

## Pharmacokinetics of enrofloxacin in inbred and outbred rabbits

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### ABSTRACT

The pharmacokinetics of enrofloxacin (ENR) were investigated after intravenous (i/v) administration in inbred (n = 10) and outbred (n = 10) healthy rabbits. After i/v ENR injection the elimination half-life ( $t_{1/2\beta}$ ), total body clearance ( $Cl_B$ ) and the area under the concentration vs. time curve (AUC) in inbred rabbits were 1.51 h,  $28.35 \pm 1.51$  ml/kg/min and  $3.01 \pm 0.16$   $\mu$ g.h/ml, respectively. The value of  $t_{1/2\beta}$  in outbred rabbits was 2.12 h. The value of  $Cl_B$  ( $21.47 \pm 1.16$  ml/kg/min) was significantly lower in outbred rabbits. There were no differences in pharmacokinetic parameters between male outbred and inbred rabbits, and only the value of  $Cl_B$  in female outbred rabbits was significantly lower in comparison to inbred animals. The phenotypic diversity, a manifestation of the complex interaction among the genotype and the environmental factors (the ENR treatment), was clearly less manifested in the inbred group than in outbreds.

**Key words:** enrofloxacin, pharmacokinetics, inbred, outbred, rabbits

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### Introduction

The influence of different factors (species, breed, gender) on the pharmacokinetics of antibacterials in animals is documented in a series of reports. The interspecies differences are well known (NAKAGAWA et al., 1995; PASHOV et al., 1997; RIVIERE et al., 1997; BREGANTE et al., 1999; LIN et al., 2000). Gender can influence antibacterials to a differing extent depending on the drug and the species (LASHEV and MIHAILOV, 1994). There are also some papers which aimed to present breed dependent differences, but they are scars (SALLOVITZ et al., 2002).

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Enrofloxacin (ENR), one of the most frequently used quinolones by veterinarians, is registered for veterinary use in many countries. Its pharmacokinetics have been studied in rabbits and has shown rapid distribution with good penetration in the tissues and relatively slower elimination (BROOME et al., 1991; CABANES et al., 1992; ARAMAYONA et al., 1996; BREGANTE et al., 1999; HARITOVA, 2001). The pharmacokinetics of ENR in inbred and outbred rabbits had not previously been studied. In this connection, the reaction of rabbits to ENR treatment depending on the degree of homozygosity and heterozygosity animals is interesting.

The aim of the present study was to compare the ENR pharmacokinetics in inbred and outbred rabbits after intravenous (i/v) administration.

### Materials and methods

*Animals.* The study was performed on 2 groups of rabbits aged 12 months. The first group comprised 10 inbred animals obtained from the copulation of 1 male rabbit and its 3 sisters - full sibs (inbred group). The second group comprised 10 rabbits that were not a product of inbreeding (outbred group). All animals were in a state of a normal health and had an average body weight of 3.38 kg (2.35 - 4.6 kg). They were housed indoors and fed an antibiotic-free diet (alfalfa hay and a commercially available concentrated grain diet). Water was available *ad libitum*. The injections were administered between 07.00 h and 07.30 h.

*Test drug.* ENR was used as a commercially available solution (2.5 % sterile solution for injection - Syvaquinol<sup>®</sup>, Syva Laboratorios, Leon, Spain, batch N<sup>o</sup> 01/04).

*Treatment.* The rabbits were subjected to one experiment. ENR was administered as an intravenous bolus via the marginal ear vein at a dose rate of 5 mg/kg bw as a bolus.

*Sampling.* Blood samples were collected before drug administration and at the following time after dosing - 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 10 hours. Blood samples after i/v injection of antibacterial agent were collected from the marginal ear vein not used for drug administration.

The blood was allowed to clot at room temperature and the serum was kept in plastic tubes at -28 °C until time of analysis.

*Drug assay.* The serum concentrations of ENR, and the antimicrobial activities associated with ENR and its active metabolite ciprofloxacin were determined using *Escherichia coli* by 14 as a test organism (ARRET et al., 1971). The standard solutions were prepared in serum obtained from untreated rabbits. The limit of quantification for the drug in serum was 0.01 µg/ml. Concentrations of the standard solutions in serum were 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 and 0.01 µg/ml. Assay validation was performed by analyzing replicates of serum fortified (n = 36) with ENR at 10 different

concentrations (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 and 0.01 µg/ml). The mean percentage recovery of ENR from the serum was 94.38 %, with an intra-assay coefficient of variation (CV) of 6.78 and an inter-assay CV of 9.72. The linearity (presented as  $r^2$ ) was 0.9952.

*Pharmacokinetic analysis.* Pharmacokinetic analysis of the data after i/v ENR administration was performed using the non-compartment model.

Pharmacokinetic parameters were calculated by using the Topfit V.2.0 computer program (HEINZEL et al., 1993). The area under serum concentration-time curve (AUC) was calculated by the method of trapezoids and extrapolation to infinity was made.

*Statistical analysis.* Pharmacokinetic parameters for ENR were presented as mean (SEM). The statistically significant differences between pharmacokinetic parameters and serum concentrations were determined using Mann-Whitney U-test. P values <0.05 were considered significant. They were calculated with the Statistica computer program, Statistica for Windows, StatSoft, Inc. 1993. Elimination half-lives were calculated as harmonic mean.

## Results

The serum concentration-time data of ENR following single i/v administration in outbred and inbred rabbits and the pharmacokinetic parameters are presented in Fig. 1 and Table 1, respectively.

Table 1. Pharmacokinetic parameters of enrofloxacin in serum after a single i/v administration at a dose rate of 5 mg/kg in inbred (n = 10) and outbred (n = 10) rabbits (mean ± SEM)

Pharmacokinetic parameters	Inbred	Outbred	P
$\beta$ ( $h^{-1}$ )	0.479 ± 0.029	0.344 ± 0.027	(P = 0.004)
$t_{1/2\beta}$ (h)	1.51 ± 0.11	2.12 ± 0.15	(P = 0.004)
$t_{1/2\beta}'$ (h)	1.45	2.06	
MRT (h)	1.92 ± 0.15	2.31 ± 0.18	
V <sub>ss</sub> (l/kg)	3.17 ± 0.12	2.92 ± 0.22	
V <sub>darea</sub> (l/kg)	3.61 ± 0.16	3.86 ± 0.25	
Cl <sub>B</sub> (ml/kg.min)	28.35 ± 1.51	21.47 ± 1.16	(P = 0.002)
AUC (µg.h/ml)	3.01 ± 0.16	3.99 ± 0.24	(P = 0.002)

$\beta$  - terminal (elimination) rate constant;  $t_{1/2\beta}$  - terminal elimination half-life;  $t_{1/2\beta}'$  - harmonic mean; AUC - area under the serum concentration-time curves for serum; V<sub>darea</sub> and V<sub>ss</sub> - apparent volume of distribution and steady-state volume of distribution; MRT - mean residence time, Cl<sub>B</sub> - total body clearance.

Table 2. Coefficient of variation (%) of pharmacokinetic parameters of enrofloxacin, administered intravenously at a dose rate of 5 mg/kg in inbred (n = 10) and outbred (n = 10) rabbits

Pharmacokinetic parameters	Inbred rabbits	Outbred rabbits
$\beta$ (h <sup>-1</sup> )	19.49	24.87
$t_{1/2\beta}$ (h)	23.56	22.23
MRT (h)	7.81	25.13
V <sub>ss</sub> (l/kg)	12.38	23.38
V <sub>darea</sub> (l/kg)	13.58	20.04
Cl <sub>B</sub> (ml/kg.min)	16.84	17.13
AUC (μg.h/ml)	17.61	19.05

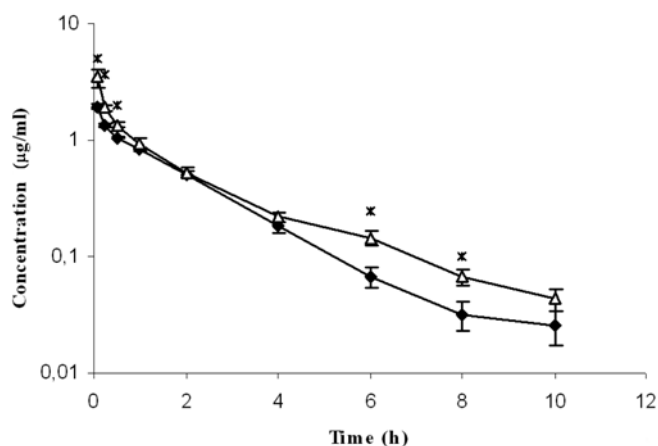


Fig. 1. Mean (SEM) serum concentrations of enrofloxacin in inbred rabbits (n = 10) (◆) and in outbred rabbits (n = 10) (Δ) after i/v administration of 5 mg/kg. \* Statistically significant differences at P<0.05

The value of elimination half-life was significantly longer in outbred rabbits than the  $t_{1/2\beta}$  in inbred animals. A relatively large apparent volume of distribution ( $V_{d_{area}}$ ) was observed in both groups. The value of total body clearance ( $Cl_B$ ) in inbred rabbits was significantly higher in comparison with the value of  $Cl_B$  in outbred animals. The value of AUC in outbred animals was significantly higher than the value of the same parameter in inbred animals. The other pharmacokinetic parameters were not changed significantly. The

values of coefficient of variation of pharmacokinetic parameters had a tendency to increase in outbred compared with inbred rabbits (Table 2). The only exception was nearly equal values of  $t_{1/2\beta}$  in the two investigated groups. Differences in CV were relatively large for values of  $V_{darea}$ ,  $V_{ss}$  and MRT.

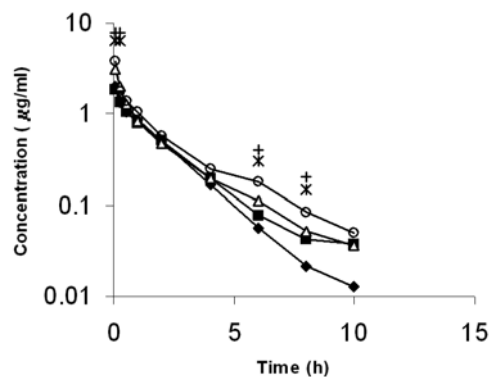


Fig. 2. Mean (SEM) serum concentrations of enrofloxacin in female (n = 5;  $\blacklozenge$ ) and male (n = 5;  $\blacksquare$ ) inbred rabbits and in female (n = 5;  $\triangle$ ) and male (n = 5;  $\circ$ ) outbred rabbits after i/v administration of 5 mg/kg, + - Statistically significant differences at  $P < 0.05$  between inbred and outbred male rabbits

Concentrations of ENR in serum in male outbred and inbred rabbits were significantly higher than in female rabbits from the two investigated groups, respectively (Fig. 2). The value of  $Cl_B$  in outbred female rabbits ( $23.38 \pm 1.15$  ml/kg/min) was significantly lower than the  $Cl_B$  found in inbred female rabbits ( $28.44 \pm 1.05$  ml/kg/min). There were no significant differences in the other pharmacokinetic parameters.

### Discussion

Our data for the elimination half-life after i/v administration of ENR in outbred rabbits were similar to those obtained in outbred animals by HARITOVA, (2001) ( $2.15 \pm 0.11$  h); ARAMAYONA et al. (1996) (1.86 h - harmonic mean); BREGANTE et al. (1999), and CABANES et al. (1992) (from 2.18 to 2.21 h) in New Zealand White rabbits. High values of  $V_{darea}$  suggest that ENR penetrates easily into tissues. Similar data were obtained in outbred rabbits (HARITOVA, 2001) and in New Zealand White rabbits (CABANES et al., 1992; ARAMAYONA et al., 1996; BREGANTE et al., 1999). In our experiments the value of  $t_{1/2\beta}$  was significantly higher in outbred rabbits than in inbred animals because of slower elimination and the lower values of body clearance.

The variability in the pharmacokinetic parameters  $t_{1/2\beta}$ ,  $V_{darea}$  and  $Cl_B$  were very low in Wistar rats: 9.17% 13.8% and 14.9%, respectively (BREGANTE et al., 1999) compared to the same calculation in our experiments. The values of  $V_{darea}$  and  $Cl_B$  in inbred rabbits were closer to the values of CV in Wistar rats than the values of CV in outbred animals. These data allow us to conclude that knowledge about the pharmacokinetic parameters in outbred animals will permit a more valid prediction of the drug's behaviour from the viewpoint of clinical pharmacology. The data obtained from outbred animals could provide information for different subpopulations.

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**SAŽETAK**

Farmakokinetika enrofloksacina istraživana je nakon intravenske (i/v) primjene u zdravih srodnih (n = 10) i nesrodnih (n = 10) kunića. U srodnih kunića poluvrijeme izlučivanja lijeka iz plazme ( $t_{1/2\beta}$ ) iznosilo je 1,51 sat, ukupni klirens lijeka iz organizma ( $Cl_B$ )  $28,35 \pm 1,51$  mL/kg/min, a područje pod koncentracijom u odnosu na vremensku krivulju (AUC)  $3,01 \pm 0,16$  µg/h/mL. Vrijednost  $t_{1/2\beta}$  u nesrodnih kunića bila je 2,12 h. Vrijednost  $Cl_B$  ( $21,47 \pm 1,6$  mL/kg/min) bila je značajno niža u nesrodnih kunića. Nisu ustanovljene razlike u farmakokinetici između srodnih i nesrodnih mužjaka. Jedino je  $Cl_B$  u nesrodnih ženki bio značajno niži u odnosu na vrijednost u srodnih ženki. Fenotipska raznolikost, pokazatelj zamršenog međudjelovanja između genotipa i okolišnih čimbenika (davanje enrofloksacina), slabije se očitovala u srodnih nego u nesrodnih kunića.

**Ključne riječi:** enrofloksacin, farmakokinetika, srodni kunići, nesrodni kunići

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