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Pharmacokinetic study of single dose intramuscular administration of enrofloxacin in barbari goats

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Abstract
Enrofloxacin is a second generation fluoroquinolone, specially developed for therapeutic purpose in veterinary practice and extensively used in goats for therapeutic purposes. The present study was planned to study the pharmacokinetic of enrofloxacin after single dose intramuscular administration in 6 healthy Barbari goats. The study was conducted by following cross-over design. The enrofloxacin was injected @ 5mg/kg body weight through intramuscular route. After drug administration, blood sample were collected at different time intervals and the plasma concentration of enrofloxacin were measured by high performance liquid chromatography (HPLC) having PDA detector. The mean peak plasma drug concentration of 0.38±0.05 μg.ml^{-1} was achieved at 1h, however, effective therapeutic concentration (0.1μg.ml^{-1}) was maintained up to 4h with mean concentration of 0.17±0.03 μg.ml^{-1} at 4 h. The mean absorption half-life of ($t_{1/2ka}$) and elimination half-life ($t_{1/2\ \beta}$) were calculated to be 0.25±0.05h, and 2.07±0.31h, respectively. The mean apparent volume of distribution (Vd_{area}), volume of distribution at steady state (Vd_{ss}) and mean residential time (MRT) was calculated to be 10851.49±1353.06ml.kg^{-1}, 12123.31±1196.69ml.kg^{-1} and 3.35±0.40h, respectively.

Keywords: Enrofloxacin, intramuscular route, goats, pharmacokinetic, HPLC

1. Introduction
Fluoroquinolones developed over the past few years having greater potency, broader spectrum of activity, greater *in vivo* efficacy against resistant organisms and better safety profile than other antimicrobial agents. Fluoroquinolones have been shown to be effective in the treatment of a wide variety of bacterial infections in both humans and animals [6, 9]. Fluoroquinolones have a broad bactericidal spectrum includes gram-negative and gram-positive bacteria, chlamydiae and mycoplasma [12]. It is mainly indicated for gastrointestinal, urogenital, skin and respiratory tract infections in various domestic animal species [1]. In fact they have been used successfully to treat infection such as pulmonary infections, urinary tract infections and digestive infections in animals [4].
Enrofloxacin is a derivative of quinolone carboxylic acid classified into the group of broad spectrum antibacterial, belong to second generation fluoroquinolones. It is widely used in veterinary medicine in cattle, sheep, goat, pigs, poultry, fish, dogs and cats; in the treatment of diseases caused by aerobic gram-negative and gram-positive bacteria. It is mainly indicated for gastrointestinal, urogenital, skin and respiratory tract infections in various domestic animal species [1]. Patel and Mody studied the pharmacokinetics of enrofloxacin alone and in combination with meloxicumin in male buffalo calves and sheep [10].

2. Materials and Methods
Enrofloxacin (Fortivir ® - 10%) - an injectable single application commercial preparation containing enrofloxacin in concentration of 100mg.ml^{-1} marketed by Virbac Animal Health India Pvt. Ltd., Mumbai, India was used in the present study. All chemicals used in this study were of HPLC grade and purchased from reputed firms.

a) Experimental animals
Six clinically healthy Barbari goats of 1-2 years of age at livestock farm, Amanala, NDVSU, Jabalpur was used in this study. The average weight of goats was between 22-25 kg. All the goats were ear tagged with identification number and kept under observation for two weeks prior to commencement of experiment. Animal were housed in hygienic conditions and provided balance ration with *ad-lib* water. 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 study was approved by Institutional Animal Ethics Committee of College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, India (No. 61/IAEC/Vety./2017, Dated: 31/05/2017). The goats were individually weighed immediately before administration of drug in order to determine the precise dose. Enrofloxacin (5mg/kg) was injected intramuscularly in each goat and blood samples (1.5ml) were collected from jugular vein in tubes containing K_3EDTA at 0 h. (before drug administration), 0.033, 0.083, 0.166, 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 18, 24, 30 and 36 hours after administration of drug following aseptic precautions. Blood samples were centrifuged at 5000 RPM for 10 minutes at 4ºC, and obtained plasma samples were stored at -20ºC until analyzed by UHPLC usually, within 24 to 36 hours of collection.

b) HPLC 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 5µ). 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 and the flow rate was 1ml.min^{-1}. The temperature of column oven was 25±0.5ºC and effluent was monitored at 278nm wavelength.

c) Sample preparation for enrofloxacin

Acetonitrile (0.75ml) was added to plasma (0.5ml) by shaking on vortex mixture, this mixture was centrifuged at 5000 RPM for 10 minutes at 5ºC and obtained supernatant was collected. To this clean supernatant, 500µl of HPLC water was added and thoroughly mixed. This mixture was filtered through 0.22µ nylon syringe filter and put into the auto-sampler of UHPLC. For sample preparation, method of Rao and co-workers was followed, with some modifications [11].

d) Preparation of standard concentration in plasma for enrofloxacin

Enrofloxacin was quantified by calibration curve drawn between the plasma having the spiked and known concentration of enrofloxacin and obtained peak area. The limit of sensitivity and quantification of enrofloxacin was 0.05µg.ml^{-1}. Mean recovery for enrofloxacin from plasma was more than 89.52%. This 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.

3. Result and Discussion

Concentrations of enrofloxacin in plasma at various time intervals following its single intramuscular injection at the dose rate of 5mg.kg^{-1} body weight have been shown (Table 1 and figure 1). For enrofloxacin, the minimum therapeutic plasma concentration against majority of pathogens in animals has been reported by Kaartinen and co-workers as 0.1µg.ml so in this discussion, the MIC of 0.1µg.ml^{-1} of enrofloxacin was taken into consideration [8]. The peak plasma concentration was observed 0.38±0.05µg.ml^{-1} at 1h after intramuscular administration of enrofloxacin. Hussain *et al.* (2014) reported the peak plasma level of 1.26±0.19µg.ml^{-1} at 1.5h after administration; the highest plasma concentration of 2.74±0.28µg.ml^{-1} was recorded by Rao and co-workers at 0.75h after intramuscular administration [7]. However, Anadon *et al.* (1995) and Rao and co-workers reported peak plasma level of 1.17±0.2328µg.ml^{-1} in pig at 1.81±0.23h by intramuscular route of administration [3, 11].

Table 1: Plasma concentration of enrofloxacin (5mg.kg^{-1}) following single intramuscular administration in Barbari goats

Time (h)	Mean ±S.E. (µg.ml^{-1})
0.033	0.06±0.01
0.083	0.08±0.01
0.166	0.10±0.01
0.25	0.13±0.01
0.5	0.23±0.02
0.75	0.36±0.04
1	0.38±0.05
1.5	0.29±0.03
2	0.24±0.03
4	0.17±0.03
6	0.07±0.01
8	0.03±0.01
10	ND

The elimination half-life ($t_{1/2\beta}$) of enrofloxacin in goat in the present study was 2.07±0.31h. In agreement to the present findings, almost similar values of $t_{1/2\beta}$ was reported in goats by Hussain *et al*. (2014), however, Al-Nazawi (2005) reported higher value of elimination half-life in goats [2,7]. Anadon *et al*. (1995) reported elimination half-life of 12.06±0.68h in pigs administered enrofloxacin at the dose rate of 2.5mg.kg^{-1} body weight, intramuscularly [3]. The low value of absorption half-life ($t_{1/2ka}$) of enrofloxacin observed in this study (0.25±0.05h) indicated the rapid absorption of drug in Barbari goats. This value of absorption half-life was shorter than the corresponding values in goat (0.88±0.190h) and pig (0.42±0.06h), respectively [3, 7].

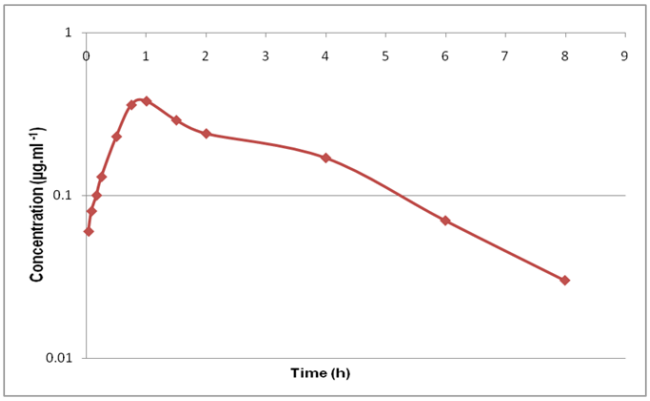

Fig 1: Semi logarithmic plot of plasma concentration of enrofloxacin by intramuscular route at different time interval in Barbari goats

In the present study, the value of Vd_{area} (10851.49±1353.06ml.kg^{-1}) in goats indicated a higher distribution of drug into various body fluids and tissues. The mean value of volume of distribution at steady state (Vd_{ss}) observed in the present study was 12123.31±1196.69ml.kg^{-1}, the same was reported as 1.51±0.16L.kg^{-1} and 2.25±0.287L.kg^{-1} respectively by Elmas *et al.* (2001) [5] and Hussain *et al*. (2014) [7] in goat; however in pig, the value was 1.66±0.28L.kg^{-1} as reported by Anadon *et al*. (1995) [3]. Following intramuscular administration of drug, the observed

values of AUC was 1.38±0.31µg.ml^{-1}.h; however, Al-Naziwa (2005) reported the same as 2.29±0.12µg.ml^{-1}.h^{-1} in goats. Rao *et al*. (2002) observed relatively higher values of AUC (7.82±0.763µg.ml^{-1}.h^{-1}) in goats following intramuscular administration of enrofloxacin at the dose rate of 5mg.kg^{-1} body weight [11]. Moreover, Anadon *et al.* (1995) reported AUC value of 19.18±3.37mg.L^{-1}.h^{-1} in pigs following intramuscular administration of enrofloxacin at the dose rate of 2.5mg.kg^{-1} body weight [3].

The MRT calculated following single dose intramuscular administration of enrofloxacin was 3.35±0.40h. Rao *et al*. (2002) reported lower value of MRT as 2.37±0.21h in goats following intramuscular administration of enrofloxacin at the dose rate of 5mg.kg^{-1} [11]. However, higher value of MRT as 5.75±0.41h was reported by Elmas *et al*. (2001) when enrofloxacin was administered by intramuscular route, in goats. Total body clearance (Cl_B) of enrofloxacin in the present study was found to be 3805.73±438.87ml.kg^{-1}.h^{-1}, whereas, Elmas *et al.* (2001) reported the value of Cl_B as 1.51±0.16 L. kg^{-1}.h^{-1} [5]. The value of total body clearance in the present study was higher to the value reported by Hussain *et al*. (2014) in goats as 0.96±0.099L.kg^{-1}.h^{-1} [7].

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

Kinetic parameters	Unit	Mean ±S.E
A	µg.ml^{-1}	3.95±2.55
B	µg. ml^{-1}	0.56±0.06
Ka	h^{-1}	1.49±0.27
β	h^{-1}	0.16±0.02
$t_{1/2ka}$	h	0.25±0.05
$t_{1/2\,\beta}$	h	2.07±0.31
$AUC_{(0-\infty)}$	µg.ml^{-1}. h	1.38±0.31
AUMC	µg.ml^{-1}. h^2	4.82±0.86
Vd_{area}	ml.kg^{-1}	10851.49±1353.06
Vd_{ss}	ml.kg^{-1}	12123.31±1196.69
Cl_B	ml.kg^{-1}.h$^{1.}$	3805.73±438.87
MRT	h	3.35±0.40
F	%	47.46±5.37

A = zero-time intercept of distribution phase; B = zero-time intercept of elimination phase; Ka = distribution constant; β = elimination constant; $t_{1/2ka}$ = half-life of distribution phase; $t_{1/2\beta}$ = half-life of elimination phase; $AUC_{(0-\infty)}$= area under the concentration-time curve; AUMC= area under the movement curve; 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, F= bioavailability.

Table 3: Dosage regimen of enrofloxacin for intramuscular administration in Barbari goats

$C_P{}^{\infty}{}_{min}$ (µg.ml^{-1})	τ (h)	Dose type	Dose (mg.kg^{-1})
0.1	8	D*	3.90
		D_0	2.82
	10	D*	5.37
		D_0	4.29
	12	D*	7.40
		D_0	6.32
0.2	8	D*	7.80
		D_0	5.63
	10	D*	10.75
		D_0	8.58
	12	D*	14.80
		D_0	12.63

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

Dosage regimen

The calculation of loading and maintenance doses of enrofloxacin after intramuscular administration were based upon the maintenance of minimum inhibitory concentration (MIC or $C_P{}^{\infty}{}_{min}$) of enrofloxacin in plasma. In this study, the loading doses (D*s) and maintenance doses (D_0 s) for enrofloxacin for 8, 10 and 12h interval in healthy goats have been presented (Table 3). For maintaining $C_P{}^{\infty}{}_{min}$ of 0.1µg.ml^{-1}, the loading doses (D*s) were calculated to be 3.90, 5.37 and 7.40mg.kg^{-1}; while maintenance doses (D_0 s) were calculated to be 2.82, 4.29 and 6.32mg.kg^{-1}, respectively. The D*s were calculated to be 7.80, 10.75 and 14.80mg.kg^{-1} while D_0s were found to be 5.63, 8.58 and 12.63mg.kg^{-1} at dose interval of (τ) 8,10 and 12h respectively, for maintaining $C_P{}^{\infty}{}_{min}$ of 0.2µg.ml^{-1}.in blood.

4. Acknowledgement

We declare that we have no conflicts of interest.

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