J. Vet. Sci. (2007), **8**(4), 357–360

JOURNAL OF
Veterinary
Science

Disposition kinetics and dosage regimen of levofloxacin on concomitant administration with paracetamol in crossbred calves

Vinod K. Dumka*

Department of Pharmacology and Toxicology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India

The disposition kinetics of levofloxacin was investigated in six male crossbred calves following single intravenous administration, at a dose of 4 mg/kg body weight, into the jugular vein subsequent to a single intramuscular injection of paracetamol (50 mg/kg). At 1 min after the injection of levofloxacin, the concentration of levofloxacin in plasma was 17.2 ± 0.36 μg/ml, which rapidly declined to 6.39 ± 0.16 μg/ml at 10 min. The drug level above the MIC_{90} in plasma, was detected for up to 10 h. Levofloxacin was rapidly distributed from blood to the tissue compartment as evidenced by the high values of the distribution coefficient, α (17.3 ± 1.65 /h) and the ratio of K_{12}/K_{21} (1.83 ± 0.12). The values of AUC and Vd_{area} were 12.7 ± 0.12 μg.h/ml and 0.63 ± 0.01 l/kg. The high ratio of the AUC/MIC (126.9 ± 1.18) obtained in this study indicated the excellent antibacterial activity of levofloxacin in calves. The elimination half-life, MRT and total body clearance were 1.38 ± 0.01 h, 1.88 ± 0.01 h and 0.32 ± 0.003 l/kg/h, respectively. Based on the pharmacokinetic parameters, an appropriate intravenous dosage regimen for levofloxacin would be 5 mg/kg repeated at 24 h intervals when prescribed with paracetamol in calves.

Key words: calves, disposition, dosage, levofloxacin, paracetamol

Introduction

Under field conditions, the management of bacterial infections with the administration of antibacterial with analgesic agents is standard treatment. Fluoroquinolones are known to interact with non-steroidal anti-inflammatory drugs at pharmacokinetic levels [20]. Fluoroquinolone resistance relates directly to the human and veterinary usage and emerging bacterial resistance poses the single greatest threat to the future survival of the fluoroquinolone drugs as a therapeutically useful antibiotic class [8]. Levofloxacin [(-) -9-Fluoro-3-methyl-10-(4-methyl-1-piprazinyl)-7-oxo-2, 3-dihydro-7 H-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 [10,22]. As compared to other fluoroquinolones, such as ofloxacin and ciprofloxacin, it also has more pronounced bactericidal activity against organisms such as Pseudomonas, Enterobacteriaceae and Klebsiella [19]. The drug distributes well to the 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 [20]. Levofloxacin is metabolized in the liver to demethyl-levofloxacin and levofloxacin-N-oxide and excreted in the urine [20]. The disposition of levofloxacin has been investigated in man [9], rabbits [11], rats [17], guinea pigs [14] and crossbred calves [12,13]. However, there is no information on the disposition of levofloxacin on concurrent administration with paracetamol in cattle. In view of the alterations in the kinetic behavior of simultaneously administered drugs, the present study was undertaken to determine the disposition and appropriate dosage of levofloxacin following a single intravenous injection when co-administered along with paracetamol in crossbred calves.

Materials and Methods

Six healthy male crossbred calves (Holstein Friesian × Sahiwal), ranging between 1-1.5 years of age with an average body weight of 87.8 ± 13.1 kg were used for this study. The animals were maintained in the departmental animal shed on seasonal green fodder and water *ad libitum* and were determined to be healthy by regular clinical examination. The experimental protocol followed the ethical guidelines on the proper care and use of animals. The average day temperature in the shed was about 25ºC during the

*Corresponding author
Tel: + 91-161-2414032; Fax: +91-161-2400822
E-mail: vkdumka@yahoo.com

experimental period. Levofloxacin (Hoechst Marion Roussel, India) was administered at a dose of 4 mg/kg body weight into the left jugular vein, immediately after intramuscular injection of paracetamol (Sarabhai Zydus Animal Health, India) at a dose of 50 mg/kg into the neck region.

Blood samples (5 ml) were withdrawn from the contralateral jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 7.5, 10, 15, 20, 30 min and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 16 and 24 h after administration of the levofloxacin. Plasma was separated by centrifugation at 2,000 × g for 15 min at room temperature, and kept at −20ºC until analysis, which was usually done on the day of collection.

The concentration of levofloxacin in the plasma samples was estimated by a standard microbiological assay technique [6] using *Escherichia* (*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. The diameter of the zone of inhibition of reference as well as study samples was measured with a Fisher Lilly Antibiotic Zone Reader (Fisher Scientific, USA). For each sample, nine replicates were analyzed and correlated with the zone of inhibition of the standard reference solution. The concentration of the drug in the samples was calculated as μg/ml of plasma.

The plasma concentration-time profile of levofloxacin after its concomitant administration with paracetamol in each animal was used to establish various disposition kinetic determinants and the mean kinetic variables were obtained by averaging the variables calculated for individual animals. Disposition kinetic parameters were calculated manually by the computed least-squares linear regression technique [15].

Results

The mean plasma concentrations of levofloxacin, following its single intravenous administration (4 mg/kg body weight) subsequent to a single intramuscular injection of paracetamol (50 mg/kg body weight), as a function of time on a semilogarithmic scale are presented in Fig. 1. At 1 min, the mean plasma drug concentration was 17.2 ± 0.36 μg/ml. The drug was detected in plasma for up to 10 h after dosing (0.16 ± 0.01 μg/ml). Evaluation of the results revealed that the disposition pattern of levofloxacin best fit a 2-compartment open model. It was adequately described by the bi-exponential equation: $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where, C_p was the plasma level of levofloxacin at time t and e represents the base of the natural logarithm; A and B are the extrapolated zero-time intercepts of the distribution and elimination phases, respectively, and α and β are the distribution and elimination rate constants, respectively. The disposition kinetic parameters that describe the distribution and elimination pattern of levofloxacin on co-administration with paracetamol in the calves were calculated and are presented in Table 1. The absolute dose of levofloxacin per day was calculated using AUIC and Cl_B values from Table 1 according to the method of McKellar *et al*. [21]. Where, AUIC is the ratio of AUC/MIC.

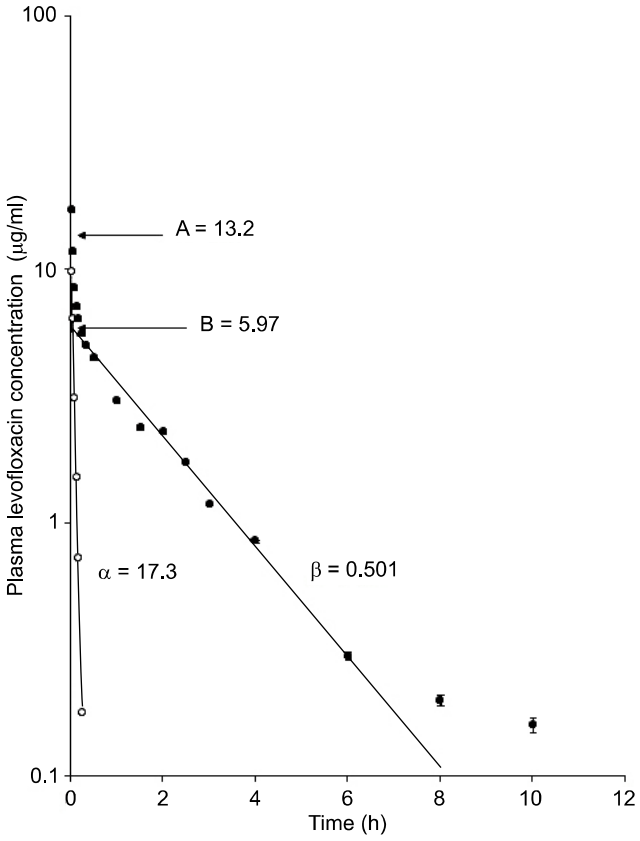

Fig. 1. Semilogarithmic plot of the plasma concentration-time profile of levofloxacin following a single intravenous injection of 4 mg/kg body weight subsequent to a single intramuscular injection of paracetamol (50 mg/kg) in crossbred calves. Values are presented as mean ± SE of six animals. The data was analyzed according to the two-compartment open model. Distribution (α) and elimination (β) phases are represented by least square regression lines. The calculated points (o) of the distribution phases were obtained by the feathering technique.

Discussion

Consistent with our findings that the disposition curve of levofloxacin administered alone in the calves [13] and another fluoroquinolone, danofloxacin, in goats after intravenous administration was reported to follow a two-compartment open model [7]. An average plasma concentration of 0.032-0.5 μg/ml has been reported to be the minimum therapeutic concentration (MIC_{90}) of levofloxacin

Table 1. Disposition parameters of levofloxacin in cross bred calves (n = 6) following its single intravenous administration of 4 mg/kg body weight subsequently with a single intramuscular injection of paracetamol (50 mg/kg)

Parameter	Unit	Mean ± SE
Cp^0	μg/ml	19.1 ± 0.83
A	μg/ml	13.2 ± 0.80
B	μg/ml	5.97 ± 0.06
α	/h	17.3 ± 1.65
β	/h	0.501 ± 0.003
$t_{1/2\alpha}$	h	0.04 ± 0.01
$t_{1/2\beta}$	h	1.38 ± 0.01
K_{12}/K_{21}	ratio	1.83 ± 0.12
AUC	μg.h/ml	12.7 ± 0.12
AUMC	$\mu g.h^2/ml$	23.8 ± 0.29
Vd_{area}	l/kg	0.63 ± 0.01
Cl_B	l/kg/h	0.32 ± 0.003
K_{el}	/h	1.51 ± 0.07
MRT	h	1.88 ± 0.01
P/C	ratio	2.01 ± 0.12
AUC/MIC	ratio	26.9 ± 1.18
td	h	7.35 ± 0.05

Cp^0=plasma drug concentration at time zero after intravenous dose; α and A=distribution rate constant from central to peripheral compartment and the zero time intercept of distribution phase, respectively; B and β=zero time intercept of the elimination phase and elimination rate constant, respectively; $t_{1/2\alpha}$=distribution half life; $t_{1/2\beta}$=elimination half life; K_{12} and K_{21} are rate constants of drug transfer from central to peripheral and from peripheral to central compartment, respectively; AUC=area under the plasma-concentration time curve; AUMC=area under the first moment of plasma-concentration time curve; $Vd_{(area)}$=apparent volume of distribution; Cl_B=total body clearance of drug; K_{el}=rate constant for elimination of drug from central compartment; MRT=mean residence time; P/C=ratio of drug present in peripheral to central compartment; MIC=minimum inhibitory concentration of levofloxacin; td=total duration of pharmacological effect.

against most gram-positive, gram negative and atypical bacteria [9] including statphylococci, citrobactor, enterobactor, *E.coli*, klebsiella, morgenella, proteus, hemophillus, ligionella, morexella, clostridium, chlamydia and mycoplasma [20]. Keeping in mind the synergistic effect of the body immune system, and other *in vivo* factors, to cover most of the susceptible organisms, in this discussion, a MIC_{90} of 0.1 μg/ml of levofloxacin was taken into consideration.

At 1 min after injection, the plasma level (17.2 ± 0.36 μg/ml) was approximately 172 fold higher than the MIC of levofloxacin and the drug was detected above the minimum therapeutic plasma level up to 10 h after administration. Levofloxacin was rapidly transferred from the central to the peripheral compartment in calves, as is evident from the low value of the distribution half-life (0.04 ± 0.01 h) and the high ratio of K_{12}/K_{21} (1.83 ± 0.12). Similar low values for the distribution half-life (0.06 h) were reported after intravenous administration of levofloxacin alone in calves [13]. However, in contrast to our findings, a long $t_{1/2\alpha}$ of 19 h was reported after intravenous administration of enrofloxacin in calves [1]. The high value of the P/C ratio (2.01 ± 0.12) and the apparent volume of distribution confirmed the extensive penetration of levofloxacin into various body fluids and tissues. The value of Vd_{area} established in the present study (0.63 ± 0.01 l/kg) was lower than the findings of Dumka and Srivastava [13] and Langtry and Lamb [20] who reported that the volume of distribution of levofloxacin, when administered alone by single intravenous injection, to be 0.74 l/kg in calves and 0.94 l/kg in man. However, the volume of distribution of other fluoroquinolones used in veterinary medicine, after intravenous administration, varied from 0.4 l/kg for enrofloxacin in calves [1] to 1.42 l/kg and 3.44 l/kg for danofloxacin in goats [7] and calves [5], respectively. The high value of AUC (12.7 ± 0.12 μg.h/ml) in the present study, which was higher than the AUC (7.66 μg.h/ml) of levofloxacin when administered alone in calves [12], reflected coverage of a vast body area by the drug concentration. High values of AUC of levofloxacin have been reported in rabbits (29.7 ± 6.3 μg.h/ml) and man (55.3 μg.h/ml) [11, 20]. Furthermore, high values of AUC have also been reported after intravenous administration of enrofloxacin in calves (17.8 μg.h/ml) and cows (7.42 μg.h/ml) [1,18] and danofloxacin (29.6 μg.h/ml) in goats [7]. The high value of AUC/MIC_{90} (126.9 ± 1.18) obtained in the present study, shows the excellent antibacterial activity of levofloxacin in calves. This ratio was higher than the values of the AUC/MIC ratio reported for levofloxacin (76.6) administered intramuscularly without paracetamol in calves [12] and for another fluoroquinolone, danofloxacin (60.5) after intravenous administration in sheep [4]. The total body clearance of levofloxacin in the present study was 0.32 ± 0.003 l/kg/h. This finding is in agreement with the Cl_B of 0.21 l/kg/h and 0.32 l/kg/h after a single intramuscular [12] and intravenous [13] administration of levofloxacin without paracetamol and 0.28 l/kg/h reported for enrofloxacin after intravenous administration in calves [1]. The elimination half-life of levofloxacin in calves calculated in this study (1.38 ± 0.01 h) was comparable to the $t_{1/2\beta}$ of 1.61 h for levofloxacin administered alone intravenously in calves [13], 2.3 h for norfloxacin in cattle [16] and 1.68 h for enrofloxacin in cows [18]. However, the elimination half-life of levofloxacin in the present study was shorter than $t_{1/2\beta}$ of 3.67 h reported for levofloxacin administered intramuscularly without paracetamol in calves. [12] It was 4.67 h and 4.01 h for danofloxain in goats [2,7],

5.37 h in camels [3] and 6.26 h in calves [5] but longer than the $t_{1/2\beta}$ of 0.95 h for enrofloxacin in calves [1] after intravenous administration.

The main aim of this disposition kinetic study was to determine the appropriate intravenous dose regimen for levofloxacin. Based on the results of the present study, the absolute dose of levofloxacin per day, with simultaneous administration of paracetamol, was calculated to be 4.9 mg/kg under field conditions. This is for most bacteria sensitive to levofloxacin (several species of staphylococci, streptococci, including *Streptococcus pneumoniae*, most enterococci, enterobacteriaceae, *E. coli*, klebsiella, proteus, pseudomonas, bacteroides, clostridium, haemophilus, moraxella, legionella, mycoplasma and chlamydia [20]). The most appropriate dose regimen for levofloxacin, would be 5 mg/kg repeated at 24 h intervals when prescribed along with paracetamol in calves. This dose was different from the intravenous dose of 3 mg/kg at 12 h intervals [13] and the intramuscular dose of 1.5 mg/kg at 8 h intervals [12] reported for levofloxacin when prescribed alone in calves.

References

1. **Ahanger AA, Srivastava AK, Raina R**. Disposition kinetics of enrofloxacin in crossbred calves. J Vet Pharmacol Toxicol 2003, **3**, 16-20.
2. **Aliabadi FS, Lees P**. Pharmacokinetics and pharmacodynamics of danofloxacin in serum and tissue fluids of goats following intravenous and intramuscular administration. Am J Vet Res 2001, **62**, 1979-1989.
3. **Aliabadi FS, Ali BH, Landoni MF, Lees P**. Pharmacokinetics and PK-PD modeling of danofloxacin in camel serum and tissue cage fluids. Vet J 2003, **165**, 104-118.
4. **Aliabadi FS, Landoni MF, Lees P**. Pharmacokinetics (PK), pharmacodynamics (PD) and PK-PD integration of danofloxacin in sheep biological fluids. Antimicrob Agents Chemother 2003, **47**, 626-635.
5. **Apley MD, Upson DW**. Lung tissue concentrations and plasma pharmacokinetics of danofloxacin in calves with acute pneumonia. Am J Vet Res 1993, **54**, 937-943.
6. **Arret B, Johnson DP, Kirshbaum A**. Outline of details for microbiological assays of antibiotics: second revision. J Pharm Sci 1971, **60**, 1689-1694.
7. **Atef M, El-Gendi AY, Aziza, Amer MM, Abd El-Aty AM**. Some pharmacokinetic data for danofloxacin in healthy goats. Vet Res Commun 2001, **25**, 367-377.
8. **Bakken JS**. The fluoroquinolones: how long will their utility last? Scand J Infect Dis 2004, **36**, 85-92.
9. **Chulavatnatol S, Chindavijak B, Vibhagool A, Wananukul W, Sriapha C, Sirisangtragul C**. Pharmacokinetics of levofloxacin in healthy Thai male volunteers. J Med Assoc Thai 1999, **82**, 1127-1135.
10. **Davis R, Bryson HM**. Levofloxacin. A review of its antibacterial activity, pharmacokinetics and therapeutic efficacy. Drugs 1994, **47**, 677-700.
11. **Destache CJ, Pakiz CB, Larsen C, Owens H, Dash AK**. Cerebrospinal fluid penetration and pharmacokinetics of levofloxacin in an experimental rabbit meningitis model. J Antimicrob Chemother 2001, **47**, 611-615.
12. **Dumka VK, Srivastava AK**. Pharmacokinetics, urinary excretion and dosage regimen of levofloxacin following single intramuscular administration in cross bred calves. J Vet Sci 2006, **7**, 333-337.
13. **Dumka VK, Srivastava AK**. Disposition kinetics, urinary excretion and dosage regimen of levofloxacin formulation following single intravenous administration in crossbred calves. Vet Res Commun 2007, **31**, 873-879.
14. **Edelstein PH, Edelstein MA, Lehr KH, Ren J**. *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 Chemother 1996, **37**, 117-126.
15. **Gibaldi M, Perrier D**. Method of residuals. In: Pharmacokinetics. Gibaldi M, Perrier D (eds.). 2nd ed. pp. 433-444, Marcel Dekker, New York, 1982.
16. **Gips M, Soback S**. Norfloxacin nicotinate pharmacokinetics in unweaned and weaned calves. J Vet Pharmacol Ther 1996, **19**, 130-134.
17. **Ito T, Yano I, Masuda S, Hashimoto Y, Inui K**. Distribution characteristics of levofloxacin and grepafloxacin in rat kidney. Pharm Res 1999, **16**, 534-539
18. **Kaartinen L, Salonen M, Alli L, Pyörälä S**. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cows. J Vet Pharmacol Ther 1995, **18**, 357-362.
19. **Klesel N, Geweniger KH, Koletzki P, Isert D, Limbert M, Markus A, Riess G, Schramm H, Iyer P**. Chemotherapeutic activity of levofloxacin (HR 355, DR-3355) against systemic and localized infections in laboratory animals. J Antimicrob Chemother 1995, **35**, 805-819.
20. **Langtry HD, Lamb HM**. Levofloxacin. Its use in infections of the respiratory tract, skin, soft tissues and urinary tract. Drugs 1998, **56**, 487-515.
21. **McKellar QA, Sanchez Bruni SF, Jones DG**. Pharmacokinetic/pharmacodynamic relationship of antimicrobial drugs used in veterinary medicine. J Vet Pharmacol Ther 2004, **27**, 503-514.
22. **North DS, Fish DN, Redington JJ**. Levofloxacin, a second-generation fluoroquinolone. Pharmacotherapy 1998, **18**, 915-935.