Bulgarian Journal of Veterinary Medicine (2011), **14**, No 3, 158–164

PHARMACOKINETICS OF ENROFLOXACIN IN DOGS WITH EXPERIMENTAL STAPHYLOCOCCAL INFECTION

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Summary

Dimitrova, D., I. Borissov, Ts. Chaprazov, T. Georgieva, S. Yanev & B. Pandova, 2011. Pharmacokinetics of enrofloxacin in dogs with experimental staphylococcal infection. *Bulg. J. Vet. Med.*, **14**, No 3, 158–164.

The study investigated the effect of experimental *Staphylococcus aureus* bacterial infection on enrofloxacin pharmacokinetics in dogs. After infection, serum blood enrofloxacin concentrations and the values of $AUC_{0\rightarrow\infty}$, C_{max}, T_{max} and $t_{1/2abs}$ were higher. Other pharmacokinetic parameters ($t_{1/2\beta}$, MRT) exhibited an insignificant decrease. The infection resulted in lower serum concentrations of ciprofloxacin, the main metabolite of enrofloxacin. Statistically significantly lower biological half-life, lower $AUC_{0\rightarrow\infty}$, T_{max}, C_{max} and MR were also observed.

Key words: ciprofloxacin, dogs, enrofloxacin, infection, pharmacokinetics, *Staphylococcus aureus*

INTRODUCTION

Enrofloxacin is a fluorinated quinolone with a broad antibacterial spectrum and high bactericidal activity (Walker & Dowling, 2006). In veterinary medicine, it is successfully used in septicaemic states and for treatment of respiratory, excretory, skin, soft tissues, joint and bone marrow bacterial infections (Walker & Dowling, 2006; Papich, 2007).

The pathological events caused by microbial agents (Meinen *et al*., 1995; De Manuelle *et al*., 1998.; McKellar *et al*., 1999; Ahangar & Srivastava, 2000; Post *et al*., 2002; Gerhardt *et al*., 2006; Plock, 2007; Fu *et al*., 2008), or administration of microbial endotoxins (Post *et al*., 2003; Elmas *et al*., 2006; 2008) could provoke an altered systemic disposition of antibacterial drugs in animals and humans.

The fever, accompanying bacterial infections, changes the physiological parameters in the diseased animal and consequently, the pharmacokinetics of applied drugs (Ahangar& Srivastava, 2000).

The purpose of the present study was to investigate the effect of *Staphylococcus aureus* infection on the pharmacokinetics of enrofloxacin in dogs.

MATERIALS AND METHODS

Experimental animals

The study was performed on 6 clinically healthy mongrel dogs (equal number of both genders) at the age of 1–3 years, weighing 12–15 kg.

The dogs were housed indoor in individual metal cages with wooden floors under controlled microclimatic conditions: 20–22 °C, mixed light regimen and air humidity 55–60 %.

A 30-day period of adaptation was allowed, during which the dogs were treated three times at 7-day intervals against ectoparasites with powder Bolfo® (Bayer, Germany) and against endoparasites with Prazimec-D (Biovet, Peshtera, Bulgaria). During the entire experimental period, they were fed dry commercial canine food.

The experiment was carried out in compliance with animal welfare standards (Regulation 15/2006).

Drugs and treatment

The drug used was Syvaquinol®-25 injectable (Syva Laboratories, Spain), containing enrofloxacin hydrochloride at a concentration of 2.5 %.

Twenty days before the inoculation of the broth *Staphylococcus aureus* culture, dogs were injected with 5 mg/kg Syvaquinol®-25 injectable. Blood samples were obtained immediately before the quinolone injection (0 h) and at hours 0.17, 0.33, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h from the cephalic vein of the right forelimb by means of venflon cannula (Venflon 2, Viggo AG, Sweden). Serum samples were stored at –20 °C until analysis.

The infection was performed by s. c. injection of 5 mL 24-hour suspension from agar culture of a standard *Staphylococcus aureus* strain with a density of 3.1×10^9 CFU/mL in the scapular region.

During the experiment, the changes in the clinical status (rectal temperature, heart and respiratory rates), and some haematological parameters: erythrocyte counts (Er), total and differential leukocyte counts (Leu), haemoglobin content (Hb), haematocrit (Hc) were monitored.

Drug analysis

The concentrations of enrofloxacin and its active metabolite ciprofloxacin were assayed on high-performance liquid chromatograph (Waters) by the method of Imre *et al.* (2003).

The limit of quantitation of enrofloxacin and its metabolite (ciprofloxacin) was 0.01 μg/mL, and the limit of detection for both substances – 0.005 μg/mL.

Intra-day and inter-day coefficients of variation for enrofloxacin were between 6.8% and 13.6%, and the precision of the assay between 0.51 and 11.21%. Intra-day и inter-day coefficients of variation for the metabolite were between 5.6% and 9.19%, and assay precision: 0.268–7.56%.

Pharmacokinetic analysis

The pharmacokinetic analysis of serum concentrations-time curve after single s.c. injection was done with the TopFit, v.2.0 software (Heinzel *et al*., 1993). The data were analysed by compartmental and non-compartmental models. The Akaike's information criterion (AIC) was used to determine the most appropriate pharmacokinetic model (Yamaoka *et al*., 1978). The area under the serum concentration curve ($AUC_{0\rightarrow\infty}$) was calculated by the trapezoid rule.

Statistical analysis

The significance of the effect of infection on clinical status and pharmacokinetics was evaluated with a statistical software (Statistica®, v. 6.0) using the non-parametric Mann-Whitney U test. Data were presented as mean ± SEM. Differences were considered significant at $P<0.05$.

RESULTS

The subcutaneous injection of the *Staphylococcus aureus* strain caused statistically significant increase in body temperature, respiratory rate and slightly increased heart rate during the first 24 hours. In all animals, a warm, generalized and painful swelling was observed on the 24th hour. In two dogs the regional lymph nodes were swollen. A statistically significant increase in total leukocyte counts was present (Table 1). Segmented neutrophils and metamyelocyte counts were insignificantly increased.

Serum levels of enrofloxacin and its metabolite ciprofloxacin in dogs before and after inoculation of *Staphylococcus aureus* are presented on Fig. 1. The lowest enrofloxacin concentrations by the 24th hour in infected dogs were 33.33% of those at the same time interval in non-infected dogs. The subcutaneously administered fluoroquinolone and its metabo-

Table 1. Some clinical and haematological indices in dogs prior to and 24 h after infection with *Staphylococcus aureus* (mean ± SEM, n=6)

	Before infection	24 h after infection
Rectal temperature (°C)	38.6±0.5	39.69±0.5**
Heart rate (min^{-1})	112±25	123±20
Respiratory rate (min^{-1})	25 ±3.0	44.67±5.39**
Erythrocyte counts (T/L)	6.00±0.27	5.89±0.42
Haemoglobin (g/L)	132.0±17.48	136.0±16.46
Haematocrit (L/L)	0.366±0.04	0.375±0.01
Leukocyte counts (G/L)	9.74±1.54	17.81±4.02**

* P<0.05; ** P<0.01 vs preinfection values by the Mann-Whitney U test.

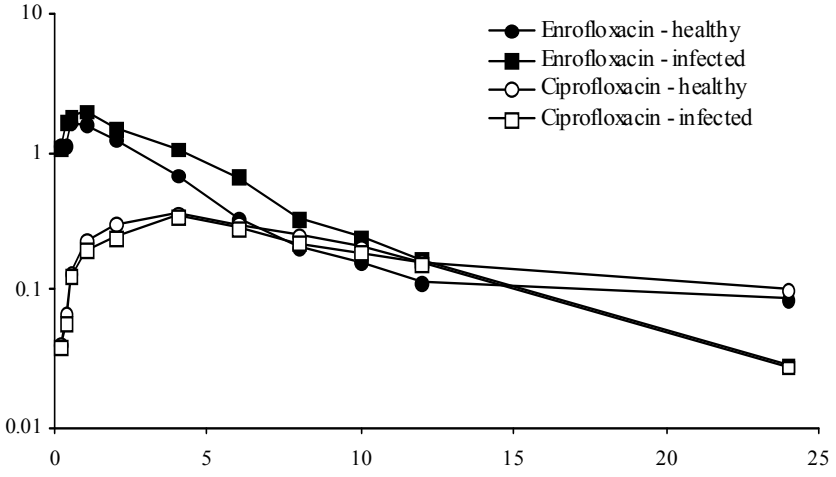

Fig. 1. Serum enrofloxacin and ciprofloxacin concentrations after single *s.c.* adminsitration of enrofloxacin to healthy dogs and dogs infected with *Staphylococcus aureus*.

lites were present in the blood very soon – after 0.17 h, in similar concentrations for healthy and infected dogs.

Serum concentrations of enrofloxacin in febrile animals were higher than those established before the infection between post administration hours 0.33 and 12.

In febrile dogs, serum ciprofloxacin levels were lower at all sampling intervals compared to preinfection values (Fig. 1). They varied between 98.74% до 28.00% of concentrations in healthy subjects from the first to the last sampling.

Mean values of pharmacokinetic parameters of enrofloxacin and its metabolite are presented in Tables 2 and 3. In febrile dogs, the absorption half-life of enrofloxacin ($t_{1/2abs}$) was significantly higher. The time to reach maximum serum concentrations (T_{max}) after s. c. admini-

Table 2. Some pharmacokinetic parameters of enrofloxacin after single s. c. administration of 5 mg/kg to dogs, before and after infection with *Staphylococcus aureus* (mean ± SEM, n=6)

Parameter	Noncompartmental analysis	
	Before infection	After infection
$t_{1/2abs}$ (h) $^{\&}$	0.34±0.03	0.80±0.37*
$t_{1/2\beta}$ (h)	4.97±0.38	3.28±0.41
MRT (h)	6.25±0.64	4.70±0.53
$AUC_{0\rightarrow\infty}$ (μg.h/mL)	8.95±0.76	10.52±1.14
T_{max} (h)	0.50±0.00	0.81±0.13
C_{max} (μg/mL)	1.66±0.08	1.99±0.13

$t_{1/2abs.}$ – absorption half-life; $t_{1/2\beta}$ – elimination half-life; MRT – mean residence time; $AUC_{0\rightarrow\infty}$ – area under the serum concentration curve from time 0 to infinity; T_{max} – time to reach maximum serum concentration; C_{max} – maximum serum concentration; $^{\&}$ determined by the compartmental pharmacokinetic method; * $p < 0.05$ vs preinfection values by Mann-Whitney U-test.

Table 3. Some pharmacokinetic parameters of the active metabolite ciprofloxacin after single s. c. administration of 5 mg/kg to dogs, before and after infection with *Staphylococcus aureus* (mean ± SEM, n=6)

Parameter	Noncompartmental analysis	
	Before infection	After infection
$t_{1/2\beta}$ (h)	10.76±1.09	5.34±0.67**
MRT (h)	16.55±1.79	9.44±0.83**
$t_{1/2f\,(h)}$ $^{\&}$	0.73±0.01	1.83±0.43*
$AUC_{0\rightarrow\infty}$ (μg.h/mL)	5.27±1.06	4.18±0.35
T_{max} (h)	0.36±0.03	0.35±0.02
C_{max} (μg/mL)	4.00±0.001	3.33±0.56
MR (%)	58.81±7.01	40.50±2.67*

$t_{1/2abs.}$ – absorption half-life; $t_{1/2\beta}$ – elimination half-life; MRT – mean residence time; $t_{1/2f}$ – time for appearance of the metabolite in the central circulation; $AUC_{0\rightarrow\infty}$ – area under the serum concentration curve from time 0 to infinity; T_{max} – time to reach maximum serum concentration; C_{max} – maximum serum concentration; MR – metabolic ratio; $^{\&}$determined by the compartmental pharmacokinetic method; * $P<0.05$; ** $P<0.01$ vs preinfection values by the Mann-Whitney U test.

stration of enrofloxacin was by 61% longer. A similar tendency was observed in C_{max} values (Table 2).

After enrofloxacin administration, the biological half-life ($t_{1/2\beta}$), mean residence time (MRT) and the area under the concentration time curve ($AUC_{0\rightarrow\infty}$) of ciprofloxacin in febrile dogs were statistically significantly lower than in healthy ones. Maximum serum concentrations (C_{max}) of the metabolite in febrile dogs were lower and 95.60% of preinfection levels. The time for appearance of the metabolite in the central circulation ($t_{1/2f}$) in infected dogs was considerable longer and numerically, was 250% of that in healthy dogs (Table 3).

DISCUSSION

Fever is one of the most important symptoms of bacterial infection in domestic animals. Many authors (van Miert, 1990; Lashev, 1997; Ahangard & Srivastava, 2000) have shown that fever, occurring as a consequence of bacterial infection or inoculation of pyrogens alters the pharmacokinetics of chemotherapeutic drugs in infected animals.

The significantly higher serum concentrations of enrofloxacin in febrile dogs, inoculated with *Staphylococcus aureus*, compared to those in healthy subjects could be explained to the enhanced blood circulation and the increased vascular permeability in soft tissues accompanying the fever, causing better, more prolonged and more complete absorption of the quinolone from the injection site. This assumption is supported by the statistically significantly higher absorption half-life values in febrile dogs. Shorter biological half-life and MRT in infected dogs indicate that enrofloxacin was eliminated more rapidly from the organism of infected dogs as compared to healthy ones. The values of these two pharmacokinetic parameters correlated to enhanced heart rate resulting in higher circulating blood volumes through the renal parenchyma.

The lower blood serum concentrations of the metabolite in febrile dogs could be explained with the inhibitory effect of fever on liver function and on the levels of liver microsomal enzymes which are responsible for converting enrofloxacin into ciprofloxacin in hepatocytes, for the altered biotransformation and the elimination of the parent substance in febrile states. The inhibited metabolic potential of the liver parenchyma are further confirmed by the lower metabolic ratios in febrile dogs (Table 3).

On the basis of the results from this study, it could be concluded that the induced experimental *Staphylococcus aureus* infection in dogs influenced the serum concentrations and the disposition of the injected fluoroquinolone and its active metabolite.

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Paper received 03.09.2010; accepted for publication 03.12.2010

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