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RESEARCH ARTICLE

Pharmacokinetics of Enrofloxacin in Emu (*Dromaius novaehollandiae*) Birds After Intravenous and Oral Bolus Administration

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Abstract Pharmacokinetics of enrofloxacin was studied after intravenous and oral bolus administration at 10 mg/kg in healthy emus aged between 18 and 24 months. Blood samples were collected from jugular vein at predetermined time intervals after drug administration. Enrofloxacin and its active metabolite ciprofloxacin in plasma were determined by HPLC. Plasma concentrations versus time were analyzed by a non-compartmental analysis. For i.v. and oral bolus dose of administration, elimination half-life ($t_{1/2\beta}$) was 4.364 ± 0.179 and 4.125 ± 0.361 h, respectively; $AUC_{0-\infty}$ was 20.085 ± 3.493 and 16.056 ± 1.436 μg h/mL, respectively; mean residence time (MRT) was 5.105 ± 0.216 and 6.616 ± 0.475 h, respectively; volume of distribution was 3.921 ± 1.005 and 3.171 ± 0.296 L/kg, respectively and total body clearance was 0.629 ± 0.164 and 0.507 ± 0.003 L/h kg, respectively. Mean absolute bioavailability for enrofloxacin after oral administration was 79.941 ± 7.147 %. The metabolite ciprofloxacin could be detected from 15 min to 24 h following i.v. and oral administration of enrofloxacin. The ratio of AUC_{0-t}cipro/AUC_{0-t}enro was 7.764 and 9.031 %, respectively for i.v. and oral administration of enrofloxacin. The $t_{1/2\beta}$ and MRT of the metabolite were longer than those of parent substance. From the PK/PD indices such as C_{max}/MIC, AUC/MIC and C_{max}/MPC, AUC/MPC study, the recommended doses of enrofloxacin in emu birds were 10 mg/kg body weight once daily for i.v. and oral routes against organisms susceptible to 0.25 and 0.125 μg/mL, respectively. Taking the PAE and active metabolite ciprofloxacin into consideration, it is recommended that enrofloxacin could be used at the dose rate of 10 mg/kg at every 24 h even against the organisms susceptible to 0.5 μg/mL.

Keywords Enrofloxacin · Emu · *Dromaius novaehollandiae* · Ratites · Pharmacokinetics

Abbreviations

β	Elimination rate constant
AUC_{0-t}	Area under the concentration vs. time curve 0 to time
$AUC_{0-\infty}$	Area under the concentration–time curve 0 to infinity
$AUMC_{0-t}$	Area under the first moment curve from 0 to time
$AUMC_{0-\infty}$	Area under the first moment curve from 0 to infinity
MRT	Mean residence time
MAT	Mean residence time
$V_{d\ area}/F$	Apparent volume of distribution after oral administration
$V_{d\ area}$	Apparent volume of distribution
V_{dss}/F	Volume of distribution at steady-state after oral
CL_B	Total body clearance
CL_B/F	Total body clearance after oral administration
$t_{1/2\beta}$	Elimination half life,
C_{max}	Maximum (peak) plasma concentration

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t_{max}	Time of maximum observed concentration in plasma
AF	Absolute bioavailability

Introduction

Enrofloxacin is a fluoroquionolone antimicrobial agent developed solely for use in animals. The relative safety of enrofloxacin, its low minimum inhibitory concentrations (MIC), broad spectrum of activity, long post antibiotic effect (PAE), good tolerance and rapid absorption after parenteral and oral administration resulting in high blood and tissue concentrations have encouraged its use in veterinary medicine. Although enrofloxacin itself is an active antimicrobial, biotransformation to active metabolite ciprofloxacin may occur in some species [1].

Pharmacokinetic studies offer highly relevant information on the time course of the drugs, their metabolites facilitate the computation of optimal dosage regimens of drugs to maintain their therapeutic concentration at the biophase [2]. The pharmacokinetic behaviour of enrofloxacin has been investigated in various animal and bird species including wild animals and aquatic species. But, pharmacokinetics of enrofloxacin remains less well understood in emu birds.

Emu *(Dromaius novaehollandiae)* belongs to ratite group of birds. Bacterial infections are important causes of morbidity and mortality in domestic emu birds [3]. *E. coli* and *Salmonella* sp. was isolated in emu birds reared in India by Kumar et al. [4]. Since research on antimicrobial therapies in ratite birds has been minimal, the determination of some drug doses for these birds is strictly empirical or based on metabolic scaling. Because drug disposition differs among species, extrapolation of dosages from domestic animals may result in sub-therapeutic or toxic level of drug [5]. The computation of an optimal dosage regimen depends on the understanding of the drugs in the target species. Hence, in the current study, it was proposed to investigate the disposition kinetics of enrofloxacin in emus following intravenous and oral bolus dose administration.

Material and Methods

Drugs and Chemicals

Enrofloxacin hydrochloride and ciprofloxacin hydrochloride purchased from Himedia Laboratories Pvt. Ltd., India were utilized for the study. For HPLC analysis, HPLC grade acetonitrile, methanol, triethyl amine and phosphoric acid were purchased from Merck Specialities Ltd., India. Water for HPLC obtained by Millipore water purification system was utilized. All solvents and solutions for HPLC analysis were filtered through 0.2 μ HNN nylon membrane filter (Nupore) and degassed using sonicator. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Preparation of Drug Solution

Enrofloxacin hydrochloride was dissolved in sterile distilled water to prepare 1 % solution for oral administration and 5 % solution for i.v. administration. For all the treatments drug solution was prepared freshly.

Experimental Design

The study was conducted in apparently healthy 8 emu birds (4 male + 4 female) aged 18–24 months with a mean (±SE) body weight of 38.20 ± 1.03 kg. The birds were under uniform conditions of housing (semi intensive system) and feeding, according to the birds requirements. Birds were offered feed and water ad libitum. Before the start of the experiment, the birds were examined clinically to rule out the possibility of any disease. No antibiotics and anthelmintics were administered 2 months prior to the start of experiment. The use of the birds and experimental design was approved by Institutional Animal Ethics Committee (IAEC), TANUVAS, Chennai.

Emu birds were randomly divided into two treatment groups. Using cross over design, the i.v. and oral bolus pharmacokinetics of enrofloxacin in emu birds was determined at a dose of 10 mg/kg body weight. Enrofloxacin was administered intravenously (bolus dose) through the jugular vein. Blood samples (2 mL) were drawn by jugular venipuncture into heparinized tubes immediately before and at 0.083, 0.167, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 18, 24 and 36 h after dosing. After 2 weeks wash out period, the same birds were administered with the same dose of enrofloxacin orally directly using a thin plastic tube attached to a syringe. Then, 2 mL of blood samples were drawn by same method at 0.25, 0.50, 0.75, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48 and 60 h after dosing.

The collected blood samples were centrifuged at $950 \times g$ for 20 min to separate the plasma. The plasma samples were stored at −40 °C until assay.

Drug Assay

Determination of enrofloxacin and ciprofloxacin was performed by high performance liquid chromatography (HPLC). The method developed by Kung et al. [6] was followed.

The HPLC system comprised of LC-20 AD double plunger pump, Rheodyne manual loop injector with a

20 μL loop, column oven CTO-10 AS vp, SPD-M20A diode array detector and a software LC Solution for data analysis. The compound separation was achieved using a reverse phase C18 column (Hibar 250-4, 6 RP-18 end-capped, Particle size 5 μm, 4.6 × 250 mm, Merck, Germany) as a stationary phase. The column was protected with 2 to 8 mm Phenomenax guard column (KJO-4282). The mobile phase consisted of a mixture of acetonitrile, methanol and water (containing 0.4 % phosphoric acid and adjusted to pH 3.0 using triethylamine) in the ratio of 17:3:80 (v/v/v). The flow rate of mobile phase was 1 mL/min and samples were analyzed for 10 min at 40 °C. The scan range of PDA was 220–400 nm, and the detection wavelength was 278 nm. The mean (±SE) retention times for ciprofloxacin and enrofloxacin were 5.65 ± 0.003 and 7.16 ± 0.006 min, respectively.

Enrofloxacin and ciprofloxacin from the plasma were subjected to liquid–liquid extraction according to the method of Nielsen and Hansen [7]. To 0.5 mL of plasma, 0.75 mL of acetonitrile was added in the ratio of 1:1.5. The mixture was vortex-mixed for 15 s and centrifuged for 15 min at 4 °C at a speed of 900×*g*. The clear supernatant was thus obtained (0.5 mL) and twice the volume of HPLC grade water (1 mL) was added in the ratio of 1:2. The aliquot was then filtered through 0.2 μ HNN nylon membrane filter and 20 μL of filtrate was injected into the HPLC system.

Working standards of enrofloxacin (0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 μg/mL) and ciprofloxacin (0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 μg/mL) were prepared from respective stock solutions after diluting with plasma collected from emus. Standard calibration curves were prepared from plasma samples containing known concentrations of enrofloxacin and ciprofloxacin separately.

The standard curves of enrofloxacin and ciprofloxacin were linear in the range of 0.01–10.0 μg/mL. The calibration curve for enrofloxacin was characterized by its regression coefficient ($r^2 = 0.999$), slope (19,070) and intercept (13,182), and was used to determine the analyte concentrations in the sample. The calibration curve for ciprofloxacin was characterized by its regression coefficient ($r^2 = 0.998$), slope (14,777) and intercept (6507.4), and was used to determine the analyte concentrations in the sample.

The concentrations of enrofloxacin and ciprofloxacin in the plasma samples were determined by substituting the respective peak areas/peak heights in the linear regression formula after calibration of standard curves.

Absence of change in the retention time was considered as the method which was found specific and selective. The mean absolute recovery was within the range of 97.778–107.45 % for plasma and the coefficient of variation (CV) was 2.129–7.676 % suggesting the suitability of the method for analysis of enrofloxacin and ciprofloxacin in emu plasma. The intra-day and inter-day CV were within the limits (<10 %) specified (enrofloxacin: 5.307–8.827 %, ciprofloxacin; 4.757–8.632 %) and hence the method was suitable for assay of both enrofloxacin and ciprofloxacin in emu plasma. The limit of detection and quantification were 0.01 and 0.025 μg/mL for enrofloxacin and 0.025 and 0.05 μg/mL for ciprofloxacin, respectively.

Pharmacokinetic Analysis

Pharmacokinetic parameters were derived from concentration vs. time curves obtained for each bird after single i.v. and p.o. administration. Non-compartmental pharmacokinetic analysis was used to describe the pharmacokinetics of enrofloxacin and ciprofloxacin using pharmacokinetic software PK function [8].

The elimination rate constant (β) was calculated from the log-linear portion of the elimination curve using linear regression analysis. The elimination half-life ($t_{1/2\beta}$) was calculated according to $t_{1/2\beta} = \ln 2/\beta$, where, ln2 − 0.693. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated using the trapezoidal rule and extrapolated to infinity by means of the elimination rate constant. The mean residence time (MRT = AUMC/AUC), total body clearance (CL_B = Dose/AUC), volume of distribution to steady state ($V_{dss} = CL_B \times MRT$) and apparent volume of distribution ($V_{darea} = Dose/\beta \times AUC_{0-\infty}$) were calculated after i.v. administration. Comparing the corresponding oral and i.v. route of administration the bioavailability (F) after oral administration was calculated as $F = AUC_{0-\infty}$.(oral)/$AUC_{0-\infty}$ (i.v.) × 100; mean absorption time as MAT = MRToral−MRTi.v.; total body clearance as $CL_B = Dose \times F/AUC_{0-\infty}$; apparent volume of distribution as $V_{darea} = Dose \times F/\beta \times AUC_{0-\infty}$).

Pharmacokinetic/Pharmacodymanic (PK/PD) Integration

The ratios C_{max}/MIC and AUC/MIC; C_{max}/MPC and AUC/MPC were calculated for hypothetical MIC_{90} (0.05, 0.125, 0.25 and 0.5 μg/mL) and MPC (0.2, 0.5, 1.0 and 2 μg/mL) values using the means of C_{max} and AUC obtained in this study.

Statistical Analysis

Statistical analysis of the data was performed by using SPSS 17.0 software. The results were expressed as mean ± SE. Harmonic mean was used with data not distributed normally. Test of significance such as *t* test and analysis of variance (one way ANOVA) were applied to find out difference between and among various groups respectively [9]. Comparison of the means of the different

subgroups was performed by Duncan's multiple range tests as described by Kramer [10].

Results and Discussion

Inter-species differences in the pharmacokinetics behaviour of enrofloxacin existed even within the ratite group at different dosage. From the available published data, it is difficult to decide the proper dosage of enrofloxacin in emus for the different routes of administration. Moreover, the data on pharmacokinetic characteristics of enrofloxacin after oral administration has not been published for emus. Hence, in the present study, pharmacokinetic parameters of enrofloxacin obtained after i.v. and oral administration are used to deduce recommendations for dosages.

The mean (±SE) plasma concentrations of enrofloxacin and its active metabolite ciprofloxacin after i.v. and oral administration of enrofloxacin at 10 mg/kg are depicted graphically in Fig. 1. After i.v. administration, enrofloxacin could be detected up to 18 h in one bird while in seven birds the drug was detected up to 24 h. The highest mean (±SE) concentration was 14.756 μg/mL at 5 min and lowest was 0.054 μg/mL at 24 h. The mean (±SE) values of plasma concentration of enrofloxacin following oral administration of enrofloxacin rapidly increased from 0.591 ± 0.073 μg/mL at 15 min to 2.207 ± 0.098 μg/mL within 1.5 h and then declined to 0.004 ± 0.004 μg/mL at 36 h. Detectable concentrations of enrofloxacin were found up to 24 h in seven birds while in one bird the drug was detected up to 36 h. The plasma concentration of the active metabolite ciprofloxacin was observed from 15 min to 24 h for the both routes of i.v. and oral administration of enrofloxacin.

Comparatively rapid absorption and excellent bioavailability was observed after oral administration in emu birds. The mean (±SE) bioavailability (79.941 ± 7.147 %) after oral administration recorded in this study is in agreement with Bugyei et al. [11] who found 80.1 % in chicken. As compared to the present study, Anadon et al. [12] in domestic fowl (64 %) and Dimitrova et al. [13] in turkeys (69.20 %) reported lesser bioavailability and Dorrestein [14] in pigeons (92 %) found higher bioavailability at the same dose of enrofloxacin. Differences in the anatomy of the digestive system are known to cause marked differences in the rate and extent of drug absorption from the oral route [14]. Retention time of particulate matter in the digestive tract of emus was 5.5 h [15], although some food items were retained commonly for one to 2 days, sometimes over 1 week [16]. Thus relatively slow intestinal transit and comparatively long intestinal tract may be the factors that could increase the absorption of orally administered drugs. The excellent bioavailability noted after oral administration was more favorable to i.v. injection.

After i.v. administration, enrofloxacin showed $AUC_{0-\infty}$ of 20.085 ± 3.493 μg h/mL with large apparent volume of distribution (3.921 ± 1.005 L/kg) (Table 1). The slower elimination half-life (4.364 ± 0.179 h) was observed with the total body clearance (Cl_B) of 0.629 ± 0.164 L/h.kg. After oral administration, enrofloxacin peak plasma concentration (C_{max}) of 2.397 ± 0.052 μg/mL was achieved at (t_{max}) 2.167 ± 0.279 h with high bioavailability of 79.941 ± 7.147 %. After i.v. and oral administration of

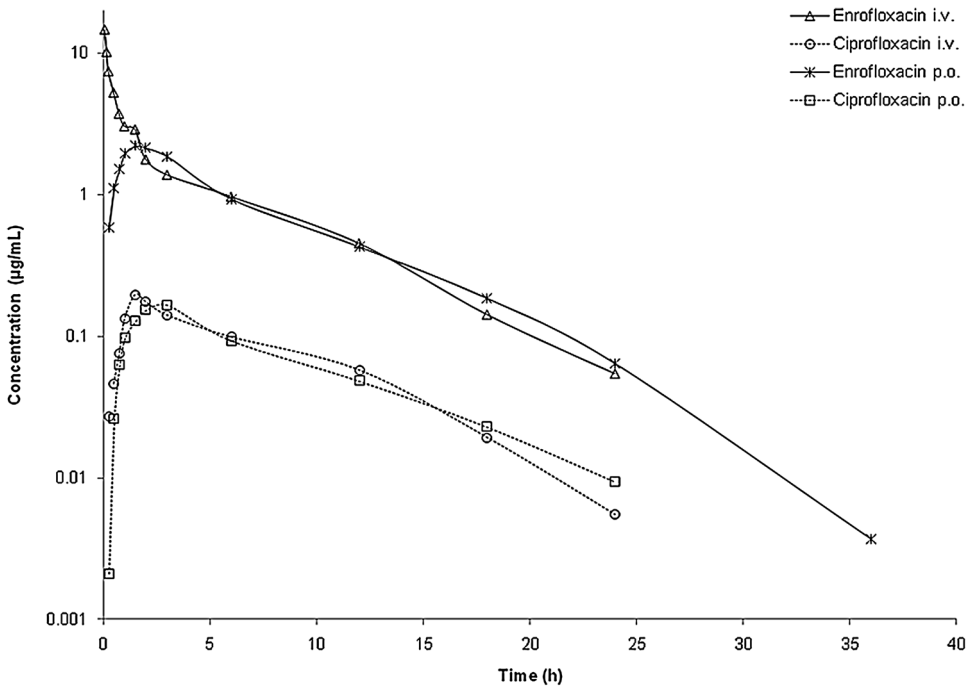

Fig. 1 Semilogarithmic plot of mean plasma concentration of enrofloxacin and its active metabolite ciprofloxacin (μg/mL) versus time in emus (n = 8) following single intravenous and oral administration of enrofloxacin (10 mg/kg)

Table 1 Pharmacokinetics of parameters of enrofloxacin and ciprofloxacin after single intravenous and oral administration of enrofloxacin (10 mg/kg) in emus

Variable	Unit	Routes of administration			
		Intravenous		Oral	
		Enrofloxacin	Ciprofloxacin	Enrofloxacin	Ciprofloxacin
β	h^{-1}	0.159 ± 0.007	0.152 ± 0.006	0.162 ± 0.015	0.129 ± 0.004
AUC_{0-t}	μg h/mL	19.553[a] ± 3.518	1.518 ± 0.258	15.756[b] ± 1.416	1.423 ± 0.130
$AUC_{0-\infty}$	μg h/mL	20.085[a] ± 3.493	1.561 ± 0.262	16.056[b] ± 1.436	1.496 ± 0.128
$AUMC_{0-t}$	μg h^2/mL	90.670 ± 19.068	10.591 ± 2.058	102.756 ± 16.766	10.575 ± 1.106
$AUMC_{0-\infty}$	μg h^2/mL	104.619 ± 19.920	11.889 ± 2.058	109.083 ± 17.395	12.892 ± 1.063
MRT	h	5.105[a] ± 0.216	7.454 ± 0.223	6.616[b] ± 0.475	8.625 ± 0.173
MAT	h	–		1.511 ± 0.475	
$V_{d\ area}/F$	L/kg	–		3.881 ± 0.234	
V_{darea}	L/kg	3.921[a] ± 1.005		3.171[b] ± 0.269	
V_{dss}/F	L/kg	–		4.168 ± 0.191	
CL_B	L/h kg	0.629 ± 0.164	8.256 ± 2.385	0.507 ± 0.003	6.897 ± 0.509
CL_B/F	L/h kg	–		0.646 ± 0.052	
$t_{1/2\beta}$	h	4.364 ± 0.179	4.595 ± 0.163	4.125 ± 0.361	5.393 ± 0.186
C_{max}	μg/mL	–	0.197 ± 0.029	2.397 ± 0.052	0.169 ± 008
t_{max}	h	–	1.417 ± 0.834	2.167[a] ± 0.279	3.167 ± 0.167
AF	%	–		79.941 ± 7.147	
AUC_{0-t} Cipro/AUC_{0-t} Enro		7.764		9.031	

[a, b] means bearing different superscripts differ significantly ($p < 0.05$)

enrofloxacin, the ciprofloxacin AUC_{0-t} was 7.764 and 9.031 % of enrofloxacin AUC_{0-t}, respectively (Table 1). The elimination half life ($t_{1/2}$) and MRT of the metabolite after i.v. and oral administration of enrofloxacin were longer than those of parent substance. The clearance of the active metabolite recorded in this study was faster as compared to the enrofloxacin.

The $t_{1/2\beta}$ of enrofloxacin recorded in this study was similar to values reported for greater rheas [17] and emus [18], but much slower than the results obtained in ostrich [19], whereas, Anadon et al. [12] in chickens, Dimitrova et al. [13] in turkey and Bailey et al. [20] in houbara bustard observed longer elimination $t_{1/2\beta}$. The $t_{1/2\beta}$ obtained in the present study indicates that emu tends to eliminate enrofloxacin slower than ostrich and faster than chickens and turkeys. The elimination half-life had the negative correlation with the body weight for all drugs studied [21]. It might be the reason that emus had the slower $t_{1/2\beta}$ in the current study. Differences between species in elimination and protein binding are other possible explanations. Baert and De-Backert [21] observed that the half-life, as the most robust parameter for interspecies scaling and point to the risk of extrapolating doses and treatments from one species to another without suitable pharmacokinetic data.

The AUC values reported in ostrich [19] and greater rheas [17] were lower while in broiler chicken [22] and houbara bustard [20] were higher as compared to the present study. The differences might be due to the difference in anatomy, dosages and species. The mean residential time reported in the present study is comparatively higher than the values found in greater rheas [17], ostrich [19] and lower than broiler chicken [12] and turkeys [13]. From these data, it appears that the persistence of enrofloxacin is longer in emus as compared to other ratite species.

The large volume of distribution obtained after i.v. and oral dosing in this study indicated good tissue penetration of enrofloxacin in emus. It is in agreement with Walker [23] who explained fluroquinolones, in general, have excellent tissue penetration as reflected by high V_{darea} in the present study. Tissue distribution studies in domestic fowls and pigeons have shown that most of organs contained higher concentrations of enrofloxacin than the corresponding blood concentrations [12, 24, 25]. Ultimately, tissue distribution studies in emus would be needed to complement plasma pharmacokinetic investigations in order to assess the distribution of enrofloxacin to the major organs. As compared to the present study value of V_{darea}, Abd-El-Aziz et al. [22] found lesser values (2.17 L/kg) in chicken while De-Lucas et al. [17] observed higher values (5.01 L/kg) in greater rheas. Bugyei et al. [11] suggested that this variability might be due to differences in protein binding. As compared to the total body clearance (Cl_B)

Table 2 Pharmacokinetic/pharmacodynamic parameters of enrofloxacin considering MICs of 0.05, 0.125, 0.25 and 0.5 μg/mL

Ratio	MIC (μg/mL)	Intravenous	Oral
C_{max}/MIC	0.05	295.11 ± 44.52[a]	47.94 ± 1.04
	0.125	118.04 ± 17.81[a]	19.17 ± 0.42
	0.25	59.02 ± 8.90[a]	9.59 ± 0.21
	0.5	29.51 ± 4.45[a]	4.79 ± 0.10
AUC_{0-24}/MIC	0.05	391.06 ± 70.35	315.11 ± 28.31
	0.125	158.42 ± 28.14	126.05 ± 11.32
	0.25	79.21 ± 14.07	63.02 ± 5.66
	0.5	39.11 ± 7.03	31.51 ± 2.83

[a] For C_{max}, a value of 14.755 μg/mL (mean peak plasma concentration at 5 min) was used for the calculation

Table 3 Pharmacokinetic/pharmacodynamic parameters of enrofloxacin considering MPCs of 0.2, 0.5, 1.0 and 2 μg/mL

Ratio	MIC (μg/mL)	Intravenous (enrofloxacin)	Oral (enrofloxacin)
C_{max}/MPC	0.2	73.78 ± 11.13[a]	11.98 ± 0.26
	0.5	29.51 ± 4.45[a]	4.79 ± 0.10
	1	14.76 ± 2.23[a]	2.40 ± 0.05
	2	7.38 ± 1.11[a]	1.20 ± 0.03
AUC_{0-24}/MPC	0.2	97.76 ± 17.59	78.78 ± 7.08
	0.5	39.11 ± 7.03	31.51 ± 2.83
	1	19.55 ± 3.52	15.76 ± 1.42
	2	9.78 ± 1.76	7.88 ± 0.71

[a] For C_{max}, a value of 14.755 μg/mL (mean peak plasma concentration at 5 min) was used for the calculation

recorded in this study, faster clearance of enrofloxacin was found in ostrich (76 mL/kg.min; at a dose of 5 mg/kg) by De-Lucas et al. [19] and in greater rheas (3.95 L/kg h) by De-Lucas et al. [17]. The Cl_B values for chickens (4.8 mL/min kg); [12] and houbara bustard (5.7 mL/min kg); [20] are comparatively lesser than the result obtained in the present study. Cox et al. [26] explained that the clearance and volume of distribution were proportional to body weight. In agreement with this statement, the clearance and volume of distribution obtained in this study are high as compared to other avian species with less body weight.

The degree of metabolism varies considerably across species [26]. In the present study, ciprofloxacin AUC_{0-t} was lower than 10 % of enrofloxacin AUC_{0-t} after i.v. and oral administration of enrofloxacin. Similar results were obtained by De-Lucas et al. [19] in ostrich. Helmick et al. [18] reported that the plasma concentration of metabolite ciprofloxacin was not consistent in emus. However, Anadon et al. [12] observed a high hepatic conversion of enrofloxacin to ciprofloxacin in the chicken. The difference between the ratio of AUC_{0-t} cipro/AUC_{0-t} enro after i.v. and oral administration were 7.764 and 9.031, respectively which indicated limited, but rapid conversion of ciprofloxacin in the liver of emu birds.

The PK/PD integration parameters of C_{max}/MIC and AUC_{0-24}/MIC were calculated from the obtained PK parameters are presented in Table 2. The pharmacodynamic ratios of mutant prevention concentration (C_{max}/MPC and AUC_{0-24}/MPC) were also determined from the obtained PK parameters which are given in Table 3.

Enrofloxacin are concentration-dependent killing agents. In addition to this, enrofloxacin exert long PAE, could well be one of the guiding factors in the optimization of dosage schedule. Fluoroquinolones exert a PAE of 4–8 h against a number of strains including *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [27] and PAE in vivo is generally longer than PAE in vitro due to post-antibiotic sub-MIC effect (PASME) and the post-antibiotic leukocyte enhancement (PALE) exerted in vivo [23].

Taking the above factors into consideration, several workers have proposed that AUC/MIC and C_{max}/MIC ratios are the best indicators for good clinical outcome. It is well established that plasma C_{max}/MIC > 8 and AUC/MIC > 100 are required for efficient and optimal pharmacotherapy of enrofloxacin [28]. In the current study, the C_{max}/MIC and AUC/MIC ratios suggested that the enrofloxacin administration at 10 mg/kg through i.v. route was effective against the organisms susceptible to MIC of 0.25 μg/mL while, the oral dosing was effective against the organisms susceptible to MIC of 0.125 μg/mL.

The mutant prevention concentration (MPC), a concept meant to face the increased prevalence of antibiotic resistance was used to calculate C_{max}/MPC and AUC/MPC. According to Drlica [29] the C_{max}/MPC_{90} and AUC/MPC_{90} ratios for enrofloxacin were 1.4 and 39, respectively, were found protective against the selection of resistant mutants of *E. coli*. From this C_{max}/MPC and AUC/MPC ratios of present study, administration of enrofloxacin through i.v. route was most useful in preventing resistance as compared to oral route of administration.

From these PK/PD results, it is obvious that use of enrofloxacin administration at 10 mg/kg through i.v. and oral route is able to produce an ideal clinical outcome against pathogens susceptible to 0.25 and 0.125 μg/mL, respectively. However, these derived values do not take into account the contribution made by the active metabolite ciprofloxacin, and therefore underestimate enrofloxacin efficacy.

Conclusion

From the present study, it can be concluded that enrofloxacin pharmacokinetic parameters after i.v. and oral bolus administration in emus at 10 mg/kg are characterized

by high volume of distribution, slower terminal elimination half life and high bioavailability. Based on the PK/PD study, the recommended doses of enrofloxacin in emu birds were 10 mg/kg body weight once daily for i.v. and oral routes against organisms susceptible to 0.25 and 0.125 μg/mL, respectively. Taking the active metabolite ciprofloxacin and PAE into consideration, it is recommended that enrofloxacin could be used at the dose rate of 10 mg/kg at every 24 h even against the organisms susceptible to 0.5 μg/mL.

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Conflict of interest The authors declare no conflict of interest.

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