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"PHARMACOKINETICS OF LEVOFLOXACIN IN CROSSBRED COW CALVES FOLLOWING INTRAMUSCULAR ADMINISTRATION"

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ABSTRACT

Pharmacokinetic study of levofloxacin was carried out in six crossbred cow calves of 9-12 months of age weighing 70-90 kg. Levofloxacin was given intramuscularly at the dose rate of 4 mg/kg body weight in six crossbred cow calves. Levofloxacin concentration was estimated at different intervals (0.042 to 24 h) by microbiological assay method using *E. coli* (ATCC 25922) as test organism. Kinetic parameters were calculated by using one compartment open models. Attempts were made to calculate the rational dosage regimens of levofloxacin on the basis of kinetic data and maintenance of therapeutic concentrations in plasma. Following intramuscular administration of levofloxacin, the peak plasma concentrations of levofloxacin (12.25 µg/ml) was observed at 1 h. The elimination half- life ($t_{1/2}$ β), mean residence time (MRT), volume of distribution (Vd_{area}) and total body clearance (Cl_B) of levofloxacin were 3.502 h, 4.82 h, 0.306 L/kg and 60.323 ml/kg/h, respectively. The therapeutic plasma concentrations of levofloxacin were maintained at or above 0.1 µg/ml from 0.083 to 24 h. For maintaining therapeutic concentration of 0.1 µg/ml, a loading dose (D*) of around 0.827 mg/kg and maintenance dose (D_0) of 0.797 mg/kg may be used at the dosage interval of 16 h for treating systemic infections in crossbred cow calves.

KEYWORDS: Pharmacokinetics, Levofloxacin, Crossbred cow calves.

INTRODUCTION

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 third-generation fluoroquinolone, possesses excellent activity against Gram-positive, Gram-negative and anaerobic bacteria.[1,2] As compared to other fluoroquinolones, ofloxacin and ciprofloxacin, it also has more pronounced bactericidal activity against organisms like *Pseudomonas*, Entero-bacteriaceae and *Klebsiella*.[3] 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.[4] The mechanism of action levofloxacin and other quinolone antibacterials involves inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV. Topoisomerases are essential in controlling the topological state of DNA replication, transcription, repair and recombination.

For a clinician, the most crucial question is the selection of the dose of an antimicrobial agent. The dosage regimen is calculated on the basis of pharmacokinetic data obtained in that particular species only. Further, the dosage regimen also varies depending on age and the sex of animals, and the environmental temperature. Accordingly, the pharmacokinetic studies of the levofloxacin have been conducted in stallion[5], goats[6], camel[7], female crossbred cow[8] and buffalo calf.[9] However, little literatures are available for the pharmacokinetics of levofloxacin in crossbred cow calves following intramuscular administration.

MATERIALS AND METHODS

The present study was undertaken to determine the pharmacokinetics of levofloxacin administered by intramuscular routes in healthy crossbred cow calves.

Experimental animals

The study was conducted on six healthy crossbred cow calves ranging 9-12 months of age and weighing between 70-90 kilograms. The animals were maintained at the Instructional Farm, College of Veterinary Science and Animal Husbandry, Mhow, Madhya Pradesh. They were kept under constant observation for fifteen days prior to commencement of the experiment. During this period they were subjected to clinical examination in order to exclude the possibility of any disease. The animals were then housed in separate pen and were provided standard ration as per the farm schedule. Water was provided *ad libitum.* Fifteen days before the start of experiment deworming carried out with broad spectrum anthelmintics. All necessary managemental procedures were adopted to keep the animals free

from stress. The experimental protocol for general procedure and use of animals for conducting the present study has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

Administration of Drugs

Levofloxacin infusion (500 mg/100 mL; ZILEE®) was marketed by Axa Parenterals Ltd. Roorkee, India. Levofloxacin (4 mg/ kg B. Wt.) was administered by intramuscular route in each crossbred cow calves. Intramuscular injection of the drug was given by using 16G x 25 mm needle. Six healthy crossbred cow calves (C1, C2, C3, C4, C5 and C6) were employed to investigate the pharmacokinetics of levofloxacin following intramuscular administration in crossbred cow calves.

Collection of blood samples

The blood samples (4-5 ml) from cow calves in clean sterilized previously added anticoagulant (10% EDTA solution) test tube were collected with the help of an intravenous catheter (Teflon, 22 × 0.9 × 25 mm) fixed into the contra lateral jugular vein. Following intramuscular administration of levofloxacin, the blood samples were collected at 0 minute (before drug administration) 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12, 16 and 24 h. Plasma was separated soon after collection by centrifugation at 5000 revolution per min (rpm) for 10 minutes (Eppendorf 5804 R, Germany). Separated plasma samples were transferred to labeled cryovials and stored in deep freezer at -20^{o}C.

Estimation of levofloxacin

The concentration of levofloxacin in plasma was determined by microbiological assay technique[10] using *E. coli* (ATCC 25922) as test organism.

Pharmacokinetic analysis

The plasma concentration-time profile of levofloxacin for each animal was used to determine the pharmacokinetics. The data of levofloxacin was subjected to one compartment open model.[11]

RESULTS

Concentrations of levofloxacin in plasma at various time intervals following its single intramuscular (i.m.) injection at the dose rate of 4 mg/kg body weight have been shown in Table 1. The mean plasma concentration of the drug at 0.042 h was found to be 0.078 ± 0.026

μg/ml and the values ranged from 0.08 to 0.16 μg/ml and drug was detectable in four animals out of six animals. The drug was detectable in all the six animals from 0.083 to 24 h and the mean concentration at 24 h was noted to be 0.131 ± 0.029 μg/ml however, the effective therapeutic concentration (≥ 0.1 μg/ml) of levofloxacin maintained from 0.083 to 24 h and with mean value of 0.708 ± 0.092 and 0.131 ± 0.029 μg/ml at 0.083 and 24 h of administration, respectively. The drug was not detectable in all the six animals at 30 h.

Table 1 Plasma concentrations (μg/ml) of levofloxacin following intramuscular administration (4 mg/kg) in crossbred cow calves.

Time after drug administration (h)	Plasma concentration (μg/ml)						
	Cow calves Number						Mean ± S.E
	C1	C2	C3	C4	C5	C6	
0.042	0.12	0.08	N.D.	0.16	N.D.	0.11	0.078 ±0.026
0.083	0.92	0.83	0.32	0.76	0.56	0.86	0.708 ± 0.092
0.167	2.31	1.98	1.92	2.02	1.99	2.02	2.040 ± 0.056
0.25	4.12	3.72	3.96	4.08	3.86	4.01	3.950 ± 0.060
0.333	7.32	6.35	6.62	6.96	7.02	7.35	6.936± 0.160
0.50	9.36	8.31	8.22	8.76	8.42	8.96	8.670±0.270
0.75	10.12	9.48	9.12	10.14	10.21	9.96	9.838 ±0.179
1	13.12	12.31	12.46	12.76	11.96	10.92	12.25±0.331
2	12.14	11.76	11.32	12.03	11.02	9.76	11.37 ± 0.361
4	7.62	7.12	6.96	8.62	6.86	6.02	7.20 ± 0.354
6	4.12	3.92	4.01	4.24	3.96	3.82	4.01 ± 0.061
8	2.12	1.96	2.22	1.99	2.04	2.01	2.056±0.039
12	1.01	0.91	1.08	0.92	0.82	0.96	0.95 ±0.036
16	0.54	0.42	0.45	0.31	0.35	0.44	0.418 ± 0.033
24	0.20	0.15	0.18	N.D.	0.10	0.16	0.131 ± 0.029

Plasma drug concentration versus time profile has confirmed the one compartment open model for levofloxacin. Table 2 shows the values of different kinetic parameters in healthy crossbred cow calves calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during absorption phase (A') and elimination phase (B) was noted to be 15.235 ± 0.348 and 12.949 ± 1.75 μg/ml, respectively. The mean absorption rate constant (K_a) and elimination rate constant (β) were 3.409±0.398 h^{-1} and 0.202 ± 0.014 h^{-1}. The mean absorption half-life ($t_{1/2\ Ka}$) and elimination half-life ($t_{1/2}\ \beta$) was calculated to be 0.222 ±0.033, and 3.502 ± 0.203 h, respectively.

The mean area under curve in plasma (AUC) and mean area under first moment curve (AUMC) were noted to be 66.316 ± 0.52 μg/ml. h and 319.2 ± 11.817 μg/ml.h^2, respectively,

with the mean residence time (MRT) of 4.82 ± 0.149 h. The mean value of bioavailability (F) was noted to be 106.3 ± 8.50%. The various values of volume of distribution calculated by different methods are shown in Table 2. The mean volume of distribution (Vd_{area}) was calculated to be 0.306 ± 0.019 L/kg. The total body clearance (Cl_B) ranged from 55.983 to 65.562 ml/kg/h with a mean of 60.323 ± 1.402 ml/kg/h.

Table 2 Pharmacokinetic parameters of levofloxacin after single intramuscular administration (4 mg/kg) in crossbred cow calves.

Parameters (Unit)	C1	C2	C3	C4	C5	C6	Mean ± SE
A' (µg/ml)	16.284	14.722	14.758	14.072	15.987	15.591	15.235±0.348
B (µg/ml)	11.033	11.165	11.145	21.536	12.718	10.098	12.949 ±1.751
Ka (h^{-1})	4.489	3.609	3.260	1.859	2.902	4.340	3.409 ± 0.398
$t_{1/2}$ Ka (h)	0.154	0.192	0.213	0.373	0.239	0.160	0.222 ± 0.033
β (h^{-1})	0.178	0.191	0.183	0.268	0.211	0.182	0.202 ± 0.014
$t_{1/2}$ β (h)	3.904	3.637	3.796	2.588	3.278	3.809	3.502 ± 0.203
AUC (µg/ml.h)	71.5	66.3	66.4	69.0	63.7	61.0	66.316± 0.520
AUMC (µg/ml.h^2)	362.1	318.5	343.2	288.8	291.2	311.2	319.2 ±11.817
MRT (h)	5.1	4.8	5.1	4.2	4.6	5.1	4.82 ± 0.149
Vd_{area} (L/kg)	0.315	0.317	0.325	0.220	0.297	0.360	0.306 ± 0.019
Cl_B (ml/kg/h)	55.983	60.362	59.326	57.935	62.770	65.562	60.323±1.402
F (%)	103.7	102.3	100.0	145.0	105.1	81.7	106.3 ± 8.50
C_{max}/MIC	131.2	123.1	124.6	127.6	119.6	109.2	122.55±3.118
AUC/MIC	715	663	664	690	637	610	663.2±15.186

The dosage regimens required to maintain the different levels of therapeutic concentration (C_p^o min = 0.1, 0.2 and 0.3 µg/ml) in plasma for i.m. route in healthy crossbred cow calves at different dosage intervals (τ) of 8, 12 and 16 h are presented in Table 3.

Table 3 shows the dosage regimens of levofloxacin for intramuscular route in crossbred cow calves.

C_P^{∞} min (µg/ml)	τ (h)	Dose (mg/kg)	Levofloxacin
0.1	8	D*	0.153 ± 0.001
		D_0	0.123 ±0.010
	12	D*	0.353 ± 0.042
		D_0	0.323 ± 0.044
	16	D*	0.827 ± 0.161
		D_0	0.797 ± 0.164
0.2	8	D*	0.307 ± 0.017
		D_0	0.247 ± 0.019
	12	D*	0.705 ± 0.084
		D_0	0.643 ± 0.087
	16	D*	1.655 ± 0.323
		D_0	1.593 ± 0.327

0.3	8	D*	0.460 ± 0.024
		D_0	0.371 ± 0.029
	12	D*	1.062 ± 0.126
		D_0	0.968 ± 0.130
	16	D*	2.483 ± 0.485
		D_0	2.391 ± 0.489

D* = Priming or Loading dose

D_0 = Maintenance dose

τ = Dosage interval

C_P^{∞} min = Minimum therapeutic concentration in plasma.

The dosage regimens required to maintain the different levels of therapeutic concentration (C_p^o min = 0.1, 0.2 and 0.3 μg/ml) in plasma for i.m. route in healthy crossbred calves at different dosage intervals (τ) of 8, 12 and 16 h are presented in Table 3. For maintaining C_p^o min of 0.1 μg/ml the loading doses (D*s) were calculated to be 0.153 ± 0.001, 0.353 ± 0.042 and 0.827 ± 0.161 mg/kg while maintenance doses (D_0s) were calculated to be 0.123 ± 0.01, 0.323 ± 0.044 and 0.797 ± 0.164 mg/kg at the dosage intervals (τ) of 8, 12 and 16 h, respectively. The D*s were calculated to be 0.307 ± 0.017, 0.705 ± 0.084 and 1.655 ± 0.323 mg/kg while D_0s were found to be 0.247 ± 0.019, 0.643 ± 0.087 and 1.593 ± 0.327 mg/kg at τ of 8, 12 and 16 h respectively, for maintaining C_p^o min of 0.2 μg/ml.

Like wise, to maintain C_p^o min of 0.3 μg/ml the D*s were calculated to be 0.460± 0.024, 1.062± 0.126 and 2.483± 0.485 mg/kg while D_0s were found to be 0.371± 0.029, 0.968± 0.130 and 2.391±0.489 mg/kg at τ of 8, 12 and 16 h, respectively.

DISCUSSION

After intramuscular administration of levofloxacin, the mean peak plasma concentration at 1 h was 12.25 ± 0.31 μg/ml was observed in crossbred cow calves. The mean therapeutic concentration (≥ 0.1 μg/ml) of levofloxacin was maintained from 5 min to 24 h in plasma of calves. Present findings are partially agreed with the findings of[12], who reported the mean therapeutic concentration of levofloxacin in buffalo calves (≥ 0.1 μg/ml) after i.m. administration (4 mg/kg) and maintained only up to 12 h in plasma.

Plasma concentrations of levofloxacin *versus* time disposition curves after intramuscular administration were best fit to one compartment open model reported in buffalo calves.[12,13]

The absorption half life, elimination half life and mean residence time (MRT) of levofloxacin in present study was noted to be 0.222 ± 0.033 h, 3.502 ± 0.203 h and 4.82 ± 0.149 h, respectively. In contrast to present study, somewhat less elimination half life 2.94 ± 0.78 h after i.m. administration of levofloxacin was observed in stallions[5], The difference in elimination half life in calves in the present study as compared to stallion may be due to differences in biotransformation and excretory processes of different species. The value of area under plasma concentration time curve (AUC) and area under first moment of plasma drug concentration time curve (AUMC) of levofloxacin in crossbred cow calves was depicted in Table 2.

The ultimate objective of the study of disposition kinetics is to determine an appropriate dose regimen of drugs. For any antimicrobial agent the dosage regimen is calculated to maintain the minimum therapeutic concentration (MIC) throughout the course of infections. 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.[14] Keeping in view of synergistic effects of the immune system and other *in vivo* factors as well as 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. Levofloxacin possessed excellent antibacterial activity (MIC for 90% of tested strains i.e. $MIC_{90} \leq 0.5$ µg/ml) against most common gram-negative aerobic pathogens, including *E. coli*, *K. pneumoniae*, *Enterobacter sp*, and *H. influenza*.[15] Watts *et al.*[16] reported that most of veterinary fluoroquinolones are active at $MIC_{90} \leq 0.17$ µg/ml against sensitive strains isolated from field of veterinary importance. Thus, in the present study dosage regimen was derived at MIC of 0.1, 0.2, and 0.3 µg/ml for levofloxacin at dosage interval of 8, 12 and 16 h in crossbred cow calves (Table 3). For maintaining therapeutic concentration of 0.1 µg/ml, a loading dose (D*) of around 0.827 mg/kg and maintenance dose (D_0) of 0.797 mg/kg may be used at the dosage interval of 16 h for treating systemic infections in crossbred cow calves.

CONCLUSION

Based on the present study, levofloxacin have better bioavailability and the loading and maintenance doses of levofloxacin are comparative lower than other fluoroquinolones groups of antimicrobial agents.

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