

Kinetic Studies of Enrofloxacin after Intravenous Administration in Yak

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Summary

The pharmacokinetic studies of enrofloxacin was investigated in yak (*Bos grunniens L.*) after intravenous administration at 5 mg.kg⁻¹ body weight. Blood samples were collected from the jugular vein at predetermined time intervals after drug administration. Plasma was separated and analysed for enrofloxacin by reverse-phase high performance liquid chromatography. Various pharmacokinetic parameters were calculated out by non-compartmental model. The elimination half-life ($t_{1/2}$), area under plasma concentration-time curve (AUC), area under the moment curve (AUMC), mean residence time (MRT), apparent volume of distribution (V_d), total body clearance (Cl_B) and apparent first-order elimination rate constant (K) of enrofloxacin were found to be 1.2757±0.3212 h, 5.7312±1.6228 µg.h.ml⁻¹, 10.5509±4.8171 µg.h².ml⁻¹, 1.8409±0.3773 h, 1.6061±0.5029.Lkg⁻¹, 872.4176 ± 223.98 ml.h⁻¹.kg⁻¹ and 0.5432±0.173 h⁻¹, respectively after administration of enrofloxacin .

Because of faster elimination rate, excellent tissue penetration, shorter half- life and low MRT after intravenous administration of enrofloxacin at 5 mg kg⁻¹, it is calculated dosage for use in yak at therapeutic level.

Key words: enrofloxacin, yak, pharmacokinetics, intravenous.

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Introduction

Enrofloxacin, a fluoroquinolone antimicrobial approved exclusively for veterinary use, has a broad spectrum of antibacterial activity with MIC value ranging from 0.008 to 0.06 µg/ml [1]. It has widespread distribution to most tissues and body fluids with a potential therapeutic application in many types of infections. Hence, it is effectively used in the treatment of septicemia, respiratory tract, urinary tract, skin, soft tissues, bone and joint infections etc. Yak (*Bos grunniens L.*), being the most ecologically sustainable animal resources of Indian Himalayas and mainstay for highlanders, provides basic needs in terms of meat, milk, hair, wool and most needful transport in hilly terrain. Pharmacokinetic profiles of enrofloxacin have been carried out in cattle [2], horses [3], and pigs [4]. The dosage regimens of enrofloxacin have been worked out for sheep, dog and goat, but such data are inadequate to warrant its effective clinical use in yak. In the light of the above reports, it was thought worthwhile to study pharmacokinetics of enrofloxacin to rationalize the dose and frequency of administration of it in yak (*Bos grunniens L.*).

Materials and Methods

Experimental animals

The study was conducted in six clinically healthy male yaks (*Bos grunniens L.*) reared in the National Research Centre on Yak, Nykmadung Farm, Dirang, Arunachal Pradesh, India. The animals were weighing 270-330 kg of 2 ½- 3 ½ years age. They were housed in the animal shed with concrete floor and were maintained on green fodder, dry grass and concentrate. Water was provided *ad libitum*.

Drugs and chemicals

Technical grade and pure enrofloxacin, which was used as external standard in HPLC assay, were generously gifted by M/S Ranbaxy India Ltd, Ghaziabad. Other reagents for HPLC were procured from M/S EMerck (India) and M/S Sigma chemicals.

Experimental design

The study was conducted in six numbers of clinically healthy male yaks. Pharmacokinetics studies of enrofloxacin was carried out after single intravenous administration at the rate of 5 mg.kg⁻¹ body weight [2]. Blood samples (2-3 ml) were collected by jugular vein puncture into heparinised tubes at 0,0.04,0.08,0.16,0.33,0.5,0.75,1,1.5,2,3,4,6,8,10,12 up to 96 hours. Plasma was harvested by centrifugation at 3000 rpm for 15 min and stored at -5°C till analysis.

Estimation of enrofloxacin

For quantitative determination of enrofloxacin in plasma, the HPLC method of Teja – Isavadharm *et al.*, [5] was followed with some modifications.

(a) Instrumentation and chromatographic conditions

Plasma analysis was performed on a HPLC system (Perkin-Elmer, series 200, USA) fitted with a quaternary pump, diode array detector, auto sampler and a data station. A 5 µm Hypersil BDS C18 (250 x 4.6 mm) HPLC column was used. The mobile phase consisted of 0.1M phosphoric acid adjusted to pH 2.5 with a solution of 45% potassium hydroxide and acetonitrile mixed in a ratio of 70:30 % (v/v). The flow rate of mobile phase was 1.2 ml. min⁻¹ and the eluent was monitored with diode array detector adjusted wavelength at 290 nm.

(b) Sample processing

Plasma samples were subjected to liquid phase extraction. To 1 ml of plasma, 1 ml of methanol was added, vortexed for 20 sec, then placed on ice for 15 min and centrifuged at 3500 rpm for 10 min. 750 μ l supernatant transferred to a test tube, 6 ml of dichloromethane was added to it and vortexed for 20 sec, followed by centrifugation at 1500 rpm for 10 min. The organic phase was transferred to a clean glass tube and evaporated to dryness at 40^oC. The residue was then reconstituted in mobile phase (500 μ l) and 10 μ l was injected into the column.

Blank plasma was spiked with standard parent compound at three different concentrations ranging from 2.5 to 10 μ g. Plasma containing drug was extracted by liquid extraction procedure as described above and 10 μ l was injected into the HPLC column to enable calibration curve to be prepared. Recovery of the drug in yak plasma ranged from 86.09 to 92.35% with an average of 88.73%. The retention time of the drug in the present study was 2.72 minute. The plasma concentrations of enrofloxacin in samples were determined by comparing the detector response for the drug in the sample with the corresponding peak area in the standard mixture.

(c) Analysis

Plasma concentrations versus time data of enrofloxacin obtained during the study were utilized for calculating various pharmacokinetic parameters using non-compartmental method of analysis [6, 7].

Results

The mean plasma concentrations at different time interval after single dose of intravenous administration at 5 mg.kg⁻¹ body weight in yak have been incorporated in Fig 1. Mean pharmacokinetic parameters such as the area under plasma concentration-time curve (AUC), area under the moment curve (AUMC), mean residence time (MRT), elimination half-life ($t_{1/2}$), apparent volume of distribution (V_d), total body clearance (Cl_B) and apparent first-order elimination rate constant (K) of enrofloxacin after i.v. administrations are presented in Table 1. Following administration of the drug, the mean pharmacokinetic parameters of enrofloxacin calculated were as follows: $t_{1/2}$, 1.2757 \pm 0.3212h; AUC, 5.7312 \pm 1.6228 μ g.h.ml⁻¹; AUMC, 10.5509 \pm 4.8171 μ g.h².ml⁻¹; MRT, 1.8409 \pm 0.3773 h; K, 0.5432 \pm 0.173 h⁻¹; Cl_B , 872.4176 \pm 223.98 ml.h⁻¹.kg⁻¹ and V_d , 1.6061 \pm 0.5029 L kg⁻¹.

Discussion

In the present study, enrofloxacin was administered intravenously at the dose of 5 mg.kg⁻¹. The same dose has been used for determining the pharmacokinetics of enrofloxacin after intravenous administration in lactating cows [2, 8] in rabbits [9], in horse [3] and in dogs [10].

Following the intravenous administration, the initial concentration was 5.5703 \pm 1.4290 μ g ml⁻¹ at 0.04 h (2.5 min) and detected up to 12 h. The plasma concentration of enrofloxacin at 12 h post administration was 0.0066 μ g ml⁻¹. Kaartinen *et al* [2] reported detectable antimicrobial activity in lactating cows up to 8 h, whereas in rabbits detectable concentration was observed up to 10 h [9].

The mean overall elimination (K) rate constant was 0.2487 \pm 0.067 h⁻¹ in the present study which was quite similar to the value of 0.283 \pm 0.024 h⁻¹ obtained after intravenous administration of enrofloxacin in goats by Roa *et al.* [11].

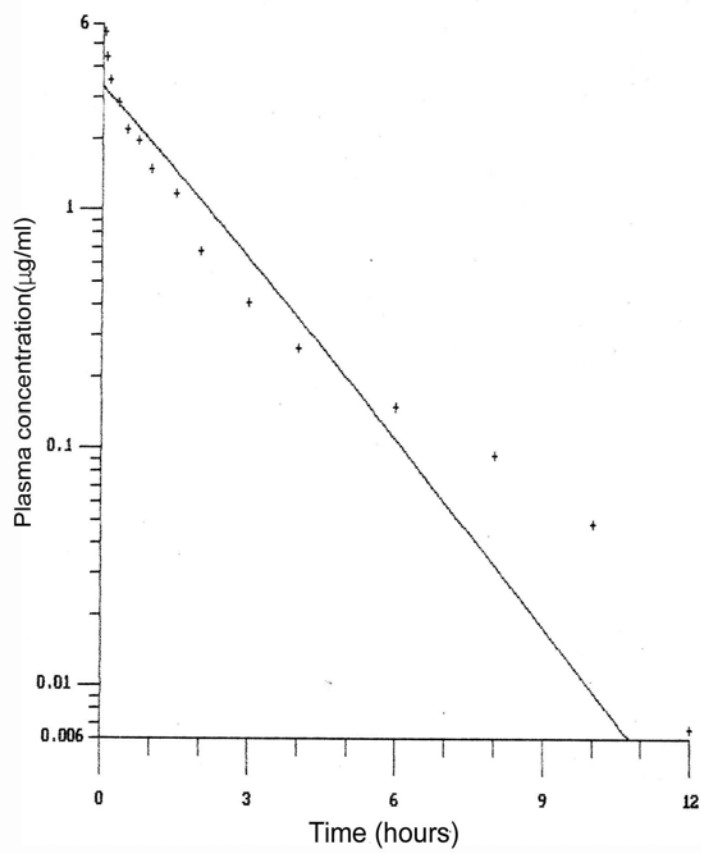


FIG-1. Semi logarithmic plot of plasma concentration ($\mu\text{g/ml}$) after intravenous (I. V.) administration of Enrofloxacin (5mg/kg body weight) in Yak.

Table 1. Pharmacokinetic determinants of enrofloxacin in Yak following intravenous administration at 5 mg /kg body weight. (n = 6, mean \pm SE)

Kinetic parameters	Determinants
AUC ($\mu\text{g. h/ml}$)	5.7312 \pm 1.6228
AUMC ($\mu\text{g h}^2/\text{ml}$)	10.5509 \pm 4.8171
MRT (h)	1.8409 \pm 0.3773
$t_{1/2}$ (h)	1.2757 \pm 0.3212
Cl_B (ml /kg/h)	872.4176 \pm 223.98
V_d (L/kg)	1.6061 \pm 0.5029
K_a (h^{-1})	0.5432 \pm 0.173

Abbreviations: AUC, area under curve; AUMC, area under moment curve; MRT, mean residence time; $t_{1/2}$, biological half life; Cl_B , total body clearance of drug; V_d , apparent volume of distribution; K_a , absorption rate constant.

The elimination half-life ($t_{1/2}$) of enrofloxacin after intravenous administration obtained in the present study was 1.2757 \pm 0.3212 h which was almost similar of dairy cows ($t_{1/2}$, 65 min) reported by Malbe *et al* [8] and lower than that of lactating cows (1.7 h) after intravenous administration of enrofloxacin [2]. But much higher elimination half-life of 6.6 h was reported by Kaartinen *et al* [12] in calves. The shorter elimination half life obtained in the present study indicates that yaks tend to eliminate enrofloxacin faster than other animal species which might be due to different anatomical and physiological features suiting their inhabitation in harsh climatic conditions in high mountain [13].

The area under the plasma concentration time-curve (AUC) of enrofloxacin after its intravenous administration was 5.7312 \pm 1.6228 $\mu\text{g.h.ml}^{-1}$. The AUC obtained in this study was comparable to that reported for ilamas of 6.95 \pm 0.93 $\mu\text{g.h.ml}^{-1}$ by Christensen *et al* [14] and slightly lower than lactating cows of 7.42 \pm 0.02 $\mu\text{g.h.ml}^{-1}$ reported by Malbe *et al* [8].

Apparent volume of distribution ($V_{d_{\text{area}}}$) obtained in the present study was 1.6061 \pm 0.5029 Lkg^{-1} . This was higher than the $V_{d_{\text{area}}}$ (1.19 Lkg^{-1}) reported by Gracia *et al* [15] in calves and lower than the $V_{d_{\text{area}}}$ of 2.12 Lkg^{-1} reported for rabbits [9]. In the present study, this comparable high $V_{d_{\text{area}}}$ reflects excellent tissue penetration of enrofloxacin after i.v. administration.

Total body clearance (Cl_B) obtained in the present study was 872.4176 \pm 223.98 $\text{ml.h}^{-1}\text{kg}^{-1}$. This value was higher than 502.53 $\text{ml.h}^{-1}\text{kg}^{-1}$ and 606.00 $\text{ml.h}^{-1}\text{kg}^{-1}$, reported in goats [16] and rabbits [9] respectively.

Mean residence time (MRT) obtained in the present study was 1.8409 \pm 0.3773 h similar to lactating cows of 1.80 h reported by Kaartinen *et al.* [2]. Mengozzi *et al.* [17] reported much higher MRT value in sheep (5.30 \pm 0.7644h) after i.v. administration of enrofloxacin. From this data, it appears that the persistence of enrofloxacin is much shorter in yaks as compared to other species

The present disposition study reveals that for maintaining MIC of 0.008 $\mu\text{g/ml}$ in plasma, enrofloxacin should be given in the dose of 5mg/kg bodyweight at an interval of 8 hours by i.v. route.

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