urine.

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