RESEARCH ARTICLE

Disposition Kinetics of Enrofloxacin Following Intramuscular Administration in Goats

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ABSTRACT

Enrofloxacin (ENRO) is a second generation fluoroquinolone that was specially developed for clinically use in veterinary practice. Most of the previous studies on the pharmacokinetics of antibiotics have emphasized that the optimal therapeutic dosage of imported drugs should be determined in the local target species. So the present study was designed to determine the disposition kinetics of ENRO in healthy adult female goats under local environment. The ENRO was injected at 5 mg/kg through intramuscular route. After ENRO administration, blood samples were collected at different time intervals and analyzed through HPLC. Kinetic parameters were determined by subjecting the plasma concentration to the two compartment open model. The value of C_{max} and T_{max} were 1.03±0.101 µg/ml and 1.47±0.14 hours, respectively. The values of A and B were (1.46±0.25 and 0.99±0.10 µg/ml), respectively. The mean half-life values of absorption ($t_{1/2}$ abs), distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) were 0.88±0.19, 0.69±0.10 and 2.84±0.30 hours, respectively. The values (mean±SE) of V_d and Cl_B were 3.96±0.66 l/kg and 0.96±0.10 l/hr/kg, respectively. The priming and maintenance doses of ENRO after intramuscular administration based upon the minimum effective concentration $C°P_{(min)}$ were determined. The calculated priming dose for goats, at $C°P_{(min)}$ 0.05 µg/ml, was 4.49 mg/kg for 8 hour dosing intervals while the maintenance dose for corresponding dosing intervals was 4.29 mg/kg.

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INTRODUCTION

Most of the developing countries like Pakistan are importing raw active substance or finished drugs for human as well as veterinary clinics. Pre-clinical and clinical drug development studies are carried out in drug exporting countries where environmental conditions and genetic make-up of animals and human beings are different than their local counterparts where drugs are to be employed clinically. These differences are manifested through the characteristic biochemical and physiological parameters which ultimately affect the bio-disposition and fate of drugs in a population. Several studies have shown that the disposition kinetics and dosage regimen of the investigated drugs in local animals under indigenous conditions were comparatively different from the literature values (Javed et al., 2003; Javed et al., 2009). As a consequence of genetic and environmental variations, the optimal therapeutic dosage of imported drugs should be determined in the local target species. Enrofloxacin is a third generation of fluoroquinolones that

was specially developed for clinical use in veterinary practices. It is a broad spectrum antibacterial drug, having activity against both of the gram-positive and gram-negative bacteria (Elsheikh et al., 2002; Sanjib et al., 2005; Narayan et al., 2009; Bimazubute et al., 2010). The synergistic effect of enrofloxacin and trimethoprim was observed against the P. hemolytica and S. typhimurium (Choi et al., 2011). Enrofloxacin has greater activity against the Anaplasma marginale than that of oxytetracyclin, so it is more effective for the treatment of bovine anaplasmosis (Facury-Filho et al., 2012). Moreover, ENRO is effective against the pathogens that are resistant to the other antibacterial drugs such as tetracyclines, aminoglycosides and β lactam (Shoorijeh et al., 2012).

Keeping in view the above mentioned particulars the present study was designed to investigate the disposition kinetics and dosage regimen of ENRO in local, adult, clinically healthy and non-lactating female goats under the indigenous environmental conditions for establishing the optimal dosage regimen in local goats.

MATERIALS AND METHODS

Experimental animals and methodology: A total of 8 healthy and dry Beetal goats were used. All the animals were kept under similar conditions at the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Before initiation of each experiment, all the animals were weighed and properly restrained. For collection of the blood samples at different time intervals, the right jugular vein of each animal was cannulated with plastic cannula No. 90 (Protex Ltd., England) under strict aseptic conditions. A commercial injectable preparation of ENRO, ENROTIL 10% injection[®], lot No. J5073, (Dae Sung Microbiological Labs. Seoul, Korea), was used in these experiments. Drug was administered through intramuscular route, in the neck region at 5 mg/kg BW. An antiseptic solution was applied on the neck region of each animal before drug administration. Before the drug administration, blank blood samples were collected in each experiment. Blood samples were collected in heparinized plastic centrifuge tubes. After drug injecting, the blood samples were collected at 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8 and 12 hours. Blood samples were centrifuged with centrifuge machine (80-2 Centrifugal Machine, China), plasma was separated and stored at -20°C for analysis. The ENRO concentration in plasma was determined by using High Performance Liquid Chromatographic (HPLC) method (Iodowu and Peggins, 2004).

Calculations of pharmacokinetic parameters and dosage regimen: Mean±SE of each concentration and parameter was calculated. Kinetic parameters were computed by using two compartment open model with the computer program MW/PHARM version 3.02 by F. Rombout, in cooperation with University Centre for Pharmacy, Department of Pharmacology and Therapeutics, University of Groningen & Medi/Ware, copy right 1987-1991. Based upon the pharmacokinetic parameters, the dosage regimen of ENRO was investigated in adult female goats (Baggot, 1977).

RESULTS

Plasma concentrations: As presented in Table 1, the plasma (mean±SE) values of ENRO in 8 goats was 0.40±0.05 µg/ml at 0.25 hours which reached maximum to 1.26±0.19 µg/ml at 1.5 hours and then declined to 0.08±0.02 µg/ml at 12 hours post intramuscular medication.

Pharmacokinetics: Pharmacokinetics of ENRO was determined in healthy adult female goats following a single intramuscular administration. Pharmacokinetic parameters (mean±SE) of ENRO in goats are presented in Table 2. The time at which the maximum concentration of ENRO was attained in the plasma of goats was presented in Table 2. The values of half-life for absorption, $t_{1/2}$ abs (0.88 hours), distribution half-life, $t_{1/2}\alpha$ (0.69 hours) and elimination half-reflecting good pharmacokinetic characteristics of the fluoroquinolones. The higher value of volume of distribution, V_d (3.96 l/kg) recorded in present study showed a typical characteristic of the fluroquinolones antibacterial agents. The greater values of the total body clearance Cl_B (0.96 l/hr/kg) of ENRO in this study showed the higher clearance rate of ENRO in local goats life $t_{1/2}\beta$ (2.84 hours) were also

Table 1: Mean±SE plasma concentration (µg/ml) of enrofloxacin following single intramuscular administration at 5 mg/kg body weight in healthy adult female goats (n=8)

Time(hours)	Mean±SE
0.25	0.40±0.05
0.5	0.69±0.09
1.0	1.19±0.20
1.5	1.26±0.19
2.0	0.97±0.11
3.0	0.73±0.07
4.0	0.55±0.06
6.0	0.36±0.05
8.0	0.23±0.04
12.0	0.08±0.02

Table 2: Mean±SE pharmacokinetic parameters of enrofloxacin after intramuscular administration at 5 mg/kg body weight in adult goats (n=8)

Kinetic Parameters	Units	Mean±SE
C_{max}	µg/ml	1.03±0.101
T_{max}	Hr	1.47±0.135
K_{abs}	hr^{-1}	0.96±0.12
$T_{1/2abs}$	Hr	0.88±0.190
A	µg/ml	1.46±0.25
α	hr^{-1}	1.17±0.17
$T_{1/2\alpha}$	Hr	0.69±0.10
B	µg/ml	0.99±0.141
β	hr^{-1}	0.39±0.133
$T_{1/2\beta}$	Hr	2.84±0.295
V_c	L/kg	2.25±0.287
V_d	L/kg	3.96±0.660
K_{el}	hr^{-1}	0.44±0.033
K_{12}	hr^{-1}	0.30±0.082
K_{21}	hr^{-1}	0.69±0.107
Cl_B	l/hr/kg	0.96±0.099

presented in Table 2. The value of $t_{1/2}\beta$ was longer than the most literature values.

Dosage Regimen: The optimal dosage regimens of ENRO based upon the pharmacokinetic parameters were calculated in healthy adult goats. The calculations of priming and maintenance doses of ENRO after intramuscular administration were based upon the minimum inhibitory concentration (MIC or $C°P_{(min)}$) of ENRO in blood. The $C°P_{(min)}$ values of ENRO 0.02, 0.05, 0.08, 0.1, 0.25, 0.5, 0.75 and 1.0 µg/ml have been used in the calculations for each dosing interval.

In this study, the priming and maintenance doses for ENRO in mg/kg BW for 8 hours interval in healthy goats have been presented in Table 3. The calculated priming dose for goats, at 0 $C°P_{(min)}$ 0.05 µg/ml, was 4.49 mg/kg for 8 hour dosing intervals while the maintenance dose for corresponding dosing intervals was 4.29 mg/kg.

DISCUSSION

Plasma concentrations: During the course of antimicrobial therapy an antibiotic must maintain a certain therapeutic level or MIC in plasma or serum. During present investigations MIC 0.02-0.05 µg/ml of ENRO (Saini and Srivastava, 2001) in goats was maintained throughout its sampling time i.e up to 12 hrs after the drug administration. In present study Cmax of 1.26 µg/ml was attained at Tmax of 1.5 hours after intramuscular administration of ENRO. Almost similar value of Cmax for ENRO in goats, 1.07 µg/ml, has been reported by Rao *et al*. (2001). Higher values of Cmax of ENRO in goats have been reported as 2.80 µg/ml (Rao *et al*., 2002); 1.33 µg/ml (Elsheikh *et al*., 2002), 2.16 µg/ml and 2.73 µg/ml after its administration alone and with diclofenac

Table 3: Dosage regimen for enrofloxacin to maintain the minimum effective concentrations in adult goats at 8, 12 and 24hrs following a single intramuscular dose at 5mg/kg body weight

Species	C_p (min) (µ/ml)	Priming Dose (mg/kg)			Maintenance Dose (mg/kg)		
		8hr	12hr	24hr	8hr	12hr	24hr
Goats	0.02	1.80	10.70	1153.06	1.72	10.60	1152.96
	0.05	4.49	26.75	2882.65	4.29	26.50	2882.40
	0.08	7.18	51.41	5541.95	6.86	50.94	5541.47
	0.10	8.98	42.71	4603.36	8.58	42.32	4602.97
	0.25	22.44	106.79	11508.41	21.45	105.80	11507.42
	0.50	44.89	213.57	23016.82	42.90	211.59	23014.84
	0.75	67.33	320.36	34525.23	64.36	317.39	34522.25
	1.00	89.77	427.15	46033.63	85.81	423.18	46029.67

sodium, respectively (Narayan *et al.*, 2009). Higher serum concentrations of ENRO were also reported in febrile dogs (Dimitrova *et al.*, 2011).

Pharmacokinetics: Kinetic parameters of ENRO were described by two compartment open model as described in sheep and goats (Ovando *et al.*, 2000; Elsheikh *et al.*, 2002; Aboubakr *et al.*, 2013) and in cow calves (Singh and Srivastava, 2000). In local goats, the absorption of ENRO was quick from the site of intramuscular administration. The value of mean $t_{1/2}$abs in the present study (0.88 hours) was found higher than 0.15 hours of ENRO investigated in two different groups of goats following 5 mg/kg intravenous and intramuscular injections (Al-Nawazi, 2005). The $t_{1/2}$abs of ENRO was reportedly longer in febrile dogs than the healthy ones (Dimitrova *et al.*, 2011). This difference might be based on the variation in genetic and environmental conditions. In present study the mean value of $t_{1/2}\alpha$ (0.69 hours) for ENRO in local goats was almost similar to the value, 0.62 hours, investigated by Elmas *et al.* (2001) in angora goats after its intravenous administration. The distribution half-life (0.69 hours) for ENRO in goats of present investigations have been found higher than the most respective literature values, 0.56, 0.30 and 0.073 hours recorded by Al-Nawazi, (2005); Elsheikh *et al.* (2002) and Rao *et al.* (2002), respectively. This indicates better absorption as well as good penetration of ENRO in indigenous goats.

The elimination half-life of a drug is a derived parameter that changes as a function of both clearance and volume of distribution (Booth and McDonald, 1988). In the present study the value of elimination half-life of ENRO in local goats was 2.84 hours which was greater than the most literature values, 1.39 hours and 0.74 hours after a single intramuscular administration (Rao *et al.*, 2001, Rao *et al.*, 2002), 1.14 hours after intravenous administration and 1.42 hours and 1.63 hours after subcutaneous administration (Raina *et al.*, 2008; Narayan *et al.*, 2009). However, elimination half-life of present investigations (2.84 hours) remained comparable with the values of 2.78 hours (Ovando *et al.*, 2000, Elsheikh *et al.*, 2002) and 2.62 hours (Javed *et al.*, 2009) in goats. Higher values for elimination half-life of ENRO were also reported in goats, 3.98 hours (Elmas *et al.*, 2001), 4.7 hours and 4.41 hours, after intravenous and intramuscular administration, respectively (Al-Nawazi, 2005) and 5.19 hours (Aboubakr *et al.*, 2013). These differences might be due to variation in mode of drug administration and values of elimination rate constants in reported studies. The mean value for volume of distribution of ENRO (3.96 l/kg) in local goats was comparable to 3.80 l/kg in goats after intravenous administration (Al-Nawazi, 2005), 3.76 l/kg for ciprofloxacin in local goats after intramuscular administration (Javed *et al.*, 2009) and 3.37 l/kg for

ciprofloxacin in goats after intravenous administration (Ovando *et al.*, 2000). Narayan *et al.* (2009) recorded the higher value of V_d 5.26 l/kg in lactating goats following a subcutaneous administration at 5 mg/kg. However, the values of V_d 0f ENRO recorded in goats, 1.94 l/kg (Elsheikh *et al.*, 2002); 1.42 l/kg (Rao *et al.*, 2001); 1.28 l/kg (Rao *et al.*, 2002); 1.5 l/kg (Griffith *et al.*, 2010) and 1.22 l/kg and 1.51 l/kg after intravenous and intramuscular administration, respectively (Elmas *et al.*, 2001) were also lower than the value of V_d (3.96 l/kg) in the present study. Besides intra species biological variations, a possible explanation for the higher value of V_d in present study may be linked to the lower extrapolated zero time drug concentration (B, 0.99 µg/ml) as compared to its higher values in above cited studies. Another study showed that in alloxan diabetic dogs osmotic diuresis lead to water loss or dehydration accompanied with higher value of B and decrease in V_d when compared with normal dogs (Nawaz, 1983). Higher value of V_d indicates the better penetration of the drug into the tissues (Baggot, 1977). This assumption is also favored by the fact that the concentrations of fluoroquinolones in most of the tissues or fluids are normally higher to those reported in plasma. Further, fluoroquinolones are lipid soluble drugs and their typical V_d varies between 2 and 4 l/kg (Brown, 1996). Nevertheless, apparent volume of distribution of a drug is a function of the volume of tissues in which drug distributes, partition coefficient of drug between tissues and circulatory blood, the blood flow to the tissues and binding of drugs to the plasma and tissue proteins.

The value of total body clearance (Cl_B) in the present study (0.96 l/hr/kg) was almost similar to the Cl_B value of ciprofloxacin in goats; 0.98 l/hr/kg, Raina *et al.* (2008), 1.09 l/hr/kg, Javed *et al.*, (2009) and 1.18 l/hr/kg, Ovando *et al.*, (2000). Some higher values of the Cl_B for ENRO have been investigated in goats; 1.33 l/hr/kg (Rao *et al.*, 2002), 2.58 l/hr/kg (Narayan *et al.*, 2009). However, the lower values of Cl_B for ENRO, in goats; 0.47 l/h/kg (Griffith *et al.*, 2010), 0.613 l/h/kg (Guo *et al.*, 2010), 0.70 l/hr/kg (Elsheikh *et al.*, 2002), 0.81 l/hr/kg (Rao *et al.*, 2000) and 0.80 l/hr/kg (Rao *et al.*, 2002 have also been reported. A higher total body clearance (Cl_B) may be related to the higher value of respective V_d (Javed *et al.*, 2009). However, the total body clearance depends upon blood flow to the organ, fraction of unbound drug in blood and maximal ability of the organ to remove the drug. Most of these factors are under genetic control.

Dosage Regimen: Analysis of unsuccessful antibiotic treatment has shown that inadequate antibiotic concentration in tissue is the frequent cause of ineffectiveness (Kiss *et al.*, 1976). The susceptibility of bacteria to the action of ENRO varies not only between species but also between strains

within the same species resulting in a very wide range of C°P$_{(min)}$ or minimum inhibitory concentration (MIC) against the susceptible microbes. For the majority of the organisms that are susceptible to ENRO reported MIC values are 0.02-0.05 µg/ml (Saini and Srivastava, 2001). During the course of therapy, plasma level of an antibiotic should not fall below a minimum inhibitory concentration at the end of a certain dosing interval (Baggot, 1977). The dose recommended by the manufacturer of the pharmaceutical preparation of ENRO (5 mg/kg/24 hours) failed to maintain the therapeutic concentrations for 24 hours in goats of present study. In view of the specificity of disposition kinetics of drugs under indigenous conditions, it is obvious that the dosage regimen needs to be defined under the conditions in which it is to be used in clinic.

Based on therapeutic plasma levels of ENRO, the present study demonstrates that the optimal dosage regimen for 8 hours dosing interval in the adult healthy goats should be 4.49 and 4.29 mg/kg BW as priming and maintenance dose, respectively.

The results in the present study demonstrate that ENRO in local goats has shown the general pharmaco-kinetic characteristics of a typical fluoroquinolone anti-bacterial agent i.e. good tissue penetration, 2-4 l/kg volume of distribution and 2-4 hours terminal half-life (Brown, 1996).

Conclusion: Based on pharmacokinetic data of ENRO in local goats, the dosage regimen was recommended as 4.49 mg/kg BW as priming dose and 4.29 mg/kg BW as maintenance dose to be repeated after 8 hours dosing interval.

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