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Asian Journal of Animal and Veterinary Advances

Pharmacokinetics of Levofloxacin Following Intravenous and Intramuscular Administration in Cattle Calves

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ABSTRACT

Levofloxacin is a third generation fluoroquinolone with excellent tissue penetration and efficacy against respiratory and urogenital bacterial infections. In the present study, disposition kinetics of levofloxacin was studied in cattle calves following an intravenous (i.v.) and intramuscular (i.m.) administration at a dose rate of 10 mg kg^{-1}. Blood was collected at predetermined time intervals and plasma was separated. Plasma concentrations of levofloxacin were determined using the HPLC assay method (R^2 = 0.999). Plasma concentration versus time data was subjected to compartmental pharmacokinetic analysis using nonlinear iterative computer software "PHARMKIT". Following intravenous and intramuscular administration of levofloxacin in cattle calves (10 mg kg^{-1}), the plasma concentration versus time data of levofloxacin was best described by two-compartment open model and one-compartment open model with first order absorption rate constant with modest bioavailability values, respectively. The mean values for $t_{\frac{1}{2}\alpha}$ and $t_{\frac{1}{2}\beta}$ were 0.05±0.01 h and 2.12±0.21 h, respectively for i.v. route. The values of AUC, Vd_{area} and Cl_B were found to be 29.32±1.19 µg mL^{-1} h, 1.05±0.10 L kg^{-1} and 0.34±0.01 L h^{-1} kg^{-1}, respectively while the ratio of drug concentrations between the tissues and plasma (T/P) was 4.47±02.09. For i.m. route, the mean (±SE) values of $t_{1/2Ka}$, $t_{\frac{1}{2}\beta}$, AUC, MAT, MRT and F were found to be 0.51±0.09 h, 2.76±0.36 h, 18.43±2.15 µg mL^{-1} h, 1.85±0.46 h, 4.72±0.72 h and 62.65±5.99%, respectively. Based on the pharmacodynamic indices, the optimal dose of levofloxacin in cattle calves may be proposed as 10 mg kg^{-1} b.wt. repeated at 24 h interval preferably by intravenous route for treating common microbial infections of veterinary importance.

Key words: Levofloxacin, pharmacokinetic parameters, cattle calves, intravenous route, plasma concentration

INTRODUCTION

Levofloxacin is an S-enantiomer (L-isomer) of ofloxacin with double the potency of ofloxacin (Tanaka *et al.*, 1993). Ofloxacin and levofloxacin share basic similarity in their core structure but their physicochemical properties, pharmacokinetic characteristics and microbial activities vary markedly. Levofloxacin is well distributed to target body tissues and fluids in the respiratory tract, skin, urine and prostate. In addition, its cellular uptake along with poor central nervous system penetration makes it more favourable for use against intracellular pathogens (Langtry and Lamb, 1998). Bacterial infections are a common occurrence in cattle calves due to contaminated feed and

water (Luga *et al.*, 2007). The clinical situation is further aggravated due to emergence of resistance against the common antimicrobials in such situation, Levofloxacin can be a promising therapeutic tool for several respiratory and urogenital tract infections (Ram *et al.*, 2008; Kumar *et al.*, 2009) in cattle calves which comprise an important component of the Indian livestock population.

The pharmacokinetic profile of levofloxacin has already been studied in large number of animal species including in cross bred calves (Dumka and Srivastava, 2006), sheep (Patel *et al.*, 2012a), buffalo calves (Ram *et al.*, 2008, 2010), goat (Ram *et al.*, 2008; Mishra and Roy, 2007), cats (Albarellos *et al.*, 2005), stallions (Goudah *et al.*, 2008), birds (Patel *et al.*, 2008a, b), rabbit (Destache *et al.*, 2001) and mice (Ender *et al.*, 2003). Thus optimization of levofloxacin dose is required to ensure that the judicious use of levofloxacin in food producing species such as cattle calves without causing any toxicities to the animal as well to avoid public health hazards. Therefore, the present study was taken up with the objective to evaluate the pharmacokinetic profile of levofloxacin in cattle calves following an intravenous and intramuscular administration at a dose of 10 mg kg^{-1}.

MATERIALS AND METHODS

Experimental design: The study was conducted over a period of 6 months (October, 2009-March, 2010) on six healthy female cattle calves aging 3-6 months (weighing 47-85 kg) quarantined at the Dairy Farm of University. The cattle calves were maintained following standard managemental practices and provided concentrate, green fodder and wheat straw along with *ad libitum* clean and fresh drinking water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

The standard curve was prepared using pure technical grade levofloxacin from Sigma-Aldrich while injectable formulation of levofloxacin (Meriflox®; Wockhardt Ltd., Mumbai) was used for animal study. Levofloxacin was injected intravenously (i.v.) by jugular venepuncture and intramuscularly on the lateral aspect of neck and blood samples were collected into heparinised tubes by contralateral venepuncture at predetermined time intervals i.e., 0, 2.5, 5, 10, 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 h post drug administration. The blood samples were centrifuged to separate plasma which were stored at -20°C until further assay.

Analytical procedure: Extraction of levofloxacin from plasma samples was carried out as per the modified method of Nielsen and Gyrd-Hansen (1997). The plasma (0.5 mL) was deproteinized by adding of 100 µL perchloric acid (0.6 M) into 0.5 mL plasma, vortexed for 1 min at high speed and then centrifuged at 10,000 xg for 5 min. The supernatant was filtered through millipore 0.22 µm filter and injected into HPLC system.

For determination of levofloxacin levels in plasma, an isocratic mobile phase of acetonitrile, water, phosphoric acid and triethylamine (16:83:0.6:0.45, by volume) with pH adjusted to 2.34 at a flow rate of 0.6 mL min^{-1} was used to elute levofloxacin from C_{18}-reverse phase column (4×125 mm; particle size 5 µm, Waters XBridge™) which was detected using a Waters 2996 photodiode array detector with software Empower at 294 nm (Gao *et al.*, 2007).

Peak areas were used for quantification of the compounds. The calibration curves prepared by spiking external standards in the pooled drug-free calf plasma were linear in the range of 0.02 to 3.2 µg mL^{-1} for levofloxacin with coefficient of variation (R^2) of 0.98. Recovery was 90%. The lowest limit of detection and quantification were 0.01 and 0.02 µg mL^{-1}, respectively. The intra and inter-assay coefficients of variation were 3.8 and 4.25, respectively.

Pharmacokinetic analysis: The plasma concentration vs. time data of levofloxacin was analyzed with the help of a non-linear iterative curve fitting computer programme (PHARMKIT) and other parameters were determined using the equations described by Baggot (1977) and Gilbadi and Perrier (1982). The best compartment model was selected on the basis of positive and negative residuals and Akaike Information criterion. The dosage regimens of levofloxacin were based on the pharmacokinetic and pharmacodynamic indices of antimicrobials (Dudley, 1991; Walker, 2000).

RESULTS

Following intravenous administration, the mean plasma concentration of levofloxacin was 24.00 ± 3.67 μg mL^{-1} at 0.04 h which initially declined to 9.65 ± 0.60 μg mL^{-1} at 0.25 h and thereafter gradually to 2.40 ± 0.13 μg mL^{-1} at 4 h (Fig. 1). Levofloxacin could be detected in plasma for up to 18 h after drug administration. An appreciable and clinically effective concentration of levofloxacin (1 μg mL^{-1}) in plasma could be detected within at 0.04 h and the peak plasma concentration of levofloxacin was observed at 1 h following i.m. administration (Fig. 1). Levofloxacin could be detected in plasma up to 18 h after drug administration.

The intravenous and intramuscular plasma concentration-time data could be best fitted to a two-compartment open model and one compartment open model, respectively and were adequately described by the following biexponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \text{ for i.v. route}$$

$$C_p = Be^{-\beta t} - A'e^{-k_a t} \text{ for i.m. route}$$

where, Cp is the plasma concentration of levofloxacin at time t, A and B are the zero time plasma drug concentration intercepts of the biphasic disposition curve and α and β are first order rate constant related to distribution (or absorption) and elimination phases, respectively and "e" is base of the natural logarithm. The resulting pharmacokinetic parameters and pharmacodynamic-pharmacokinetic indices have been mentioned in Table 1 and 2.
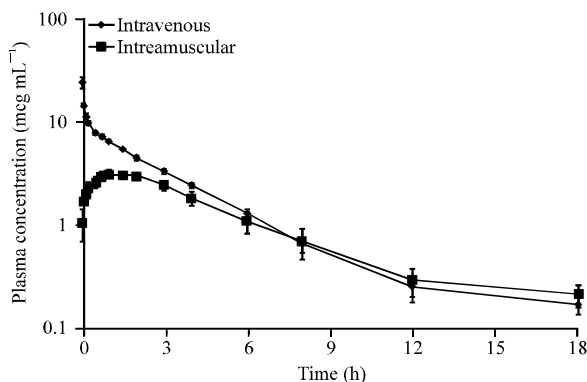

Fig. 1: Semilogarithmic plot of plasma concentrations of levofloxacin vs. time data following a single intravenous and intramuscular administration of levofloxacin (10 mg kg^{-1}) in goats, each point represents the Mean±SE of 6 animals

Table 1: Pharmacokinetic parameters (Mean±SE) of a single intravenous and intramuscular administration of levofloxacin (10 mg kg^{-1}) in cattle calves

Pharmacokinetic parameters	Intravenous administration	Intramuscular administration
A (µg mL^{-1})	52.26±29.92	-
B (µg mL^{-1})	9.24±0.75	5.72±0.62
α/Ka (h^{-1})	20.20±6.22	1.66±0.37
β/Ke (h^{-1})	0.34±0.03	0.27±0.03
$t_{\frac{1}{2}\alpha/Ka}$ (h)	0.05±0.01	0.51±0.09
$t_{\frac{1}{2}\beta/Ke}$ (h)	2.12±0.21	2.76±0.36
AUC (µg mL^{-1} h^{-1})	29.32±0.19	18.43±2.15
AUMC (µg mL^{-1} h^{-2})	84.57±1.17	93.55±26.77
MAT (h)	-	1.85±0.46
MRT (h)	2.87±0.31	4.72±0.72
Vd$_{area}$ (L kg^{-1})	1.05±0.10	2.19±0.22
Vd$_{ss}$ (L kg^{-1})	0.98±0.10	-
Cl$_B$ (L h^{-1} kg^{-1})	0.34±0.01	0.57±0.05
K$_{12}$ (h^{-1})	14.55±5.40	-
K$_{21}$ (h^{-1})	3.97±0.38	-
K$_{12}$/K$_{21}$ (ratio)	3.98±1.79	-
Vc (L kg^{-1})	0.30±0.07	-
Vp (L kg^{-1})	0.68±0.07	-
T/P (ratio)	4.47±2.09	-
f$_c$ (ratio)	0.28±0.06	-
F (%)	-	62.65±5.99

Table 2: Calculation of pharmacodynamic-pharmacokinetic indices for dosage regimen computation

Pharmacodynamic determinants	Intravenous (MIC$_{90}$)		Intramuscular (MIC$_{90}$)	
	0.06 µg mL^{-1}	0.12 µg mL^{-1}	0.06 µg mL^{-1}	0.12 µg mL^{-1}
AUC/MIC	488.7	244.3	251.7	125.8
C$_{max}$/MIC	1025	512.5	24	48

DISCUSSION

In the present study, the pharmacokinetic profile of levofloxacin following its intravenous administration in cattle calves was in perfect accordance with the two compartment disposition model. Two compartment pharmacokinetic behavior of levofloxacin has already been reported in cross bred calves (Dumka and Srivastava, 2006), sheep (Patel *et al.*, 2012a), buffalo calves (Ram *et al.*, 2008, 2010), goat (Ram *et al.*, 2008; Mishra and Roy, 2007), cats (Albarellos *et al.*, 2005), stallions (Goudah *et al.*, 2008), birds (Patel *et al.*, 2008a, b), guinea pigs (Edelstein *et al.*, 1996), rabbit (Destache *et al.*, 2001) and mice (Ender *et al.*, 2003).

A higher value of distribution rate constant (α) 20.20±6.22 h^{-1} indicated rapid distribution of levofloxacin in cattle calves. However, longer distribution half lives have been reported in male camels (0.26±0.21 h), lactating goats (0.31±0.11 h) and stallion (0.21±0.13 h) (Goudah and Abo-El-Sooud, 2009; Goudah *et al.*, 2008; Goudah, 2009). The results indicated that the distribution of levofloxacin was faster in calves in comparison to camels, goats and stallion. The differences may be attributed to the physiological variations among the species including the variations in plasma protein binding of the drug. Variations in the plasma microenvironment can significantly alter the bound fraction of levofloxacin and thus result in major kinetic alterations

(Sheikh *et al.*, 2001). Following single intramuscular administration at the dose rate of 10 mg kg^{-1} b.wt. the absorption half-life ($t_{1/2Ka}$) following i.m. administration in calves was quite comparable to that reported in cattle calves (0.35±0.03 h; Dumka and Srivastava, 2006), camels (0.43±0.23 h; Goudah, 2009) and lactating goats (0.54±0.10 h; Goudah and Abo-El-Sooud, 2009); thus suggesting insignificant species variation in absorption kinetics.

The biological half life ($t_{1/2\beta}$) of levofloxacin in cattle calves following i.v. administration suggest rapid elimination of levofloxacin from cattle calves as comparatively higher biological half life values have been reported in camel and lactating goats (Goudah, 2009; Goudah and Abo-El-Sooud, 2009). Elimination half-life ($t_{1/2\beta}$) of levofloxacin after i.v. and i.m. administration in calves was almost comparable to that of 2.15±0.07 h in febrile sheep (Patel *et al.*, 2012b) and 3.05±0.17 h in cross bred calves (Kumar *et al.*, 2009), 3.27±0.31 h in buffalo calves (Ram *et al.*, 2008), 3.47±0.86 h in camel (Goudah, 2009) and 2.94±0.78 h in stallions (Goudah *et al.*, 2008).

The AUC value of levofloxacin in cattle calves was similar to earlier reported values in goats (Mishra and Roy, 2007), stallion (Goudah *et al.*, 2008). However, much lower value of AUC have been observed in layer and broiler birds (11.07-11.33 µg h mL^{-1}) after i.v. administration (Patel *et al.*, 2008a, b). The AUC values for i.m. route were comparable to that of 21.31±1.24 µg mL^{-1} h in lactating goats (Goudah and Abo-El-Sooud, 2009). Lower values have been observed in cross bred calves (7.66±0.72 µg mL^{-1} h) (Dumka and Srivastava, 2006), buffalo calves (8.81±0.37 µg mL^{-1} h) (Ram *et al.*, 2008) and camels (13.63±3.11 µg mL^{-1} h) (Goudah, 2009).

The value of Mean Residence Time (MRT) of levofloxacin in cattle calves was 2.87±0.31 h and was much shorter compared to the MRT values in lactating and nonlactating goats (Ram *et al.*, 2008; Goudah and Abo-El-Sooud, 2009; Mishra and Roy, 2007) and camels (Goudah, 2009). The mean value of apparent volume of distribution (Vd$_{area}$) was comparable to the volume of distribution in febrile sheep (1.30±0.12 L kg^{-1}) (Patel *et al.*, 2012b), cats (1.75±0.42 L kg^{-1}) (Albarellos *et al.*, 2005), stallion (0.81±0.26 L kg^{-1}) (Goudah *et al.*, 2008) but lower compared to that in layer birds (4.02±0.08 L kg^{-1}) (Patel *et al.*, 2008b). Based on the results of the present study, it may be inferred that levofloxacin is widely distributed into body tissues and fluids of cattle calves as is true for fluoroquinolones in general in different species of animals (Brown, 1996; McKellar, 1996).

The ratio of transfer rate constant of the drug from the central to the peripheral compartment and vice versa (3.98±1.79) indicated a faster rate of drug transfer from the central to the peripheral compartment than redistribution from the peripheral to the central compartment. The fraction of drug in central compartment (f_c) was 0.28±0.06 and the calculated value of T/P was (4.47±2.09). This was expected as the K_{12}/K_{21} ratio was more than 1. Levofloxacin, as with several fluoroquinolones, is amphoteric because of the presence of a carboxylic acid and basic amine functional groups. Passive diffusion across biological membranes is a function of lipophilicity relative to the pKa values of the two ionizable moieties. These observations suggest that apart from wide distribution of drug from the central compartment, it is retained in peripheral tissues, a desirable feature for the treatment of deep seated infections.

Clearance of a drug indicates the volume of biological fluid (plasma/blood) from which the drug has been removed per unit of time (L min^{-1} kg^{-1}) to account for its elimination. Total body clearance (Cl_B) is a measurement of the ability of the body to eliminate drug and represent the sum of different clearance processes of the drug from the body e.g., hepatic biotransformation and renal excretion etc. Levofloxacin undergoes active renal tubular secretion (Waheed *et al.*, 2002). In the present study, the clearance was comparable to earlier reports in sheep (Patel *et al.*, 2012a, b) but were much higher as compared to human volunteers (Iqbal *et al.*, 2000; Waheed *et al.*, 2002).

Bioavailability (F) is one of the important determinants of drug therapeutic efficacy. In the present study, the overall bioavailability was 62.55± 5.99%. Lower bioavailability values have been reported to be in calves (44.3-56.6%) in earlier studies (Dumka and Srivastava, 2006; Ram *et al.*, 2010). Much higher nonvascular bioavailability has been reported in camels and sheep (90-94% approximately) (Goudah, 2009; Patel *et al.*, 2012a, b).

It has been established that for concentration-dependent fluoroquinolones, the AUC/MIC ratio is the most important predictor of efficacy with a clinical cure rate greater than 80% when this ratio is higher than 100-125 (Lode *et al.*, 1998). A second predictor of efficacy for concentration-dependent antibiotics is the C_{max}/MIC ratio, considering that values>10 lead to better clinical results (Toutain *et al.*, 2002). It has also been now accepted that high C_{max}/MIC values are necessary in order to avoid the emergence of bacterial resistance (Walker, 2000). The critical breakpoints that determine the efficacy of fluoroquinolones are suggested as C_{max}/MIC>0 and AUC/MIC>125 (Walker, 2000; Toutain *et al.*, 2002). The MIC of levofloxacin has not yet been determined for bacteria isolated from cattle calves. To cover most of the susceptible organism, in the cattle population, the MIC_{90} of 0.06 to 0.12 µg mL^{-1} of levofloxacin has been taken into consideration (Marshall and Jones, 1993). Based on the pharmacokinetic data generated in this study and taking the extremes of MIC_{90}, a dosage of 10 mg kg^{-1} levofloxacin i.v. in cattle calves would result in AUC/MIC and C_{max}/MIC ratios in Table 2. Based on the calculated C_{max}/MIC and AUC/MIC, levofloxacin dose of 10 mg kg^{-1} once daily can effectively be used by intravenous route of administration.

CONCLUSION

On the basis of the present study, a dose of 10 mg kg^{-1} of levofloxacin once daily intravenously or intramuscularly may be recommended for the effective eradication of bacterial pathogens in cattle calves.

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