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Pharmacokinetics of enrofloxacin after single intravenous and intramuscular administration in young domestic ostrich (*Struthio camelus*)

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Enrofloxacin is a fluoroquinolone with a broad spectrum of antibacterial activity against gram-negative bacteria, some gram-positive bacteria and mycoplasma species (Walker, 2000). Bacterial infections are prevalent in ostriches and can cause significant morbidity and death, particularly in young animals. Enteric infections occur commonly in ostrich chicks. Pathogenic organisms frequently cultured from cases of enteritis include *Clostridium* spp., *Escherichia coli*, *Campylobacter* spp. and *Proteus* spp. Enrofloxacin exerts activity against the most significant pathogens in ostriches, including those resistant to *β*-lactams, tetracyclines, aminoglycosides and macrolides (Shane, 1998).

The lack of pharmacokinetic studies in ostriches obliges the clinician to use empirical approaches based on allometric calculations or to extrapolate doses from other species (Jensen, 1998). However, in previous studies with other drugs, different pharmacokinetic profiles between ostriches and other avian species have been found (Helmick *et al.*, 1997; Clarke *et al.*, 2001; Baert & De Backert, 2003). For this reason, efficacy cannot be guaranteed with allometric calculations or extrapolation, and the risk of therapeutic failure, toxicity and/or bacterial resistance could also be increased. The aim of this study was to determine the pharmacokinetic properties of enrofloxacin after single intravenous (i.v.) and intramuscular (i.m.) administration in domestic ostriches.

The study was performed in five healthy ostriches (6–7 months; 34–53 kg b.w.). No antibiotics or anthelmintics were administered for at least 2 months prior to the start of the study. The Ethics in Animal Experimentation Committee of the Veterinary Faculty (Universidad Complutense de Madrid) gave approval.

The enrofloxacin formulation used was Baytril 10% injectable solution (Batch TA 4734; Bayer AG, Leverkusen, German). Enrofloxacin (Bayer AG: batch: R-177-3), ciprofloxacin (Bayer AG: batch R-124-02) and ofloxacin (Sigma-Aldrich Corporation, St Louis, MO, USA: batch 58H0572) internal standard were used as reference standards for the high performance liquid chromatography (HPLC) analysis.

Ostriches were randomly allocated into two treatment groups. Using a cross-over design, the i.v. and i.m. pharmacokinetics of enrofloxacin in ostriches was determined at 5 mg/kg b.w. dose level. A 2-week washout period was used. No influence of sequence and period effect was observed after ANOVA test evaluation. Enrofloxacin was administered intramuscularly in the iliotrochanterici muscle and i.v. in the brachial vein. Blood samples (2 mL) were drawn from the right jugular vein at 0, 5, 10, 15, 30, 45, 60 and 90 min and 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h after dosing. Plasma was separated and stored at −20 °C until assay.

Plasma concentrations of enrofloxacin and ciprofloxacin were simultaneously quantified using HPLC/u.v. according to a method described by Waxman *et al.* (2000). The limit of quantification (LOQ) was 0.019 μg/mL for enrofloxacin and 0.05 μg/mL for ciprofloxacin, and the method was linear up to 10 μg/mL. The recoveries of enrofloxacin and ciprofloxacin from plasma samples were 80 ± 5.0 and 74 ± 2.4%, respectively. The inter- and intra-assay reproducibility was below 5%.

Plasma concentrations of enrofloxacin after i.v. administration were subjected to compartmental analysis using a nonlinear least-squares regression analysis using PCnonlin V4.0 software package (Statistical Consultants Inc., Lexington, USA). Akaike's Information Criterion (AIC), residual sum of squares (Rs) and analysis of residual's plots were used to discriminate between models. *AUC*, *MRT* and *MAT* were calculated by noncompartmental methods.

The statistical analysis was performed using the SPSS® 10.0 software package (SAS, Cary, NC, USA). The nonparametric Wilcoxon test was used to compare the parameters obtained after i.v. and i.m. administration. A value of $P < 0.05$ was considered significant.

The plasma concentrations vs. time curves of enrofloxacin and ciprofloxacin after i.v. and i.m. administration in young ostrich and the pharmacokinetic parameters are shown in Fig. 1 and Table 1, respectively. The disposition of i.v. administered enrofloxacin in ostriches was best described by a two-compartment model.

After i.m. administration, the absorption of enrofloxacin was relatively rapid (t_{max} = 1.05 ± 0.57 h; *MAT* = 56 ± 15 min). The time required to achieve maximum plasma concentration was similar to values described in chickens (0.79 h, Bugyei *et al.*, 1999), ducks (0.94 h, Intorre *et al.*, 1997) and hawks (1.1 h, Harrenstien *et al.*, 2000) but less than that reported for bustards and owls (1.72 and 2.1 h, respectively; Bailey *et al.*, 1998, Harrenstien *et al.*, 2000). The bioavailability of enrofloxacin in ostriches was high (F = 91 ± 5%) and this was a similar finding to results reported in other avian species, which ranged from 87 to 98% (Abd el-Aziz *et al.*, 1997; Bailey *et al.*, 1998).

Peak plasma concentration of 0.44 ± 0.12 μg/mL was reached after i.m. administration. This value is lower than the value previously reported for broilers 2.1 ± 0.21 μg/mL given the same dose (Bugyei *et al.*, 1999). The lower concentrations obtained are probably a consequence of the high volume of distribution and/or a high clearance in ostriches.

In ostriches, enrofloxacin was widely distributed after i.v. administration. This high volume of distribution (V_{ss} = 3.4 ± 0.41 L/kg) suggests good tissue penetration. The V_{ss} of enrofloxacin in bird species is variable, ranging among 1.49–3.9 L/kg. Some authors have suggested that this variability may be due to differences in protein binding; although, the modest level of protein binding observed in the domestic chicken suggests that protein binding should not significantly affect the therapeutic activity of enrofloxacin (Bugyei *et al.*, 1999).

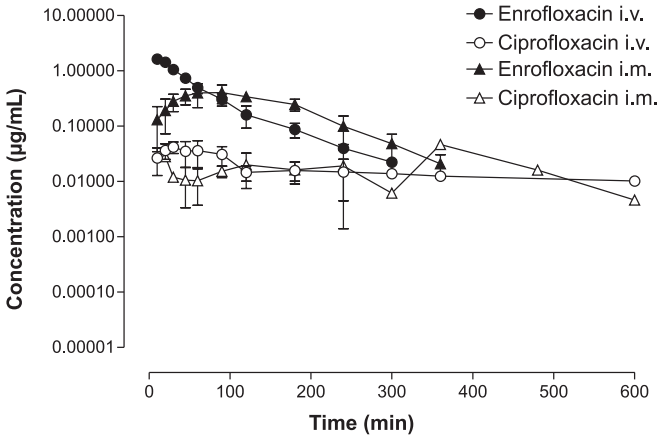

Fig. 1. Mean (±SD) plasma enrofloxacin and its active metabolite ciprofloxacin concentration vs. time following a single i.v. and i.m. dose of enrofloxacin (5 mg/kg) in five young ostriches.

Table 1. Pharmacokinetic parameters (mean ± SD) of enrofloxacin and its active metabolite ciprofloxacin after i.v. and i.m. administration of 5 mg/kg enrofloxacin to five young ostriches

	i.v. Administration	i.m. Administration
Enrofloxacin		
A (μg/mL)	1.5 ± 0.30	
B (μg/mL)	0.49 ± 0.29	
$t_{1/2\alpha}$ (min)	15 ± 4.5	
$t_{1/2\beta}$ (min)	47 ± 3.6	52 ± 22
V_c (L/kg)	2.5 ± 0.20	
V_{ss} (L/kg)	3.4 ± 0.41	
Cl (mL/kg·min)	76 ± 5.3	
AUC_t (μg·h/mL)	0.96 ± 0.10	0.91 ± 0.12
MRT_t (min)	42 ± 7.1	93 ± 21
MAT (min)		56 ± 15
T_{max} (min)		63 ± 34
C_0 (μg/mL)	1.6 ± 0.05	
C_{max} (μg/mL)		0.44 ± 0.12
F (%)		91 ± 4.8
Ciprofloxacin		
MRT_t (min)	103 ± 81	162 ± 108
AUC_t (μg·h/mL)	0.095 ± 0.057	0.078 ± 0.052
T_{max} (min)	26 ± 14	152 ± 131
C_{max} (μg/mL)	0.047 ± 0.010	0.036 ± 0.014

A, intercept of distribution phase; B, intercept of elimination phase of curve; $t_{1/2\alpha}$, distribution phase half-life; $t_{1/2\beta}$, elimination phase half-life; V_c, volume of distribution of the central compartment; V_{ss}, volume of distribution at steady-state; *Cl*, total plasma clearance; AUC_t, area under plasma concentration–time curve from time zero to last time; MRT_t, mean residence time from time zero to last time; *MAT*, mean absorption time; C_{max}, peak drug concentration; C_0, estimated initial (zero-time) drug concentration in plasma; T_{max}, time to C_{max}; from time zero to last time.

Enrofloxacin clearance rate (76 ± 5.3 mL/kg·min) is high, accounting for the low *AUC* (0.96 ± 0.10 μg h/mL) and a short residence after i.v. administration ($t_{1/2\beta}$ = 47 ± 3.6 min and *MRT* = 42 ± 7.1 min). The $t_{1/2\beta}$ of enrofloxacin in ostriches was much shorter than that reported in emus (3.3 h), bustards (5.6 h), broilers (5–10 h approximately) and hawks (19.4 h) (Anadon *et al.*, 1995; Abd el-Aziz *et al.*, 1997; Helmick *et al.*, 1997; Bailey *et al.*, 1998; Harrenstien *et al.*, 2000).

The clearance value obtained in this study was 7–35-fold higher than that described in other avian species such as broilers, emus and bustards, in which clearance ranged from 2.16 to 10.3 mL/kg·min. Similar findings have has been observed by Baert and De Backert (2003) in a comparative pharmacokinetic study of three nonsteroidal anti-inflamatory drugs in five bird species. These authors observed that clearances of flunixin were 0.009 and 0.50 L/kg·h in chickens and ostriches, respectively, and concluded that these nonsteroidal anti-inflammatory drugs (NSAIDs) are rapidly eliminated in most bird species, especially in ostriches. Baert & De Backert (2003) also observed that the half-life, as the most robust parameter for interspecies scaling, showed a negative correlation with the body weight for all drugs studied. This was mainly because the largest species (the ostrich) had the fastest elimination half-life and this

Table 2. PK/PD surrogate markers of enrofloxacin against the most significant pathogens in young ostriches after i.v. and i.m. administration of 5 mg/kg

Pathogen	MIC_{90} (μg/mL)	C_{max}/MIC_{90} i.v.	C_{max}/MIC_{90} i.m.	AUC/MIC_{90} (h) i.v.	AUC/MIC_{90} (h) i.m.
Pasteurella spp.*	0.03	54.42 ± 1.66	14.50 ± 4.12	32.04 ± 3.34	30.42 ± 4.00
*E. coli**	0.06	27.21 ± 0.83	7.25 ± 2.06	16.02 ± 1.67	15.25 ± 2.00
Mycoplasma spp.†	0.1	16.32 ± 0.50	4.35 ± 1.24	9.61 ± 1.0	9.13 ± 1.20
Salmonella spp.‡	0.13	12.56 ± 0.38	3.35 ± 0.95	7.39 ± 077	7.02 ± 0.92

*USP DI-United State Pharmacopeia Drug Information (2000).
†Hannan *et al.* (1997).
‡Salmon and Watts (2000).

contravenes an allometric relationship with weight. The reasons for these findings are not known, but differences between species in elimination and protein binding are possible explanations.

In this study, ciprofloxacin *AUC* was relatively low after i.m. and i.v. administration of enrofloxacin; the value obtained was less than 10% of enrofloxacin *AUC*. This finding supports with the results obtained by García Ovando *et al.* (1999) in chickens. Plasma ciprofloxacin concentrations were found to be generally low, ranging from 0.047 ± 0.010 (i.v.) to 0.036 ± 0.014 (i.m.) μg/mL. Similar results were described by Knoll *et al.* (1999) in chickens (0.02–0.08 μg/mL). However, Anadon *et al.* (1995) observed a high hepatic conversion of enrofloxacin to ciprofloxacin in the chicken (0.43 μg/mL). Our data could indicate limited hepatic conversion, and rapid urinary excretion of this metabolite in ostriches and/or a sequential first pass.

The values obtained for calculated pharmacokinetic/pharmacodynamic ratios (C_{max}/MIC_{90} and AUC/MIC_{90}) are presented in Table 2. MIC_{90} breakpoints of most susceptible pathogens were obtained from turkeys and chickens. These pathogens also are responsible for bacterial infections in ostriches. The data were used because there are no published data for this species (Hannan *et al.*, 1997; Salmon & Watts, 2000; USP DI-United State Pharmacopeia Drug Information, 2000). Fluoroquinolones are active against bacterial pathogens in a concentration-dependent manner. The effective use of the fluoroquinolones against clinically important animal pathogens is dependent on designing dosages that attain serum C_{max}/MIC_{90} ratios of 10:1 or AUC_{0-24}/MIC_{90} ratios of 125:1 (Walker, 2000). According to the data shown in Table 2, a dose of 5 mg/kg, recommended in this species, may not be effective in treating infections in ostriches caused by these pathogens and a dose increase could be necessary to assure efficacy and more particularly, to ensure that resistance emergence is minimized. However the targeted C_{max}/MIC and AUC/MIC ratios suggested have been based on *in vitro* and *in vivo* studies performed with inmunosupressed laboratory animals or on clinical studies involving humans with serious illness. Clinical observations in veterinary patients reveal that a cure is often achieved using standard doses, although we may not achieve these targeted ratios. Perhaps a competent immune system or less serious infections account for this difference between laboratory studies and clinical observations in veterinary medicine (Papich & Riviere, 2001). Moreover, the fact that an agent is effective in many clinical subjects does not mean that the dose is optimal either for bacteriological cure or minimizing opportunities for resistance to occur. The present data suggest that a dosage of 5 mg/mg will be ineffective in the ostrich against all common bacterial pathogens, with the possible exception of *Pasteurella* species.

We conclude that interspecies differences are important in enrofloxacin elimination and emphasize to the risk of extrapolating dosage regimens from between species without regard to pharmacokinetic differences.

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