IJPT
1735-2657/05/42-91-94
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 4:91-94, 2005

Pharmacokinetic Studies of Enrofloxacin in Yak after Intramuscular Administration

SANJIB KHARGHARIA, CHANDANA CHOUDHURY BARUA, PRITAM MOHAN and MOHAN BHATTACHARYA

Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Assam, India (S.K., C.C.B., P.M.); National Research Centre on Yak, Dirang, Arunachal Pradesh, India (M.B.).

Received July 27, 2005; Revised October 20, 2005; Accepted December 26, 2005

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

The pharmacokinetic studies of enrofloxacin were investigated in yak *(Bos grunniens L.)* after administration of enrofloxacin by intramuscular route 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 using a non-compartmental model. The elimination half-life ($t_{½}$), 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 2.79±0.60 h, 5.35±1.29 μg·h·mL^{-1}, 21.50±8.60 μg·h^2·mL^{-1}, 4.02±0.87 h, 3.76±0.83 L·kg^{-1}, 935.09 ± 236.45 mL·h^{-1}·kg^{-1} and 0.25±0.07 h^{-1}, respectively after the administration of enrofloxacin. Because of faster elimination time, excellent tissue penetration, shorter half-life, low MRT after intramuscular administration of enrofloxacin at a dose rate of 5 mg·kg^{-1}, it is recommended for use in yaks at therapeutic dose.

Keywords: *Enrofloxacin, Yak, Pharmacokinetics, Intramuscular*

Enrofloxacin, a fluoroquinolone antimicrobial is approved exclusively for veterinary use, has a broad spectrum of antibacterial activity with MIC value ranging from 0.008 to 0.06 μg/mL. It has widespread distribution to most tissues and body fluids with a potential therapeutic application in many types of infections. Hence, it is used effectively 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 is also mainstay for highlanders, provides basic needs in terms of meat, milk, hair, wool and most needful transport in hilly terrain. For control of disease in various animals pharmacokinetic properties of enrofloxacin have been investigated in cattle [1], horses [2] and pigs [3]. The dosage regimens of enrofloxacin have been worked out for sheep, dog, and goat also, 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 enrofloxacin in yak (*Bos grunniens* L.).

MATERIALS AND METHODS

Experimental Animals

The study was conducted in six male yaks (*Bos grunniens* L) reared in the National Research Centre on Yak, Nykmadung Farm , Dirang, Arunachal Pradesh,. The animals weighed between 270-330 kg and 2½-3½ years of age. The animals were examined clinically to evaluate health status and to rule out the possibility of any diseases. 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 Ranbaxy India Ltd, Ghaziabad. Other reagents for HPLC were procured from EMerck and Heparin from Sigma chemicals.

Experimental Design

The study was conducted in six clinically healthy male yaks. Pharmacokinetics studies of enrofloxacin

was carried out after its single intramuscular administration of 5 mg.kg^{-1} body weight enrofloxacin. Blood samples (2-3 mL) were collected by jugular vein puncture into heparinised tubes. The samples prior to and after administration of drug were collected at various time intervals up to 96 hr following enrofloxacin administration. Plasma was harvested by centrifugation at 3000 rpm for 15 min and stored at –5°C till analysis for enrofloxacin.

Estimation of Enrofloxacin

For quantitative determination of enrofloxacin in plasma, the HPLC method of Teja–Isavadharm *et al.*, [4] was followed with some modifications.

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 (250×4.6 mm) HPLC column was used. The mobile phase consisted of 0.1 M 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. The chromatograms were integrated on a data station.

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 µL supernatant was 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 40ºC. The residue was then reconstituted in mobile phase (500 µL) and 10 µL is injected into the column.

Blank plasma was spiked with standard parent compound at three different concentrations ranging from 2.5 to 10 µg. Plasma containing drug was extracted by liquid extraction procedure as described above and 10 µL was injected into the HPLC column to enable calibration curve to be prepared. 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.

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 [5, 6]. Differences between respective means for Pharmacokinetic parameters were evaluated using Student's't' test. P values <0.01 were considered to be statistically significant.

RESULTS

The mean plasma concentrations at different time interval after single dose of intramuscular administration at 5 mg·kg^{-1} body weights in yak have been incorporated in Fig 1 and presented in Table 1.

Evaluation of the results on plasma level of enrofloxacin was adequately described by non-compartmental method. Mean pharmacokinetic parameters such as the area under plasma concentration-time curve (AUC), area under the moment curve (AUMC), mean maximum plasma concentration (C_{max}), mean time of maximum plasma concentration (t_{max}), mean residence time (MRT), elimination half-life ($t_{½}$), apparent volume of distribution (V_d), total body clearance (Cl_B) and apparent first-order elimination rate constant (K) of enrofloxacin after i.m administration of enrofloxacin are presented in Table 2. Following administration of the drug, the mean pharmacokinetic parameters of enrofloxacin calculated were $t_{½}$, 2.79±0.60 h; AUC, 5.35±1.29 µg·h·mL^{-1}; AUMC, 21.50±8.60 µg·h^2.mL^{-1}; MRT, 4.02±0.87 h; K, 0.25±0.07 h^{-1}; Cl_B, 935.09±236.45 mL·h^{-1}·kg^{-1} and V_d, 3.76±0.83 L kg^{-1} respectively.

DISCUSSION

In the present study, enrofloxacin was administered intramuscularly at the dose of 5 mg·kg^{-1}. The same dose has been used for determining the pharmacokinetics of

Table 1. Plasma concentration (µg/mL) after intramuscular administration of enrofloxacin (5 mg/kg) in Yak (mean ± SEM).

Time in hours (minutes)	Plasma concentration (µg/ml)
0	0
0.04 (2.5)	0.1667±0.014
0.08 (5.0)	0.3315±0.0291
0.16 (10)	0.5505±0.0629
0.33 (20)	1.6048±0.1748
0.5 (30)	2.0946±0.2164
0.75 (45)	2.1807±0.5712
1 (60)	1.4596±0.1288
1.5 (90)	0.7974±0.0766
2 (120)	0.6664±0.0616
3 (180)	0.6478±0.0621
4 (240)	0.3800±0.0363
6 (360)	0.2323±0.0228
8 (480)	0.1440±0.0200
10 (600)	0.0792±0.0165
12 (720)	0.0350±0.0077
24 (1440)	0.0096±0.0011

Table 2. Pharmacokinetic determinants of enrofloxacin in Yak following intramuscular administration at 5 mg /kg body weight (n=6, mean ± SEM).

Kinetic parameters	Determinants
AUC (µg. h/mL)	5.3471±1.286
AUMC (µg h^2/mL)	21.5019±8.5968
MRT (h)	4.0212±0.8680
$t_{1/2}$ (h)	2.7866±0.6006
Cl_B (ml /kg/h)	935.0863±234.450
V_d (L/kg)	3.7599±0.8297
C_{max} (µg/mL)	2.1807±0.5712
t_{max} (min)	45±8.66
K_a (h^{-1})	0.8602±0.099

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; C_{max}, concentration maximum of drug ; t_{max}, time of maximum appearance of drug ; K_a, absorption rate constant.

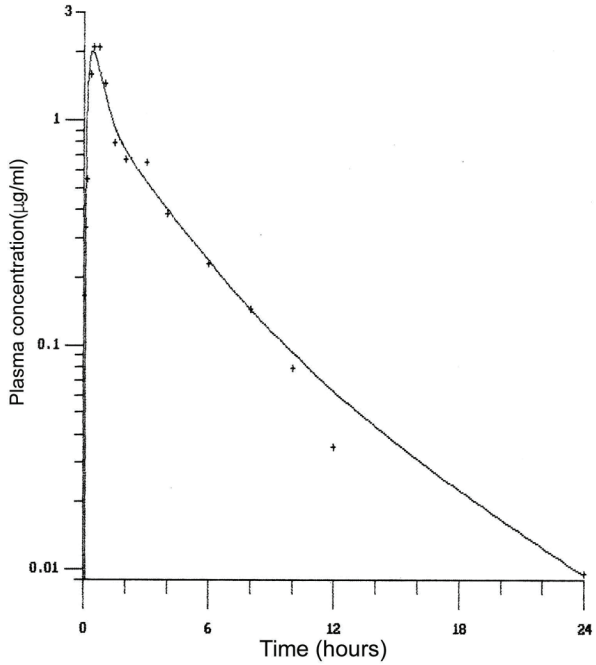

Fig 1. Semi logarithmic plot of plasma concentration (µg/mL) after intramucular (I.M.) administration of enrofloxacin (5 mg/kg body weight) in Yak.

enrofloxacin after intramuscular administration in horses [2], rabbits [7], and buffalo bulls [8].

Following the intramuscular administration, the therapeutic concentration was achieved within 0.04 h (2.5 min) and detected up to 24 h. The plasma concentration of enrofloxacin at 0.04 h (2.5 min) post administration was 0.1667 ± 0.0143 $\mu g \cdot mL^{-1}$.

The maximum concentration of enrofloxacin was 2.1807±0.5712 $\mu g \cdot mL^{-1}$ recorded at 0.75 hr (45 min) following intramuscular administration to healthy yaks. The peak plasma level obtained in this study was lower to C_{max} of 2.8±0.289 $\mu g \cdot mL^{-1}$ reported by Rao [9] after intramuscular administration of enrofloxacin at the same dose rate in goat. But Kaartinen et al [1] reported much lower C_{max} of 0.7 $\mu g \cdot mL^{-1}$ after i.m. administration of enrofloxacin in lactating cows.

The mean time of maximum plasma concentration (t_{max}) obtained in the present study was 0.75±0.14 h (45±8.66 min). This was comparable to t_{max} of 0.875±0.55 h obtained in rabbit after i.m. administration of enrofloxacin reported by Broome et al [10].

The mean overall elimination (K) rate constant was 0.2487±0.067 h^{-1} in the present study was quite similar to the value (0.283±0.024 h^{-1}) obtained after intramuscular administration of enrofloxacin in goats [9].

The elimination half-life ($t_{½}$) of enrofloxacin after intramuscular administration obtained in the present study was 2.7866±0.6006 h which was higher than that of goats ($t_{½}$, 1.396 h) reported by Rao [9] but much lower than that of horse (9.9 h) after intramuscular administration of enrofloxacin [2].

The area under the plasma concentration time-curve (AUC) of enrofloxacin after its intramuscular administration was 5.3471±1.286 $\mu g \cdot h \cdot mL^{-1}$. The AUC obtained in this study was comparable to that reported for goats (7.516 $\mu g \cdot h \cdot mL^{-1}$) [9] after i.m. administration of enrofloxacin at 5 $mg \cdot kg^{-1}$ body weight and cattle (7.1 $\mu g \cdot h \cdot mL^{-1)}$ after subcutaneous administration [11].

Apparent volume of distribution (Vd_{area}) obtained in the present study was 3.7599±0.8297 $L \cdot kg^{-1}$. This was comparatively higher than the Vd_{area} (1.19 $L \cdot kg^{-1}$) reported by Gracia et al [12] in calves and 1.52 $L \cdot kg^{-1}$ in goat [9]. In the present study the high Vd_{area} reflected excellent tissue penetration of enrofloxacin.

The bioavailability of enrofloxacin after intramuscular administration was compared by the ratio of $AUC_{i.m.}$ / $AUC_{i.v.}$ In this study, a mean bioavailability of 93.29 % was obtained which indicated almost complete absorption of the drug after intramuscular administration. However, Rao [9] reported higher bioavailability of 119 % in goat after intramuscular administration. But the present value is higher than that of the values of sheep (85%) after i.m. administration as reported by [13] Mengozzi et al.

The present disposition study reveals that for maintaining MIC of 0.06 µg/mL in plasma of yak, enrofloxacin should be given in the dose of 5 mg/kg bodyweight at 10 hours intervals by i.m. route.

REFERENCES

1. Kaartinen L, Salonene M, Alli L, Pyorala S. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cows. *J Vet Pharmacol Therap* 1995;**9:**254-63.
2. Kaartinen L, Pam S, Pyorola S. Pharmacokinetics of enrofloxacin in horses after single intravenous and intramuscular administration. *Equine Vet J* 1997;**29:**378-81.
3. Zeng Z, Geng Q, Zeng Z L, Feng QH. Pharmacokinetics and bioavailability of enrofloxacin in pigs. *Chin J Vet Sci* 1996;**19:**606-12.
4. Teja Isvadharm, P Keratithakul, D Wattl, G Webster HK, Edstein MD. Measurement of Ciprofloxacin in human plasma, whole blood and erythrocytes by high performance liquid chromatography. *Therap Drug Monitoring* 1991;**13:**263-7.
5. Goodman Gilman A, Rall TW, Nies AS, Taylor P. The Pharmacological Basis of Therapeutics. 8th ed. New York: McGraw-Hill; 1995. p. 3-32.
6. Singh B. Non-compartmental pharmacokinetics analysis of plasma level data through statistical moment approach. A worksheet instance. ICAR Short Course on 'Recent approaches in clinical pharmacokinetics and therapeutic monitoring of drugs. 1999.
7. Cabanes A, Margarita Josh M, Garcia A, Franscissa P. Pharmacokinetics of enrofloxacin after intravenous and intramuscular injection in rabbits. *Are J Vet Res* 1992;**53:**2090-3.
8. Verma HK, Pangwakar GR, Chaudhary RK, Srivastava AK. Pharmacokinetics and dosage regimen of enrofloxacin in buffalo bulls after intramuscular administration. *Vet Res Commun* 1999;**23:**501-5.
9. Rao GS. Pharmacokinetic studies of enrofloxacin in normal and febrile goats. [dissertation]. Izatnagar: Indian Veterinary Research Institute Deemed University.; 1999.
10. Broome LR, Brooks LD, Babish GI., Copeland DD , Conzelman GM. Pharmacokinetics of enrofloxacin in rabbits. *Am J Vet Res* 1991;**52:**1835-41.
11. Stegemann M, Wollen TS, Ewert KM, Terhune TN, Copeland DD. Plasma pharmacokinetics of enrofloxacin administration to cattle at a dose of 7.5 mg/kg. *J Vet Pharmacol Therap* 1997;**20 Suppl 1:**22-3.

12. Gracia OH, Errecalde C, Prieto G, Luders C, Pulles I, Berecochea C, Fernandez M. Pharmacokinetic of enrofloxacin in calves. *Archivos De Veternaria* 1996;**28:**107-11.
13. Mengozi G, Intorre L, Bertine S, Sodani. Pharmacokinetics of enrofloxacin and its metabolite, ciprofloxacin after intravenous and intramuscular administration in sheep. *Am J Vet Res* 1996;**57:**1040-104.

Address correspondence to: Dr Sanjib Khargharia, Deptartment of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Guwahati-781022, India.
E-mail: kharukharu@sify.com