Bulgarian Journal of Veterinary Medicine (2011), **14**, No 4, 221–230

ENROFLOXACIN DISPOSITION IN AQUEOUS HUMOUR AFTER SUBCUTANEOUS ADMINISTRATION IN DOGS

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Summary

Krastev, S. Zh., A. M. Haritova, L. D. Lashev & H. D. Hubenov, 2011. Enrofloxacin disposition in aqueous humour after subcutaneous administration in dogs. *Bulg. J. Vet. Med.*, **14**, No 4, 221–230.

Enrofloxacin, a fluoroquinolone drug, is often used in treatment of bacterial infections in dogs because of its potent bactericidal activity and wide distribution in tissues. The aim of the present study was to determine the penetration of enrofloxacin and its main active metabolite ciprofloxacin in aqueous humour (AH) by means of population pharmacokinetics. A single dose of enrofloxacin 5% sterile solution for injections (Baytril®, Bayer HealthCare) was administered subcutaneously to 24 dogs at a dose rate of 7.5 mg/kg. Concentrations of enrofloxacin and its main metabolite ciprofloxacin in plasma and AH were analyzed by high performance liquid chromatography. Enrofloxacin and ciprofloxacin were found in the studied fluids 24 h after parent drug administration. Maximum concentrations were lower in AH compared to plasma. The absorption rate constant of enrofloxacin was lower for AH (0.12 h^{-1}) than for plasma (0.62 h^{-1}). A lower value for this constant was obtained for the main metabolite ciprofloxacin: 0.07 h^{-1} for AH. The data from the current study with healthy animals showed that enrofloxacin and its active metabolite ciprofloxacin penetrated AH after subcutaneous administration and reached therapeutic levels for sensitive microorganisms as *Staphylococcus intermedius* and *Escherichia coli* with MIC values less than 0.25 μg/mL but concentrations were below the MIC_{90} for *Pseudomonas aeruginosa*.

Key words: aqueous humour, dogs, enrofloxacin and ciprofloxacin disposition

INTRODUCTION

Fluoroquinolones are a class of antibacterial drugs with a broad spectrum of activity mainly against Gram-negative but also against some Gram-positive bacteria (Zhanel *et al.*, 2002). They act as DNA-gyrase inhibitors with concentration-dependent manner of bactericidal activity (Hawkey, 2003). Good tissue penetration of fluoroquinolones and achievement of effective concentrations in tissues make this class of drugs applicable in treatment of wide range of infections, including ocular bacterial inflammation. Since fluoroquinolones have been reported to penetrate extensively aqueous humour (AH) they were used in prophylaxis and treatment of ocular infections in humans (García-Vázquez *et al.*, 2007; Lai *et al.*, 2007; Ong-Tone, 2007). Fluoroquinolones are also applied in veterinary practice and their disposition has been studied in dogs after systemic and topical administration (Frazier *et al.*, 2000; Sarkozy, 2001). Ciprofloxacin has a long half-life of eli-

mination in tears of healthy brachycephalic and mesocephalic dogs after topical ophthalmic application (Hendrix & Cox, 2008). Corneal penetration and ability of ciprofloxacin and ofloxacin to exceed MIC_{90} of common ocular contaminants in AH after topical administration in dogs has also been investigated (Yu-Speight *et al.*, 2005). Marbofloxacin administered intravenously can also penetrate the AH of canine eyes and may be suitable for prophylaxis or treatment of certain anterior chamber infections (Regnier *et al.*, 2003). Despite the fact that fluoroquinolones are increasingly used in prophylaxis of veterinary patients little is known about their disposition in the canine eye. The knowledge about their disposition and concentrations at the site of infection is even more valuable with regard to proper decision making in clinical cases.

Enrofloxacin is licensed for use in small animals and has the typical characteristics of fluoroquinolones. It is metabolized to ciprofloxacin in several animal species, including dogs (Bidgood & Papich, 2005). Enrofloxacin and its metabolite ciprofloxacin have low protein binding (34.74 % and 18.48 %, respectively), high volume of distribution and relatively slow clearance in dogs (Bidgood & Papich, 2005). Antibacterial activity of enrofloxacin plus ciprofloxacin exceeds that of enrofloxacin alone (McKellar *et al.*, 1999; Lautzenhiser *et al.*, 2001). Therefore, enrofloxacin may be useful in the therapy of eye infections. So far, studies on separate measurement of enrofloxacin and its main active metabolite ciprofloxacin in eye of dogs are insufficient.

The aim of this study was to determine the penetration of enrofloxacin and its active metabolite ciprofloxacin, after subcutaneous administration of the parent drug, in the aqueous humour of dogs and to evaluate its potential usefulness in prevention and treatment of intraocular infection. Another objective of the present study was also to assess inter-individual variability of pharmacokinetic parameters by means of population pharmacokinetics.

MATERIALS AND METHODS

Drug

Enrofloxacin as a 5% sterile solution for injection (Baytril ®, Bayer HealthCare) was used.

Experimental animals

The experiments were performed with 24 crossbred clinically healthy dogs aged 3–5 years, weighing 18–25 kg, housed in individual cages with an area of 1.5 m² and height of 2.2 m. During the 14-day period of adaptation, each animal was provided with food in individual bowl and had free access to water. The housing conditions were uniform. The animals were free of active ocular disease which was confirmed by complete ophthalmic examination.

Experimental design

A single dose of enrofloxacin was administered subcutaneously at a dose rate of 7.5 mg/kg. The analgesia was achieved by local anaesthesia with Alcaine® 0.5% ophthalmic solution (S.A. Alcon - Couvreur N.V., Belgium) by repeated instillation before puncture of the anterior chamber. The experiments were approved by the Ethical Committee at the Faculty of Veterinary Medicine, Trakia University (License No 9/13.02.2007).

Individual blood and aqueous humour samples (one per eye) were obtained at 0.5 and 6^{th} h; at 1 and 9^{th} h; at 2 and 12^{th} h; 4 and 24 h after antibiotic administra-

tion. Blood was sampled from the cephalic vein in heparinized tubes, blood plasma was separated after centrifugation (10 min; 1200×*g*) and frozen at −18°C. Aqueous humour (200 μL) was obtained via puncture of the anterior ocular chamber with a 29G sterile needle (Momina krepost PLC, Veliko Tarnovo, Bulgaria) and sterile 2 mL syringe. The samples were stored in Eppendorf tubes at −18 °C. They were analyzed within 2 months.

Drug assay

The concentrations of enrofloxacin in plasma and AH were determined by high performance liquid chromatography with fluorescence detection (Imre *et al.* 2003). The standard solutions were prepared in plasma collected from untreated dogs and in phosphate buffer (pH 7). The plasma concentrations of both compounds have been calculated from standard curves of blank plasma spiked with known concentrations of enrofloxacin and ciprofloxacin. The concentrations of both compounds in AH were determined by the same method and the calibration standard solutions were prepared in phosphate buffer (pH 7). The Waters reverse phase liquid chromatography system used for the analyses was equipped with a quaternary pump 626E; a fluorescence detector 474 set at λ_{ex} = 275 nm and λ_{em} = 445 nm; a guard-column Lichrosphere 100 RP-18, 3.9×20 mm, 5 μm, Beckman; analytical column: Lichrosphere 100 RP-18, 4.0×125 mm, 5 μm, Beckman with a working temperature of 45 °C. The mobile phase consisted of acetonitrile/water (12:88 v:v), containing 25 mM potassium phosphate buffer (adjusted at pH 4.0 with 85% phosphoric acid), 14 mM triethylamine and 5 mM tetrabutylammonium hydroxide. The flow rate was 0.9 mL/min. Instrument control and integration was performed with a Pentium 566 computer by Waters Empower software and the chromatogrammes were stored and reproduced by the same system. The limits of quantitation of enrofloxacin and ciprofloxacin were 0.01 μg.mL^{-1}. The limit of detection was 0.005 μg.ml^{-1} for both compounds. In the concentration range 0.01–5 μg.mL^{-1} for enrofloxacin and 0.01–0.5 μg.mL^{-1} for ciprofloxacin, the dependency of peak area to concentration was shown to be linear. The recovery was nearly 100%. Intra-day and inter-day coefficients of variations for enrofloxacin were between 6.8% and 13.6% and accuracy between 0.51 and 11.21%; these numbers for ciprofloxacin were between 5.6% and 9.19%; 0.268% and 7.56%, respectively.

Pharmacokinetic analysis

Plasma and AH concentrations were included in the population pharmacokinetic analysis in two stages. The concentrations in plasma and aqueous humour were pooled for all animals in the first stage and pharmacokinetic parameters were computed following a procedure termed "naïve pooling". Population pharmacokinetic analysis was employed with nonlinear mixed effects modeling with the Monolix v2.2 (Monolix group, http://group.monolix.org). Population parameters were estimated using the stochastic approximation expectation maximization (SAEM) algorithm. Variances of Pk parameters were also computed.

The following general nonlinear mixed effects model was considered:

$$y_{ij} = f(x_{ij}, \phi_i) + g(x_{ij}, \phi_i)\varepsilon_{ij},$$
$$1 \le i \le N, 1 \le j \le ni$$

where y_{ij} – the j^{th} observation of subject i, N – the number of subjects; n_i – the number of observations of subject i; x_{ij} – re-

gression variables; ε_{ij} – within-group errors.

One-compartmental model was selected for analysis of the concentration-time courses of antibacterial drug on the basis of the lowest value of information criteria as BIC and AIC. A basic model with no covariates on total body clearance (Cl/F) or volume of distribution (V/F) was used. Pharmacokinetic parameters of Cl/F, V/F and the absorption rate constant (k_{ab}) corresponding to the proposed model from the PK library of the computer programme (first order oral absorption with one compartment model function) were determined. The distribution of the random effects was set to a log-normal distribution. A constant error model was selected. The value of the estimated log-likelihood together with its standard error was also computed.

RESULTS

Plasma and aqueous humour concentrations

The mean concentration-time curves of enrofloxacin and its main metabolite ciprofloxacin in plasma and AH are presented in Fig. 1. The concentrations of both compounds in plasma showed considerable inter-animal variation. The concentrations of the parent drug in plasma reached a peak at approximately 1 to 4 h after administration. The main metabolite

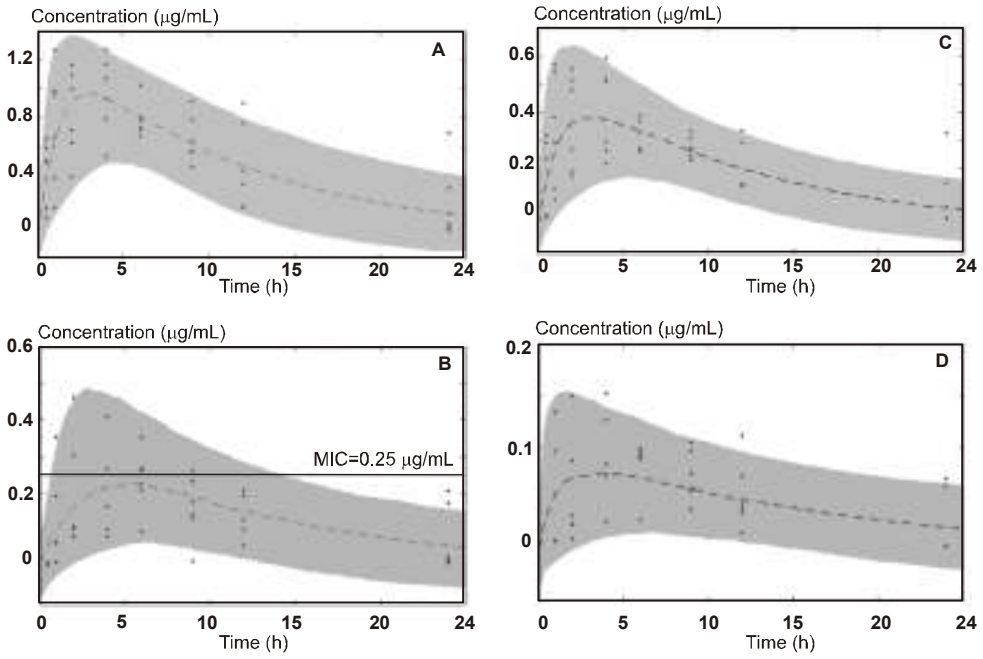

Fig. 1. Scatterplot of observed concentrations with the median and the prediction interval of enrofloxacin (dose rate of 7.5 mg/kg, subcutaneously) in plasma (a) and aqueous humour (b) of dogs and of its main metabolite ciprofloxacin (c, d, respectively). ♦ – measured concentrations; — ▪ — predicted median concentrations; ☐ – prediction interval 90%.

Table 1. Population pharmacokinetic parameters of enrofloxacin and its main metabolite ciprofloxacin administered subcutaneously at a dose rate of 7.5 mg/kg in healthy dogs (n=24)

Pharmacokinetic parameters (mean ± SE)	Enrofloxacin		Ciprofloxacin	
	Naïve pool estimation	Population mean	Naïve pool estimation	Population mean
Plasma				
Number of samples	48	48	48	48
k_{01} (h^{-1})	0.88	0.62±0.17	–	–
V/F (l/kg)	5.97	4.95±0.51	–	–
Cl/F (l/h/kg)	0.54	0.55±0.05	1.14	1.27±0.11
ω^2 k_{01} (%)		63.80		4.57
ω^2 V/F (%)		2.06		–
ω^2 Cl/F (%)		9.51		2.13
Aqueous humour				
Number of samples	42	42	39	39
k_{01} (h^{-1})	0.13	0.12±0.07	0.04	0.07±0.02
V/F (l/kg)	6.86	7.40±5.53		–
Cl/F (l/h/kg)	2.22	1.93±0.29		–
ω^2 k_{01} (%)		38.70		8.59
ω^2 V/F (%)		44.60		–
ω^2 Cl/F (%)		6.67		–

k_{01} – absorption rate constants for central compartment or for aqueous humour; V/F – volume of distribution, Cl/F – total body clearance.

was found at highest concentration between the 2nd and the 4th h after enrofloxacin administration. Both fluoroquinolone drugs were found in plasma above the limit of quantification till the 24th hour after enrofloxacin injection.

Concentrations of enrofloxacin and ciprofloxacin in AH were lower than these in plasma at all sampling intervals. Ciprofloxacin was not found at detectable levels in AH at hour 0.5 and measurable concentrations were detected in three out of six samples by the 1st h after treatment. The concentrations of enrofloxacin and ciprofloxacin in AH showed similar pattern to those in plasma with high inter-animal variation, although maximum concentrations were reached later, between the 4th and the 6th h.

Pharmacokinetic modeling

Time courses of the concentrations of enrofloxacin and ciprofloxacin in plasma (Fig. 1a and Fig. 1c, respectively) and AH (Fig. 1b and Fig 1d) were scattered and analyzed with a model assuming first-order absorption and elimination of both drugs. A summary of mean population kinetic parameters is given in Table 1. The mean pharmacokinetic parameters found by the population analysis were similar to the initial values found by the naïve pooling method. The population analysis indicated that there was considerable inter-subject variation in the kinetic parameters. The rate constant of absorption of enrofloxacin into plasma had a higher value than the rate constant of

transfer into AH. Lower absorption constant was computed for ciprofloxacin in comparison to the parent drug and smaller coefficients of variation were observed for the pharmacokinetic parameters.

DISCUSSION

Pharmacokinetics of enrofloxacin and its main active metabolite in the dog has been previously described and it has been shown that the fractions of metabolized parent drug were similar after intravenous and oral administrations of enrofloxacin (Cester & Toutain, 1997). Complete pharmacokinetic analysis of the time course of these drugs in the eye is not possible as numerous AH samples could not be obtained from one animal due to ethical reasons. Population PK analysis is very useful in situations in which the data are insuffient and when ethical and logistic issues must be taken into account. Therefore, in the present study this approach has been used to describe the intraocular disposition kinetics of systemically administered enrofloxacin and its main metabolite ciprofloxacin in dogs and to investigate the possibility to use a systemic route of administration for treatment of eye infections. Such an approach has been applied in evaluation of disposition of marbofloxacin in dogs (Regnier *et al.*, 2003) and systemically administered ciprofloxacin in humans and rabbits (Drusano *et al.*, 1995; Morlet *et al.*, 2000). In the current study individual estimates were not correlated with other factors that could have influence on pharmacokinetic parameters and the data were analyzed after assuming that enrofloxacin is not metabolized to ciprofloxacin in the eye.

In our investigation, measurable concentrations of enrofloxacin and ciprofloxacin were found in plasma and AH after subcutaneous administration and they were used for estimation of inter-subject variability in a population pharmacokinetics. A large inter-animal coefficient of variation was found for the absorption rate constant for enrofloxacin in plasma and in AH. Large variability in absorption of the parent drug in plasma could be taken as a determinant for large inter-subject variability in plasma concentrations of this drug and in its penetration in AH. A lower variability in absorption rate in AH was computed for ciprofloxacin in comparison to enrofloxacin, which could be explained with the time needed for the metabolic transformation of the parent drug. Despite the high inter-individual variability in concentrations of both drugs in the eye compartment, levels of these drugs could not exceed the safe margin for fluoroquinolones (Thompson, 2007). Volume of distribution and total body clearance could not be discussed without taking into account the influence of bioavailability after extra venous administration. However, the values of population means for these parameters do not differ from previously published data in dogs (Bidgood & Papich 2005). Interindividual variations in enrofloxacin disposition in AH of dogs have to be kept in mind when enrofloxacin is applied in clinical practice. These differences could be partly explained by the function of ABC transporter proteins which have been recently identified in the cells of eye compartments (Dey, 2004; Yang *et al.*, 2007). Moreover, it has been recognized that they played a role in drug disposition in the eye and the ocular bioavailability of their substrates can be significantly enhanced during inflammation and by proper selection of inhibitors of their function (Liu *et al.*, 1998; Dey, 2004; Ghelardi *et al.*, 2004; Yang *et al.*, 2007).

The lower concentrations of enrofloxacin and ciprofloxacin in AH than in plasma indicate limited distribution of these fluoroquinolones in AH after subcutaneous administration of enrofloxacin. Similar concentrations of ciprofloxacin in AH (0.17 μg/mL) were reported by Yu-Speight *et al.* (2005) after topical administration of 0.3% ciprofloxacin solution in dogs. After repeated drug instillation, a 2-fold higher concentration was found in AH. Comparable data were obtained after oral administration of ciprofloxacin in humans (Keren *et al.*, 1991) and after subcutaneous administration in dogs (Haritova *et al.*, 2009). Maximum concentrations in AH did not exceed 0.18–0.22 μg/mL. These levels were marginally higher than MIC_{90} for most of eye pathogens in human patients (Keren *et al.*, 1991). The subcutaneous administration of enrofloxacin to dogs in our study resulted in higher enrofloxacin plus ciprofloxacin concentrations in AH after a single injection in comparison to reported data after topical administration of ciprofloxacin. Similar disposition was observed for marbofloxacin after intravenous administration in dogs (Regnier *et al.*, 2003). Bidgood & Papich (2005) determined the disposition of three fluoroquinolone drugs in interstitial fluid in dogs and found higher concentrations of enrofloxacin in plasma than in interstitial fluid and comparable levels of ciprofloxacin in both fluids. Our data showed that these drugs had a specific distribution in AH and that lower concentrations than these in interstitial fluid could be expected but the absorption rate for AH does not differ from the rate for the other interstitial compartments (Bidgood & Papich, 2005). Moreover, a changed penetration could be expected during inflammation of the eye (Bronner *et al.*, 2003; Regnier *et al.*, 2008). Therefore, proper choice of drugs should be done in each case, taking into account the immune status of the patient, the inflammatory process, the sensitivity of bacterial pathogen and the pharmacokinetic properties of drugs. In veterinary practice additional complication could arise from the unavailability of a wide range of drug formulations registered for use in dogs.

Peak aqueous concentration of enrofloxacin was higher than the MIC_{90} for *Staphylococcus intermedius* and *Escherichia coli* (<0.25 μg/mL) (Cekic *et al.*, 1999; Walker, 2000), but below the MIC_{90} for *Pseudomonas aeruginosa* (0.5–2 μg/mL) (Piddock *et al.*, 1999; Ledbetter *et al.*, 2004; Tolar *et al.*, 2006). Despite that a better antibacterial activity could be expected due to the additive effect of enrofloxacin plus ciprofloxacin combination, it should be acknowledged that these fluoroquinolones could not penetrate to a sufficient extent in AH. At the same time, s.c. administered enrofloxacin showed better penetration in comparison to ciprofloxacin and a similar one with regard to marbofloxacin. These data suggest a potential role of systemic enrofloxacin administration in the prophylaxis or treatment of bacterial infections of the eye caused by susceptible pathogens. It could be used in combination with topical administration of ciprofloxacin in clinical practice. These results should be discussed carefully since the function of blood-aqueous barriers could be altered during the inflammation and changes in penetration profile of enrofloxacin and ciprofloxacin could be expected. The risk of surgical complications such as postoperative endophthalmitis and keratitis accentuates the need for information on pharmacokinetics of both fluoroquinolones in clinical cases and evaluation of their efficacy for prevention and treatment

of potentially sight-threatening ocular infections.

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Paper received 04.01.2011; accepted for publication 03.06.2011

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