Pharmacokinetics of Enrofloxacin and its Metabolite Ciprofloxacin after Intracoelomic administration in Tortoises *(Testudo hermanni)*

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ABSTRACT

Enrofloxacin belongs to the fluoroquinolone class of antibiotics. It is commonly used in a variety of reptile species due to its wide spectrum of efficacy, partly due to its formation of an active metabolite ciprofloxacin. Enrofloxacin shows wide disposition variability among all species resulting in large differences in the plasma concentrations of both enrofloxacin and ciprofloxacin. The aim of this study was to evaluate the pharmacokinetics of enrofloxacin and ciprofloxacin after a single intracoelomic injection of 10 mg/kg of enrofloxacin in 9 tortoises (*Testudo hermanni*). Blood samples were collected at 0, 0.5, 2, 4, 10, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240 and 264 h and analyzed using a validated high performance liquid chromatography (HPLC) florescence method. Plasma concentrations of enrofloxacin were quantifiable in all subjects for up to 240 h, while ciprofloxacin was detected in all subjects up to 120 h. The C_{max} (s) of enrofloxacin and ciprofloxacin were 8614 ± 1116 ηg/mL obtained at 2.19 h and 605 ± 43 ηg/mL obtained at 4.23 h, respectively. The values of C_{max}/MIC ratio and AUC_{0-24}/MIC ratio of enrofloxacin with a MIC value of 0.5 μg/mL were 17.23 and 132.78, respectively. In conclusion, an administration of 10 mg/kg of enrofloxacin via the intracoelomic route in *Hermann's* tortoises produced optimal pharmacodynamic parameters.

Keywords: Enrofloxacin; Ciprofloxacin; Pharmacokinetics; Intracoelomic Administration; *Testudo hermanni* Tortoises.

SHORT COMMUNICATION

Fluoroquinolone antibiotics belong to a group of synthetic antimicrobials that are widely used in veterinary medicine. Their spectrum of activity includes Gram positive, Gram negative and Mycoplasma species responsible for a vast array of pulmonary, urinary and digestive infections (1). Enrofloxacin is a prototypical fluoroquinolone, showing treatment efficacy for the major bacterial conditions in several animal species (2, 3). The enhanced efficacy demonstrated by enrofloxacin is due to the formation of an active metabolite, ciprofloxacin which exhibits potency and spectrum of activity similar to that of the parental drug (1). The pharmacokinetics of enrofloxacin following various routes of administration have been investigated in different species of turtles and tortoises with plasma concentrations of enrofloxacin and ciprofloxacin showing wide disposition variability among the species (3-5). Considering tortoise species are more closely related to one another than to other orders of animals, this underlines the importance of conducting pharmacokinetic studies for individual species rather than extrapolating doses from data generated in other reptile species (6).

The treatment of bacterial infections should be based on

a rational scientific approach. The most common pharmacokinetic/pharmacodynamic (PK/PD) approach for antimicrobial agents uses plasma concentration as the PK input value and minimum inhibitory concentration (MIC) as the PD input value (7). Fluoroquinolones are considered to be well-tolerated drugs in both humans and animals, however, their intensive use has led to a significant increase in antimicrobial resistance (1). Therefore, several PK/PD indices such as C_{max}/MIC and AUC_{0-24}/MIC have been included in the present study to evaluate the clinical efficacy of enrofloxacin.

The aim of this study was to evaluate the PK of enrofloxacin and its metabolite ciprofloxacin in *Testudo hermanni* after a single intracoelomic injection of 10 mg/kg enrofloxacin, and to establish if 10 mg/kg is an optimal dosage for treatment of different bacterial infections.

Nine tortoises of undetermined age, including both sexes (five males and four females), with a body weight range from 0.4 to 2.95 kg, were used. The tortoises were housed indoors, divided equally into three glass containers, with access to indirect sun light and heat lamps (UVB 5%). Animals were maintained at 30 to 33°C with 250-W infrared heat lamps suspended 0.5 m above the floor to allow turtles to regulate their own body temperature. Tortoises were conditioned for a 2-week period prior the commencement of the study. Tortoises were judged to be in good health based on physical examinations, normal activity, and routine acceptance of food. These observations were made by specialized veterinary personnel. All the tortoises were fed a mixture of vegetables, and given access to fresh water *ad libitum*.

Animal care and handling was performed according to the provision of the EC council Directive 86/609 EEC. The study protocol was approved by the University of Pisa's ethics committee for animal welfare (CEASA) and transmitted to the Italian Ministry of Health.

Enrofloxacin as the commercial injectable solution (Enrovet® 25mg/mL, Bio98, Milan Italy), was diluted with saline to 10 mg/mL and given as a 10 mg/kg bolus by intracoelomic injection in the left prefemoral fossa using a sterile 22-gauge, 3.75-cm needle. The dose used in the present study was selected based on previous studies in turtles (3, 5, 8). The drug was diluted because a previous study demonstrated that a 10 mg/kg intracoelomic injection of 10 mg/mL in yellow bellied slider turtles did not cause local irritation and soft tissue necrosis (5). These changes did occur when the same dose was used at higher concentrations (25 mg/mL) (9-10).

Blood samples (0.5 mL or 0.25 mL in subjects greater than or less than 0.5 kg body weight, respectively) were collected from the subcarapacial venipuncture site at 0, 0.5, 2, 4, 10, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240 and 264 h after enrofloxacin administration. Although subcarapacial blood collection could be considered suboptimal because of potential lymph contamination, enrofloxacin has been reported to be equally distributed in blood and lymph (7), and thus the pharmacokinetic data were not expected to be affected by sampling method. The blood samples were immediately transferred to tubes containing heparin, centrifuged and stored at -20°C until they were analyzed. Sample analysis was completed within 30 days of collection. The analytical method was based on a previous method using high performance liquid chromatography (HPLC) with a fluorescence detector (5). Pharmacokinetic analysis of enrofloxacin and ciprofloxacin was performed using WinNonlin 5.3.1 software program according to a non-compartmental model.

No adverse effects at the point of injection and no behavioural or health alterations were observed in the animals during or after the study. Some transient, self-resolving side effects such as uncoordinated movements were noticed in an earlier study (5) in yellow bellied slider turtles. Species differences might have triggered this distinction in side effects.

Blood levels of enrofloxacin were quantified in all subjects up to 240 h following injection. Blood levels of ciprofloxacin were detected in all subjects up to 120 h. The semi-logarithmic blood concentration *vs.* time average curves for enrofloxacin and ciprofloxacin are reported in Figure 1. The pharmacokinetic parameters are reported in Table 1.

The mean maximum blood concentration of enrofloxacin (C_{max} 8614.64±1116.36 ηg/mL) was reached at 2.19 h. This value, if normalized for the dose, was within the range of peak concentrations shown in previous studies on intramuscular injection of 5 mg/kg enrofloxacin in Gopher tortoises (2.4 μg/mL) (4) and Indian star tortoises (3.59 μg/mL) (11). Similar trends were noticed also in the ciprofloxacin concentration. The C_{max} of ciprofloxacin was 605.16±43.04 ηg/mL attained at 4.23 h. This value was comparable to that shown in Indian star tortoises (0.35 μg/mL) after intramuscular injection of 5 mg/kg enrofloxacin, if normalized for the dose (11), but higher than that shown in yellow-bellied slider turtles (0.32 μg/mL) following an intracoelomic injection of 10 mg/kg enrofloxacin (5). The values of apparent terminal half-life ($T_{1/2}\lambda z$) of enrofloxacin and ciprofloxacin were 37.00±11.97 h
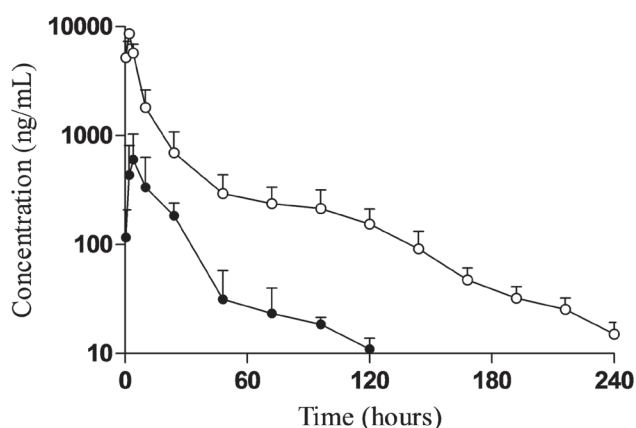
Figure 1: Mean semi-logarithm plasma concentrations of enrofloxacin (-○-) and ciprofloxacin (-●-) *vs* time curves following intracoelomic injection of enrofloxacin (10 mg/kg) in tortoises (n=9). Bars represent the standard deviations.

Table 1: Pharmacokinetic parameters of enrofloxacin and ciprofloxacin after 10 mg/kg enrofloxacin intracoelomic injection in tortoises (*Testudo hermani*) (*n*=9)

Parameter	Units	Enrofloxacin Mean		SD	Ciprofloxacin Mean		SD
r^2		0.99	±	0.01	0.97	±	0.01
λz	1/hr	0.02	±	0.03	0.01	±	0.01
$T_{1/2}\lambda z$	hr	37.00	±	11.97	49.06	±	5.82
T_{max}	hr	2.19	±	0.58	4.23	±	0.93
C_{max}	ng/mL	8614	±	1116	605.16	±	43.04
AUC_{0-24}	hr*ng/mL	66388	±	4647	7952	±	318
$AUC_{0-\infty}$	hr*ng/mL	102123	±	9476	12835	±	1244
Vz/F	mL/kg	5227	±	926	55140	±	443
CL/F	mL/hr/kg	97.92	±	19.98	779.08	±	88.17
$AUMC_{0-\infty}$	hr*hr*ng/mL	3383764	±	42011	412688	±	58959
$MRT_{0-\infty}$	hr	33.13	±	1.69	32.15	±	1.28

r^2 = correlation coefficient.
λz = terminal phase rate constant.
$T_{1/2}\lambda z$ = terminal half-life.
T_{max} = time of peak.
C_{max} = peak plasma concentration.
Vz/F = apparent volume of distribution.
CL/F = apparent clearance.

AUC_{0-24} = area under the plasma concentration-time from 0-24 h curve.
$AUC_{0-\infty}$ = area under the plasma concentration-time from 0 h to infinity curve.
$AUMC_{0-\infty}$ = area under the first moment curve.
$MRT_{0-\infty}$ = mean resident time.

and 49.06 ± 5.82 h, respectively. $T_{1/2}\lambda z$ of enrofloxacin was longer than that reported in earlier studies: Gopher tortoises (23.1 h) (4) and Indian star tortoises (5.1 h) (11). This difference might be due to the different routes of administration (intracoelomic *vs.* intramuscular) and the previously mentioned wide variability in pharmacokinetic parameters among tortoise species. In agreement with this speculation, a recent study involving a 10 mg/kg intracoelomic injection of enrofloxacin in yellow-bellied slider turtles (5) has shown that intracoelomic administration significantly increased the drug $T_{1/2}\lambda z$ compared to the same parameter after intramuscular and oral administration in red-eared slider turtles (8) and after oral administration in Loggerhead sea turtles (3).

The reported MICs of enrofloxacin for most susceptible Gram negative, Gram positive bacteria and Mycoplasma isolated from domestic animals were < 0.1 µg/mL, with some additional moderately susceptible isolates having MICs of 0.125-0.5 µg/mL (4). C_{max}/MIC ratio > 10 and AUC_{0-24}/MIC ratio of 100 and 125, are required for fluoroquinolones to have antibiotic activity and to limit the development of bacterial resistance, respectively (7, 12-13). In the present study, considering a bacterium with a MIC value of 0.5 µg/mL, the C_{max}/MIC ratio of enrofloxacin was 17.23 and the average AUC_{0-24}/MIC ratio was higher (132.78) than the required safety value. In contrast, C_{max}/MIC ratio and AUC_{0-24}/MIC ratio of ciprofloxacin were below the target ranges. These results could be due to the limited extent to which ciprofloxacin is produced in reptiles (<15%), as compared to mammals (35%) (14). This finding is in line

with the low contribution of ciprofloxacin shown in reptiles (5,8). It has been postulated that this minimal presence of ciprofloxacin could be due to the slow metabolism of turtles and tortoises. In fact, cytochrome P450 3A, the enzyme that metabolizes enrofloxacin to ciprofloxacin, has been found to be poorly expressed in reptiles and fish (15-16).

In conclusion, the plasma concentrations of enrofloxacin achieved in this study after intracoelomic administration of 10 mg/kg enrofloxacin are adequate to reach the target end points associated with efficacy of fluoroquinolones in tortoises (*Testudo hermanni*).

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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REFERENCES

1. Giguere, S. and Dowling, P.M.: Fluoroquinolones. In Giguere, S., Prescott, J.F. and Dowling, P.M. (Eds.). Antimicrobial therapy in veterinary medicine. Wiley Blackwell, Oxford UK, pp. 295-314, 2013.

2. Lizodo, M., Pons, M., Gallardo, M. and Estelrich, J.: Physicochemical properties of enrofloxacin. J. Pharm. Biomed. Anal. 15:1845-1849, 1997.

3. Jacobson, E., Gronwall, R., Maxwell, L., Merrit, K. and Harman, G.: Plasma concentrations of enrofloxacin after single-dose oral administration in loggerhead sea turtles (*Caretta caretta*). J. Zoo Wildlife Med. 36:628-634, 2005.

4. Prezant, R.M., Isaza, R. and Jacobson, E.R.: Plasma concentrations and disposition kinetics of enrofloxacin in Gopher tortoises (*Gopherus polyphemus*). J. Zoo Wildl. Med. 25:82-87, 1994.

5. Giorgi, M., Rota, S., Giorgi, T., Capasso, M. and Briganti, A.: Blood concentrations of enrofloxacin and the metabolite ciprofloxacin in yellow-bellied slider turtles (*Trachemys scripta scripta*) after a single intracoelomic injection of enrofloxacin. J. Exotic Pet. Med. 22:192-199, 2013.

6. Isazar, R. and Jacobson, E.R.: Antimicrobial drug use in reptiles. In Giguere, S., Prescott, J.F. and Dowling, P.M. (Eds.). Antimicrobial therapy in veterinary medicine. Wiley Blackwell, Oxford UK, pp. 623-636, 2013.

7. Liu, P., Müller, M. and Derendorf, H.: Rational dosing of antibiotics: the use of plasma concentrations versus tissue concentrations. Int. J. Antimicrob. Agents19. :285-290, 2002.

8. James, S.B., Calle, P.P., Raphael, B.L., Papich, M., Breheny, J. and Cook, R.A.: Comparison of injectable versus oral enrofloxacin pharmacokinetics in red-eared slider turtles, *Trachemys scripta elegans*. J. Herpetol. Med. Surg. 13:5-10, 2003.

9. Maxwell, L. K. and Jacobson, E. R.: Preliminary single-dose pharmacokinetics of enrofloxacin after oral and intramuscular administration in green iguanas (*Iguana iguana*). Houston, TX: Proc. Am. Assoc. ZooVet. 25, 1997.

10. Young, L.A.,Schumacher, J., Papich, M.G. and Jacobson, E.R.: Disposition of enrofloxacin and its metabolite ciprofloxacin after IM injection in juvenile Burmese pythons (*Python molurus bivittatus*). J. Zoo Wildl. Med. 28:71-79, 1997.

11. Raphael, B. L., Papich, M. and Cook, R.A.: Pharmacokinetics of enrofloxacinafter a single intramuscular injection in Indian star tortoises (*Geochelone elegans*). J. Zoo Wildl. Med. 25: 88-94, 1994.

12. Mckellar Q. A., Sanchez Bruni, S.F. and Jones D.G.: Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. J. Vet. Pharmacol. Ther. 27:503-514, 2004.

13. Wright, D.H., Brown, G. H., Peterson, M. L. and Rotschafer, J.C.: Application of fluoroquinolone pharmacodynamics. J. Antimicrob. Chemother. 46:669-683, 2000.

14. Reo, G. S., Ramesh, S., and Ahmad, A.H.: Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intramuscular administration of enrofloxacin in goats. Vet. Res. Commun. 25:197-204, 2001.

15. Ertl, R.P. and Winston, G.W.: The microsomal mixed function oxidase system of amphibians and reptiles: components, activities and induction. Comp. Biochem. Physiol. C Pharmacol. 121:85-105, 1998.

16. Vaccaro, E., Giorgi, M., Longo, V., Mengozzi, G. and Gervasi P.G.: Inhibition of cytochrome P450 enzymes by enrofloxacin in the sea bass (*Dicentrarchuslabrax*). Aquatic Toxicol. 62:27-33, 2003.