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

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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 MIC90 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, \( \alpha (17.3 \pm 1.65 /h) \) and the ratio of \( K_{12}/K_{21} (1.83 \pm 0.12) \). The values of AUC and \( V_{d,rs} \) 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 intra-cellular pathogens [20]. Levofloxacin is metabolized in the liver to demethyl-levofoxacin 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 25°C during the
 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: \( C_p = A e^{-\alpha t} + B e^{-\beta t} \), 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 \( \alpha \) and \( \beta \) 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 ClH values from Table 1 according to the method of McKellar et al. [21]. Where, AUIC is the ratio of AUC/MIC.

**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 (MIC90) 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   |
|--------------------|-------------|-------------|
| Cp₀                | 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 |
| t₁/₂a              | h           | 0.04 ± 0.01 |
| t₁/₂β              | h           | 1.38 ± 0.01 |
| K₁₂/K₂₁           | ratio       | 1.83 ± 0.12 |
| AUC                | µg.h/ml     | 12.7 ± 0.12 |
| AUMC              | µg.h²/ml    | 23.8 ± 0.29 |
| Vd(area)          | l/kg        | 0.63 ± 0.01 |
| Cl₈              | l/kg.h     | 0.32 ± 0.003 |
| K₄               | /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 |

Cp₀=plasma drug concentration at time zero after intravenous dose; α and B=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; t₁/₂a=distribution half-life; t₁/₂β=elimination half-life; K₁₂ and K₂₁ 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; Cl₈=total body clearance of drug; K₄=rate constant for elimination of drug from central compartment; MRT=mean residence time; P/C=rate of drug present in peripheral to central compartment; MIC=minimum inhibitory concentration of levofloxacin; td=total duration of pharmacological effect.

c against most gram-positive, gram negative and atypical bacteria [9] including staphylococci, citrobacter, enterobacter, E.coli, klebsiella, morgenella, proteus, hemophilus, 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 MIC₀ 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 K₁₂/K₂₁ (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 t₁/₂β 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/MIC₀ (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 Cl₈ 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 t₁/₂β 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 t₁/₂β of 3.67 h reported for levofloxacin administered intramuscularly without paracetamol in calves. [12] It was 4.67 h and 4.01 h for danofloxacin in goats [2,7],
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