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Pharmacokinetic Study of Single Dose Intravenous Administration of Enrofloxacin in Barbari Goats

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ABSTRACT

Pharmacokinetic studies of enrofloxacin were performed after single intravenous (IV) administrations of 5 mg/kg body weight (BW) to 6 healthy Barbari goats. The study was performed by cross-over design. Blood samples were collected from jugular vein at predetermined time intervals after drug administration. Plasma enrofloxacin concentrations were measured by high performance liquid chromatography. Various pharmacokinetic parameters were calculated using non-compartmental model. The drug showed distribution half life ($t_{1/2\alpha}$) of 0.11±0.02 h and elimination half life ($t_{1/2\beta}$) of 1.34±0.06 h. Large volume of distribution (Vd_{area}) of 3289.92±278 $ml.kg^{-1}$ in goats indicated high distribution of drug into various body fluids and tissues. The average values for area under plasma drug concentration-time curve $AUC_{(0-\infty)}$ and area under first moment curve (AUMC) was 2.97±0.19 $\mu g.ml^{-1}.h$ and 5.03±1.41 $\mu g.ml^{-1}.h^2$, with mean residence time (MRT) of 1.68±0.24 h respectively.

Keywords: Enrofloxacin, Intravenous, Pharmacokinetic, Goat

Enrofloxacin is a synthetic antimicrobial agent of fluoroquinolone group developed exclusively for veterinary use (Altreuher, 1987). It is a derivative of quinolone carboxylic acid classified into the group of broad spectrum antibacterial agents related to second generation fluoroquinolone. It is widely used in veterinary medicine in cattle, goat, pigs, poultry, fish, dogs and cats; in the treatment of diseases caused by aerobic gram-negative and gram-positive bacteria and pathogens such as mycoplasma, chlamydia and rickettsia (Brown, 1996). It is mainly indicated for gastrointestinal, urogenital, skin and respiratory tract, soft tissues, bone and joint infections in various domestic animal species (Aboubakr, 2013).

Enrofloxacin acts by interfering with DNA synthesis by inhibiting DNA gyrase activity (Zechiedrich and Cozzarelli, 1995). It possesses bactericidal activity at relatively low concentration, having high bioavailability, following oral or parental administration in most animal species and achieves good penetration into body tissue and fluids (Dorfman *et al.,* 1995; Schroder, 1989). The objective of this study was to determine the plasma concentration and pharmacokinetics of enrofloxacin following single dose IV administration in Barbari goats.

MATERIALS AND METHODS

Location and place of work

This study was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P).

Experimental animals

The experiment was conducted in 6 healthy Barbari goats of 1-2 years of age at livestock farm, Amanala,

NDVSU, Jabalpur. The average weight of goats was 22-25 kg. All the goats were ear tagged with identification numbers and kept under observation for two weeks prior to commencement of experiment. Animals were housed in hygienic condition and provided with balance ration and water *ad-lib*. Animals were subjected to regular clinical examination during entire period of experimentation. All necessary management procedures were adopted to keep the animals free from undue stress and CPCSEA guidelines were followed for care and management of animals. The experimental protocol and use of animals in experiment was approved by Institutional Animal Ethics Committee (No. 61/IAEC/Vety./2017, Dated: 31/05/2017).

Drugs

In this research work, injectable commercial preparation Fortivir® (Virbac Animal Health India Pvt. Ltd) containing enrofloxacin in concentration of 100mg.ml^{-1} was used. All the chemicals used in this study were of HPLC grade and purchased from reputed firms.

Experimental design

The study was undertaken in total 6 healthy Barbari goats using cross over design. Before initiation of experiment, animals were weighed and enrofloxacin was administered @ 5 mg/kg body weight, IV.

Collection of blood samples

Blood samples (1.5ml) were collected from jugular vein in tubes containing K_3EDTA, at 0 minutes (before drug administration), 0.033, 0.083, 0.166, 0.25, 0.5, 0.75, 2, 4, 6, 8, 10, 12, 18, 24, 30 and 36 hours after administration of drug following all aseptic precautions.

Preparation of plasma from blood

Blood samples were centrifuged at 5000 RPM for 10 minutes at 4ºC, and obtained plasma samples were stored at -20ºC until analyzed by UHPLC within 24 to 36 hours after collection.

UHPLC assay procedure

Plasma concentration of enrofloxacin were measured by Ultra High Performance Liquid chromatography system (Shimadzu Corporation, Japan) equipped with binary gradient solvent delivery pump (SIL-30AC) and Photo diode array Detector (SPD-M20A) using C_{18} reverse phase column (Supelco Discovery Column 25cm x 4.6mm, particle size 5 µ).

For enrofloxacin estimation, mobile phase consist of acetonitrile: methanol: HPLC water (17: 3: 80 V/V) containing 0.4% phosphoric acid (85% V/V) and 0.4% triethylamine (V/V), was used. The pH of mobile phase was 2.4. Mobile phase was filtered by 0.22µ nylon syringe filter before use and the flow rate was 1ml.min^{-1}. The temperature of column oven was 25±0.5ºC and effluent was monitored at 278 nm wavelength.

Sample preparation for enrofloxacin

Acetonitrile (0.75 ml) was added to plasma (0.5 ml) by shaking on vortex mixture, this mixture then centrifuged at 5000 RPM for 10 minutes at 5ºC and supernatant was collected. To this clean supernatant, 500 µl of HPLC water was added and thoroughly mixed. This mixture was filtered through 0.22 µ nylon syringe filter and put into the auto-sampler of UHPLC. For sample preparation, method of Rao *et al*. (2002) was followed, with some modifications.

Preparation of standard concentration of enrofloxacin in plasma

Enrofloxacin was quantified from the peak area, and the concentrations in plasma samples were determined by means of calibration curve. This calibration curve was drawn between the areas of spiked plasma samples (external standards) and their respective concentration.

The limit of sensitivity and quantification of enrofloxacin was 0.05 µg.ml^{-1}. Mean recovery for enrofloxacin from plasma was more than 89.52%. The method was found to be linear and reproducible in the concentration range from 0.01-10 µg.ml^{-1} with coefficient of correlation (r^2) of 0.99.

RESULTS AND DISCUSSION

No local or systemic adverse reaction was observed, to the IV administration of enrofloxacin in Barbari goats. The concentrations of enrofloxacin in plasma of goats at various time intervals following single IV administration have been shown in Table 1 and Fig. 1 and its pharmacokinetic parameters have been shown in Table 2, respectively.

Table 1: Plasma concentration of enrofloxacin (5 mg.kg^{-1}) following single intravenous administration in Barbari goats at different time interval

Time (h)	Mean ±S.E. (µg.ml^{-1})
0.033	3.57±0.15
0.083	2.27±0.12
0.166	1.74±0.11
0.25	1.50±0.11
0.5	1.20±0.06
0.75	0.99±0.04
1	0.86±0.04
1.5	0.57±0.03
2	0.43±0.02
4	0.16±0.02
6	0.07±0.01
8	0.02±0.0
10	ND

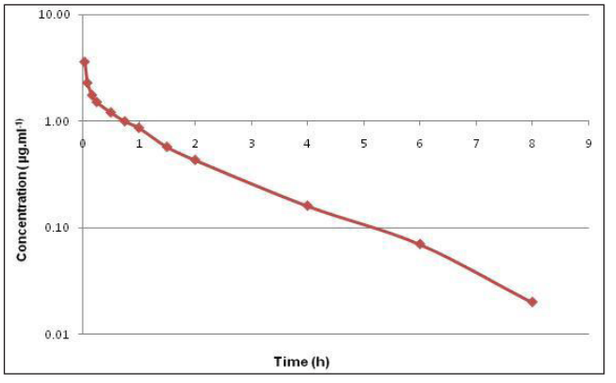

Fig. 1: Semi logarithmic plot of plasma concentration of enrofloxacin by intravenous route at different time interval in Barbari goats

To evaluate pharmacokinetic pattern of enrofloxacin in Barbari goat, enrofloxacin was administered by single IV injection at the dose rate of 5mg.kg^{-1} body weight. The dose of enrofloxacin used in the study was comparable to the dosage used in goat by researchers as Rao *et al*. (2000), Elmas *et al*. (2001) and Al-Nazawi (2005). In the present study, pharmacokinetic parameters following single dose IV administration were calculated using non compartmental approach in order to exclude any assumption regarding compartmental analysis.

The highest plasma concentration of enrofloxacin (3.57±0.15 µg.ml^{-1}) was obtained at 0.033 h. This concentration declined rapidly to 0.57±0.03 µg.ml^{-1} at 1.5 h and after that enrofloxacin gradually disappeared from plasma and a concentration of 0.02±0.00 µg.ml^{-1} was detected at 8h. The peak plasma level of enrofloxacin was approximately 35.7 fold higher than minimum therapeutic level (0.1 µg.ml^{-1}) and the drug was detected above minimum inhibitory concentration for up to 4h. However Rao *et al.* (2000) observed the concentration of 5.86±0.53 µg.ml^{-1} in plasma at 0.03 h after IV administration of enrofloxacin at the dose rate of 5 mg.kg^{-1} b.w. in goats. Varma *et al*. (2003) reported the peak plasma level of 2.7±0.40 µg.ml^{-1} at 2 minutes after single IV administration in cow when enrofloxacin was administered at the dose rate of 5 mg.kg^{-1}. Moreover, in sheep, Rahal *et al*. (2006) reported peak plasma level of 3.42±0.33 µg.ml^{-1} at the dose rate of 5mg.kg^{-1} following IV route.

The low value of distribution half-life ($t_{1/2\alpha}$) of enrofloxacin (0.11±0.02 h) observed in this study indicates, rapid distribution of drug from central to peripheral compartment in goats. However, the value of distribution half-life ($t_{1/2\alpha}$) obtained in the present study was similar to the value reported by Rao *et al*. (2000) as 0.122±0.04 h in goats. This value of distribution half-life was shorter than the corresponding values of 0.62±0.13 h observed in goat by Elmas *et al*. (2001).

In the present study, the value of Vd_{area} (3289.92±278 ml.kg^{-1}) in goats indicated high distribution of drug into various body fluids and tissues. In contrast to the present findings, lower value of Vd_{area} (2.34±0.54 L.kg^{-1}) has been reported after single IV administration of enrofloxacin in goats by Kumari *et al*. (2004). However, still lower values of Vd_{area} (1.292±0.04 L.kg^{-1}) have been observed by Rao *et al*. (2000) in goat and Anadon *et al*. (1999) (1.41±0.36 L.kg^{-1}) in pig. The high value of Vd_{area} observed in this study suggested that enrofloxacin is principally distributed in extracellular fluid space.

The mean value of volume of distribution at steady state (Vd_{ss}) was higher (2833.70±271 ml.kg^{-1}) than the values of 1.213±0.084 L.kg^{-1} reported by (Rao *et al*., 2000) in goats. However, in cow, the value of Vd_{ss} was 0.447±0.012 L.kg^{-1} as reported by Varma *et al*. (2003). Our finding indicates good extra-vascular distribution of drug in goats.

Following IV administration of drug in present study, the values of $AUC_{(0-\infty)}$ was 2.97±0.19 µg.ml^{-1}.h in goats, which is consistent to the values (2.23±0.16 µg.ml^{-1}.h) reported by Al-Nazawi (2005) (2.23±0.16 µg.ml^{-1}.h). Rao *et al*. (2000) have reported higher values of $AUC_{(0-\infty)}$ (6.715±0.751 µg.ml^{-1}.h) in goats following IV administration. Similarly, still higher values of AUC (21.12±0.21 µg.ml^{-1}.h) have been reported in goats following IV administration of enrofloxacin by Elmas *et al*. (2001).

The elimination half-life ($t_{1/2\beta}$) is the time taken for plasma concentration in the body to be reduced by half. The elimination half-life of enrofloxacin in goat in the present study was 1.34±0.06 h. In partial agreement to the present findings, little lower values of $t_{1/2\beta}$ (1.13±0.05 h) were reported in goats by Rao *et al*. (2000). The value of elimination half life ($t_{1/2\beta}$) obtained in the present study is lower than the value reported in goat (3.98±0.18 h) by Elmas *et al*. (2001) and in sheep (6.32±0.72 h) by Rahal *et al*. (2006) following single IV administration.

In present study, the elimination rate of enrofloxacin (K_{el}) from central compartment was 1.13±0.25 h^{-1}, which is comparable to the value of 1.035±0.10 h^{-1} and 1.0±0.05 h^{-1} in goat reported by Rao *et al*. (2000) and Al-Nazawi (2005) respectively. However, comparatively lower value of K_{el} (0.577±0.137 h^{-1}) was observed by Kumari *et al*. (2004) in goats. Similarly lower value of K_{el} (0.33±0.06 h^{-1}) was observed by Anadon *et al*. (1999) in pig.

Total body clearance (Cl_B) of enrofloxacin, which represents the sum of metabolic and excretory processes was 1719.99±251 ml.kg^{-1}.h^{-1} in Barbari goats. Lower value of Cl_B (806.78±114.70 ml.kg^{-1}.h^{-1}) in goat was reported by Rao *et al*. (2000). The value of total body clearance in the present study was lower to the value reported by Elmas *et al*. (2001) as 0.24±0.01 L.kg^{-1}.h^{-1} in goats and 0.86±0.10 L.kg^{-1}.h^{-1} in sheep by Rahal *et al*. (2006).

The MRT calculated following single dose IV administration of enrofloxacin was 1.68±0.24 h, however, lower values of MRT (1.5±0.104 h) was reported by Rao *et al*. (2000). Elmas *et al*.(2001) and Al-Nazawi (2005) have reported higher values of MRT as 4.13±0.16 h and 5.33±0.04 h, respectively, in goats following IV administration of enrofloxacin at the dose rate of 5 mg. kg^{-1}b.w. Likewise, Rahal *et al*. (2006) observed higher values of MRT as 3.43±0.41 h in sheep for enrofloxacin at the dose rate of 5mg.kg^{-1}.

Dosage regimen

The main objective of pharmacokinetic study of any drug is to compute the rational dosage regimen. The dosage regimen has been suggested on the basis of maintenance of therapeutic concentration (MIC) ranging from 0.1 to 0.2 µg.ml^{-1} with an average of 0.10 to 0.14 µg.ml^{-1}, has been reported to be the minimum therapeutic concentration of fluoroquinolone against various species of pathogens (Kumari *et al.,* 2004). The dosage regimens required to maintain the different levels of therapeutic concentration ($C_P{}^{\infty}{}_{min}$ = 0.1 and 0.2 µg.ml^{-1}) in plasma for IV route in goats at different dosage intervals (τ) of 8, 10 and 12 h have been presented in Table 3.

For IV route, the loading dose for 8 and 10 h dose interval was 2.07 and 3.27 mg.kg^{-1}, respectively for $Cp^{\infty}{}_{min}$ of 0.1 µg.ml^{-1}, this dose increased up to 4.13 mg.kg^{-1} and 6.54mg. kg^{-1}, when $Cp^{\infty}{}_{min}$ was increased to 0.2 µg.ml^{-1}. The maintenance dose, for 8 and 10 h dose interval was 1.74 and 2.94 mg.kg^{-1}, respectively for $Cp^{\infty}{}_{min}$ of 0.1 µg.ml^{-1}. Based on the maintenance of therapeutic concentration (>0.1 µg.ml^{-1}) in plasma, enrofloxacin may be administered at the dose rate of 5mg/kg, IV every 8, 10 and 12 h for treating septicaemia, mammary gland infections, urinary tract infection etc. caused by susceptible organisms.

Table 2: Pharmacokinetic parameters of enrofloxacin (5 mg.kg^{-1}) following single intravenous administration in Barbari goats

Kinetic parameters	Unit	Mean± S.E.
Cp^0	µg.ml^{-1}	3.24±0.24
A	µg.ml^{-1}	1.87±0.26
B	µg.ml^{-1}	1.36±0.04
α	h^{-1}	3.44±0.68
β	h^{-1}	0.23±0.01
$t_{1/2\,\alpha}$	H	0.11±0.02
$t_{1/2\,\beta}$	H	1.34±0.06
$AUC_{(0-\infty)}$	µg. ml^{-1}.h	2.97±0.19
AUMC	µg. ml^{-1}. h^2	5.03±1.41
K_{12}	h^{-1}	3.84±2.59
K_{21}	h^{-1}	3.47±1.09
K_{el}	h^{-1}	1.13±0.25
Vd_{area}	ml.kg^{-1}	3289.92±278
Vd_{ss}	ml.kg^{-1}	2833.70±271
Cl_B	ml.kg^{-1}.h^{-1}	1719.99±251
MRT	H	1.68±0.24

Cp^0 = Theoretical concentration of drug in plasma at zero time; A = zero-time intercept of distribution phase; B = zero-time intercept of elimination phase; α = distribution rate constant; β = elimination rate constant; $t_{1/2\alpha}$ = half-life of distribution phase; $t_{1/2\beta}$ = half-life of elimination phase; AUC = area under the concentration-time curve; AUMC= area under the movement curve; K_{12} = rate constant from central to peripheral compartment; K_{21} = rate constant from peripheral to central compartment; Kel: elimination rate from central compartment; Vd_{area} = volume of drug distribution; Vd_{ss}=Volume of distribution in steady state; Cl_B = total body clearance of the drug; MRT = mean residence time.

Table 3: Dosage regimen of enrofloxacin following intravenous administration in Barbari goats

$C_{P^{min}}^{\infty}$ (μg.ml^{-1})	τ (h)	Dose type	Dose (mg.kg^{-1})
0.1	8	D*	2.07
		D_0	1.74
	10	D*	3.27
		D_0	2.94
	12	D*	5.18
		D_0	4.85
0.2	8	D*	4.13
		D_0	3.47
	10	D*	6.54
		D_0	5.89
	12	D*	10.36
		D_0	9.71

D* - Loading dose and D^0 – Maintenance dose.

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