RESEARCH PAPER

Research Journal of Animal Husbandry and Dairy Science
Volume 4 | Issue 1 | June, 2013 | 28-32

Comparative pharmacokinetics study of three commercial preparation of 10% enrofloxacin in goats

V.K. GOND, R.K NIRALA, ARCHANA **AND** C. JAYACHANDRAN

ABSTRACT : Antimicrobial therapy constitutes a major component of modern medical and veterinary practices. Enrofloxacin has been developed exclusively for veterinary. In the present investigation, five clinically healthy female goats of non-descript were used. Three commercial preparations of enrofloxacin (10%) were used@5mg/kg b.wt .The samples of plasma were collected at different time interval *i.e.* 0.042, 0.083, 0.125, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after IM administration of drugs. Estimation of enrofloxacin were done by HPLC method at the flow rate was 0.6 $ml.min^{-1}$,. Loop size was 200 ml, injection volume was 400 ml, the chart speed was 0.25 $mm.min^{-1}$ and the detector sensitivity was 2.000 A.U.F.S. The mobile phase comprised of acetonitrile : methanol : water (17 : 3 : 80 v/v/v) containing 0.4% phosphoric acid (85% v/v) and 0.4% triethylamine (v/v). The pH of mobile phase was 3 (approx). The drug is present significantly at a lower concentration in brand II (0.07 ± 0.02 $\mu g.ml^{-1}$) as compared to brand I (1.58 ± 0.41$\mu g.ml^{-1}$) at 0.042 h. Similarly, brand II show lower concentrations upto 12 h .The drug maintained its therapeutic concentration (^{3} 0.125 $\mu g.ml^{-1}$) upto 12 h in all the three brands. The value of absorption half-life ($t_{1/2}$ Ka) of brand I, II and III were noted to be non-significant with a mean of 0.31 ± 0.04, 0.66 ± 0.13 and 0.83 ± 0.31 h, respectively. Brand I showed rapid absorption as compared to brand II and III but statistically it is non-significant. Elimination half life ($t_{1/2}$ â) of brand I (3.10 ± 0.34 h) was found to be lower as compared to brand II and brand III (4.25 ± 0.71 and 3.84 ± 0.55 h, respectively), Mean residential time (MRT) of brand I, brand II and brand III were noted to be non-significant with a mean of 5.54 ± 1.13, 7.22 ± 0.86 and 6.52 ± 0.83 h, respectively. The values of mean absorption time (MAT) of brand I, brand II and brand III were noted to be non significant with a mean of 2.61 ± 0.98, 2.70 ± 0.92 and 2.61 ± 1.26 h, respectively. Maximum attainable concentrations (C_{max}) was found to be significantly lower in brand II (1.94 ± 0.16 $\mu g.ml^{-1}$) as compared to brand I (5.35 ± 0.87 $\mu g.ml^{-1}$). However in case of time to reach maximum concentration (T_{max}) there was no significant difference between different brands.In conclusion,all these three brands of enrofloxacin interchangeable and substituted for each other.

KEY WORDS : Pharmacokinetics, Enrofloxacin, Antibacterial, Brands, HPLC, Goats

HOW TO CITE THIS PAPER : V.K. Gond, R.K Nirala, Archana and C. Jayachandran (2013). Comparative pharmacokinetics study of three commercial preparation of 10% enrofloxacin in goats, *Res. J. Animal Hus. & Dairy Sci.,* **4**(1) : 28-32.

INTRODUCTION

Fluoroquinolones (FQs) are antibacterial agents related to nalidixic acid. They are used in both human and veterinary medicine to treat a variety of infections (Brown, 1996). Like other FQs, enrofloxacin(ENR) exhibits a broad spectrum bactericidal activity and exclusively used in veterinary medicine(Sheer,1990;Vancutsen *et al*.,1990). The drug has an excellent antibacterial activity against most pathogenic bacterial that are resistant to other bacterial agents (Bauditz, 1987; Elmas *et al*., 2000). Pharmacokinetic studies have indicated that ENR is rapidly absorbed and well distributed throughout the body following oral and and intramuscular administrationin animals(Soliman, 2000). In the United States, enrofloxacin is approved for use in beef cattle and calves (excluding veal calves), chickens and turkeys not laying eggs for human consumption. Because of high prevalence of enrofloxacin sensitive bacterial infection and high cost of the pioneer product, there has been tremendous increase in the use of other brand of enrofloxacin with increase availability use of generic enrofloxacin product from different pharmaceutical companies, practitioner are faced with dilemma

MEMBERS OF RESEARCH FORUM

Address for correspondence :
V.K. Gond, Department of Animal Husbandry and Veterinary Science, Tirhut College of Agriculture (R.A.U.), Dholi, MUZAFFARPUR (BIHAR) INDIA
Email : vijayrau2012@gmail.com

Associated Authors' :
R.K. Nirala, Archana and J. Jayachandran, Department of Veterinary Pharmacology and Toxicology, Bihar Veterinary College, PATNA (BIHAR) INDIA

of therapeutic failure and side effects following the use of some of these array of multisource product in the market. Since these clinical condition results in great economic losses to farmer and the pioneer formulations and few brand have severally proven effective..Keeping in view of above facts the present study was undertaken and compare with each other with the respect of pharmacokinetics parameters.

MATERIAL AND METHODS

In the present investigation, five clinically healthy female goats of non-descript breed between 18-24 months of age and 17-20 kg body weight were used. The goats were housed in the animal shed with concrete floor in the Department of Veterinary Pharmacology and toxicology, Bihar Veterinary college Patna-14. The goats were maintained on dry fodder concentrate and green grasses apart from routine grazing of about 4 to 5 hours. Deworming was done a fortnight prior to the experiment with Analogon (albendazole) 5 mg. kg^{-1} body weight.

Experimental design:

Three commercial preparations of enrofloxacin were used in the present investigation. First commercial product of enrofloxacin (ENR) was administered in each of five female healthy goats through intramuscular(I.M) routes an interval of 15 day, respectively, was allowed to elapse before administration of next dose of the drug. After conducting the kinetic study of first commercial product, the next two commercial products were administered in the same goats alternate way a wash out period of 15 days was allowed before each administration by the above noted routes.

Drug used:

Three commercial products of 10% ENR were used in present experiment Brand I (10%), Brand II (10%) & Brand III (10%), marketed by Intervet India Pvt. Limited, Pune, Ranbaxy Laboratories Limited India & Shellwell Pharmaceutical Limited, Indore, respectively.

Collection of samples and their timings:

The samples of plasma were collected following I.M administration of drugs in goats. The samples of blood were collected at 0.042, 0.083, 0.125, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h**.** The plasma samples were then kept in a refrigerator until assay was carried out. For the preparation of standards normal blood prior to drug administration was also collected.

Estimation of enrofloxacin:

Apparatus:

Estimation of enrofloxacin were done simultaneously by HPLC method described by Nielsen and Gyrd-Hansen (1997) and Kung *et al*. (1993) with slight modification as described below The HPLC equipment used comprised of a HPLC pump (Model 515-Waters), a dual wavelength absorbance detector (Model 2487 – Waters), a rheodyne manual injector with a 200 mg loop size and a data module (Model 746 – Waters). Chromatographic separations were performed using column 3.9 x 300 mm (m Bondapak^{TM} C_{18} – Waters).

Chromatographic conditions:

The flow rate was 0.6 $ml.min^{-1}$, the effluent wavelength was monitored at 278 mm. Loop size was 200 ml, injection volume was 400 ml, the chart speed was 0.25 $mm.min^{-1}$ and the detector sensitivity was 2.000 A.U.F.S (Absorbance under full scale) were adopted for HPLC analysis for enrofloxacin

Mobile phase:

The mobile phase comprised of acetonitrile : methanol : water (17 : 3 : 80 v/v/v) containing 0.4% phosphoric acid (85% v/v) and 0.4% triethylamine (v/v). The pH of mobile phase was 3 (approx).

Preparation of standards of enrofloxacin :

In water:

Three commercial preparation containing enrofloxacin in concentration of 100 $mg.ml^{-1}$ was diluted in sterile triple distilled water to make different strengths *viz.*, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 $mg.ml^{-1}$.

In plasma:

From each standard solution of enrofloxacin in water, 0.1 ml was added to a sterile vial containing 0.9 ml of plasma collected prior to drug administration. This yielded enrofloxacin standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 $mg.ml^{-1}$ in plasma. Blank plasma containing no drug was also prepared.

Analytical method/procedure:

– In a clean and dry centrifuge tube 400 ml of plasma samples was taken and 600 ml of acetonitrile was added for precipitation of plasma proteins (1:1.5).

– The mixture was shaken on a vortex mixer for 1 min. and centrifuged for 15 min at 3000 rpm.

– Then, 300 ml of supernatant was transferred to a clean tube and mixed with 600 ml of triple distilled water/mobile (1: 2).

– An aliquot of this mixture (up to 400 ml) was injected directly into the loop of injector and the integrator recorded (print out) retention time and area.

Pharmacokinetic parameters of enrofloxacin after intramuscular administration were calculated from semilog plot of plasma drug concentration versus time curve. The experimental data were analyzed one-compartment open model by following formula-

$C_p = A_e^{-\alpha t} - B_e^{-\beta t}$……..(One-compartment open

model)

Statistical analysis :

Statistical analysis was done by using single factor Anova (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

In the present investigation on pharmacokinetics studies of different brands of enrofloxacin following intramuscular administration in goat the finding are as follow:

Distribution of enrofloxacin in plasma:

Mean ± S.E.M.(n=5) concentrations of enrofloxacin at various time intervals of three brands after intramuscular administration (5 $mg.kg^{-1}$) are shown in Table- 1.The drug appeared in all goats at 2.5 min. (0.042 h) with a mean value of 1.58 ± 0.41 $\mu g.ml^{-1}$ in brand-I which is significantly higher as compared to brand II (0.07 ± 0.02) and brand III (0.42 ± 0.21 $\mu g.ml^{-1)}$. Brand II maintained lower concentrations upto 12 h and more or less similar level at 24 h (0.07 $\mu g.ml^{-1)}$. The therapeutic concentration(^{3} 0.125 $\mu g.ml^{-1}$) of enrofloxacin was maintained upto 12 h in all the three brands. Elmas *et al.* (2001) noted more or less similar value of 0.09 $\mu g.ml^{-1}$ at 24 h after i.m. injection of enrofloxacin (5 $mg.kg^{-1}$)in goat.

Kinetic parameters of enrofloxacin:

Table 2 depicts the Mean ± S.E.M.(n=5) of kinetic parameters of enrofloxacin of three different brands in goats calculated by one-compartment open model after i.m. administration (5 $mg.kg^{-1}$).The mean extrapolated zero time concentration of the drug in plasma during absorption phase (A) is noted to be non-significant for all the three brands where as during elimination phase (B) brand I shows significantly higher value as compared to brand II. The value of absorption half-life ($t_{1/2}$ Ka) of brand I, II and III were noted to be non-significant with a mean of 0.31 ± 0.04, 0.66 ± 0.13 and 0.83 ± 0.31 h, respectively. Brand I showed rapid absorption as compared to brand II and III but statistically it is non-significant. More or less similar $t_{1/2}$ Ka of 0.25 h (Elmas *et al.*, 2001) after i.m. injection of enrofloxacin in goat was noted. In contrast, 0.26 h in breeding bull (Verma *et al.*, 1999) and 0.36 h (Abdel-Aziz *et al.*, 1997) in chickens were noted after i.m. injection of enrofloxacin. Elimination half life ($t_{1/2}$ b) of brand I (3.10 ± 0.34 h) was found to be lower as compared to brand II and brand III (4.25 ± 0.71 and 3.84 ± 0.55 h, respectively), though the data between brands were noted to be non significant. More or less similar $t_{1/2}$ b of 3.87 h (Haritova *et al.*, 2003), 3.65 ± 0.31 h (Mengozzi *et al.*, 1996) after i.m. injection of enrofloxacin in sheep and 4.00 to 4.71 h (Elmas *et al.*, 2001) in goat were noted.The absorption half life ($t_{1/2}$ Ka) and elimination half life ($t_{1/2}$ b) of enrofloxacin denote rapid absorption and comparatively slower elimination of enrofloxacin after i.m. administration.Mean residential time (MRT) of brand I, brand II and brand III were noted to be non-significant with a mean of 5.54 ± 1.13, 7.22 ± 0.86 and 6.52 ± 0.83 h, respectively. In contrast, lower MRT of 4.52 h (Haritova *et al.*, 2003) was noted in sheep after i.m. administration of

Table 1 : Mean ± S.E.M.(n=5) of plasma concentrations ($\mu g.ml^{-1}$) of enrofloxacin of three different commercial preparation in goats following single intramuscular dose of 5 $mg.kg^{-1}$

Time (h)	Intramuscular route		
	Brand I	Brand II	Brand III
0.042	1.58^{a} ± 0.41	0.07^{b} ± 0.02	0.42^{c} ± 0.21
0.083	1.82^{a} ± 0.37	0.11^{b} ± 0.01	0.55^{b} ± 0.27
0.125	2.04^{a} ± 0.38	0.15^{b} ± 0.01	0.60^{b} ± 0.29
0.25	2.47^{a} ± 0.37	0.20^{b} ± 0.02	0.77^{b} ± 0.29
0.333	2.73^{a} ± 0.42	0.24^{b} ± 0.03	0.88^{b} ± 0.31
0.50	3.00^{a} ± 0.42	0.34^{b} ± 0.05	1.28^{b} ± 0.38
0.75	3.34^{a} ± 0.48	0.46^{b} ± 0.05	1.51^{a} ± 0.39
1	3.61^{a} ± 0.37	0.73^{b} ± 0.09	2.65^{a} ± 1.00
1.5	4.52^{a} ± 0.66	1.53^{b} ± 0.20	2.50^{ab} ± 0.13
2	4.35^{a} ± 0.96	1.70^{b} ± 0.23	2.61^{ab} ± 0.55
3	2.96^{a} ± 0.57	1.34^{b} ± 0.20	1.75^{ab} ± 0.70
4	2.05^{a} ± 0.34	1.02^{a} ± 0.18	1.46^{a} ± 0.61
6	1.14^{a} ± 0.14	0.68^{a} ± 0.15	1.08^{a} ± 0.44
8	0.64^{a} ± 0.12	0.43^{a} ± 0.11	0.70^{a} ± 0.29
10	0.43^{a} ± 0.09	0.27^{a} ± 0.06	0.40^{a} ± 0.17
12	0.24^{a} ± 0.07	0.15^{a} ± 0.04	0.30^{a} ± 0.18
24	0.07^{a} ± 0.03	0.07^{a} ± 0.01	0.06^{a} ± 0.01

Different superscripts denote significant difference (P < 0.05)

Table 2 : Mean ± S.E.M.(n=5)of kinetic parameters of enrofloxacin of three different commercial preparation in goats calculated by one-compartment open model following single intramuscular administration (5 mg.kg^{-1})

Parameter (Unit)	Intramuscular route		
	Brand I	Brand II	Brand III
A (µg.ml^{-1})	4.17^{a} ± 0.58	2.43^{a} ± 0.44	4.00^{a} ± 1.15
B (µg.ml^{-1})	5.08^{a} ± 0.47	2.16^{b} ± 0.43	4.05^{ab} ± 1.35
Ka (h^{-1})	2.45^{a} ± 0.35	1.23^{a} ± 0.24	1.87^{a} ± 0.90
$t_{1/2}$ Ka (h)	0.31^{a} ± 0.04	0.66^{a} ± 0.13	0.83^{a} ± 0.31
β (h^{-1})	0.23^{a} ± 0.02	0.19^{a} ± 0.04	0.21^{a} ± 0.05
$t_{1/2}$ β (h)	3.10^{a} ± 0.34	4.25^{a} ± 0.71	3.84^{a} ± 0.55
AUC (mgL^{-1}h)	19.04^{a} ± 1.84	9.29^{a} ± 1.81	14.62^{a} ± 3.80
AUMC (mg.L^{-1}.h^{2})	105.19^{a} ± 22.02	71.23^{a} ± 17.52	98.72^{a} ± 32.99
MRT (h)	5.54^{a} ± 1.13	7.22^{a} ± 0.86	6.52^{a} ± 0.83
MAT (h)	2.61^{a} ± 0.98	2.70^{a} ± 0.92	2.61^{a} ± 1.26
C_{max} (µg.ml^{-1})	5.35^{a} ± 0.87	1.94^{b} ± 0.16	3.34^{ab} ± 0.77
T_{max} (h)	1.60^{a} ± 0.09	1.80^{a} ± 0.12	1.15^{a} ± 0.15
Vd_B (L.kg^{-1})	1.01^{a} ± 0.07	2.75^{b} ± 0.56	1.89^{ab} ± 0.50
Vd_{area} (L.kg^{-1})	1.21^{a} ± 0.15	3.52^{b} ± 0.48	2.30^{ab} ± 0.55
Cl_B (mg.kg^{-1}.min)	4.53^{a} ± 0.43	10.90^{a} ± 2.59	6.84^{a} ± 1.16

Different superscripts denote significant (P<0.05)

Table 3 : Mean ± S.E.M.(n=5) of dosage regimen of enrofloxacin of three different commercial preparation for intramuscular route in goat

C_p^{∞} min (µg.ml^{-1})	γ (h)	Dose (mg.kg^{-1})	Intramuscular route		
			Brand I	Brand II	Brand III
		D*	0.58 ± 0.11	1.75 ± 0.76	1.04 ± 0.33
0.125	8	D_0	0.46 ± 0.10	1.46 ± 0.74	0.85 ± 0.34
		D*	1.37 ± 0.39	5.06 ± 3.14	3.28 ± 1.92
	12	D_0	1.26 ± 0.39	4.67 ± 3.06	3.10 ± 1.93
		D*	1.16 ± 0.21	3.37 ± 1.57	2.08 ± 0.67
	8	D_0	0.93 ± 0.21	2.92 ± 1.49	1.70 ± 0.60
0.25		D*	2.74 ± 0.78	10.12 ± 6.34	6.57 ± 3.84
	12	D_0	2.52 ± 0.78	9.35 ± 6.12	6.20 ± 3.87
		D*	2.32 ± 0.43	7.02 ± 3.05	3.76 ± 1.39
	8	D_0	1.86 ± 0.42	5.83 ± 2.98	3.41 ± 1.38
0.50		D*	5.49 ± 1.57	20.25 ± 12.68	13.30 ± 7.18
	12	D_0	5.04 ± 1.56	18.69 ± 12.24	12.41 ± 7.75

All data are non-significant.

enrofloxacin. More or less similar MRT of 7.98 ± 1.17 h (Kaartinen *et al.*, 1995) in cow after i.m. administration was observed.The values of mean absorption time (MAT) of brand I, brand II and brand III were noted to be non significant with a mean of 2.61 ± 0.98, 2.70 ± 0.92 and 2.61 ± 1.26 h, respectively. In contrast, higher MAT of 6.18 ± 1.24 h was noted in cow (Kaartinen, *et al.*, 1995) after i.m. administration of enrofloxacin.Maximum attainable concentrations (C_{max}) was found to be significantly lower in brand II (1.94 ± 0.16 µg.ml^{-1}) as compared to brand I (5.35 ± 0.87 µg.ml^{-1}). However in case of time to reach maximum concentration (T_{max}) there was no significant difference between different brands.In case of volume distribution (Vd_B and Vd_{area}), significantly increased values were obtained in case of brand II as compared to brand I. More or less similar Vd_{area} of 1.42 L.kg^{-1} (Rao *et al.*, 2001) after i.m. administration of enrofloxacin in goat was noted.

The mean value of total body clearance (Cl_B) varied from 4.53 – 10.90 ml.kg^{-1}.min^{-1} in the present study. The values between different brands for Cl_B did not differ significantly.

Dosage regimen:

The calculated dosage regimen of enrofloxacin of three different brands for i.m. route are shown in Table 3. The dosage regimen was calculated at three different therapeutic concentration (C_p ther = C_p^{∞} min of 0.125, 0.25 and 0.50 µg.ml^{-1})

at convenient dosage interval (g) of 8 and 12 h. Though brand II shows higher doses (D* and D_0) but they are not statistically significant. Thus, the three brands are expected to be equally effective when given through intramuscular.

So on above fact we conclude that three different commercial preparations of enrofloxacin along with same strength manufacture by different pharmaceuticals companies are interchangeable and substituted for each other. Enrofloxacin may be administered intramuscular at the dose rate of 5mg/kg body weight every 12 hrly for treating systemic as well as local infection in goats.

LITERATURE CITED

Abdal-Aziz, M.I., Aziaz, M.A., Soliman, F.A. and Afify, N.A. (1997). Pharmacokinetic evaluation of enrofloxacin in chickens. *Br. Port. Sci.,* **38** (5) : 614.

Bauditz,R.(1987). Results of clinical studies with Baytril™ .Poultry. *Vet. Med. Rev*, **2**:130-136.

Brown, S.A. (1996). Fluoroquinolones in animal health. *J.Vet. Pharmacol. Ther.,* **19** : 1- 4.

Elmas, M., Yazar, E., Tras, B., Bas, A.L. and Eryavuz, A. (2000). Pharmacokinetics and oral Bioavailability of enrofloxacin in faunated and defaunated Angora Goats. *Revue Med.Vet.*, **6** (151):507-510.

Elmas, M, Traso, Kaya, S., Bas, A.L., Yazar, E. and Yarsan, E. (2001). Pharmacokinetics of enrofloxacin after intravenous and intramuscular administration in Angora Goats. *Can. J. Vet. Res.,* **65** (1) : 64-7.

Haritova, A, Lashev, L. and Pashov, D. (2003). Pharmacokinetic of enrofloxacin in Lactating sheep. *Res. Vet. Sci.,* **74** (3) : 241-5.

Kaartinen, L., Salonen, M., Alli, L. and Pyorala, S. (1995). Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cow. *J. Vet. Pharmacol. Ther.,* **18** : 357-62.

Mengozzi, G., Intorre, L., Bertini, S. and Soldini, G. (1996). Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and intramuscular administration in sheep. *Am. J. Vet. Res.,* **57** : 1040-1043.

Rao, G. S., Ramesh, S., Ahmad, A. H., Tripathi, H. C., Sharma, L. D. and Malik, J.K. (2001). Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intramuscular administration of enrofloxacin in goats. *Vet. Res. Com.,* **25** : 197-204.

Scheer, M., Bauditz, R., Peeters, J. and Okerman, L. (1990). Baytril (enrofloxacin) : Antimicrobial activity and pharmacokinetics in rabbits. 7. Arbeeit-stagung uber Haltung and Krankheiten der Kaninchen, Pelztiere and Heimtiere. 31. Mai bis 1. Juni 1990 in celle 1990, 279-86.

Soliman, G.A. (2000). Tissue distribution and disposition kinetics of enrofloxacin in healthy and E. coli infected broilers. *Dtsch Tierarztl Wochenschr,* **107**(1): 23-27

Verma, H.K., Pangawkar, G.R., Chaudhary, R.K. and Srivastava , A.K. (1999). Pharmacokinetics and dosage regimen of enrofloxacin in buffalo bulls after intramuscular administration. *Vet. Res. Com*. **23** : 501-5.

Vancutsem, M., Babish, J.G. and Schwark, W.S (1990). The Fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Corn. Vet.,* **80**: 173-186.

Received : *01.04.2013;* ***Accepted :*** *28.05.2013*