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Pharmacokinetics, dosage regimen and *in vitro* plasma protein binding of intramuscular levofloxacin in buffalo calves

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Summary

The pharmacokinetics of levofloxacin following its single intramuscular administration (3 mg/kg) was investigated in six male buffalo calves. Peak plasma level of 2.95 ± 0.13 μg/ml was observed at 1 h and the drug level above MIC_{90} in plasma was detected up to 12 h of administration. The bioavailability was 68.1 ± 5.4% and levofloxacin was bound to the plasma proteins to the extent of 19.1 ± 1.5%. High values of AUC (8.81 ± 0.37 μg.h/ml) and Vd_{area} (1.06 ± 0.04 L/kg) reflected a vast area of body covered by drug concentration and appropriate penetration of levofloxacin into various body fluids and tissues. The elimination half-life and mean residence time were 3.27 ± 0.31 h and 5.4 ± 0.59 h, respectively. The total body clearance was 343.2 ± 14.1 ml/kg/h. An appropriate intramuscular dosage regimen for levofloxacin in buffalo calves would be 1.7 mg/kg repeated at 12-h intervals.

Key words: Buffalo calves, Dosage, Intramuscular, Levofloxacin, Pharmacokinetics

Introduction

Fluoroquinolone resistance relates directly to human and veterinary usage and emerging bacterial resistance poses the single greatest threat to the future survival of the fluoroquinolone drugs as an antibiotic class (Bakken, 2004). Levofloxacin is a recently introduced second generation fluoroquinolone, which possesses excellent activity against Gram-positive, Gram-negative and anaerobic bacteria (Davis and Bryson, 1994; North *et al*., 1998). In human clinical trials, levofloxacin has been found to be very effective in the treatment of infections of upper and lower respiratory tract, genitourinary system, skin and soft tissue (Davis and Bryson, 1994). Several species of staphylococci, streptococci, including *Streptococcus pneumoniae*, most enterococci, Enterobacteriaceae, *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Bacteroides*, *Clostridium*, *Haemophilus*, *Moraxella*, *Legionella*, *Mycoplasma* and *Chlamydia* are susceptible to levofloxacin (Langtry and Lamb, 1998). As compared to other fluoroquinolones, ofloxacin and ciprofloxacin, it also has more pronounced bactericidal activity against organisms like *Pseudomonas*, Enterobacteriaceae and *Klebsiella* (Klesel *et al*., 1995). The drug distributes well to target body tissues and fluids in the respiratory tract, skin, urine and prostate and its uptake by cells makes it suitable for use against intracellular pathogens (Langtry and Lamb, 1998). The pharmacokinetics of levofloxacin has been investigated in man (Chulavatnatol *et al*., 1999), calves (Dumka and Srivastava, 2006), rabbits (Destache *et al*., 2001), rats (Ito *et al*., 1999) and guinea pigs (Edelstein *et al*., 1996). However, there is no information available on the pharmacokinetics of levofloxacin in buffalo

species. In view of the marked species variation in the pharmacokinetic data of antimicrobial drugs, the present study was undertaken to determine the pharmacokinetics, *in vitro* plasma protein binding and an appropriate dosage regimen of levofloxacin, following its single intramuscular administration in cross bred calves.

Materials and Methods

Experimental animals and drug administration

Six healthy male buffalo calves of non-descript breed, ranging between 1-1.5 years of age and 82-128 kg body weight were used. The animals were maintained on seasonal green fodder, wheat straw and water *ad libitum*. The average day temperature in the shed was about 25°C during the experimental period. The experimental protocol followed the ethical guidelines on the proper care and use of animals. Levofloxacin [Tavanic (0.5% levofloxacin), Hoechst Marion Roussel Ltd., India] was administered intramuscularly at the dose rate of 3 mg/kg body weight into the neck region.

Collection of samples

Blood samples (5 ml) were withdrawn from the jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 10, 15, 30 min and 1, 2, 4, 6, 8, 10, 12, 16 and 24 h after administration of drug. Plasma was separated by centrifugation at 1300 g for 15 min at room temperature and kept at -20°C until analysis, which was being done usually on the day after collection.

Estimation of drug concentration

The concentration of levofloxacin in plasma samples was estimated by a standard microbiological assay technique (Arret *et al*., 1971) using *E. coli* (ATCC 10536) as the test organism. This method estimated the level of drug having antibacterial activity, without differentiating between the parent drug and its active metabolites. The assay could detect a minimum of 0.1 μg/ml of levofloxacin (Dumka and Srivastava, 2006). For each sample, 9 replicates were analysed and compared with the zone of inhibition of reference levofloxacin solution (0.3 μg/ml). The concentration of levofloxacin in the samples was calculated as μg/ml of plasma.

In vitro plasma protein binding

In vitro binding of levofloxacin to plasma proteins was determined by employing the equilibrium dialysis technique (Kunin *et al*., 1959). Various concentrations of levofloxacin (0.5, 1, 5, 10 and 20 μg/ml) were prepared in pooled plasma taken from untreated animals. Each dialyzing bag (4 Aº pore size) filled with 5 ml of plasma containing known amount of drug and then immersed in a separate tube containing 5 ml of phosphate buffer (0.2 M; pH = 7.4), the tubes were then incubated at 37ºC for 24 h with occasional shaking. At the end of incubation period, buffer as well as contents of the dialyzing bags were separately analysed for the concentration of levofloxacin. For each concentration three separate sets of experiments were conducted. The extent of *in vitro* plasma protein binding of levofloxacin was calculated by the following equation:

$$\text{Percent of levofloxacin bound to plasma protein} = \frac{CP' - CB}{CP} \times 100$$

where, CP′ is the concentration of levofloxacin in plasma after incubation, CB is the concentration of levofloxacin in phosphate buffer after incubation and CP is the concentration of levofloxacin in plasma before incubation. The free drug concentration of levofloxacin was plotted against the constant I_i which was obtained by the following equation:

$$I_i = \frac{P}{T/L - W}$$

where, P = protein content of plasma, T = total concentration of drug, L = free concentration of drug and W = water content of plasma. β_i was calculated by least squares regression technique and its negative intercept with the ordinate was equal to K_β.

Pharmacokinetic variables and dosage regimen

The plasma concentration-time profile of levofloxacin after intramuscular administra-

tion in each animal was used to establish various pharmacokinetic determinants and mean kinetic variables were obtained by averaging the variables calculated for individual animals. Overall systemic bioavailability was calculated by using the values of AUC and β (10.5 ± 0.1 μg.h/ml and 0.27 ± 0.01 /h, respectively) obtained after single intravenous administration of levofloxacin in the same animals which were used for intramuscular study of levofloxacin at an interval of 30 days. Pharmacokinetic parameters were calculated manually by the computed least squares linear regression technique (Gibaldi and Perrier, 1982). Using convenient dosage interval and using the values of β and Vd_{area} from Table 1, the priming (D) and maintenance (D') doses of levofloxacin were calculated from the following equations:

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

$D' = Cp\ (min)^{\alpha}.\ Vd\ (e^{\beta\tau}-1)$

where, $Cp\ (min)^{\alpha}$ is the minimum therapeutic concentration of levofloxacin, β is the elimination rate constant and τ is the dosing interval (Baggot, 1977).

Results

The mean plasma levels of levofloxacin at different time intervals following its single intramuscular injection at the dose rate of 3 mg/kg body weight in buffalo calves are presented on a semilogarithmic scale in Fig. 1. Intramuscular injection resulted in appreciable plasma concentration of drug (0.280 ± 0.003 μg/ml) at 1 min and peak plasma level of 2.95 ± 0.13 μg/ml was attained at 1 h post-administration. The plasma levels then declined gradually to 0.18 μg/ml at 12 h. Evaluation of the results revealed that the disposition pattern of levofloxacin was best fitted to one-compartment open model and it was adequately described by the equation:

$Cp = Be^{-\beta t} - A'e^{-Ka.t}$

where, Cp is the plasma level of levofloxacin at time t and e represents the base of natural logarithm, A' and B are the extrapolated zero-time intercepts of the absorption and elimination phases, respectively, Ka and β are the absorption and elimination rate constants, respectively.

Table 1 shows the pharmacokinetic parameters that describe the absorption and elimination pattern of levofloxacin in buffalo calves. By taking various dosage intervals, the different desired plasma concentrations ranging from 0.06 to 0.14 μg/ml and using the values for β and Vd_{area} from Table 1, the required doses of levofloxacin were calculated and are presented in Table 2. Table 3 summarizes the parameters of *in vitro* plasma protein binding of levofloxacin. At plasma concentrations of 0.5 to 20 μg/ml the extent of plasma protein binding of levofloxacin ranged from 11.9 to 27.0% with an overall mean of 19.1 ± 1.5%. The values of association rate constant (β_i) and

Table 1: Pharmacokinetic parameters of levofloxacin in buffalo calves (n = 6) following its single intramuscular dose of 3 mg/kg body weight

Parameter	Unit	Mean ± SE
A'	μg/ml	2.41 ± 0.45
Ka	/h	1.88 ± 0.15
$t_{1/2Ka}$	h	0.38 ± 0.03
B	μg/ml	2.25 ± 0.21
β	/h	0.22 ± 0.02
$t_{1/2\beta}$	h	3.27 ± 0.31
AUC	μg.h/ml	8.81 ± 0.37
AUMC	$\mu g.h^2/ml$	46.0 ± 6.5
Vd_{area}	L/kg	1.06 ± 0.04
Cl_B	ml/kg/h	343.2 ± 14.1
MRT	h	5.40 ± 0.59
td	h	16.0 ± 1.5
C_{max}	μg/ml	2.95 ± 0.13
t_{max}	h	1.00 ± 0.00
F	%	68.1 ± 5.4
AUC/MIC	ratio	88.1 ± 3.7
C_{max}/MIC	ratio	29.5 ± 1.3

A′ and B = zero-time plasma drug concentration intercepts of the regression lines of absorption and elimination phases, respectively; Ka and β = absorption and elimination rate constants, respectively; $t_{1/2Ka}$ = absorption half-life; $t_{1/2\beta}$ = elimination half-life; AUC = area under the plasma concentration-time curve; AUMC = area under the first moment curve; Vd_{area} = apparent volume of distribution; Cl_B = total body clearance; MRT = mean residence time; td = duration of therapeutic effect; C_{max} and t_{max} = peak plasma drug concentration and time required to attain the peak concentration, respectively; MIC = minimum inhibitory concentration of drug in plasma; F = overall systemic bioavailability

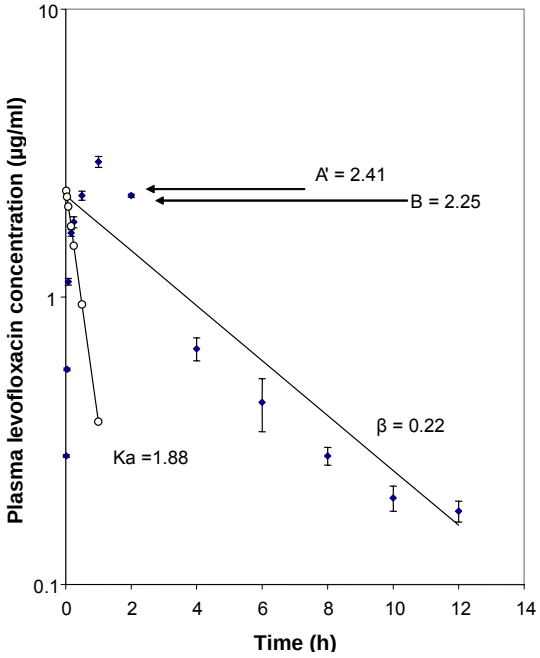

Fig. 1: Semilogarithmic plot of plasma concentration-time profile of levofloxacin following its single intramuscular injection of 3 mg/kg body weight in buffalo calves. Values are presented as mean ± SE of 6 animals. Data were analysed according to one-compartment open model. Absorption and elimination phases are represented by least square regression lines. The calculated points (○) of absorption phase were obtained by residual technique. Constants A' and B are zero-time intercepts of absorption and elimination phases, respectively

dissociation rate constant (K_β) were 2.6 × 10^{-8} mol/g and 2.4 × 10^{-7} mol, respectively.

Discussion

The rapid appearance of levofloxacin in plasma following its intramuscular administration in buffalo calves suggested that the drug rapidly entered into the systemic circulation. The high value of absorption rate constant further confirmed rapid absorption of levofloxacin. Rapid absorption after intramuscular injection has also been reported for another fluoroquinolone used in veterinary practice, marbofloxacin (Shem-Tov *et al*., 1997; Schneider *et al*., 2004) in cattle. An average plasma concentration of 0.032-0.5 µg/ml has been reported to be the minimum therapeutic concentration (MIC_{90}) of levofloxacin against most Gram-positive, Gram-negative and atypical bacteria (Chulavatnatol *et al*., 1999). Keeping in mind the synergistic effect of the body immune system and other *in vivo* factors, and to cover most of the susceptible organisms, in this discussion, the MIC_{90} of 0.1 µg/ml of levofloxacin has been taken into consideration. The peak plasma level of levofloxacin attained in the present study was approximately 30 fold higher than the MIC of levofloxacin and the drug was detected above the minimum therapeutic plasma level up to 12 h of administration.

Table 2: Calculated intramuscular dosage regimen of levofloxacin (mg/kg) at various intervals for different MICs in buffalo calves

MIC (µg/ml)	Dose	Dosage interval (h)		
		8	12	16
0.06	D	0.40	1.04	2.79
	D'	0.33	0.98	2.72
0.08	D	0.53	1.39	3.73
	D'	0.45	1.30	3.64
0.10	D	0.66	1.73	4.66
	D'	0.56	1.63	4.55
0.12	D	0.80	2.08	5.59
	D'	0.61	1.95	5.45
0.14	D	0.93	2.43	6.53
	D'	0.78	2.28	6.36

D = priming dose and D' = maintenance dose

Table 3: *In vitro* binding and kinetic constants of levofloxacin to plasma proteins of buffalo calves

Exp. No	Extent of binding (%)					Association rate constant, β_i (mol/g)	Dissociation rate constant, K_β (mol)
	Concentration of levofloxacin (µg/ml)						
	0.5	1	5	10	20		
1	15.3	17.8	15.5	26.3	25.2		
2	12.8	13.6	17.6	22.3	26.4		
3	10.6	14.5	16.2	23.7	29.5		
Mean ± SE	11.9	14.3	16.4	24.1	27.0		
	±1.4	±1.3	±0.6	±1.2	±1.3	2.6×10^{-8}	2.4×10^{-7}

Overall Mean ± SE of extent of binding (%) = 19.1 ± 1.5

The high value of AUC (8.81 ± 0.37 μg.h/ml) and Vd_{area} (1.06 ± 0.04 L/kg) obtained in the present study reflected vast area of body covered by drug concentration and good penetration of levofloxacin into various body fluids and tissues after intramuscular injection. These observations are in accordance with the high values of AUC for marbofloxacin (7.7 μg.h/ml) in cows (Schneider *et al*., 2004) and Vd_{area} for danofloxacin (1.42 L/kg) in goats (Aliabadi and Lees, 2001) reported after intramuscular injection. The high values of AUC/MIC_{90} (88.1 ± 3.7) and C_{max}/MIC_{90} (29.5 ± 1.3) obtained in the present study, gave an indication of good antibacterial activity of levofloxacin in buffalo calves. High ratio of AUC/MIC has also been reported after intramuscular injection of marbofloxacin (40.7) in cows (Schneider *et al*., 2004) and danofloxacin (55.9) in sheep (Aliabadi *et al*., 2003). In agreement with the present results, a C_{max}/MIC ratio of more than 10 has been reported following subcutaneous administration of danofloxacin and enrofloxacin in calves (TerHune *et al*., 2005). The total body clearance of levofloxacin (343.2 ± 14.1 ml/kg/h) in buffalo calves was in accordance with the Cl_B of 204.9 ml/kg/h reported after intramuscular injection of levofloxacin in calves (Dumka and Srivastava, 2006). The elimination half-life (3.27 ± 0.31 h) in the present study was comparable to the $t_{1/2\beta}$ of 2.53 h for marbofloxacin (Schneider *et al*., 2004) and 2.4 h for norfloxacin (Gips and Soback, 1996) in cattle and 4.41 h for danofloxacin in goats (Aliabadi and Lees, 2001) observed after intramuscular administration. Among various pharmacokinetic parameters, bioavailability plays an important role in the therapeutic efficacy of a drug. On the basis of AUC and β after single intravenous (10.5 ± 0.1 μg.h/ml and 0.27 ± 0.01 /h, respectively) and intramuscular administration (Table 1) in buffalo calves, the systemic bioavailability of levofloxacin was calculated to be 68.1 ± 5.4%. This finding was comparable to the systemic bioavailability of 56.6% for levofloxacin and 73% for norfloxacin reported after their intramuscular administration in cattle (Gips and Soback, 1996; Dumka and Srivastava, 2006).

The extent of binding of levofloxacin to the plasma proteins of buffalo calves (19.1 ± 1.5%) in the present study was in accordance with the corresponding values of 24-38% for levofloxacin in man (Langtry and Lamb, 1998) and 26% for danofloxacin (Giles *et al*., 1991) and 36-45% for enrofloxacin (Kaartinen *et al*., 1995) in cattle. The values of β_i and K_β were 2.6×10^{-8} mol/g and 2.4×10^{-7} mol, respectively, in the present study, reflecting that the binding of levofloxacin to the plasma proteins of buffalo calves was weak and reversible.

On the basis of the present study, the priming and maintenance doses of levofloxacin, at a dosage interval of 12 h, were calculated to be 1.73 and 1.63 mg/kg, respectively. Under field condition, for most bacteria sensitive to levofloxacin the most appropriate dosage regimen of levofloxacin, would be 1.7 mg/kg repeated at 12-h intervals. These regimens are suitable for the treatment of respiratory, gastrointestinal, urinary tract and other infections in buffalo calves. Lack of local reaction or any other adverse effect, rapid absorption, moderate bioavailability and large volume of distribution of levofloxacin observed in the present study revealed that levofloxacin may be effectively employed by intramuscular route in the treatment of bacterial infections in buffalo calves.

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