

Pharmacokinetics of Enrofloxacin Following Intravenous and Intramuscular Administration in Kilis Goats

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Abstract

Enrofloxacin (ENR) is a wide broad-spectrum antibacterial drug used widely in veterinary medicine. It was aimed to investigate the pharmacokinetic behavior of ENR in Kilis goats at single dosage of 2.5 mg/kg per goat body weight (bw) in the present study. A total of 10 healthy Kilis male goats were divided into 2 groups: each including 5 animals for the pharmacokinetic studies Group 1, received 2.5 mg/kg via vena jugularis by IV route; Group 2 received the same dose by intramuscular route through administration at musculus semitendinosus at formulation. Group 1 blood samples were taken at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24, 36 and 48 hours after administration of drug. ENR and its metabolite, ciprofloxacin (CPR) analysis was performed by high-performance liquid chromatography. Maximal plasma concentration (C_{max} 0.77 ± 0.05 µg/ml for IM) was reached at 1.00 h (T_{max}) for IM. The elimination half-life ($T_{1/2}$) of ENR was of 21.09 ± 0.87 h for IV and 29.75 ± 2.50 h for IM. The values of area under concentration-time curve (AUC) of ENR were of 6.46 ± 0.52 and 4.11 ± 0.35 µg.h/ml. ENR bioavailability (F) was calculated as 63.62%. The elimination half-life of CPR was of 19.31 ± 2.15 h for IV and 33.42 ± 1.28 h for IM.

Keywords: Pharmacokinetics, Kilis goats, enrofloxacin, ciprofloxacin.

INTRODUCTION

Kilis goat is a hybrid of Hair goat constituting 97% of the goat population in Turkey (TUIK, 2015). Kilis goat have higher milk yield, reproductive performance and better toleration for warm environments compared to other goats (Keskin et al., 2017). Although Kilis goat's population is smaller than the other goat populations, it has become remarkable in terms of its characteristics (Gül et al., 2016). It is estimated that number of Kilis goat is approximately 0.5 million in Turkey. The production of Kilis goats is officially carried out in Adana, Adiyaman, Kahramanmaraş and Mersin, especially in Gaziantep, Hatay and Kilis provinces (Aksoy and Kaya, 2007; Guzelier et al., 2010; Alizadehasl and Ünal, 2011; Paksoy and İriadam, 2012).

Fluoroquinolones are used in the treatment of diseases caused by pathogens including Gram-positive and Gram-negative bacteria, chlamydia and mycoplasma (Wolfson and Hooper, 1989; Moellering, 1996; Watts et al., 1997; Hooper, 1998; Al-Nazawi, 2005). Enrofloxacin (ENR) is a subfamily of synthetic fluoroquinolone antimicrobial agent. Ciprofloxacin (CPR) is the major active metabolite of ENR and it is used a drug product on human and veterinary market (widely human medicine). CPR is less lipophilic than ENR (EMA, 1998; Al-Nazawi, 2005; Troughon and Lefebvre, 2016).

ENR inhibit bacterial DNA replication by inhibiting the action of type II topoisomerases, topoisomerase IV and DNA gyrase (Ruiz, 2003). Minimum inhibitory concentration (MIC) values of ENR and CPR for pathogenic organisms such as *Actinomyces pyogenes*, *Pasteurella haemolytica* and *Haemophilus parasuis* ranges from 0.001 to 1 µg/mL (Prescott and Yielding, 1990). ENR distribute well to most tissues and body fluids and penetrate excellent intracellularly. It is effective against many disease agents. Therefore, it is used efficacious for the treatment of infections in many

different body systems such as respiratory and urinary tract, soft tissues, skin, joint and bone infections (Khargharia et al., 2005; Troughon and Lefebvre, 2016).

Pharmacokinetic properties of ENR have been investigated in cattle (De Lucas et al., 2008), sheep (Mengozzi et al., 1996; Haritova et al., 2003), calve (Kartinen et al., 1997), Yak (Khargharia et al., 2005) and pig (Anadon et al., 1999). However, there is very little data about the pharmacokinetic behavior of ENR in goats. The purpose of our study was to determine some of pharmacokinetic parameters of ENR in healthy Kilis goats following intramuscular (IM) and intravenous (IV) administration of a single dose.

MATERIALS AND METHODS

ENR preparations were used as 2.5 mg/kg per goats for 10 healthy Kilis male goats, 22-30 kg weight range and around 12-13 months-old were used in the present study where parasitic load pre-tested animals. All goats were subjected to clinical examination for possibility of any disease before study and they were clinically normal. Animals were given water *ad libitum* and were fed without any additives twice daily. Spray paint was used to number the animals for a clear distinguish. The animals were divided into 2 groups, each including 5 animals for the pharmacokinetic studies. Ethical committee approval for study was taken from the Adana Veterinary Control Institute Ethics Board (22.05.2014/29).

Group 1 received 2.5 mg/kg (Vil-flox, Vilsan) via *V. jugularis*; Group 2 was given at the same amount at *M. semitendinosus*. 5 ml blood samples from Group 1 was transferred to the Ca EDTA tubes after administration drug time of 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24, 36 and 48 hours. Five ml blood samples from Group 2 was transferred to the Ca EDTA tubes after administration drug time of 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24, 36 and

48 hours. All samples have been transported to laboratory under cold chain. The samples were centrifuged at 5000 rpm for 15 minutes (Hettich U320R) on the same day and then plasmas were removed from the samples. The method of (Anon, 2018) was used for ENR and CPR determination of plasma samples. Mobile phase consisted 0.005 M Heptane-1-sulfonic acid sodium, 0.002 M Potassium dihydrogen phosphate, acetonitrile and deionized water (5:5:20:70, v/v) and was applied at a flow rate of 1.0 ml/min (Thermo Finnigan, ChromQuest 4.1. version). Column oven temperature was set to 40°C degrees. A Hypersil™ BDS C18 analytical column (250 mm × 4.6 mm, 5 μm, Thermo Scientific™) was applied with Photo Diode Array (PDA) detection at wavelength of 278 nm.

Pharmacokinetic parameters were calculated by pharmacokinetic software program (Pharsight, WinNonlin 5.0). The bioavailability of ENR administered IM was calculated using the following formula. (%) F = (AUC_{im} / AUC_{iv}) × 100. Pharmacokinetic parameters of ENR and CPR are expressed as mean ± S.D.

Table 1. The comparison of the kinetic parameters in the Kilis Goat Group 1 (IV) and Group 2 (IM) (at 2.5 mg/kg dose) for ENR.

	IV Group (Mean ± SD)	IM Group (Mean ± SD)
Kinetic Parameters		
T _{1/2αZ} (h)	21.09 ± 0.87	29.75 ± 2.50
T _{max} (h)	-	1.00 ± 0.00
C _{max} (μg/ml)	-	0.77 ± 0.05
T _{last} (h)	48.00 ± 0.00	48.00 ± 0.00
C _{last} (μg/ml)	0.03 ± 0.00	0.02 ± 0.00
AUC ₀₋₄₈ (μg.h/ml)	6.46 ± 0.52	4.11 ± 0.35
AUC _{0-∞} (μg.h/ml)	7.24 ± 0.67	5.15 ± 0.53
V _{z obs} (L/kg)	10.57 ± 0.85	20.93 ± 1.94
Cl (L/kg/h)	0.35 ± 0.03	0.49 ± 0.05
AUC _{INF pred} (μg.h/ml)	7.18 ± 0.63	5.14 ± 0.53
V _{z pred} (L/kg)	10.65 ± 0.81	20.97 ± 1.89
Cl _{pred} (L/kg/h)	0.35 ± 0.03	0.49 ± 0.05
AUMC ₀₋₄₈ (μg.h ² /ml)	54.26 ± 6.13	47.01 ± 4.79
AUMC _{0-∞} (μg.h ² /ml)	115.19 ± 18.94	141.44 ± 24.90
AUMC _{INF pred} (μg.h ² /ml)	110.35 ± 15.96	140.61 ± 24.67
MRT ₀₋₄₈ (h)	8.38 ± 0.29	11.41 ± 0.26
MRT _{0-∞} (h)	15.84 ± 1.14	27.32 ± 2.7
MRT _{INF pred} (h)	15.32 ± 0.87	27.21 ± 2.08
V _{ss} (L/kg)	5.48 ± 0.18	-
F (%)	-	63.62%

In Table 2, ciprofloxacin kinetic parameters for Group 1 and Group 2 are shown.

Table 2. The comparison of the kinetic parameters in the Kilis Goat Group 1 (IV) and Group 2 (IM) for CPR.

	IV Group (Mean ± SD)	IM Group (Mean ± SD)
Kinetic Parameters		
T _{1/2αZ} (h)	19.31 ± 2.15	33.42 ± 1.28
T _{max} (h)	1.50 ± 0.00	1.50 ± 0.00
C _{max} (μg/ml)	0.28 ± 0.05	0.07 ± 0.01
T _{last} (h)	48.00 ± 0.00	48.00 ± 0.00
C _{last} (μg/ml)	0.02 ± 0.00	0.03 ± 0.00
AUC ₀₋₄₈ (μg.h/ml)	3.08 ± 0.54	1.97 ± 0.23
AUC _{0-∞} (μg.h/ml)	3.73 ± 0.65	3.17 ± 0.34
AUC _{INF pred} (μg.h/ml)	3.62 ± 0.66	3.14 ± 0.33
AUMC ₀₋₄₈ (μg.h ² /ml)	43.81 ± 5.17	39.79 ± 4.73
AUMC _{0-∞} (μg.h ² /ml)	93.58 ± 16.74	155.50 ± 14.88
AUMC _{INF pred} (μg.h ² /ml)	85.22 ± 17.33	152.51 ± 13.71
MRT ₀₋₄₈ (h)	14.35 ± 0.87	20.19 ± 0.11
MRT _{0-∞} (h)	25.11 ± 1.85	49.06 ± 1.82
MRT _{INF pred} (h)	23.51 ± 2.10	48.61 ± 1.70

RESULTS

Retention time for ENR were found as 6.1 minutes; with a limit of detection (LOD) as 6.25 ppb and limit of quantitation (LOQ) as 12.5 ppb. Average recovery rate was %93.6. Retention time for CPR were found as 4.8 minutes; with a limit of detection (LOD) as 6.25 ppb and limit of quantitation (LOQ) as 12.5 ppb. Average recovery rate was %94.2.

The average plotted regression line for ENR concentration standards were calculated from 0, 6.25, 12.5, 25, 50, 100, 250, 500, 1000, 2500 and 5000 ppb concentrations with correlation coefficient as r²= 0.999 using “y = 161460x – 42154” formula. The average plotted regression line for CPR concentration standards were calculated from 0, 6.25, 12.5, 25, 50, 100, 250, 500, 1000, 2500 and 5000 ppb concentrations with correlation coefficient as r²= 0.999 using “y = 16146x – 42154” formula.

In Table 1, ENR kinetic parameters for Group 1 and Group 2 are shown.

In Figure 1, the IV plasma drug concentration-time curve of ENR and CPR is given.

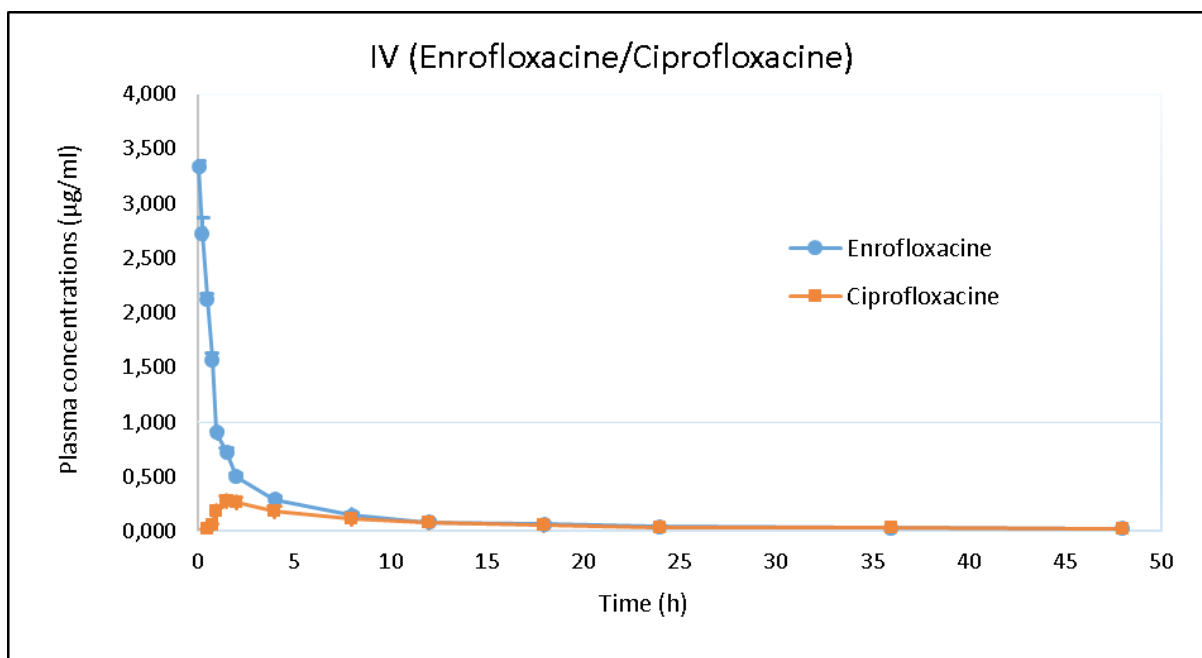


Figure 1. Plasma drug concentration-time curve of ENR and CPR (IV).

In Figure 2, the IM plasma drug concentration-time curve of ENR and CPR is given.

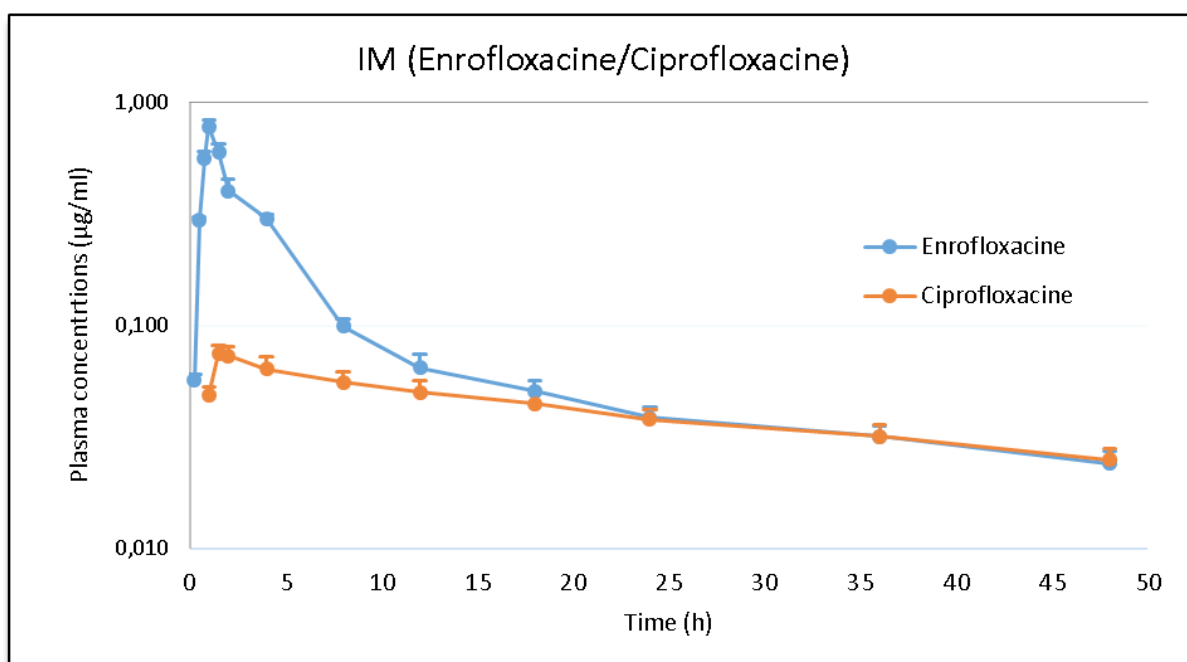


Figure 2. Plasma drug concentration-time curve of ENR and CPR (IM).

DISCUSSION

The pharmacokinetics of ENR was investigated after administration of it IV and IM at a dose rate of 2.5 mg/kg bw in 10 healthy Kilis goats. In this study, Maximal plasma concentration (C_{max} , 0.77 ± 0.05 µg/ml for IM) was reached at 1.00 h (T_{max}) for IM. The higher plasma level of ENR was observed at 0.88 h in goat (C_{max} , 2.8 ± 0.289 µg/mL) (Rao et al., 2001) and at 1 h in calves (Garcia et al., 1996) (C_{max} , 1.66 ± 0.61 µg/mL) given the same IM dose of the drug.

ENR has a longer elimination half-life of 21.09 ± 0.87 h

for IV and 29.75 ± 2.50 h for IM in Kilis goats compared to the other goats and sheep. After IV and IM administration of ENR, our data for the elimination half-life ($t_{1/2\beta}$) were higher than those by Haritova et al. (2003) (3.30 ± 0.36 h for IV and 3.87 ± 0.1 h for IM in sheep), Otero et al. (2009) (4.57 ± 1.08 h for IV in sheep), Aboubakr (2013) (5.39 ± 0.96 h for IM in goat), Daundkar et al. (2015) (8.20 ± 0.53 h for IM and 8.12 ± 0.31 h for IV in buffalo calves at dose of 7.5 mg/kg bw) and Rao et al. (2002b) (0.73 ± 0.047 h for IV in goat).

The values of area under concentration-time curve

(AUC) after IV and IM administration of ENR (6.46 ± 0.52 and 4.11 ± 0.35 $\mu\text{g}\cdot\text{h}/\text{ml}$) in the present study were similar to those by Rao et al. (2002a) (7.82 ± 0.763 $\mu\text{g}\cdot\text{h}/\text{ml}$ for IM in goat at dose of 5 mg/kg bw) and Aboubakr (2013) (5.79 $\mu\text{g}\cdot\text{h}/\text{ml}$ for IM in goat) but higher than those reported by Rao et al. (2001) (2.09 ± 0.22 $\mu\text{g}\cdot\text{h}/\text{ml}$ for IM in goat) and Rao et al (2002b) (1.916 ± 0.140 $\mu\text{g}\cdot\text{h}/\text{ml}$ for IV in goat).

Based on the ratio between AUCs of CPR and ENR, plasma levels of CPR accounted for 48% of the parent drug. The metabolic conversion of ENR to CPR in Kilis goats was higher in other goat (24% IV) (Rao et al., 2002b) and sheep (36% for IV) (Mengozzi et al., 1996). The elimination half-life of CPR in this study (19.31 ± 2.15 h for IV and 33.42 ± 1.28 h for IM) was longer than that of a goat (0.92 ± 0.13 h for IV and 1.82 ± 0.209 for IM) (Rao et al., 2002a; Rao et al., 2002b) and sheep (4.8 h for IV) (Mengozzi et al., 1996). This longer elimination half-life suggest that Kilis goats eliminate CPR slower than other goats and sheep. Higher values (for IM) for the elimination half-life of CPR than those for ENR indicate that the active metabolite can increase the pharmacokinetic properties of ENR in the treatment of bacterial diseases in Kilis goats.

Although ENR levels remained above the suggested therapeutic concentration (0.1 $\mu\text{g}/\text{ml}$) (Kaartinen et al., 1997) in the plasma for up to 8 h, CPR levels did not remain above the concentration after IM administration of the drug. Although ENR levels remained above the therapeutic concentration in the plasma for up to 10 h, CPR levels remained above the concentration for up to 9 h after IV administration of the drug. According to the results of the study, IV administration of the drug is recommended for better therapeutic efficacy in Kilis goats. It has been known that Fluoroquinolones generate a significant post-antibiotic effects lasting for up to 4-8h (Rao et al., 2001). The drug given at 2.5 mg/kg IM and IV at 8 h intervals seems to be suitable for the treatment of diseases of Kilis goats caused by the bacteria that are sensitive to the ENR and its metabolite.

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