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INFLUENCE OF TRIKATU PRETREATMENT ON THE PHRMACOKINETICS 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 resident 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 0.2 µg/ml, a very lower loading dose (D*) of around 0.71 mg/kg and maintenance dose (D_0) of 0.64 mg/kg may be used at the dosage interval of 12 h for treating systemic infections in crossbred cow calves.

KEYWORDS: Levofloxacin, Trikatu, Crossbred cow calves, Intramuscular administration

INTRODUCTION

In recent times, two or more drugs are simultaneously administered for treating clinical cases suffering from diseases of multiple origins. It is well established that the combination of drugs when given concurrently causes drug interactions, which in turn may alter the pharmacokinetic and pharmacodynamic behavior of drugs. The knowledge of drug interactions, acquired from the experiments on animals has been used to therapeutic advantages in animals and man or enable a clinician to minimize or prevent drug toxicity by adjustment of dosages.

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.[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.

Trikatu is a herbal bioenhancer compound having Indian long pepper, black pepper and ginger. It provides a natural and safe support system for impaired gastric function associated with gaseous distension. Trikatu is a safe digestive, carminative, anti-flatulent and is effective in dyspepsia. It also improves gastric function.

Pharmacokinetic interaction is result of alterations of drug absorption, distribution, metabolism and elimination in combination therapy. The present study is planned to determine effect of pretreated trikatu on the pharmacokinetics of levofloxacin in crossbred cow calves.

MATERIALS AND METHODS

The present study was undertaken to determine the effect of trikatu on 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 kg. 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®, Axa Parenterals Ltd. Roorkee, India and Trikatu (60 capsules, each 250mg Himalaya health care Ltd., India) were procured from Khandewal Chemist, Mhow. Levofloxacin was administered at a dose rate of 4 mg/kg of body weight. Injectable levofloxacin infusion (500 mg/100ml) was used for intramuscular administration. Trikatu pretreatment was done at the dose rate of 0.2 g/kg of body weight orally for 7 days. Present study was conducted in a cross over design with an interval of fifteen days between successive administrations of the drug/s. Six healthy crossbred cow calves (C1, C2, C3, C4, C5 and C6) were employed to investigate the pharmacokinetics of levofloxacin administration (intramuscular) alone and in combination with pretreated trikatu (orally) in crossbred cow calves.

Plan of work

The present study was conducted broadly in two different phases to study the influence of trikatu pretreatment on pharmacokinetics of levofloxacin in crossbred cow calves.

Phase I

Levofloxacin (4 mg/kg B. Wt) was administered by intramuscular route in crossbred cow calves.

Phase II

Levofloxacin (4 mg/kg B. Wt) was administered by intramuscular route in trikatu (0.2 g/kg B. Wt orally for seven days) pretreated crossbred cow calves.

Collection of blood samples

In each phase, 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 intravenous/intramuscular administration of levofloxacin (alone and trikatu pretreated crossbred cow calves) 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

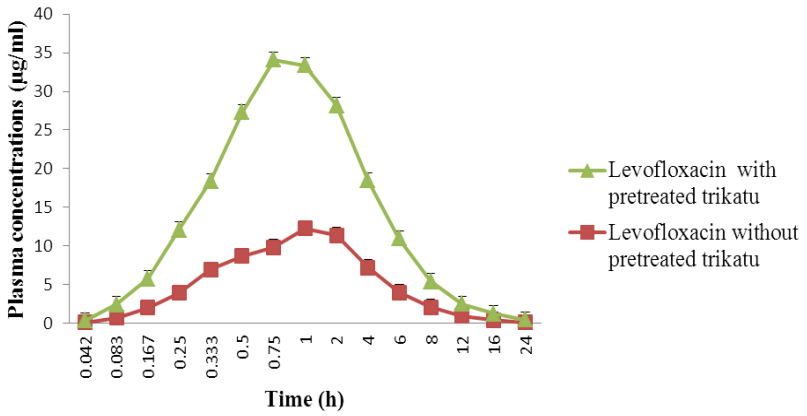
The plasma concentration-time profile of levofloxacin for each animal was used to determine the pharmacokinetics. The data of levofloxacin was subjected to one compartment open model.[11]

RESULTS

Comparison of plasma concentration of levofloxacin (4 mg/kg body weight, i.m.) alone and pretreated trikatu (0.2 g/kg body weight, orally for seven days) in crossbred cow calves are presented in Figure 1.

Significantly higher plasma concentration of levofloxacin was observed from 0.042 h to 24 h in trikatu pretreated group as compared to levofloxacin alone group. The MIC of levofloxacin ≤ 0.1 µg/ml was maintained from 0.042 h to 24 h and 0.083 to 24 h in trikatu pretreated group as compared to levofloxacin alone group, respectively.

Figure 1: Showing comparison of plasma concentrations (µg/ml) of levofloxacin (4 mg/kg, i.m.) alone and with pretreated trikatu (0.2 g/kg, orally for seven days) in crossbred cow calves



Comparison of different pharmacokinetic parameters of levofloxacin (4mg/kg body weight, i.m.) alone and with pretreated trikatu (0.2 g/kg body weight, orally for seven days) in crossbred cow calves are presented in Table 1. The value for extrapolated zero time concentration during absorption phase (A'), zero time concentration during elimination phase (B) were significantly higher in trikatu pretreated group as compared to single administration of levofloxacin. Non-significant difference were observed in case of absorption rate constant (Ka), absorption half life ($t_{1/2}$ Ka), elimination rate constant (β), elimination half life ($t_{1/2}$ β) and mean residence time (MRT) in trikatu pretreated group. Area under curve (AUC) and area under first moment curve (AUMC) were found to be significantly higher while the bioavailability (F) were observed to be non-significantly higher in trikatu pretreated group as compared to single administration of levofloxacin. Volume of distribution (Vd_{area}) and total body clearance (Cl_B) were found to be significantly lower in trikatu pretreated group as compare to levofloxacin alone group which indicated the low distribution and slow elimination of levofloxacin from the body in trikatu pretreated crossbred cow calves.

Table 1 Comparison of pharmacokinetic parameters of levofloxacin (4 mg/kg, i.m.) alone and with pretreated trikatu (0.2 g/kg, orally for seven days) in crossbred cow calves

Parameters (Unit)	Levofloxacin without pretreated trikatu	Levofloxacin with pretreated trikatu
A' (µg/ml)	15.24 ±0.35	36.86±0.089 **
B (µg/ml)	12.95±1.75	17.42±0.62**
Ka (h^{-1})	3.409±0.40	3.133±0.06+
$t_{1/2}$ Ka (h)	0.22±0.03	0.22±0.004+
β (h^{-1})	0.202±0.014	0.178±0.003+
$t_{1/2}$ β (h)	3.50 ±0.20	3.89±0.07+
AUC (µg/ml.h)	66.32±1.52	111.40±2.10**
AUMC (µg/ml.h^2)	319.02±11.82	560.00±10.23**
MRT (h)	4.82±0.15	5.03±0.06+
Vd_{area} (L/kg)	0.31±0.02	0.20±0.006**
Cl_B (ml/kg/h)	60.32±1.40	35.94±0.65**
F (%)	106.3±8.5	116.1±4.3+
C_{max}/MIC	122.55±3.12	242.30±5.43**
AUC/MIC	663.17±15.19	1114.80±21.03**

+ = Non Significant ** = $p < 0.01$

The comparison of calculated dosage regimen of levofloxacin alone and pretreated trikatu group for different therapeutic level (C_P^0 mean 0.1, 0.2 and 0.3 µg/ml) and different dosages intervals (τ) of 8 and 12 h have been shown in Table 2.

All calculated data for loading (D*) and maintenance (D_0) doses for different therapeutic level at different dosage intervals (τ) were noted to be significantly lower in trikatu pretreated group as compared to levofloxacin alone group.

Table 2 Comparison of dosage regimen of levofloxacin alone and with pretreated trikatu group for intramuscular route in crossbred cow calves

C_P^∞ min (µg/ml)	τ (h)	Dose (mg/kg)	Levofloxacin without pretreated trikatu	Levofloxacin with pretreated trikatu
0.1	8	D*	0.15± 0.001	0.09± 0.002**
		D_0	0.12± 0.01	0.07± 0.002**
	12	D*	0.35± 0.04	0.17± 0.003**
		D_0	0.32± 0.04	0.15± 0.004**
0.2	8	D*	0.31± 0.02	0.17± 0.003**
		D_0	0.25± 0.02	0.13± 0.002**
	12	D*	0.71± 0.09	0.34± 0.008**
		D_0	0.64± 0.09	0.31± 0.008**
	8	D*	0.46± 0.02	0.25± 0.005**

0.3		D_0	0.37± 0.03	0.19± 0.003**
	12	D*	1.06± 0.13	0.52± 0.01**
		D_0	0.97± 0.13	0.46± 0.01**

** = $p < 0.01$

D* = Priming or Loading dose

D_0 = Maintenance dose

τ = Dosage interval

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

DISCUSSION

Mean plasma concentrations of levofloxacin at all time intervals after post i.m. administrations of levofloxacin alone (4 mg/kg) and with trikatu pretreated group (0.2 g/kg, orally for seven days) differed significantly. Higher concentrations were found in trikatu pretreated group as compared to its alone i.m. administration in crossbred cow calves (Figure 1).

After intramuscular administration of levofloxacin the mean peak plasma concentration at 1 h was 12.25 ± 0.31 µg/ml in levofloxacin alone group whereas at 0.75 h it was 24.22 ± 0.55 µg/ml in trikatu pretreated group. Levofloxacin was detectable from 0.042 h to 24 h in both the groups.

The higher concentration of levofloxacin in trikatu pretreated group indicates that pretreatment with trikatu may influence the plasma levels of levofloxacin all time intervals and also influenced the metabolism of levofloxacin in crossbred cow calves.

No kinetic study pertaining to influence of trikatu pretreatment on pharmacokinetics of levofloxacin has been carried out in crossbred cow calves so far.

In contrast to present study, Dama *et al.*[12] investigated the pharmacokinetics of orally administered pefloxacin to evaluate the bio-enhancing effect of the herbal bio-enhancer, trikatu, in mountain Gaddi goats (n = 6). The findings of the study revealed a decreased plasma concentration ($p > 0.05$) of pefloxacin following trikatu administration during the absorption phase (10, 15, 20 min post pefloxacin administration).

The difference in plasma concentration of levofloxacin in goats reported by the above workers as compare to plasma level of levofloxacin in crossbred calves of present study may

be due to the drug factors (different fluoroquinolones) apart from physiological differences in species.

The peak plasma level of levofloxacin attained in the present study was approximately 51 fold higher in trikatu pretreated calves. The mean therapeutic concentration (≥ 0.1 μg/ml) of levofloxacin was maintained from 5 min to 24 h in plasma of calves when levofloxacin was given alone however, in trikatu pretreated group it was maintained from 2.5 min to 24 h.

Present findings are partially agreed with the findings of Ram *et al.*[13], who reported approximately 30 fold higher 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.[13, 14] Most of the kinetic parameters of levofloxacin (4 mg/kg, i.m.) when given alone and with pretreated trikatu (0.2 g/kg, orally for seven days) differed significantly except absorption rate constant (K_a), absorption half life ($t_{1/2}$ K_a), elimination half life (β) mean residence time (MRT) and bioavailability (F) (Table 1). The above findings clearly indicate that pretreated trikatu influences kinetic processes of levofloxacin in crossbred cow calves. The result was more or less in close agreement with the findings of Dama *et al.*[12] who reported that effect of trikatu pretreatment on the pharmacokinetics of pefloxacin administered orally in mountain gaddi goats showed significant changes in some kinetic parameters such as β, B, $t_{1/2}$ β, AUC, AUMC, Vd_{area}, MRT and F as well as Dahikar[15] who reported that the effect of *Withania somnifera* on the pharmacokinetics of amikacin in buffalo calves showed significant changes in most of kinetic parameters except AUMC and T ≈ P.

Significantly higher values of extrapolated zero time concentration during absorption phase (A') and extrapolated zero time concentration during elimination phase (B) was obtained in trikatu pretreated group as compared to levofloxacin alone group. Whereas non-significant difference of theoretical zero time concentration (C_P^0) was reported in both the groups.

Significantly lower volume of distribution during area under curve (Vd_{area}) was found in trikatu pretreated group as compared to levofloxacin alone group. Non-significant difference was observed in absorption half life, elimination half life and mean residence time (MRT) in

trikatu pretreated group as compared to levofloxacin alone group. This is also supported by significantly lower values of total body clearance in trikatu pretreated group as compared to levofloxacin alone group. 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 were significantly higher in trikatu pretreated group as compared to levofloxacin alone administration in crossbred cow calves (Table 1). It reflects coverage of a vast body area by the drug in trikatu pretreated calves than levofloxacin alone. The present findings are in contrast with the findings of Dama *et al.*[12] who reported that effect of trikatu pretreatment on the pharmacokinetics of pefloxacin administered orally in mountain gaddi goats showed significantly lower AUC, AUMC and MRT in pefoxacin with trikatu pretreated than pefloxacin alone group.

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.[16] 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.*[17] Watts *et al.*[18] 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. Significantly lower loading (D*s) and maintenance (D_0s) doses were observed for levofloxacin at all dosage intervals in levofloxacin with trikatu pretreated as compared to its alone administration following intramuscular administration in crossbred cow calves (Table 2) which suggested that levofloxacin dose has to be reduced for safe and effective combination with trikatu pretreated for treating systemic microbial infections. Dama *et al.*[12] also reported that dosage regimen of pefloxacin could be low in

trikatu pretreated group as compared to pefloxacin alone in mountain gaddi goats. 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 and 12 h in crossbred cow calves (Table 2). For maintaining therapeutic concentration of 0.2 µg/ml, a loading dose (D*) of around 0.71 mg/kg and maintenance dose (D_0) of 0.64 mg/kg may be used at the dosage interval of 12 h for treating systemic infections in crossbred cow calves.

CONCLUSION

Based on the present study, trikatu non-significantly enhances the bioavailability of levofloxacin, which clearly shows that the loading and maintenance doses of levofloxacin are lower in levofloxacin with trikatu as compared to levofloxacin without trikatu.

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