Iranian Journal of Veterinary Research, University of Shiraz, Vol. 8, No. 4, Ser. No. 21, 2007

Kinetic disposition, urinary excretion and dosage regimen of subcutaneously administered levofloxacin in cross bred calves

Dumka, V. K.[1*] and Srivastava, A. K.[2]

[1]Department of Pharmacology and Toxicology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India; [2]Faculty of Veterinary Sciences and Animal Husbandry, SKUAST(J), R S Pura, Jammu-181102, India

[*]**Correspondence:** V. K. Dumka, Department of Pharmacology and Toxicology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India. E-mail: vkdumka@yahoo.com

(Received 5 Nov 2006; revised version 5 Feb 2007; accepted 24 Feb 2007)

Summary

The present study was conducted on five healthy male cross bred calves to study the kinetic disposition and urinary excretion of levofloxacin following its single subcutaneous administration at the dose of 4 $mg.kg^{-1}$. The concentration of levofloxacin in plasma and urine samples was estimated by microbiological assay. Peak plasma level of levofloxacin (2.8 ± 0.3 $\mu g.ml^{-1}$) was observed at 45 min and the drug level above MIC_{90} in plasma, was detected up to 12 hrs of administration. The bioavailability of levofloxacin was $41.9 \pm 3.2\%$. High value of AUC (9.88 ± 0.1 $\mu g.ml^{-1}.hr$) reflected a vast area of body covered by drug concentration. Good distribution of the drug was reflected by the high value of Vd_{area} (0.73 ± 0.04 $L.kg^{-1}$). The elimination half-life and MRT were 3.0 ± 0.2 hr and 4.79 ± 0.2 hr, respectively. A suitable subcutaneous dosage regimen for levofloxacin was calculated to be 1.25 $mg.kg^{-1}$ repeated at 12-hr intervals in calves.

Key words: Calves, Dosage, Levofloxacin, Subcutaneous, Urinary excretion

Introduction

Fluoroquinolone antibacterials are increasingly being employed in veterinary medicine for the treatment of mild to severe bacterial infections. 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 [(-) -9-Fluoro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-2, 3-dihydro-7H-pyrido [1, 2, 3-de] [1,4]-benzoxazine-6-carboxylic acid] a recently introduced second-generation fluoroquinolone, possesses excellent activity against Gram-positive, Gram-negative and anaerobic bacteria (Davis and Bryson, 1994; North *et al*., 1998). As compared to other fluoroquinolones, ofloxacin and ciprofloxacin, it also has more pronounced bactericidal activity against organisms like *Pseudomonas*, Entero-bacteriaceae 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). Levofloxacin is metabolized in the liver to demethyl-levofloxacin and levofloxacin-N-oxide and excreted in urine (Langtry and Lamb, 1998). Levofloxacin has been found to alter the pharmacokinetic behaviour and urinary excretion of meloxicam in calves (Dumka and Srivastava, 2006a). The disposition of levofloxacin has been investigated in man (Chulavatnatol *et al*., 1999), guinea pigs (Edelstein *et al*., 1996) and after intramuscular administration in calves (Dumka and Srivastava, 2006b). However, information on the pharmacokinetics of levofloxacin in calves is still meagre. In view of the marked species variation in the kinetic data of

antimicrobial drugs, the present study was undertaken to determine the kinetic disposition, urinary excretion and an appropriate dosage regimen of levofloxacin, following its single subcutaneous administration in cross bred calves.

Materials and Methods

The experiments were performed on 5 healthy male cross bred calves (Holstein Friesian × Sahiwal), ranging between 1-1.5 years of age with an average weight of 86 ± 12.5 kg. The animals were acclimatized to the experimental animal house conditions for 2 weeks prior to the commencement of the study and were provided 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 subcutaneously at the dose of 4 $mg.kg^{-1}$ body weight into the neck region.

To conduct the pharmacokinetic study, the animals were kept in metabolic stalls of standard size, designed in such a way that whole amount of urine excreted naturally by the animals within a period is automatically collected without contamination or spillage in the containers placed beneath the stalls. Blood samples (5 ml) were withdrawn from the jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 7.5, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16 and 24 hrs after administration of drug. Plasma was separated by centrifugation at 2000 g for 15 min at room temperature and kept at -20°C until analysis, which was usually done on the next day after collection. Urine samples were also collected simultaneously from the same animals at various predetermined time intervals of 2, 4, 6, 8, 10, 12, 16 and 24 hrs after administration of drug. At the end of the given time interval, the volume of total urine voided and collected in the container, was measured for each animal and after filtration, 10 ml of samples were taken for analysis.

The concentration of levofloxacin in plasma and urine samples was estimated by a standard microbiological assay technique (Arret *et al*., 1971) using *Escherichia 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 $\mu g.ml^{-1}$ of levofloxacin. For each sample, 9 replicates were analysed and compared with the zone of inhibition of standard reference solution (0.3 $\mu g.ml^{-1}$). Preliminary experiments were conducted to determine the concentration of standard reference solution of levofloxacin required to obtain the required optimum dimensions (15-16 mm diameter) of the zone of inhibition. The concentration of levofloxacin in the samples was calculated as $\mu g.ml^{-1}$ of plasma or urine.

The plasma concentration-time profile of levofloxacin after subcutaneous administration in each animal was used to establish various disposition kinetic 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 obtained after single intravenous administration of levofloxacin in the same animals which were used for subcutaneous study of levofloxacin at an interval of 30 days. Disposition kinetic parameters were calculated manually by the computed least-squares linear regression technique (Gibaldi and Perrier, 1982). The maintenance dose (D') of levofloxacin in calves was calculated according to the equation:

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

where, Cp $(min)^{\propto}$ is the minimum therapeutic concentration of levofloxacin, Vd is the apparent volume of distribution, β is elimination rate constant and τ is the dosing interval. The priming dose was obtained by omitting -1 from the above equation.

Results

The mean plasma concentrations of levofloxacin, following its single sub-

cutaneous administration at the dose of 4 $mg.kg^{-1}$ body weight as a function of time on a semilogarithmic scale, are presented in Fig. 1. Subcutaneous injection resulted in appreciable plasma concentration of drug (0.28 ± 0.02 $\mu g.ml^{-1}$) at 2.5 min and peak plasma level of 2.8 ± 0.3 $\mu g.ml^{-1}$ was attained at 45 min post administration. The drug was detected in plasma for up to 12 hrs after dosing (0.19 ± 0.01 $\mu g.ml^{-1}$). Evaluation of the results revealed that the disposition pattern of levofloxacin was best fitted a mono-compartment open model and

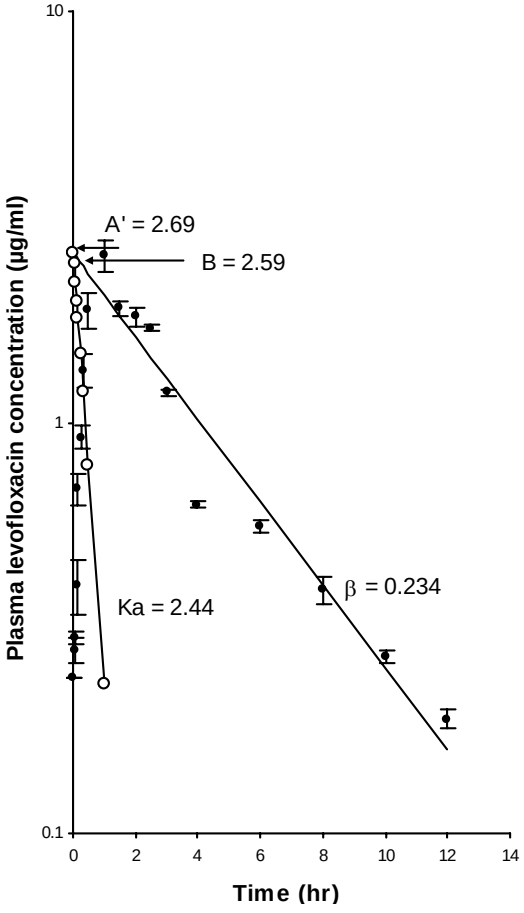

Fig. 1: Semilogarithmic plot of plasma concentration-time profile of levofloxacin following its single subcutaneous injection of 4 $mg.kg^{-1}$ body weight in cross bred calves. Values are presented as mean ± SE of 5 animals. Data were analysed according to one-compartment open model. Absorption and elimination phases are represented by least square regression lines. The calculated points (o) of absorption phase were obtained by residual technique. Constants A' and B are zero-time intercepts of absorption and elimination phases, respectively

it was adequately described by the bi-exponential equation: $C_p = Be^{-\beta t} - A'e^{-Kat}$, where, C_p 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. The disposition kinetic parameters that describe the absorption and elimination pattern of levofloxacin in calves, were calculated and presented in Table 1. The mean cumulative amount and per cent of levofloxacin excreted in urine at different time intervals are shown in Table 2.

Table 1: Disposition kinetic parameters of levofloxacin in cross bred calves (n = 5) following its single subcutaneous dose of 4 $mg.kg^{-1}$ body weight

Parameter	Unit	Mean ± SE
A'	$\mu g.ml^{-1}$	2.69 ± 0.2
Ka	hr^{-1}	2.44 ± 0.4
$t_{½Ka}$	hr	0.31 ± 0.05
B	$\mu g.ml^{-1}$	2.59 ± 0.2
β	hr^{-1}	0.23 ± 0.01
$t_{1/2\beta}$	hr	3.0 ± 0.2
AUC	$\mu g.\ ml^{-1}.hr$	9.88 ± 0.1
AUMC	$\mu g.ml^{-1}.hr^2$	47.2 ± 2.1
$Vd_{(area)}$	$L.kg^{-1}$	0.73 ± 0.04
Cl_B	$L.kg^{-1}.hr^{-1}$	0.17 ± 0.01
MRT	hr	4.79 ± 0.2
td	hr	16 ± 0.8
C_{max}	$\mu g.ml^{-1}$	2.8 ± 0.3
t_{max}	min	45 ± 0.0
F	%	41.9 ± 3.2
AUC/MIC	ratio	98.8 ± 1.3
C_{max}/MIC	ratio	28.1 ± 2.5

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; $t_{½Ka}$ = absorption half-life; $t_{½\beta}$ = 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

Table 2: Urinary excretion of levofloxacin in cross bred calves following its single subcutaneous dose of 4 mg.kg^{-1} body weight

Time interval (hr)	Amount excreted (mg)	Per cent of total dose excreted	Time interval (hr)	Cumulative amount excreted (mg)	Cumulative per cent of total dose excreted
0-2	13.7 ± 10.6	4.26 ± 3.0	0-2	13.7 ± 10.6	4.26 ± 3.0
2-4	48.8 ± 10.3	16.6 ± 3.4	0-4	59.1 ± 16.3	19.8 ± 4.7
4-6	56.4 ± 27.4	14.3 ± 4.4	0-6	104 ± 21.2	30.2 ± 4.5
6-8	21.2 ± 5.9	5.78 ± 1.0	0-8	125 ± 26.4	36.0 ± 0.9
8-10	10.4 ± 1.5	3.16 ± 0.5	0-10	135 ± 27.2	39.1 ± 5.1
10-12	10.8 ± 1.9	3.07 ± 0.3	0-12	146 ± 28.5	42.2 ± 5.0
12-16	8.47 ± 1.3	2.47 ± 0.3	0-16	154 ± 28.9	44.7 ± 4.8
16-24	6.55 ± 1.6	1.97 ± 0.5	0-24	161 ± 29.6	46.7 ± 5.0

The values given at different time intervals are mean ± SE of the results obtained from 3-5 animals

Discussion

The rapid appearance of levofloxacin in plasma following its subcutaneous administration suggested that the drug rapidly entered into systemic circulation. The high value of absorption rate constant further confirmed rapid absorption of levofloxacin. Rapid absorption after extravascular injection has also been reported for other fluoroquinolones, marbofloxacin (Shem-Tov *et al*., 1997; Schneider *et al*., 2004) and ciprofloxacin (Singh and Srivastava, 2000) in cattle. An average plasma concentration of 0.032-0.5 μg.ml^{-1} 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^{-1} of levofloxacin has been taken into consideration. The peak plasma level of levofloxacin attained in the present study was approximately 28 fold higher than the MIC of levofloxacin and the drug was detected above the minimum therapeutic plasma level up to 12 hrs of administration. Similar to our findings, a peak plasma concentration of 3.4 μg.ml^{-1} was attained after single intraperitoneal injection of levofloxacin in penumonic guinea pigs (Edelstein *et al*., 1996). However, a lower C_{max} of 0.23 ± 0.05 μg.ml^{-1} was reported after subcutaneous administration of danofloxacin in calves (McKellar *et al*., 1999).

The high value of AUC (9.88 ± 0.1 μg.ml^{-1}.hr) and apparent volume of distribution (0.73 ± 0.04 L.kg^{-1}) obtained in the present study reflected vast area of body covered by drug concentration and extensive penetration of levofloxacin into various body fluids and tissues after subcutaneous administration. These observations are in accordance with the similar values for AUC of levofloxacin (7.66 μg.ml^{-1}.hr) in calves (Dumka and Srivastava, 2006b) and marbofloxacin (7.648 μg.ml^{-1}.hr) in cows (Schneider *et al*., 2004) and the high values for Vd_{area} of levofloxacin (1.02 L.kg^{-1}) in calves (Dumka and Srivastava, 2006b) and danofloxacin (1.42 L.kg^{-1}) in goats (Aliabadi and Lees, 2001) reported after intramuscular injection.

The high values of AUC/MIC_{90} (98.8 ± 1.3) and C_{max}/MIC_{90} (28.1 ± 2.5) obtained in the present study, give an indication of the excellent antibacterial activity of levofloxacin in calves. High value of AUC/MIC (23.05) has also been reported after subcutaneous administration of danofloxacin in calves (McKellar *et al*., 1999). In agreement to the present results, a C_{max}/MIC ratio of more than 10 has been reported following subcutaneous administration of both danofloxacin and enrofloxacin in calves (TerHune *et al*., 2005). The total body clearance of levofloxacin (0.17 ± 0.01 L.kg^{-1}.hr^{-1}) and the elimination half-life (3.0 ± 0.2 hr) in the present study, was comparable to the Cl_B of 0.2 L.kg^{-1}.hr^{-1} for levofloxacin after intramuscular injection in calves (Dumka and Srivastava, 2006b) and the $t_{½\beta}$ of 2.53 hrs for marbofloxacin (Schneider *et al*., 2004) and 2.4 hrs for norfloxacin (Gips and

Soback, 1996) in cattle and 4.41 hrs for danofloxacin in goats (Aliabadi and Lees, 2001), observed after extravascular 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 (12.76 ± 0.169 $\mu g.ml^{-1}.hr$ and 0.436 ± 0.021 hr^{-1}, respectively) and subcutaneous (Table 1) administration in calves, the systemic bioavailability of levofloxacin was calculated to be 41.9 ± 3.2 per cent in the present study. Comparable value of bioavailability (56.6%) was obtained after single intramuscular dose of levofloxacin in calves (Dumka and Srivastava, 2006b). This finding was however, greater than the systemic bioavailability of ciprofloxacin (21.6%) but lower than the value of 73% for norfloxacin and 91% for danofloxacin reported after their extravascular administration in cattle (Apley and Upson, 1993; Gips and Soback, 1996; Singh and Srivastava, 2000).

The amount of levofloxacin-equivalent inhibitory units excreted in the urine of calves was very high (6.55 ± 1.6 mg), even at 16-24 hrs after administration. High urinary concentration of danofloxacin (58.58 $\mu g.ml^{-1}$) has been reported after intravenous dose of 1.25 $mg.kg^{-1}$ in goats (Atef *et al*., 2001). Approximately 46.7% of the microbiological activity of the administered drug was recovered in the urine of calves within 24 hrs. This was relatively higher than the urinary recovery of 26.6% of levofloxacin after intramuscular injection in calves (Dumka and Srivastava, 2006b). These findings suggest that levofloxacin may be an appropriate drug for treating urinary tract infections in cattle.

On the basis of the present study, the priming and maintenance doses of levofloxacin, at a dosage interval of 12 hrs, were calculated to be 1.27 and 1.19 $mg.kg^{-1}$, respectively by subcutaneous route, or under field condition; for most bacteria sensitive to levofloxacin, the most appropriate subcutaneous dosage regimen for levofloxacin, would be 1.25 $mg.kg^{-1}$ repeated at 12-hr intervals for the treatment of respiratory, gastrointestinal, urinary tract and other infections in calves. Lack of local reaction or any other adverse effect, rapid absorption, moderate bioavailability, large volume of distribution and high urinary concentration of levofloxacin observed in the present study revealed that levofloxacin may be effectively employed by subcutaneous route in the treatment of bacterial infections in calves.

References

Aliabadi, FS and Lees, P (2001). Pharmacokinetics and pharmacodynamics of danofloxacin in serum and tissue fluids of goats following intravenous and intramuscular administration. Am. J. Vet. Res., 62: 1979-1989.

Apley, MD and Upson, DW (1993). Lung tissue concentrations and plasma pharmacokinetics of danofloxacin in calves with acute pneumonia. Am. J. Vet. Res., 54: 937-943.

Arret, B; Johnson, DP and Kirshbaum, A (1971). Outline of details for microbiological assay of antibiotics: second revision. J. Pharm. Sci., 60: 1689-1694.

Atef, M; El-Gendi, AY; Aziza Amer, MM and El Aty, AM (2001). Some pharmacokinetic data for danofloxacin in healthy goats. Vet. Res. Commun., 25: 367-377.

Bakken, JS (2004). The fluoroquinolones: how long will their utility last? Scand. J. Infect. Dis., 36: 85-92.

Chulavatnatol, S; Chindavijak, B; Vibhagool, A; Wananukul, W; Sriapha, C and Sirisangtragul, C (1999). Pharmacokinetics of levofloxacin in healthy Thai male volunteers. J. Med. Assoc. Thailand. 82: 1127-1135.

Davis, R and Bryson, HM (1994). Levofloxacin. A review of its antibacterial activity, pharmacokinetics and therapeutic efficacy. Drugs. 47: 677-700.

Dumka, VK and Srivastava, AK (2006a). Pharmacokinetics and urinary excretion of subcutaneously administered meloxicam in cross bred calves on co administration with levofloxacin. Int. J. Cow Sci., 2: 24-27.

Dumka, VK and Srivastava, AK (2006b). Pharmacokinetics, urinary excretion and dosage regimen of levofloxacin following a single intramuscular administration in cross bred calves. J. Vet. Sci., 7: 333-337.

Edelstein, PH; Edelstein, MA; Lehr, KH and Ren, J (1996). *In vitro* activity of levofloxacin against clinical isolates of *Legionella* spp, its pharmacokinetics in guinea pigs, and use in experimental *Legionella pneumophila* pneumonia. J. Antimicrob. Chemoth., 37: 117-126.

Gibaldi, M and Perrier, D (1982). Methods of residuals. *Pharmacokinetics*. 2nd. Edn., New York, Marcel Dekker Inc., PP: 433-444.

Gips, M and Soback, S (1996). Norfloxacin nicotinate pharmacokinetics in unweaned and weaned calves. J. Vet. Pharmacol. Ther., 19: 130-134.

Klesel, N; Geweniger, KH; Koletzki, P; Isert, D; Limbert, M; Markus, A; Riess, G; Schramm, H and Iyer, P (1995). Chemotherapeutic activity of levofloxacin (HR 355, DR-3355) against systemic and localized infections in laboratory animals. J. Antimicrob. Chemoth., 35: 805-819.

Langtry, HD and Lamb, HM (1998). Levofloxacin: its use in infections of the respiratory tract, skin, soft tissues and urinary tract. Drugs. 56: 487-515.

McKellar, Q; Gibson, I; Monterio, A and Bregante, M (1999). Pharmacokinetics of enrofloxacin and danofloxacin in plasma, inflammatory exudates and bronchial secretions of calves following subcutaneous administration. Antimicrob. Agents Chemother. 43: 1988-1992.

North, DS; Fish, DN and Redington, JJ (1998). Levofloxacin, a second-generation fluoro-quinolone. Pharmacotherapy. 18: 915-935.

Schneider, M; Valle, M; Woehrle, F and Boisrame, B (2004). Pharmacokinetics of marbofloxacin in lactating cows after repeated intramuscular administrations and pharmacodynamics against mastitis isolated strains. J. Dairy Sci., 87: 202-211.

Shem-Tov, M; Ziv, G; Glickman, A and Saran, A (1997). Pharmacokinetics and penetration of marbofloxacin from blood into milk of cows and ewes. J. Vet. Med. Assoc., 44: 511-519.

Singh, K and Srivastava, AK (2000). Plasma levels, pharmacokinetics, urinary excretion and dosage regimen of ciprofloxacin in cross bred cow calves. J. Pun. Acad. Sci., 1: 105-107.

TerHune, TN; Skogerboe, TC; Shostorm, VK and Weigel, DJ (2005). Comparison of pharmacokinetics of danofloxacin and enrofloxacin in calves challenged with *Mannheimia haemolytica*. Am. J. Vet. Res., 66: 342-349.