THE PLASMA KINETICS AND TISSUE DISTRIBUTION OF ENROFLOXACIN AND ITS METABOLITE CIPROFLOXACIN IN THE MUSCOVY DUCK

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ABSTRACT

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The disposition and tissue distribution of enrofloxacin and of its main metabolite ciprofloxacin were investigated in ducks after oral or intramuscular administration of a single dose of 10 mg/kg enrofloxacin. Plasma and tissue concentrations were determined by a HPLC method. The peak concentrations of enrofloxacin after intramuscular administration (1.67 µg/ml at 0.9 h) were higher than after an oral dose (0.99 µg/ml at 1.38 h). The relative bioavailability of enrofloxacin after administration directly into the crop was 68%, while the metabolic conversion of enrofloxacin to ciprofloxacin was quite low (< 10%) with both routes of administration. High tissue concentrations and high tissue:plasma concentration ratios were demonstrated for enrofloxacin and ciprofloxacin 24 h after treatment. It was concluded that a dose of 10 mg/kg per day provides serum and tissue concentrations sufficiently high to be effective in the control of many infectious diseases of ducks.

Keywords: antibiotic, ciprofloxacin, distribution, enrofloxacin, Muscovy ducks, pharmacokinetics

Abbreviations: AUC, area under the concentration–time curve; AUMC, area under the first moment curve; Cl, total body clearance; C_{max}, plasma peak concentration; $C_{ss(max,min,av)}$, concentration at steady-state (maximum, minimum, average); HPLC, high-performance liquid chromatography; K_{el}, terminal elimination rate constant; i.m., intramuscular administration; MRT, mean residence time; p.o., oral; T_{max}, time to reach the peak concentration; $V_{d(area)}$, apparent volume of distribution

INTRODUCTION

Enrofloxacin (1-cyclopropyl-7-(ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid) is a fluorinated quinolone carboxylic acid derivative which was developed exclusively for use in animals (Altreuther, 1987; Chu and Fernandes, 1989).

The drug is well absorbed in a variety of domestic animals after either oral or parenteral administration (Scheer, 1987a, 1990; Dorrestein and Verburg, 1990; Flammer *et al.*, 1991; Cabanes *et al.*, 1992; Kung *et al.*, 1993; Froyman *et al.*, 1994; Anadon *et al.*, 1995). The high bioavailability and the good tissue penetration of

enrofloxacin result in tissue concentrations equal to or significantly higher than those achieved in serum (Scheer, 1987a, 1990; Anadon et al., 1995). The drug is partially metabolized in the liver to ciprofloxacin, a primary metabolite which is itself a potent antimicrobial agent used in human therapy (Bergan et al., 1988; Tyczkowska et al., 1989). Renal excretion is the major route of elimination by both filtration and tubular excretion (Hooper and Wolfson, 1991).

Enrofloxacin is a broad-spectrum antibiotic active against both Gram-negative (*Escherichia coli*, *Salmonella* spp., *Haemophilus* spp., *Pasteurella* spp.) and Gram-positive bacteria (*Streptococcus* spp., *Staphylococcus* spp., *Clostridium* spp., *Erysipelothrix rhusiopathiae*) and also against mycoplasmas at very low concentrations (MIC range of 0.01–0.5 µg/ml) (Scheer, 1987b, 1990; Bauditz, 1990). These antimicrobial properties indicate that this antibiotic might be suitable in avian medicine (Stipkovits, 1988; Bauditz, 1990). Enrofloxacin is currently available in a variety of formulations marketed as medicaments to be given in drinking water to poultry (trade name Baytril, Bayer, AG).

Pasteurellosis and respiratory colibacillosis are very common infectious diseases which are responsible for high rates of mortality and morbidity in many avian species, including ducks, with important economic implications for breeding. Although enrofloxacin may be effective in such infections, its kinetics and tissue distribution in ducks have not been fully investigated. Studies have been reported on the kinetics of enrofloxacin in poultry (Scheer, 1987a; Anadon et al., 1995) and homing pigeons (Dorrestein and Verburg, 1990). However, as species differences in absorption and disposition of drugs can vary widely (Baggot, 1992), pharmacokinetic studies in each target species are needed in order to refine the required dosages and administration rates for each species.

The aim of the present study was to investigate the plasma kinetics and tissue distribution of enrofloxacin and its metabolite ciprofloxacin after single intramuscular or oral administration of enrofloxacin in the duck.

MATERIALS AND METHODS

Animals

Sixteen 14-week-old male Muscovy ducks (*Cairina moschata*) weighing 3.5–4.2 kg were obtained from a commercial farm. On arrival, the birds were placed in individual cages and an acclimatization period of 1 week was allowed before starting the treatment. During this period their health status was checked by daily observations without any clinical signs of disease being seen. Food and water were supplied *ad libitum* except on the day of experiment, when food was withheld overnight. Animal care and handling were performed according to the provisions of the EC Council Directive 86/609 EEC, recognized and adopted by the Italian Government (DL 27/1/1992, no. 116).

Study design

On the day of the treatment, the birds were divided into two groups of 8 ducks each. Those in the first group were given a single i.m. injection of 10 mg/kg enrofloxacin (Baytril 2.5%, Bayer, Milan, Italy) into the leg muscle. The birds in the second group received a single p.o. dose of 10 mg/kg enrofloxacin (Baytril 0.5%, Bayer, Milan, Italy) into the crop by means of a feeding tube and a syringe.

Sampling

Serial blood samples were collected in heparinized syringes from the cutaneous ulnar or medial metatarsal veins of each bird at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after treatment. After the last blood sample had been collected, each duck was euthanized by electronarcosis followed by exsanguination. Tissue samples were taken from the liver (the entire organ), kidney (the entire organ), muscle (pectoral) and skin (50 g in natural proportions). The blood samples were centrifuged at 3000 rpm for 10 min within 1 h after sampling; the plasma was collected and stored at –20°C until assayed. Tissue samples were packaged and frozen at –20°C pending analysis.

Analytical method

Plasma and tissue concentrations of enrofloxacin and its metabolite ciprofloxacin were assayed by a HPLC method. The system consisted on a Reodyne 7125 injector, a Jasco 880 PU pump, a Jasco 821 FP fluorescence detector set at 278 nm and 440 nm as excitation and emission wavelengths respectively and a Spectra Physics SP 4270 integrator. The isocratic mobile phase consisted of a phosphate buffer–acetonitrile (85:15, v/v) solution, adjusted to pH 3 with triethylamine. Separation was achieved with an Ultropac Lichrosolv RP 18/5 column (LKB, Bromma, Sweden). The flow rate was 0.9 ml/min. The external standards were enrofloxacin and ciprofloxacin (Bayer, Milan, Italy). For both enrofloxacin and ciprofloxacin, the assay was linear between 0.01 and 10 µg/ml or µg/g; the detection limit was 0.01 µg/ml or µg/g; the recovery was 91% and 82% in plasma and 70% and 65% in tissues for enrofloxacin and ciprofloxacin, respectively. Extraction of the compounds from plasma was achieved with 1% acetic acid in ethanol. After incubation for 10 min at room temperature, the samples were centrifuged for 15 min at 3000 rpm. The supernatant was dried using a vortex evaporator (Rotavapor RE 111 and Water Bath 461, Buchi, Switzerland), reconstituted in 1 ml of the mobile phase and, after filtration through a 0.45 µm filter (Sartorius, Gottingen, Germany), 50 µl was injected onto the HPLC system. Extraction of the compound from tissues was achieved with 20 ml of 10% acetic acid in ethanol. After incubation for 10 min at room temperature, 5 g of each tissue sample was homogenized (T 25 Top Ultra Turrax, IKA, Staufen, Germany) and centrifuged for 15 min at 3000 rpm. The supernatant was transferred into a clean centrifuge tube, while the residue was re-extracted with a further 20 ml of 10% acetic acid in ethanol. The combined

supernatants were dried using a vortex evaporator, reconstituted in 2 ml of the mobile phase and, after filtration through a 0.45 μm filter, 50 μl was injected onto the HPLC system.

Pharmacokinetic analysis

The individual plasma enrofloxacin concentration–time data were analysed using non-compartmental analysis based on statistical moment theory (Gibaldi and Perrier, 1982). A regression line was fitted to the data for the terminal phase using the Easy-Fit (Mario Negri Institute, Milan, Italy) program for a Macintosh computer. The K_{el} was then obtained from the slope of the regression line. The program calculates the AUC and the AUMC using the linear trapezoidal rule and extrapolation to infinite time. From these data, values were calculated for MRT, $V_{d(area)}$ and Cl. Predicted drug concentrations following multiple dosing were calculated using the principle of super-position (Gibaldi and Perrier, 1982).

Statistical analysis

The results are expressed as mean values $\pm$ SE. Comparisons among the mean values were performed by one-way analysis of variance for independent samples. When a significant overall difference was detected, a Dunnett test was used (Winer, 1971); $p < 0.05$ was considered to be significant.

RESULTS

Plasma concentration–time profiles and pharmacokinetic parameters for enrofloxacin and ciprofloxacin after a single i.m. or p.o. dose of 10 mg/kg of enrofloxacin are shown in Figure 1 and in Tables I and II. The concentrations of enrofloxacin and ciprofloxacin in the tissues are shown in Table III.

Enrofloxacin was rapidly absorbed after both i.m. and p.o. administration. Plasma enrofloxacin concentrations in birds which had been dosed i.m. were constantly higher than after a p.o. dose. The enrofloxacin concentrations in the plasma peaked soon after i.m. administration and then declined with MRTs of 8.35 ± 0.65 h and 6.01 ± 0.54 h, reaching 0.05 ± 0.01 μg/ml and 0.02 ± 0.01 μg/ml 24 h after i.m. and p.o. dosing, respectively (Table I). The relative oral availability of the drug, calculated as the ratio between the AUCs, was 68%. The values for AUC, MRT, C_{max} and Cl differed significantly following i.m. and p.o. dosing (Table I).

Ciprofloxacin concentrations rose rapidly after administration of enrofloxacin and remained above the detection limit for at least 12 h after both p.o. and i.m. administration (Table II). The ciprofloxacin concentrations paralleled those of enro-floxacin but they were always lower than those of the parent drug, after both i.m. and p.o. dosing. The ciprofloxacin:enrofloxacin ratio increased progressively during the
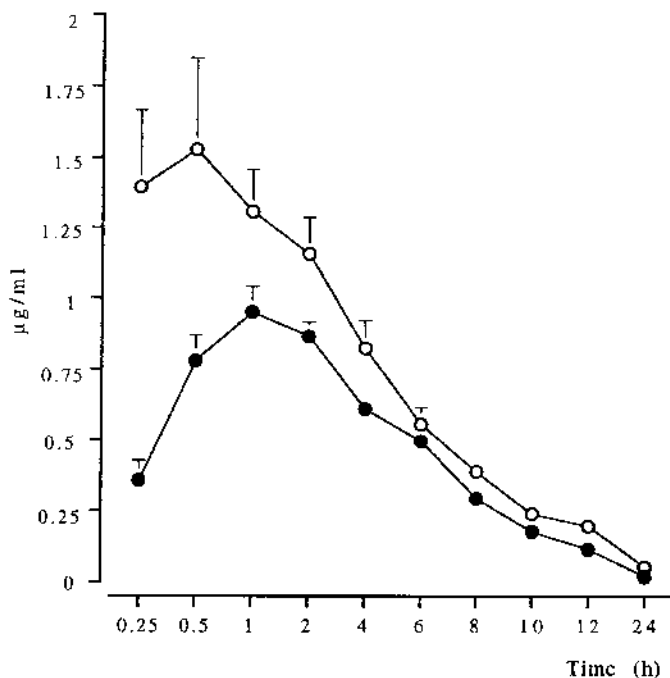
Figure 1. Plasma concentrations of enrofloxacin following a single p.o. (●) or i.m. (○) dose of 10 mg/kg enrofloxacin in ducks. Values expressed as mean ± SE (n = 8)

experiment, reaching 0.12 at 10 h (i.m.) and 0.22 at 12 h (p.o.). The plasma concentrations of ciprofloxacin, calculated as the ratio between the AUCs of ciprofloxacin and enrofloxacin, accounted for 8.9% (p.o.) and 7.2% (i.m.) of the parent drug.

Enrofloxacin and its metabolite ciprofloxacin were also present in the tissue samples collected at 24 h. The highest average tissue concentrations for ciprofloxacin and enrofloxacin combined after either i.m. or p.o. administration of enrofloxacin were found in the kidney. The tissue concentrations of the drugs after either p.o. or i.m. administration were graded as kidney > liver > muscle > skin > plasma (Table III). The concentrations of ciprofloxacin exceeded those of enrofloxacin in the skin, muscle and liver after the p.o. dose but only in the kidney after i.m. administration. The mean combined tissue concentrations for enrofloxacin and ciprofloxacin also differed significantly following the p.o. and i.m. doses, but there were no significant differences in the tissue:plasma drug concentration ratios following i.m. or p.o. dosing (Table III). The predicted steady-state concentrations of enrofloxacin in the serum, based on the mean pharmacokinetic parameters determined in the present study, are presented in Table I.

TABLE I

Mean pharmacokinetic values ($\pm$ SE) for enrofloxacin following a single i.m. or p.o. dose of 10 mg/kg enrofloxacin in ducks ($n = 8$)

	Route	
	i.m.	p.o.
AUC (μg.h/ml)	10.11 ± 0.87	6.65 ± 0.44*
MRT (h)	8.35 ± 0.65	6.01 ± 0.54*
T_{max} (h)	0.94 ± 0.18	1.38 ± 0.18
C_{max} (μg/ml)	1.67 ± 0.29	0.99 ± 0.08*
K_{el} (h^{-1})	0.16 ± 0.02	0.18 ± 0.01
$V_{d(area)}$ (L/kg)	7.45 ± 1.76	8.89 ± 1.04
Cl (L/kg/h)	1.03 ± 0.07	1.56 ± 0.14*
$C_{ss(max)}$ (μg/ml)	1.73	1.00
$C_{ss(min)}$ (μg/ml)	0.05	0.02
$C_{ss(av)}$ (μg/ml)	0.42	0.27

*$p < 0.05$ for differences between the i.m. and p.o. routes

TABLE II

Mean plasma concentrations (μg/ml $\pm$ SE) of enrofloxacin (EF) and ciprofloxacin (CF), following single i.m. or p.o. doses of 10 mg/kg of enrofloxacin ($n = 8$)

	Route			
	i.m.		p.o.	
Time (h)	EF	CF	EF	CF
0.25	1.39 ± 0.27	–	0.36 ± 0.07	–
0.5	1.53 ± 0.32	0.04 ± 0.01	0.78 ± 0.09	0.04 ± 0.00
1	1.31 ± 0.15	0.06 ± 0.01	0.95 ± 0.09	0.04 ± 0.00
2	1.16 ± 0.13	0.07 ± 0.01	0.87 ± 0.05	0.05 ± 0.00
4	0.82 ± 0.10	0.07 ± 0.01	0.61 ± 0.04	0.06 ± 0.01
6	0.56 ± 0.06	0.06 ± 0.01	0.50 ± 0.03	0.05 ± 0.00
8	0.39 ± 0.03	0.04 ± 0.00	0.29 ± 0.03	0.03 ± 0.00
10	0.24 ± 0.02	0.03 ± 0.00	0.18 ± 0.03	0.02 ± 0.00
12	0.19 ± 0.01	0.02 ± 0.00	0.11 ± 0.02	0.02 ± 0.00
24	0.05 ± 0.01	–	0.02 ± 0.00	–

TABLE III

Mean tissue concentrations (μg/g ± SE at 24 h) of enrofloxacin (EF), ciprofloxacin (CF), and tissue:plasma ratios (T/P) following a single i.m. or p.o. dose of 10 mg enrofloxacin/kg in ducks (n = 8)

	Skin			Muscle			Liver			Kidney		
	EF	CF	T/P	EF	CF	T/P	EF	CF	T/P	EF	CF	T/P
Intramuscular route												
	0.18 ± 0.03	0.09 ± 0.02	5.60 ± 0.79	0.16 ± 0.04	0.12 ± 0.02	6.22 ± 1.40	0.41 ± 0.09	0.31 ± 0.04	17.10 ± 4.06	0.48 ± 0.10	0.66 ± 0.06	25.28 ± 4.02
Oral route												
	0.03* ± 0.01	0.07 ± 0.02	7.47 ± 2.10	0.05* ± 0.02	0.06* ± 0.01	6.93 ± 1.34	0.08* ± 0.04	0.24 ± 0.06	22.71 ± 5.43	0.34 ± 0.08	0.32 ± 0.10	46.86 ± 14.93

*p < 0.05 for differences between the i.m. and p.o. routes

DISCUSSION

The study showed that enrofloxacin is well absorbed and widely distributed throughout the body following the administration of a single p.o. or i.m. dose of 10 mg/kg in ducks.

Enrofloxacin is rapidly absorbed by both the p.o. and i.m. routes; its kinetics after i.m. administration were characterized by a greater degree of absorption and a slower rate of elimination than after it was given by the oral route, as shown by the differences in the pharmacokinetic parameters relating to bioavailability, C_{max}, MRT and Cl. The C_{max} achieved with a 10 mg/kg p.o. dose in the present study (1.05 µg/ml for enrofloxacin and ciprofloxacin combined), was lower than that observed with the same dosage of enrofloxacin in broilers by Scheer (1987a) (1.4 µg/ml) and Anadon et al. (1995) (2.44 µg/ml) and in homing pigeons (3.62 µg/ml) by Dorrestein and Verburg (1990). These differences may be explained by variations in drug formulation and/or in drug disposition due to differences in the anatomy and physiology of the digestive system and in the metabolic transformation and drug distribution among avian species (Dorrestein, 1991).

As previously observed in both mammalian (Tyczkowska et al., 1989; Richez et al., 1994) and non-mammalian (Flammer et al., 1991; Anadon et al., 1995) species, enrofloxacin is also partially metabolized to ciprofloxacin in ducks. Although the extent of this metabolic conversion was quite low in the present study (< 10% after either p.o. or i.m. dosing), it could have therapeutic importance because ciprofloxacin contributes to the in vivo activity of enrofloxacin (Flammer et al., 1991; Brown, 1996). Indeed, studies on the MICs and kinetics of bacterial killing have shown that ciprofloxacin is more active than enrofloxacin against the respiratory pathogen *Pasteurella multocida* (Gicquel et al., 1994) responsible for field outbreaks of pasteurellosis in ducks and other animals.

The analytical method used in the present study (HPLC coupled with fluorescence detection) allowed simultaneous detection of both enrofloxacin and ciprofloxacin. This represents a clear advantage compared with microbiological analysis, which does not discriminate between enrofloxacin and its active metabolite ciprofloxacin (Scheer, 1987a; Dorrestein and Verburg, 1990). The latter studies can be compared with the present one by calculating the total combined enrofloxacin and ciprofloxacin concentrations.

The large V_d values observed after both p.o. and i.m. doses indicate that enrofloxacin is widely distributed in the extravascular compartments. This is supported by the high tissue concentrations and the high tissue:plasma ratios observed 24 h after treatment. The enrofloxacin and ciprofloxacin tissue concentrations observed in the present study were higher than those reported in broilers given an oral dose of 10 mg/kg of enrofloxacin (Scheer, 1987a), but lower than those observed in a residue study in broiler chickens treated orally with enrofloxacin at 10 mg/kg for 4 days (Anadon et al., 1995). In the latter study, enrofloxacin and ciprofloxacin concentrations in tissues were cleared slowly and substantial concentrations of ciprofloxacin were still detected 12 days after termination of enrofloxacin treatment (Anadon et al., 1995). There is no clear explanation for the differences observed in the pattern of distribution of enrofloxacin as between ducks and broilers. It is well known that changes in the

volume of distribution may occur where membrane permeability is altered or when the binding of a drug to plasma proteins and/or extravascular binding sites is changed (Baggot, 1994). However, from the available data, it is not possible to predict the influence of these factors in enrofloxacin distribution. Taken together, these findings suggest that, in many tissues, enrofloxacin reaches long-lasting concentrations which are above those in plasma. Accordingly, a clinical evaluation of enrofloxacin cannot be based only on plasma concentrations, but should also include an evaluation of the concentrations of enrofloxacin and ciprofloxacin in infected tissues.

From a practical point of view, for mass treatment over several days, enrofloxacin is administered most easily by mixing the drug with the drinking water. In a study concerning the treatment of ducks with drinking water supplemented with enrofloxacin at a dose of 100 ppm (Froyman *et al.*, 1994), the mean peak concentrations and the persistence of serum enrofloxacin concentrations bactericidal for avian *Pasteurella* spp. and avian coliforms ($\geqslant 0.1$ µg/ml) were similar to those observed in the present study. This indicates some similarities in the enrofloxacin kinetic patterns attainable after individual p.o. or i.m. administration of 10 mg/kg or treatment of a flock with medicated water at 100 ppm.

The efficacy of an antibiotic at the site of infection depends on maintaining the plasma and tissue concentrations above the MIC for bacterial pathogens for the entire dosing period. In the present study, plasma and tissue concentrations of enrofloxacin achieved after either p.o. or i.m. administration were above the MIC values of enrofloxacin-sensitive bacteria by 24 h after drug administration. On the basis of the pharmacokinetic values obtained in this study, a dose regimen of 10 mg/kg per day of enrofloxacin should provide average steady-state plasma concentrations of 0.42 µg/ml (i.m.) and 0.27 µg/ml (p.o.), sufficiently high to be effective in many infectious diseases in ducks.

In conclusion, this study demonstrated that enrofloxacin is well absorbed and widely distributed after p.o. or i.m. administration in ducks, providing tissue concentrations several times higher than those in the plasma by 24 h after treatment. On the basis of the present results, enrofloxacin seems to be an antimicrobial drug with favourable pharmacokinetic properties for the treatment of infectious diseases in ducks.

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