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PHARMACOKINETIC INTERACTIONS AND PHARMACOKINETIC-PHARMACODYNAMIC SURROGATE RELATIONSHIPS OF ENROFLOXACIN AND DICLOFENAC IN BUFFALO CALVES FOLLOWING INTRAVENOUS ADMINISTRATION

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ABSTRACT

Clinical relevance of pharmacokinetic interactions of enrofloxacin and diclofenac were studied in five female buffalo calves to know about the merit of the combination. Enrofloxacin (4 mg/kg) were administered intravenously separately and in combination. Between each injection an interval of 3 weeks were allowed in order to eliminate the drug(s) from the body. Samples of plasma and urine were collected at predetermined time intervals, extracted and analyzed by High Performance Liquid Chromatography while urine samples of enrofloxacin were analyzed by microbiological assay. Pharmacokinetic evaluation of plasma concentrations versus time profile of enrofloxacin and diclofenac was performed by using two-compartment open model whereas ciprofloxacin (active metabolite of enrofloxacin) was analyzed by using non-compartmental analysis. All kinetic parameters of enrofloxacin and its metabolite ciprofloxacin when enrofloxacin given alone and together with diclofenac did not differ significantly which denote that diclofenac may not have any influence in distribution, metabolism and elimination of enrofloxacin. The ratios of enrofloxacin metabolized to ciprofloxacin were 0.47 and 0.42 when enrofloxacin was given alone and together with diclofenac, respectively. In contrast, some of the important kinetic parameters of diclofenac differed significantly in combined administration. The rate of distribution of diclofenac was not affected by enrofloxacin as shown by non-significant

difference in distribution half life values when diclofenac was given alone and together with enrofloxacin. Significantly ($p<0.01$) higher elimination half life, mean residence time and volume of distribution values of 12.84 ± 1.29 h, 18.07 ± 1.92 h and 1.34 ± 0.04 L/kg were obtained when diclofenac was given together with enrofloxacin as compared to the values of 4.06 ± 0.59 h, 4.72 ± 0.85 h and 0.54 ± 0.10 L/kg respectively, when diclofenac was given alone. No effect of diclofenac on the pharmacokinetics of enrofloxacin in buffalo calves when both drugs were given in combination. In contrast, enrofloxacin influenced some of the important kinetic parameters of diclofenac when both the drugs were given together. Therefore, we conclude that enrofloxacin can be used safely and effectively with diclofenac in clinical cases of drug sensitive microbial infections accompanied by any other inflammatory conditions in buffalo species.

Key words: Pharmacokinetic interactions, Pharmacodynamic efficacy, enrofloxacin, diclofenac, buffalo calves.

INTRODUCTION

Antimicrobials and non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used concomitantly and pharmacokinetic interactions between them have been described [1, 2]. The antimicrobial fluoroquinolone enrofloxacin has received wide spread acceptance in veterinary practice. The drug has a broad spectrum of activity, being active against both gram-positive and gram-negative bacteria and also against mycoplasmas. Enrofloxacin is bactericidal and acting by inhibiting the DNA gyrase enzyme. It is biotransformed in the body by N-dealkylation into a pharmacologically active metabolite, ciprofloxacin, which is also a potent bactericidal agent used in human medicine [3]. The usefulness of enrofloxacin in the treatment of gastrointestinal, respiratory and urogenital tract infections has been well documented and its pharmacokinetic properties have been extensively examined in a variety of domestic species other than buffalo calves after oral and parentral administration [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. To the best of our knowledge, the pharmacokinetic behaviour of enrofloxacin and its active metabolite ciprofloxacin has not been described in buffalo calves following intravenous (i.v.) administration of enrofloxacin.

Diclofenac is a potent NSAID, which also possesses good analgesic, antipyretic with uricosuric properties. It produces its effect by irreversibly inhibiting cyclooxygenase pathway of prostaglandin synthesis, which is the main mediator of pain, fever and inflammation. It is used in degenerative joint diseases, rheumatoid arthritis, ankylosing spondylitis and allied

conditions [18]. The pharmacokinetic studies of diclofenac have been examined in man [19] and in yaccatan mini pigs [20]. However, there seems to be no information available on the pharmacokinetic interaction of diclofenac with enrofloxacin in buffalo calves. The purpose of the present study was to determine the plasma and urine concentrations as well as the detailed kinetic data and also to know the pharmacokinetic interactions of enrofloxacin and diclofenac in buffalo calves in order to find out the suitability of the combined use of both the drugs in clinical practice.

MATERIALS AND METHODS

Experimental animals and drugs

The study was conducted on five clinically healthy female buffalo calves of indigenous breed between 12 to 18 months of age and 102 to 175 kg body weight. The animals were housed in climate-controlled rooms, with a 12-h light/12-h dark cycle. The experiment was approved by the institute ethical committee which follow care and maintenance of animals as per CPCSEA guidelines and the synopsis advisory committee of Rajendra Agricultural University, Bihar as a part of post graduate degree programme of the first author. The animals were housed for the duration of the experiment and maintained on dry fodder, concentrates along with routine grazing and supply clean water *ad libitum*.

Enrofloxacin and diclofenac were used in the present experiment. ENROCIN® 10% - an injectable commercial preparation containing enrofloxacin in concentration of 100 mg/mL (marketed by Ranbaxy Laboratories Limited, India) was used in the present study. Similarly, ZOBID® - an injectable preparation containing diclofenac sodium in concentration of 25 mg/mL (marketed by Ambalal Sarabhai Enterprises Limited, Gujarat, India) was used. Enrofloxacin (4 mg/kg, i.v.) was injected in each of five buffalo calf and in the same animals diclofenac (1 mg/kg, i.v.) was injected after a gap of 3 weeks interval. After conducting kinetic studies of the above two drugs alone, both drugs were administered together in the same set of animals at similar dose rate in two different syringes one after another immediately after an interval of three weeks.

Collection and storage of blood and urine samples

Enrofloxacin (4 mg/kg, i.v.) was injected into the jugular vein of each buffalo calf. Samples of blood were collected from contra-lateral jugular vein into heparinised glass centrifuge tubes before and at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 30, 36 and 48 h post i.v. administration. Similarly, diclofenac (1 mg/kg, i.v.) was injected

into the jugular vein and the blood samples were collected at the above noted times. Likewise, both the above drugs were given simultaneously one after another immediately at similar dose rate and samples of blood were collected at the above noted times. Simultaneously, samples of urine were also collected at similar times post i.v. administration of the drug(s). For collection of urine, a Foley's balloon catheter (No. 12) was introduced into the bladder through the urethra and kept in position by inflating the balloon by giving 30 ml of water. Plasma was separated after centrifugation (3000 rpm for 15 min) at room temperature and both plasma and urine samples were kept in refrigerator at -4^{o}C until they were analysed, usually within three days of collection.

Estimation of drugs

HPLC analysis

Concentrations of enrofloxacin and its active metabolite, ciprofloxacin were simultaneously measured in plasma using reverse phase partition chromatography technique using High Performance Liquid Chromatography (HPLC) as per the method with slight modifications [5, 21]. A Waters HPLC system with a pump (model 515, USA), a dual wavelength U-V detector (model 2487, USA), rheodyne manual injector with a 200 µL loop size and a data module/integrator (model 746, USA) was used. Chromatographic separation was performed using column 3.9 × 300 mm (µ Bondapak^{TM} C_{18}, Ireland). The flow rate was 0.6 mL/min, the wavelength was monitored at 278 nm, injection volume was 200 µL, chart speed was 0.25 mm/min and detector sensitivity was 2.000 A.U.F.S. (absorbance under full scale) were adopted for HPLC analysis for both enrofloxacin and ciprofloxacin. The mobile phase consisted of acetonitrile : methanol : water (17 : 3 : 80 v/v/v) containing 0.4 % ortho-phosphoric acid (85 % v/v) and 0.4 % triethylamine (v/v). The pH of mobile phase was 3 (approx.). All the reagents were of HPLC grade.

Stock solutions of 1 mg/mL enrofloxacin and ciprofloxacin (obtained from Cadila Health Care Limited, Ahmadabad, Gujarat, India) were prepared in acetonitrile. From stock solutions working standards of different strengths were prepared using the same diluent. The relationship between concentrations and peak area was linear from 0.01 to 4 µg/mL for enrofloxacin and 0.03 to 4 µg/mL for ciprofloxacin. The samples of higher concentrations were diluted and remeasured. The detection and quantitation limits for enrofloxacin and ciprofloxacin were noted to be 0.01 and 0.03 µg/mL, respectively. The coefficient of variation was 0.5 % for plasma samples. The retention time for ciprofloxacin was 10.08 min

and enrofloxacin was 12.60 min. No interfering peaks in all blank samples were noted in the elution position of ciprofloxacin and enrofloxacin. From the standard curve constructed based on the peak areas (Fig. 1), concentrations in plasma samples collected post i.v. administration was estimated.

Concentrations of diclofenac in plasma and urine were estimated by HPLC method as described with some modifications [22]. The above HPLC system was used. Here, the loop size was 20 µL, the flow rate was 1.5 mL/min and the wavelength was monitored at 280 nm. All other conditions were same as described above. The mobile phase comprised of acetonitrile : water (50 : 50 % v/v), adjusted to pH 3.3 with glacial acetic acid.

A stock solution of 1 mg/ml of diclofenac was prepared in acetonitrile. Working concentrations (0.01 to 4 µg/ml) were prepared using the same diluent and peak areas were noted to be linear. The detection and quantification limit of the method for diclofenac was 0.01 µg/ml. The coefficient of variation was 0.8 % for both plasma and urine samples. The retention time for diclofenac was 6.07 min. No interfering peaks in all blank samples were noted in the elution position of diclofenac. A linear relationship existed in the calibration curve at both low and high concentrations within the concentration range of the study.

Fig. 1: Representative HPLC chromatogram obtained from dual wavelength UV detector at 278 nm (enrofloxacin and ciprofloxacin) and at 280 nm (diclofenac)

C = 1 µg/ml of ciprofoxacin, E = 1 µg/ml of enrofoxacin, D = 0.5 µg/ml of diclofenac

Microbiological assay

Estimation of both enrofloxacin and ciprofloxacin could not be standardised in urine samples by HPLC since various constituents of urine are known to interfere [23]. Hence, a micobiological assay technique (cylinder plate diffusion method) was used using *Escherichia coli* (ATCC 25922) as the test organism [24, 25]. Since it is difficult to differentiate and estimate enrofloxacin and ciprofloxacin separately by microbial assay, the estimation of antimicrobial activity of enrofloxacin (including ciprofloxacin) corresponding to the concentrations of enrofloxacin in urine samples were estimated. Various standard samples (0.02 to 8 µg/ml) in urine were prepared and used simultaneously with test samples in assay plates for obtaining the zone of inhibition (measured as diameter in mm). The relationship between standards and zone of inhibition was linear from 0.05 to 4 µg/ml. The test samples with higher concentrations were diluted and remeasured. The sensitivity of the assay method was as low as 0.05 µg/ml.

Pharmacokinetic analysis

Calculation of kinetic parameters of enrofloxacin and diclofenac

The log plasma drug concentration versus time profile showed a biphasic curve and hence, followed a 2-compartment open model. Various kinetics parameters were obtained by least square regression method [26, 27].

Calculation of kinetic parameters of ciprofloxacin

The log plasma drug concentration versus time profile showed a non-linear curve and hence, non-compartmental analysis was done through statistical moment approach as described [28].

Pharmacodynamic efficacy / Efficacy predictors

Clinical and microbiological outcomes of enrofloxacin therapy can be predicted by the site of infection and in terms of pharmacokinetic-pharmacodynamic (PK/PD) surrogate relationships based on C_{max} : MIC and the AUC : MIC ratio [29]. The pharmacodynamic efficacy of enrofloxacin was determined by calculating C_p^o / MIC_{90} and AUC/MIC ratios following i.v. administration of drugs. In order to calculate the PK/PD efficacy predictors hypothetical MIC values were used. The minimum therapeutic concentration (MIC_{90}) value of enrofloxacin for different species of microorganisms ranged between 0.001 to 1.0 µg/ml [30]. Keeping in mind the synergistic effect of the body immune system and other *in vivo* factors as well as to cover most of the susceptible organisms, the MIC_{90} of 0.125, 0.25

and 0.5 µg/ml are taken into consideration. In the present investigation, a MIC_{90} of 0.125 µg/ml of enrofloxacin was taken into consideration for discussion.

Statistical analysis

Comparison of concentrations of the drugs in plasma and urine at various time intervals as well as various kinetic parameters of the drugs when the drugs were given alone and when given together in combination in buffalo calves were compared by using paired *t*-test [31].

RESULTS

Comparison of drug concentrations in plasma and urine

Enrofloxacin appeared in plasma at 0.042 h and was detectable up to 12 h in both groups of buffalo calves (Fig. 2). On the other hand, ciprofloxacin appeared in plasma at 0.042 h in both groups but it was detectable up to 12 h in plasma when enrofloxacin was given alone whereas for a shorter period of 10 h in plasma when enrofloxacin was given in conjunction with diclofenac. All data differed non-significantly which reveal that there may not be any significant effect of diclofenac in the kinetics of enrofloxacin and ciprofloxacin. The mean therapeutic concentration in plasma (0.12 µg/ml - combined enrofloxacin + ciprofloxacin) was maintained up to 6 h in both groups.

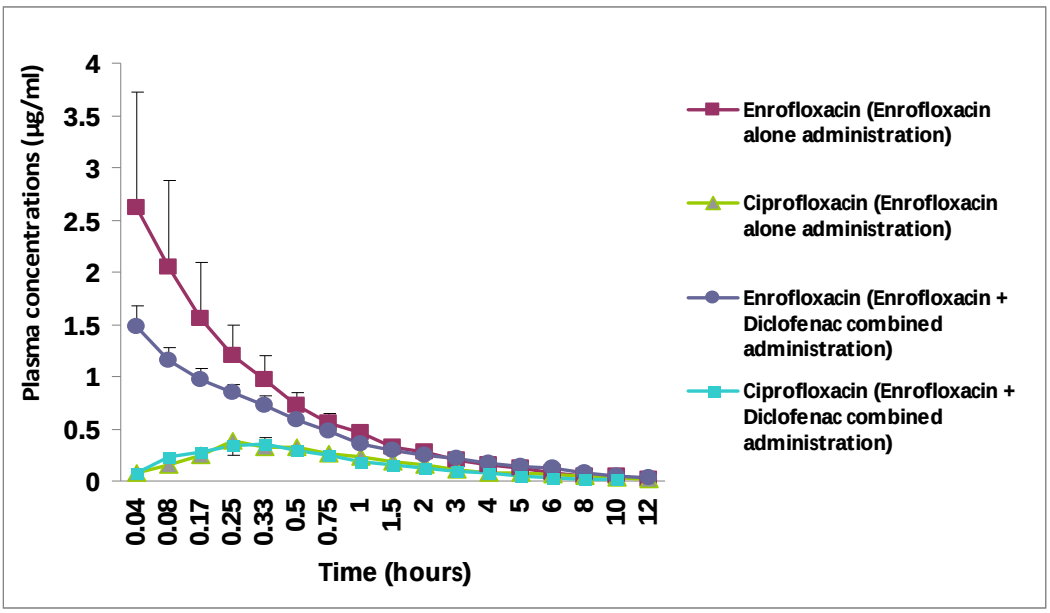

Figure 2: Comparison of plasma concentration of enrofloxacin and its active metabolite ciprofloxacin estimated by HPLC method when enrofloxacin (4 mg/kg, i.v.) given alone and together with diclofenac (1 mg/kg, i.v.) in buffalo calves

Enrofloxacin (including its active metabolite, ciprofloxacin) appeared in urine at 0.042 h and was detectable even beyond 48 h in both groups (Fig. 3). The peak drug concentration in urine was attained at 0.5 h (161.6 ± 12.20 µg/ml) when enrofloxacin was given alone while at 0.333 h (160.2 ± 11.92 µg/ml) when enrofloxacin was given together with diclofenac. The mean therapeutic concentration in urine (0.12 µg/ml) was maintained from 0.042 to 48 h in both groups. All data are non-significant which denote that diclofenac may not interfere in urinary excretion of enrofloxacin in buffalo calves.

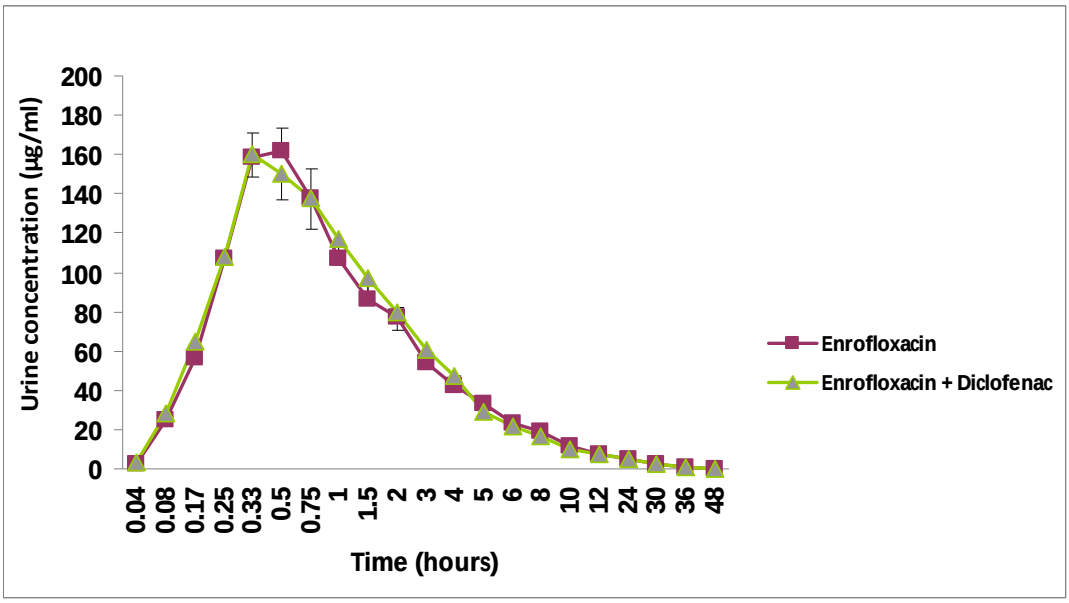

Figure 3: Comparison of urine concentration of enrofloxacin (including ciprofloxacin) estimated by microbiological assay technique when enrofloxacin (4 mg/kg, i.v.) given alone and together with diclofenac (1 mg/kg, i.v.) in buffalo calves

Fig. 4 depicts the comparison of plasma and urine concentration of diclofenac when given alone or in combination with enrofloxacin in buffalo calves at various time intervals following i.v. administration. Significantly lower ($p < 0.05$) diclofenac concentrations in plasma were obtained in combined administration of diclofenac with enrofloxacin (0.042 to 3 h) whereas significantly higher ($p < 0.05$) diclofenac concentrations in plasma were obtained at later period (8 to 24 h). Significantly lower levels ($p < 0.05$) of diclofenac concentrations in urine were obtained from 0.167 to 1.5 h whereas significantly higher levels ($p < 0.01$) of urine diclofenac concentrations were obtained at later period (4 to 48 h) when diclofenac was given in combination with enrofloxacin. The peak diclofenac concentration in urine was attained earlier at 0.167 h (30.01 ±6.58 µg/ml) when diclofenac was given alone and at 4 h (22.80 ± 1.34 µg/ml) when it was given together with enrofloxacin.

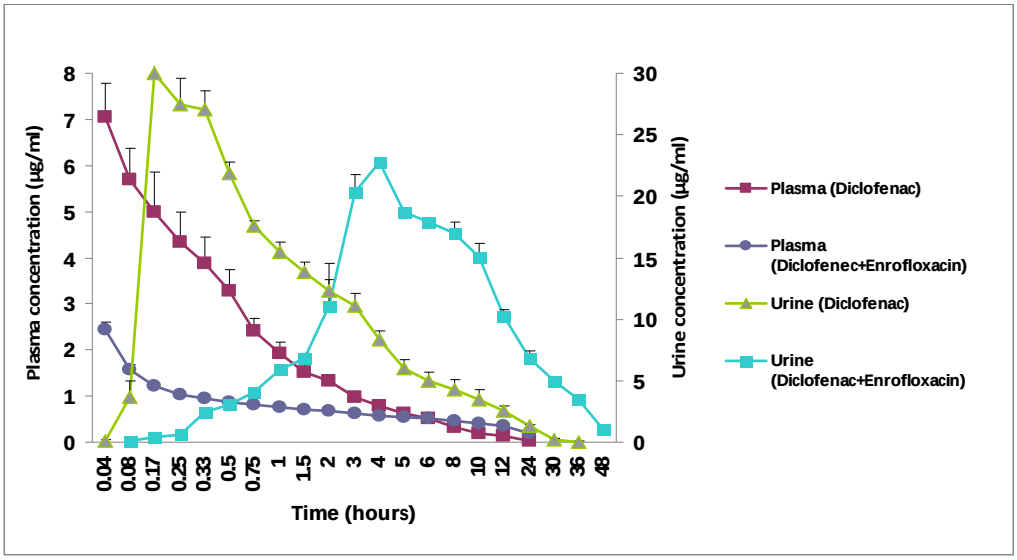

Figure 4: Comparison of plasma and urine concentration of diclofenac (1 mg/kg) when given alone and together with enrofloxacin (4 mg/kg) in buffalo calves following intravenous administration

Comparison of kinetic parameters

Statistical comparison of various kinetic parameters of enrofloxacin and ciprofloxacin when enrofloxacin was given alone and when given together with diclofenac in buffalo calves following i.v. administration are shown in Table 1. None of the kinetic parameters differed significantly which indicates that diclofenac may not have any influence over the kinetics of enrofloxacin as well as ciprofloxacin when given by i.v. route.

Table – 1: Comparison of kinetic parameters of enrofloxacin and ciprofloxacin when enrofloxacin (4 mg/kg) given alone and when given together with diclofenac (1 mg/kg) in buffalo calves following intravenous administration

Parameter (Unit)	Enrofloxacin given alone		Enrofloxacin + diclofenac combined administration	
	Enrofloxacin	Ciprofloxacin	Enrofloxacin	Ciprofloxacin
A ($\mu g.\ ml^{-1}$)	1.56 ±0.58		0.98 ±0.16	
B ($\mu g.\ ml^{-1}$)	0.41 ±0.06		0.43 ±0.05	
C_p^0 ($\mu g.\ ml^{-1}$)	1.97 ±0.55		1.41 ±0.17	
α (h^{-1})	2.65 ±0.41		3.34 ±0.60	
$t_{½}\ \alpha$ (h)	0.28 ±0.04		0.23 ±0.03	
β (h^{-1})	0.25 ±0.03	0.31 ±0.05	0.26 ±0.05	0.41 ±0.06
$t_{½}\ \beta$ (h)	2.92 ±0.41	2.40 ±0.33	3.12 ±0.62	1.87 ±0.03
AUC ($mg.L^{-1}.h$)	2.37 ±0.45	1.10 ±0.27	2.28 ±0.37	0.82 ±0.07
AUMC ($mg.\ L^{-1}.h^2$)	7.44 ±1.67	4.29 ±1.56	10.14± 3.40	2.22 ±0.41

MRT (h)	3.05 ±0.20	3.47 ±0.47	3.93 ±0.94	2.70 ±0.48
K_{12} (h^{-1})	1.17 ±0.27		1.59 ±0.39	
K_{21} (h^{-1})	1.06 ±0.09		1.29 ±0.28	
Kel (h^{-1})	0.68 ±0.15		0.72 ±0.19	
Fc	0.43 ±0.06		0.38 ±0.03	
T ≈ P	1.53 ±0.35		1.70 ±0.23	
Vdc ($L.kg^{-1}$)	2.44 ±0.38		3.02 ±0.40	
Vd_B ($L.kg^{-1}$)	10.63 ±1.40		9.83 ±1.23	
Vd_{area} ($L.kg^{-1}$)	7.47 ±0.69		7.87 ±0.67	
Vd_{SS} ($L.kg^{-1}$)	5.33 ±1.04	13.60 ±1.23	6.66 ±0.79	13.49 ±2.49
Cl_B ($ml.kg^{-1}.min^{-1}$)	32.40 ±5.69	72.38 ±13.17	33.67 ±7.22	84.88 ±9.05
% conversion of enrofloxacin to ciprofloxacin (AUC_{cipro}/AUC_{Enro})		46.96 ± 5.60		42.17 ±9.85

All data are non-significant.

The individual PK/PD ratios for the assumed MIC_{90} values for enrofloxacin and its active metabolite ciprofloxacin when enrofloxacin was given alone and together with diclofenac in buffalo calves are summarized in Table 2. The threshold AUC/MIC_{90} for a successful clinical/microbiological outcome of >20 was achieved at MIC_{90} of 0.125 µg/ml and optimal C_p^o/MIC (>10) was attained at MIC_{90} of 0.125 µg/ml for Gram-negative and Gram-positive pathogens when enrofloxacin was given alone and together with diclofenac in buffalo calves.

Table 2: Comparison of antimicrobial efficacy predictors (C_{max}/MIC and AUC/MIC) estimated for enrofloxacin and its active metabolite ciprofloxacin when enrofloxacin was given alone and together with diclofenac in buffalo calves using MIC value = 0.125 µg/ml

Parameter (Unit)	ENROFLOXACIN		ENROFLOXACIN + DICLOFENAC	
	Enrofloxacin	Ciprofloxacin	Enrofloxacin	Ciprofloxacin
C_{max}(µg/ml)	2.61 ± 1.12	0.54 ± 0.23	1.47 ± 0.20	0.37 ± 0.06
C_{max}/MIC	21.72 ± 9.37	4.47 ± 1.93	12.22 ± 1.63	3.08 ± 0.48
AUC/MIC	19.72 ± 3.76	9.20 ± 2.21	18.96 ± 3.05	6.80 ± 0.61

Table 3 depicts the comparison of various kinetic parameters of diclofenac when given alone and when given together with enrofloxacin in buffalo calves. The values observed for the extrapolated zero time concentration during distribution phase (A), theoretical zero time

concentration (C_p^0) and elimination rate constant (β) were significantly lower ($p < 0.05$) whereas the values of elimination half life ($t_{1/2}$ β), area under first moment curve (AUMC), mean residential time (MRT) and various values of volume of distribution (Vd_C, Vd_B, Vd_{area} and Vd_{SS}) were significantly higher ($p < 0.05$) in combined administration of diclofenac with enrofloxacin as compared to its alone administration. All other kinetic parameters are almost similar in both groups.

Table – 3: Comparison of kinetic parameters of diclofenac when given alone (1 mg.kg^{-1}) and together with enrofloxacin (4 mg.kg^{-1}) in buffalo calves following intravenous administration

Parameter (Unit)	Diclofenac given alone	Enrofloxacin + diclofenac combined administration
A (μg. ml^{-1})	5.74 ± 1.20	1.21 ± 0.22*
B (μg. ml^{-1})	1.65 ± 0.35	0.73 ± 0.03^{+}
C_p^0 (μg. ml^{-1})	7.38 ± 1.49	1.94 ± 0.22*
α (h^{-1})	2.76 ± 0.81	4.47 ± 1.09^{+}
$t_{½}$ α (h)	0.34 ± 0.08	0.21 ± 0.06^{+}
β (h^{-1})	0.19 ± 0.03	0.06 ± 0.01**
$t_{½}$ β (h)	4.06 ± 0.59	12.84 ± 1.29**
AUC (mg.L^{-1}.h)	11.24 ± 0.48	13.99 ± 1.59^{+}
AUMC (mg. L^{-1}.h^2)	51.78 ± 7.30	264.8 ± 58.10*
MRT (h)	4.72 ± 0.85	18.07 ± 1.92**
K_{12} (h^{-1})	1.48 ± 0.53	2.64 ± 0.79^{+}
K_{21} (h^{-1})	0.83 ± 0.22	1.75 ± 0.41^{+}
Kel (h^{-1})	0.64 ± 0.12	0.14 ± 0.01*
Fc	0.30 ± 0.03	0.40 ± 0.04^{+}
T ≈ P	2.43 ± 0.32	1.59 ± 0.30^{+}
Vdc (L.kg^{-1})	0.17 ± 0.05	0.54 ± 0.05*
Vd_B (L.kg^{-1})	0.72 ± 0.13	1.38 ± 0.06*
Vd_{area} (L.kg^{-1})	0.54 ± 0.10	1.34 ± 0.04**
Vd_{SS} (L. kg^{-1})	0.43 ± 0.10	1.31 ± 0.03**
Cl_B (ml.kg^{-1}.min^{-1})	1.52 ± 0.07	1.24 ± 0.15^{+}

\+ Non-significant * $p < 0.05$ ** $p < 0.01$

The comparison of calculated dosage regimen of enrofloxacin when given alone and when given together with diclofenac to maintain the different levels of therapeutic concentration in plasma for i.v. route in buffalo calves at different dosage intervals are presented in Table 4. For maintaining C_P^∞ min of 0.125 μg/mL, the loading doses (D*) were calculated to be 6.07 ± 1.67 and 20.72 ± 7.46 mg/kg while maintenance doses (D_0) were calculated to be 5.45 ±

1.65 and 20.10 ± 7.44 mg/kg at dosage interval (γ) of 8 and 12 h, respectively were noted for enrofloxacin given alone. Similarly, for maintaining C_P^{∞} min of 0.125 µg/mL, the loading doses (D*) were calculated to be 7.14 ± 2.31 and 27.69 ± 12.64 mg/kg while maintenance doses (D_0) were calculated to be 6.49 ± 2.32 and 27.04 ± 12.65 mg/kg at dosage interval (γ) of 8 and 12 h, respectively were noted for enrofloxacin given together with diclofenac. Like wise, D*s and D_0s were derived for maintaining C_P^{∞} min of 0.25 and 0.5 µg/mL at γ of 8 and 12 h are summarized (Table 4).

Table – 4: Comparison of calculated dosage regimen of enrofloxacin when given alone and when given together with diclofenac in buffalo calves following intravenous administration

C_p^{∞} min (µg. ml^{-1})	γ(h)	Dose (mg.kg^{-1})	Enrofloxacin given alone	Enrofloxacin + diclofenac given together
0.125	8	D*	6.07 ± 1.67	7.14 ± 2.31
		D_0	5.45 ± 1.65	6.49 ± 2.32
	12	D*	20.72 ± 7.46	27.69 ± 12.64
		D_0	20.10 ± 7.44	27.04 ± 12.65
0.25	8	D*	12.15 ± 3.35	14.28 ± 4.61
		D_0	10.90 ± 3.30	12.99 ± 4.63
	12	D*	41.44 ± 14.92	55.38 ± 25.27
		D_0	40.20 ± 14.88	54.08 ± 25.29
0.50	8	D*	24.30 ± 6.70	28.56 ± 9.22
		D_0	21.80 ± 6.60	25.98 ± 9.26
	12	D*	82.88 ± 29.84	110.8 ± 50.55
		D_0	80.40 ± 29.76	108.2 ± 50.59

All data are non-significant.

D* = Priming or Loading dose.

D_0 = Maintenance dose

γ = Dosage interval

C_p^{∞} min = Minimum therapeutic concentration in plasma (MIC).

DISCUSSION

Concentration of enrofloxacin in plasma and urine at various time interval post i.v. injection (4 mg/kg) did not differ significantly (Fig. 2 and 3). Similarly, the plasma concentrations of ciprofloxacin also did not differ significantly at all time intervals. This fact denotes that diclofenac may not have any influence in altering the plasma levels of enrofloxacin as well as influencing the metabolic conversion of enrofloxacin to ciprofloxacin in buffalo calves. In

contrast to the present study, Varma et al [32] noted lower maintenance of therapeutic concentration of enrofloxacin when given alone as compared to combined administration of enrofloxacin with diclofenac in cattle. Further they observed that the conversion of enrofloxacin to ciprofloxacin was reduced to lower plasma concentration of ciprofloxacin when enrofloxacin was given together with diclofenac. In the present study, the conversion of enrofloxacin to ciprofloxacin was observed in similar quantities when enrofloxacin was given alone and together with diclofenac. The differences in plasma concentrations of enrofloxacin and ciprofloxacin in cattle [32] as compared to the present study in buffalo calves may be due to differences in physiological and bio-chemical status between the two species.

On the other hand, concentrations of diclofenac were found to be significantly lower initially from 0.042 to 3 h (plasma) and 0.167 to 1.5 h (urine) and significantly higher later from 8 to 24 h (plasma) and 4 to 48 h (urine) in buffalo calves when diclofenac was administered in combination with enrofloxacin as compared to its single i.v. administration (Fig. 4). Peak concentration in urine was noted earlier at 0.167 h in case of alone administration of diclofenac as compared to 4 h noted in case of its combined administration with enrofloxacin.

Various kinetic parameters of enrofloxacin when given alone and when given with diclofenac did not differ significantly (Table 1). This indicates that diclofenac may not have any influence over distribution and elimination of enrofloxacin in buffalo calves. Similarly, kinetic parameters of ciprofloxacin also did not differ significantly. In contrast, Varma et al [32] showed various changes in kinetic parameters of enrofloxacin such as $t_{1/2}$ β, AUC, Vd_{area}, MRT and total body clearance (Cl_B) when enrofloxacin was given alone (5 mg/kg, i.m.) as compared to combined administration with diclofenac (0.8 to 1 mg/kg, i.m.). This may be due to species differences and it is well known that physiological status of bufflo is widely differed with other ruminants including cattle.

In the present study, $t_{1/2}$ α (Table 1) is more or less similar to that of 0.23 ± 0.05 h in pigs [11] while higher value of 0.60 ± 0.01 h in goats [33], 0.63 to 0.68 h horses [12] and a very low value of 0.07 ± 0.001 h in chicken [10] were noted.

The value obtained for $t_{1/2}$ β in goats (2.82 ± 0.33 h) [33], in rabbits (2.5 and 2.19 h) [34] and [4], respectively, and in dogs (2.4 and 3 h) [5] and [9], respectively are similar to the present value (Table 1) obtained in bufflo calves after i.v. administration of enrofloxacin. However, lower $t_{1/2}$ β of 0.734 h (enrofloxacin) and 0.934 h (ciprofloxacin) [35], 0.734 h [6] and 1.7 h [7] were

noted in cows after i.v. administration of enrofloxacin. Slightly lower $t_{1/2}$ β of 1.97 ± 0.23 h was noted in bufflo bulls [15] after i.m. administration of enrofloxacin. On the other hand, higher $t_{1/2}$ β of 3.73 ± 0.44 h in sheep [14], 5.94 to 6.09 h [12], 6.5 h [36] and 4.4 h [8] in horses, 5.33 ± 1.05 h [16] in mare, 17.10 ± 0.09 h [37] in foals and 9.64 ± 1.49 h [11] in pigs were observed after i.v. administration of enrofloxacin.

The calculated $t_{1/2}$ β value of diclofenac when it was given alone and when it was given with enrofloxacin (Table 3) are higher as compared to 1.1 h after i.v. administration [19] and 1.15 h after IM injection [38] of diclofenac in man which denote that the drug is expected to be removed at a slower rate from the body of bufflo calves as compared to man. Significantly ($p < 0.01$) higher $t_{1/2}$ β was obtained for diclofenac in combined administration of diclofenac with enrofloxacin as compared to its alone administration. This indicates that there are definite kinetic interactions and enrofloxacin has influenced in its slow removal from the body of buffalo calves. This fact is further supported by lower Kel value obtained in animals when diclofenac was co-administered with enrofloxacin.

Volume distribution of 0.6 L/kg [35, 6] and 1 L/kg [7] in cows, 0.61 ± 0.13 L/kg in buffalo bulls [15], 2 L/kg [36], 0.77 ± 0.11 to 1.22 ± 0.07 L/kg [12] and 2.3 L/kg [8] in horses, 2.49 ± 0.43 L/kg in foals [37], 3.02 ± 0.22 L/kg in sheep [14] and 2.34 ± 0.54 L/kg in goats [33] were reported whereas, higher Vd_{area} and Vd_{ss} was obtained in the present study (Table 1). The above findings denote that enrofloxacin is well distributed in different tissues and body fluids in all the above species of animals including buffalo calves.

The reported value for the volume of distribution of 0.17 ± 0.11 L/kg in man [19] is significantly lower to that of 0.54 ± 0.10 L/kg observed when diclofenac was given alone in the present study. The Vd_{area} obtained in combined administration of diclofenac with enrofloxacin in the present investigation (1.34 ± 0.04 L/kg) is significantly ($p < 0.01$) higher than that of diclofenac given alone. This indicates that there may be extensive penetration of diclofenac in various body fluids and inflammatory tissues during combined administration with enrofloxacin, which may be beneficial to the animals.

The metabolite (ciprofloxacin) formation in buffalo calves when enrofloxacin given alone was 47.0 % and when given in conjunction with diclofenac was 42.2%. Variations in the metabolism of enrofloxacin in other species and the extent of ciprofloxacin formation was 43 % in dogs [5], 36 % and 28.8 % [17] in goats, 29.9 % in dairy cows [13], 20 to 35 % in horses [8]

and <10 % in ducks [39]. Variation among sex, breed, species, age and different methods for estimating the drug and kinetic parameters may contribute to the wide discrepancies in kinetics parameters reported by various workers [40].

The effect of diclofenac on the pharmacokinetic of antimicrobial agents is well documented. The effect of diclofenac on cephalosporins (ceftriaxone, cefotiam, and cefmenoxime) in rabbits was studied [41]. Diclofenac increased significantly ($p < 0.05$), the $t_{1/2}$ β of ceftriaxone and non-significantly that of cefotiam but not for cefmenoxime. The pharmacokinetic parameters of cyclosporins were unchanged during co-administered with diclofenac in man [42]. Similarly no effect of diclofenac on the pharmacokinetics of cloxacillin was shown in man [43].

In contrast, the influence of antimicrobials including enrofloxacin on the pharmacokinetics of diclofenac was little studied in animals. In the present study, it is clearly demonstrated that diclofenac may not influence the kinetics of enrofloxacin while many changes in the kinetic parameters of diclofenac were noted when both drugs are given together as compared to its alone administration. Significantly lower values for A, C_p^0, β and Kel and significantly higher values for $t_{1/2}$ β, AUMC, MRT and various values of volume of distribution (Vd_{area} and Vd_{ss}) were noted for diclofenac when administered with enrofloxacin as compared to its alone administration in buffalo calves (Table 3).

Similarly, no significant change in kinetic parameters was observed for enrofloxacin while theophylline clearance and concentration-time profile were significantly changed leading to change in many kinetic parameters when theophylline was co-administered with enrofloxacin in rabbits. Significantly increase in theophylline concentrations occurred when enrofloxacin was given together in rabbits leading to kinetic interactions of enrofloxacin with theophylline [44]. The inhibition of metabolic pathways involving cytochrome P- 450 related isoenzymes seems to be the major mechanism by which quinolones intract with theophylline. Study state systemic diclofenac concentrations were elevated 1.4 to 2.5 times when monkeys were pretreated with L- 754, 394 (10 mg/kg, i.v.), an inhibitor of cytochrome P- 450 (CYP) 3A [45]. It is known that metabolic effect of quinolones are attributable to the selective inhibition exerted by quinolones on the cytochrome P- 450. The significantly longer $t_{1/2}$ β, MRT and significantly lower value of Kel were obtained when diclofenac was co-administerted with enrofloxacin as compared to its alone administration. This may be due to the the possible

inhibion of cytochrome P- 450 by enrofloxacin leading to the above significant changes in kinetic parameters of diclofenac.

One of the main objectives of conducting kinetic studies of antimicrobial agents is to suggest appropriate dosage regimen for treating microbial infections. It is suggested for ciprofloxacin that C_{max}/MIC ratio obtained of 10 or greater is predictive of a successful clinical outcome [46] or, alternatively, that an AUC for a 24 hour dosing period divided by the MIC of 125 or greater is predictive of bacterial eradication in pneumonic patients [47]. In the present study the value of enrofloxacin 21.72 ± 9.37 and 12.22 ± 1.63 obtained for C_{max}/MIC is quite higher than the value reported in cattle (8.17) while AUC/MIC ratio of 19.72 ± 3.76 and 18.96 ± 3.05, respectively when enrofloxacin given alone and together with diclofenac (Table 2) is lower than the value of 52 noted in cattle [25]. By considering the above facts and maintenance of therapeutic concentration in plasma (0.125 µg/ml-combined enrofloxacin and ciprofloxacin) for a period of 8 h as well as possible PAE, the drug can be used at the dose of 7 mg/kg, i.v. twice daily for treating systemic infections (Table 4).

There is general consensus that the clinical and microbiological outcomes of fluoroquinolone treatment are favourable and selection of a mutant subpopulation is preventable if an AUC/MIC ≥ 100–125 and a $C_{max,}$/MIC of 10 are achieved in Gram-negative infections[47, 48]. For Gram-positive pathogens, the minimum required $C_{max,}$/MIC is also 10, while the optimum AUC/MIC target values are still a topic of debate [48]. An AUC/MIC of 30–50 is claimed to be optimal in numerous studies performed mainly in *in vitro* or animal models [49]. Other studies conducted on different patient populations suggested a minimum AUC/MIC of 87-125 to achieve a favourable outcome and to avoid development of resistance regardless of whether the organism is Gram-positive or Gram-negative [47].

Considering the AUC/MIC_{90} and C_p^o/MIC_{90} ratios obtained in the present study, it can be stated that enrofloxacin administered i.v. in the dosing schedule applied is efficacious against bacteria with MIC_{90} values under 0.125 µg/ml in buffalo calves. The value of AUC/MIC_{90} (18.96) and C_p^o/MIC_{90} (12.22) obtained in the present study, provides support for excellent clinical and bacteriological efficacy of enrofloxacin together with diclofenac in buffalo calves. In agreement with 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 [50]. The AUC/MIC ratio was lower in present study than the values of 76.6 reported for levofloxacin administered intramuscularly in calves [51] and for other fluoroquinolones, 40.7 for marbofloxacin in cows [52]. However, it is necessary to note that the numerical values of AUC/MIC_{90} and C_{max}/MIC_{90} used as a surrogate marker to predict optimal dosage, have been generated in experimental infections in laboratory animals or in human clinical trials [53].

The therapeutic concentration in urine (0.125 mg/ml) was maintained > 48 h after i.v. administration of enrofloxacin (4 mg/kg) and hence, it can be given at similar dose rate every alternate day for treating susceptible microorganisms causing urinary tract infections in buffalo calves. The combination of diclofenac with enrofloxacin is also highly beneficial in inflammatory conditions caused by microorganisms since significantly higher ($p < 0.01$) $t_{1/2}\beta$, MRT and Vd_{area} were obtained for diclofenac when combined with enrofloxacin as compared to alone administration. Diclofenac is expected to persist in the body and distributed to a greater extent in buffalo calves in combined therapy with enrofloxacin as compared to alone administration.

Thus, the present study clearly demonstrates the usefulness of combined therapy of enrofloxacin in combination with diclofenac for effective therapy of systemic and local (UTI, mammary gland infections etc.) infections caused by microbes along with other inflammatory conditions.

CONCLUSION

In conclusion, the fact that general adverse reactions were not observed in any buffalo calves and favourable pharmacokinetic properties of enrofloxacin and diclofenac, such as long half-life with wide penetration into different body fluids and tissues from blood, were found. Based on the calculated AUC/MIC_{90} and C_p^o/MIC_{90}, a dosage of 7 mg/kg of enrofloxacin could be effective in buffalo calves $MIC_{90} \geq 0.125\mu g/ml$ along with 1 mg/kg of diclfenac following i.v. route.

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