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# Pharmacokinetics of enrofloxacin following intravenous administration to greater rheas: a preliminary study

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**Abstract**

The pharmacokinetic behaviour of enrofloxacin (ENR) and its active metabolite ciprofloxacin (CIP) were determined in six greater rheas following a single intravenous (i.v.) dose of 15 mg/kg bw. Plasma concentrations of ENR and CIP were simultaneously determined by a HPLC/u.v. method. Following i.v. administration, the plasma drug concentrations were best fitted by an open two-compartment model with a rapid distribution phase. The high volume of distribution ($V_{ss}$ = 5.01 L/Kg) suggests good tissue penetration. ENR presents a high clearance (3.95 L/kg h) explaining the low AUC values (3.57 mg h/L) and a short permanence ($t_{1/2\beta}$ = 2.66 h and MRT = 1.23 h). Ciprofloxacin comprised 14% of the total fluoroquinolone (ENR + CIP).


*Keywords:* Pharmacokinetic; Enrofloxacin; Ciprofloxacin; Fluoroquinolones; Rhea; Avian

The ratite industry is recent in comparison with poultry. One of the most challenging problems for ratite producers is the high mortality rate of young chicks caused by enteric infections (Navarro and Martella, 2002). Enrofloxacin shows activity against the most significant pathogens in ostriches, including those resistant to β-lactams, tetracyclines, aminoglycosides and macrolides. Since research on antimicrobial therapies in ratite birds has been minimal, the determination of some drug doses for these animals is strictly empirical or based in metabolic scaling (Jensen, 1998). The aim of this study was to determine the pharmacokinetic behaviour of enrofloxacin and its active metabolite ciprofloxacin after a single intravenous (i.v.) administration in young domestic rheas.

The Committee of Ethics in Animal Experimentation of the Faculty of Veterinary U.C.M. approved the work. Six healthy greater rheas (*Rhea americana albescens*) (4 months of age, 2.97 ± 0.26 kg bw), obtained from the experimental breeding farm of Centro de Zoología Aplicada, Universidad Nacional de Córdoba (Argentine), were used. No antibiotics or anthelmintics were administered for at least 2 months prior to starting the study. Enrofloxacin (Baytril® 5%, Bayer AG) was injected intravenously in the brachial vein at a single dose of 15 mg/kg bw. Blood samples (1 ml) were collected from the yugular vein at 0, 5, 10, 15, 30, 45, 60, 90 min, 2, 3, 4, 6, 8, 10, 24 and 48 h after dosing. The samples were protected from light to avoid degradation during all the experiments. The plasma was separated and stored at −20 °C until assay (analyses were performed within 4 weeks after sample collection).

Plasma concentrations of enrofloxacin and its active metabolite 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-

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(1-piperazinyl)-3-quinolinecarboxylic acid (ciprofloxacin) were simultaneously quantified in all samples using high performance liquid chromatography (HPLC/u.v.), according to the previously modified methods (Cester et al., 1996). The plasma samples (300 μL) were added with internal standard (75 μL ofloxacin) mixed (1000 rpm/2 min) and shaken with chloroform (4.5 ml) at 800 rpm/10 min. After centrifugation (at 10 °C, 4000 rpm/7 min.) the organic phase was collected (repose 5 °C/10 min) and dried under nitrogen (<40 °C). The extracted samples was injected directly into the HPLC/uv apparatus (Spectra System® AS1000 autosamplers, Thermo Separation Products, Fl. USA) where there separation was accomplished using an ion-pairing reverse-phase column (PR C-18 5 μm 150 × 4.6 mm. Precolumn: PR C-18 5 μm 15 × 4.6 mm). Mobile phase comprised buffer pH 2.7:methanol:acetonitrile:acetic acid:triethylamine (74:20:4:1:1, v/v/v/v/v) Flow rate: 1 ml/min. Enrofloxacin and ciprofloxacin were detected using ultraviolet spectrophotometry at 279 nm. The limit of quantification (LOQ) was 0.030 mg/L for enrofloxacin and 0.022 mg/L for ciprofloxacin, and the method was linear up to 10 mg/L. The mean percentage recoveries of enrofloxacin and ciprofloxacin from plasma samples were 85.94% and 78.62%, respectively. The inter- and intra-assay reproducibility was below 5%.

Plasma levels of enrofloxacin after i.v. administration were subjected to compartmental analysis using a non-linear least-squares regression analysis with the help of PCnonlin V4.0 software package (Statistical Consultants Inc., Lexintong, USA). Akaike's Information Criterion (AIC), residual sum of squares (Rs) and analysis of residuals' plots were used to discriminate between models. The statistical analysis was performed using the SPSS® 10.0 software package (SAS, Cary, NC, USA).

After i.v. injection, the kinetic behaviour of enrofloxacin (Fig. 1) showed a very rapid initial distribution phase ($t_{1/2\alpha}$ = 0.31 h; Table 1) followed by a fast ($t_{1/2\beta}$ = 2.66 h) elimination phase (two-compartment open model). In ostriches and buzzards, fluoroquinolones are widely distributed (De Lucas et al., 2001; García-Montijano et al., 2001). The high volume of distribution ($V_{ss}$ = 5.01 L/kg) observed in our rheas suggests good tissue penetration, similar to the results observed in young ostriches (3.4 L/kg; De Lucas et al., 2004) and emus (1.49 L/kg; Helmick et al., 1997).

The value of the total body clearance for rheas was high (3.95 L/Kg h), explaining the low $AUC_t$ (3.57 mg h/L) and the short permanence after i.v. administration ($MRT_t$ = 1.23 h). The clearance value obtained in rheas (3.95 L/Kg h) was close to that described in ostriches (4.56 L/Kg h; De Lucas et al., 2004), but it was 10- and 35-fold higher, respectively, than those described in emus (0.36 L/Kg h; Helmick et al., 1997) and chickens (0.13 L/Kg h; Abd El-Aziz et al., 1997). Reasons for these findings are not known, but differences among species in elimination and protein binding are a possible explanation.

The $t_{1/2\beta}$ of enrofloxacin in rheas was longer than that observed in ostriches (0.78 h; De Lucas et al., 2004), and slightly shorter than the one of emus (3.3 h; Helmick et al., 1997), but it was much shorter than that observed in chickens (10.3 h; Anadon et al., 1995). Similar findings have been reported by Clarke et al. (2001) who observed that mean values for elimination half-life and mean residence time of penicillin G were significantly higher in emus than in ostriches.

In our study, the active metabolite of enrofloxacin, ciprofloxacin, shows a low AUC (Table 1) after i.v. administration of enrofloxacin, this value representing

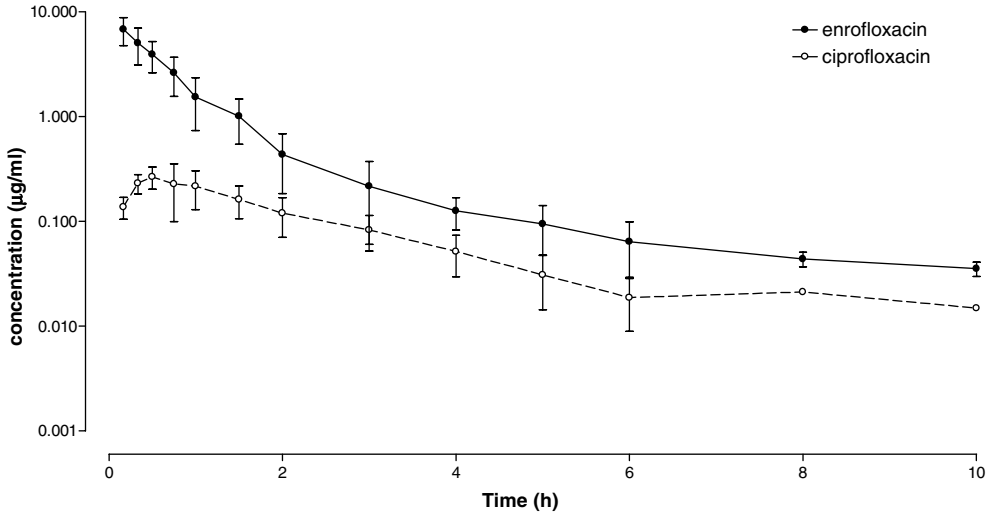


Fig. 1. Mean (±SD) plasma enrofloxacin and its active metabolite ciprofloxacin concentration vs. time, following a single i.v. dose of enrofloxacin (15 mg/kg) in six young greater rheas.

Table 1
Pharmacokinetic parameters (mean ± SD) of enrofloxacin and its active metabolite ciprofloxacin, after a single i.v. administration of enrofloxacin (15 mg/kg bw) to six young greater rheas

| | |
|---|---|
| *Enrofloxacin* | |
| $A$ (mg/L) | 6.95 ± 2.25 |
| $B$ (mg/L) | 0.27 ± 0.09 |
| $t_{1/2\alpha}$(h) | 0.31 ± 0.05 |
| $t_{1/2\beta}$(h) | 2.66 ± 0.46 |
| $V_c$ (L/kg) | 2.22 ± 0.61 |
| $V_{ss}$ (L/kg) | 5.01 ± 1.18 |
| Cl (L/Kg h) | 3.95 ± 1.07 |
| $AUC_t$(mg h/L) | 3.57 ± 1.54 |
| $MRT_t$ (h) | 1.23 ± 0.21 |
| *Ciprofloxacin* | |
| $t_{1/2\beta}$(h) | 1.60 ± 0.57 |
| $AUC_t$(mg h/L) | 0.50 ± 0.22 |
| $MRT_t$ (h) | 1.62 ± 0.40 |
| $T_{max}$ (h) | 0.37 ± 0.14 |
| $C_{max}$ (mg/L) | 0.27 ± 0.07 |

14% of the enrofloxacin AUC. This finding is similar to the results obtained in chickens by Garcıa Ovando et al. (1999). Plasma ciprofloxacin concentrations were generally low, ranging between 0.03 and 0.14 mg/L (Fig. 1). These values are slightly higher than those described in broilers (0.02–0.08 mg/mL) by Knoll et al. (1999) or in ostriches (0.047 mg/mL) by De Lucas et al. (2004), but lower than those observed by Anadon et al. (1995) in broilers (0.47 μg/ml).

Notorious inter-specific differences in the pharmacokinetics behavior of enrofloxacin exist even within the ratite group. The present experiment showed that plasma clearance in the greater rheas was very high. As clearance is the genuine kinetic parameter controlling drug exposure (i.e. AUC), the currently recommended dosage regimen in ratite (5 mg/kg twice daily for 2 days, intramuscularly) (Jensen, 1998) does not allow to achieve the recommended peak value for quinolone in domestic animals (Walker, 2000). Further pharmacokinetic and pharmacodynamic studies should be carried out to establish therapeutic dosages that are effective and safe for the bird and the consumer.

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