Pharmacokinetics of enrofloxacin in the red pacu (*Colossoma brachypomum*) after intramuscular, oral and bath administration

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The intramuscular (i.m.), oral (p.o.), and bath immersion disposition of enrofloxacin were evaluated following administration to a cultured population of red pacu. The half-life for enrofloxacin following i.m. administration was 28.9 h, considerably longer than values calculated for other animals such as dogs, birds, rabbits, and tortoises. The 4 h maximum concentration (C_{max}) of 1.64 μg/mL following a single 5.0 mg/kg dosing easily exceeds the *in vitro* minimum inhibitory concentration (MIC) for 20 bacterial organisms known to infect fish. At 48 h post i.m. administration, the mean plasma enrofloxacin concentration was well above the MIC for most gram-negative fish pathogens. The gavage method of oral enrofloxacin administration produced a C_{max} of 0.94 μg/mL at 6–8 h. This C_{max} was well above the reported *in vitro* MIC. A bath immersion concentration of 2.5 mg/L for 5 h was used in this study. The C_{max} of 0.17 μg/mL was noted on the 2 hour post-treatment plasma sample. Plasma concentrations of enrofloxacin exceeded published *in vitro* MIC's for most fish bacterial pathogens 72 h after treatment was concluded. Ciprofloxacin, an active metabolite of enrofloxacin, was detected and measured after all methods of drug administration. It is possible and practical to obtain therapeutic blood concentrations of enrofloxacin in the red pacu using p.o., i.m., and bath immersion administration. The i.m. route is the most predictable and results in the highest plasma concentrations of the drug.

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INTRODUCTION

Keeping and breeding of tropical fishes is a popular hobby throughout the world. Over sixty million dollars worth of tropical freshwater fishes are raised annually on farms in Florida and this represents only a fraction of the entire industry (Florida Agricultural Statistics Service, 1994). For many years veterinarians have had little impact on the pet fish hobby (Purchase *et al.*, 1993; Smith, 1994). Most sick fish are treated by aquaculturists, hobbyists, and pet store clerks with chemotherapeutics which have been purchased over the counter. In an effort to curb the flow of pet fish medications to the food fish aquaculture industry in the United States, the FDA is currently considering placing restrictions on the wide availability of these compounds. If these regulations are imposed, many more veterinarians will become more involved in treating ornamental fishes whether they be client owned pets or fishes at the retail, wholesale, and aquaculture levels of the industry.

The fluoroquinolone antimicrobials have attracted much interest in the past ten years. They provide broad spectrum antibacterial activity and are particularly active against gram-negative pathogens (Neer, 1988; Vancutsem *et al.*, 1990). Enrofloxacin kills bacteria at low concentrations by interrupting the normal coiling of DNA within the nucleus through inhibition of DNA-gyrase activity. This unique mechanism of action makes enrofloxacin effective against bacteria that are resistant to other antibiotics (Sheer, 1987).

Results of pharmacokinetic studies of enrofloxacin in domestic animals (cats, cattle, chickens, dogs, pigeons, pigs, rabbits, turkeys) and exotic species (African grey parrots, Florida gopher tortoises, radiated tortoises) have been reported (Walker *et al.*, 1989; Dorrestein & Verburg, 1990; Flammer *et al.*, 1990; Vancutsem *et al.*, 1990; Flammer *et al.*, 1991; Cabanes *et al.*, 1992; Prezant *et al.*, 1994; Raphael *et al.*, 1994). The *in vitro* antimicrobial activity and clinical efficacy of enrofloxacin against gram-negative bacterial pathogens of ornamental and

food fishes have also been reported (Barnes *et al.*, 1990; Reimlinger *et al.*, 1990; Dalsgaard & Bjerregaard, 1991; Martinsen *et al.*, 1992; Stoffregen *et al.*, 1993). Several related papers discuss earlier quinolones, oxolinic and nalidixic acid, but do not describe pharmacokinetics of these compounds in fishes (Austin *et al.*, 1983; Hastings & McKay, 1987; Stamm, 1989; Tsoumas *et al.*, 1989; Bowser & House, 1990). A single paper on enrofloxacin pharmacokinetics in fishes exists (Bowser *et al.*, 1992) and it deals with a salmonid, the rainbow trout (*Oncorhynchus mykiss*).

The objectives of this study were to determine the maximum serum concentrations, elimination half-life, and relative bioavailability of enrofloxacin in the red pacu following intramuscular (i.m.), oral (p.o.), and bath administration. Results of this research can be compared with existing information on the clinical efficacy of this drug and will provide a foundation for determining p.o., i.m., and bath immersion treatment protocols for enrofloxacin in ornamental fishes. This data can be correlated with *in vitro* minimum inhibitory concentration (MIC) for given bacterial pathogens in order to design safe and effective clinical treatment protocols.

MATERIALS AND METHODS

Animals

We used 88 red pacu for the study. Fish were of uniform age and size ($\approx$ 8 months old and weighing 35–50 g each). All animals were quarantined and acclimatised to their environment for 30 days prior to the study. Fish were maintained in 22 57-L aquariums which all shared a common water supply via a recirculating system. Each aquarium housed four research fish. Important water quality parameters such as temperature (25°C), pH (7.2), total alkalinity (51.0 mg/L), and specific gravity (1.000) were constant and tightly controlled.

Experimental design

Intramuscular and oral dosing. All fish were weighed immediately prior to dosing. Eighty-eight fish were randomly divided into two groups; a two-way cross-over design was employed. Fish in group A received 5.0 mg/kg enrofloxacin i.m. while those in group B received the same dose p.o. (via gavage tube). The i.m. route was essential to this study since it is the only one which theoretically approximated 100% bioavailability of the drug. Three treated fish and one control fish were kept in each tank. Controls were given sterile water and fish were individually identified by clipping pectoral and pelvic fins. Approximately 0.4 mL of blood was taken from the caudal vein from three fish in each group and one control fish at the following times post drug administration: 0, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h. Each fish was sampled prior to dosing and once per experiment. Following a 21-day washout period, fish in group B received 5.0 mg/kg enrofloxacin i.m. while those in group A received the same dose p.o.. Sampling was performed as previously described.

Bath administration

Following a 21-day washout period (O'Grady *et al.*, 1988) 60 fish were divided into 10 aquariums. Fifty fish were given enrofloxacin by immersing the fish in water containing 2.5 mg enrofloxacin/L of water for 5 h. Ten fish (one per group) served as controls and were immersed in clean, unmedicated water. Therefore, each tank housed five treated fish and one control fish. Approximately 0.4 mL of blood was taken from the caudal vein from each group before treatment and at the following times post-treatment: 2, 4, and 8 h, and 1, 3, 6, 10, 14, 17, and 21 days.

Sample and data analysis

Plasma was immediately separated from blood and stored at -70°C until sample analysis was performed. All samples were analyzed by high performance liquid chromatography (HPLC) using modifications of previously published methods. The HPLC system consisted of a LC-10AD pump, SPD-10AV ultraviolet detector, SIL–9 A autosampler (Schimadzu Scientific Instruments, Columbia, MD). Samples were injected onto a guard column (Zorbax C_{18} 4 mm $\times$ 1.25 cm, MAC-MOD Analytical Inc., Chadds Ford, PA). Chromatographic separations were performed on a Zorbax SB-C_8 column (4.6 mm $\times$ 15 cm, MAC-MOD Analytical, Chadds Ford, PA). The mobile phase was 86% phosphoric acid (HPLC grade chemicals, Fisher Scientific, Pittsburgh, PA). Elution was isocratic (1 mL/min) at room temperature; the detection wavelength was 279 nm. Stock solutions of standards were made by reconstituting powdered enrofloxacin, ciprofloxacin (Miles Inc., Shawnee Mission, Kansas) or ofloxacin (Sigma Chemicals Co., St. Louis, MO) in methanol (1 μg/mL). Standards were further diluted in mobile phase and stored at 4°C for up to 1 month.

To polypropylene tubes containing 200 μL of plasma, were added 25 μL of internal standard (10 μg ofloxacin/mL) and 0.5 mL of methanol. The samples were mixed by vortexing for 20 s, placed on ice for 15 min, and then centrifuged at a high rate of speed for 10 min. The supernatant (750 μL) was transferred to centrifuge tubes. Dichloromethane (6 mL, Fisher Scientific, Pittsburgh, PA) was added and the contents mixed by vortexing for 20 s followed by centrifugation at 1,000 ***g*** for 10 min. After discarding the aqueous phase, the organic phase was transferred to a clean glass tube and evaporated to dryness at 40°C under a steady stream of nitrogen. The residue was then reconstituted in 50 mL of mobile phase; 50 μL of which was injected into the column for HPLC analysis.

Retention times of ofloxacin, ciprofloxacin, and enrofloxacin were 5.7, 6.9 and 9.0 min, respectively. Standard curves of enrofloxacin:ofloxacin and ciprofloxacin:ofloxacin (determined by chromatographic areas) were used to calculate enrofloxacin and ciprofloxacin concentrations. For both ciprofloxacin and enrofloxacin, the limit of detection was 5 ng/mL and the range of linearity was 5 ng/mL to 1 μg/mL. The coefficient of variations for enrofloxacin and ciprofloxacin were 8.1% and 9.3%, respectively. The mean recoveries of enrofloxacin, ciprofloxacin and ofloxacin were 92%, 76% and 100%, respectively.

Pharmacokinetic calculations

A non-compartmental model analysis using Quattro Pro™ (Borland, Scotts Valley, CA) was used to generate pharmacokinetic data. The mean concentration at each time point was used for calculation of elimination half-life, C_{max}, and area under the time–concentration curve (*AUC*). The *AUC* was calculated using the trapezoidal rule. Relative bioavailability of i.m. and p.o. routes of drug administration were calculated using *AUC* data. Half-life values were calculated from linear regression of log transformed data. Data from the bath experiment was used to determine the duration of time which serum levels of enrofloxacin greater than the MIC for most susceptible fish pathogens were maintained.

RESULTS

Intramuscular study

Figure 1 demonstrates the plasma concentration–time profile for enrofloxacin following i.m. administration. The corresponding pharmacokinetic parameters are listed in Table 1. The mean C_{max} of enrofloxacin was 1.64 (± 0.92) μg/mL obtained at 4 h following injection and declined to 0.2 (± 0.09) μg/mL by 96 h. The elimination half-life for enrofloxacin was 28.9 h.

Ciprofloxacin, a major metabolite of enrofloxacin, was present in the plasma at much lower concentrations than enrofloxacin. The mean C_{max} of ciprofloxacin was 0.05 (± 0.01) μg/mL at 4 h and declined to 0.002 (± 0.003) μg/mL by 96 h (Table 1). The elimination half-life for ciprofloxacin was 53 h.

Oral study

Figure 2 demonstrates the plasma concentration–time profile for enrofloxacin following oral administration. The corresponding pharmacokinetic parameters are listed in Table 1. The mean C_{max} of enrofloxacin was 0.8 (± 1.17) μg/mL obtained at 36 h following gavage and declined to 0.19 (± 0.08) μg/mL by 120 h.

The mean C_{max} of ciprofloxacin was 0.02 (± 0.008) μg/mL at 36 h and declined to 0.008 (± 0.007) by 96 h (Table 1).

Bath administration study

The plasma concentration–time profile following 5 hour bath administration of enrofloxacin is demonstrated in Fig. 3. The

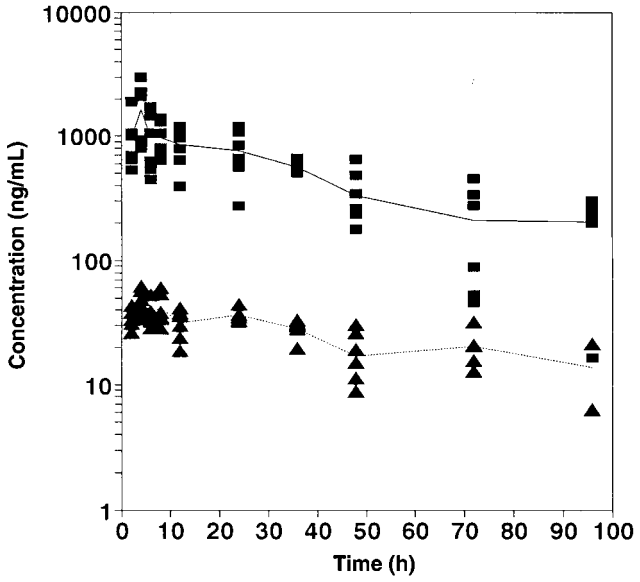

Fig. 1. Mean plasma concentrations of enrofloxacin (solid line) and ciprofloxacin (dotted line) in red pacu following i.m. administration of enrofloxacin at a dose of 5.0 mg/kg body weight.

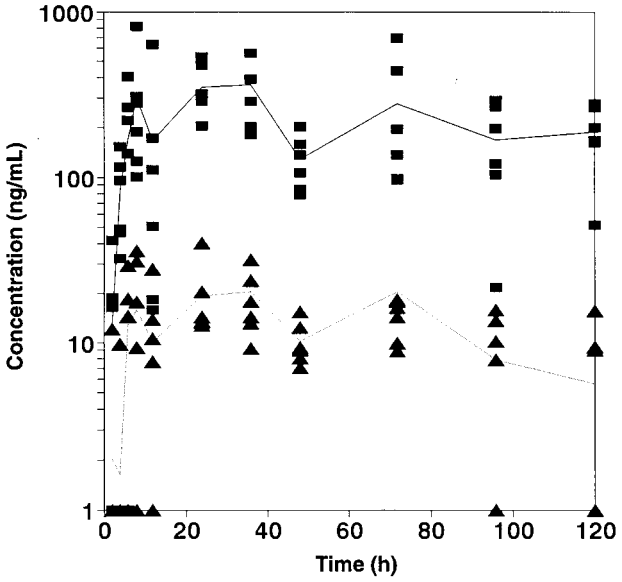

Fig. 2. Mean plasma concentrations of enrofloxacin (solid line) and ciprofloxacin (dotted line) in red pacu following p.o. administration of enrofloxacin at a dose of 5.0 mg/kg body weight.

	Intramuscular		Oral	
	ENRO	CIPRO	ENRO	CIPRO
AUC (μg·h/mL)	46.3	2.36	26.5	1.56
AUMC (μg·h²/mL)	1,481.0	93.6	1,523.0	86.3
$t_{1/2}$ (h)	28.9	53		
Relative F (%)			57.2	
C_{max} (μg/mL)*	1.64 ± 0.92	0.05 ± 0.01	0.8 ± 1.17	0.02 ± 0.008
T_{max}	4	4	36	36

*Mean (SD)

Table 1. Mean pharmacokinetic values calculated for enrofloxacin and ciprofloxacin following i.m. and p.o. administration of 5.0 mg/kg enrofloxacin to red pacu

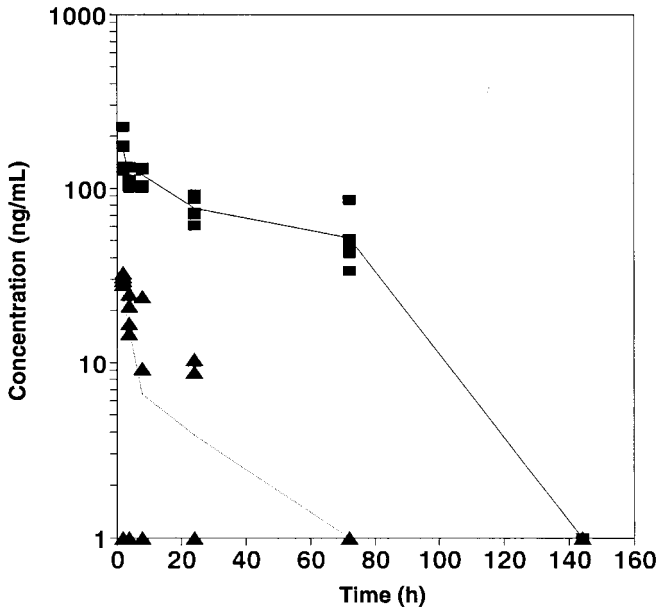

Fig. 3. Mean plasma concentrations of enrofloxacin (solid line) and ciprofloxacin (dotted line) in red pacu following bath administration of enrofloxacin for a 5 hour duration.

mean C_{max} of enrofloxacin was 0.17($\pm$ 0.04) μg/mL obtained at 2 h following removal from the bath and declined to 0.052 ($\pm$ 0.02) μg/mL by 72 h. No detectable levels of enrofloxacin existed at 144 h post bath administration.

The mean C_{max} of ciprofloxacin was 0.024 ($\pm$ 0.013) μg/mL at 2 h and declined to 0.004 ($\pm$ 0.005) by 24 h.

DISCUSSION

Three different routes of enrofloxacin administration were analysed in order to determine selected pharmacokinetic parameters for enrofloxacin in the red pacu. All three regimens are used frequently when treating ornamental fishes.

The half-life for intramuscularly administered enrofloxacin was considerably longer than values calculated for other animals such as dogs, birds, rabbits, and tortoises (Sheer, 1987; Flammer *et al.* 1991; Cabanes *et al.*, 1992; Prezant *et al.*, 1994; Raphael *et al.*, 1994). Among these animals, the gopher tortoise study (Prezant *et al.*, 1994) most closely approximated the results of the pacu study with an elimination half-life of 23.1 h and a C_{max} of 2.4 μg/mL. The gopher tortoises in the study were given the same 5 mg/kg dose as the pacu. In rainbow trout given 5 mg/kg enrofloxacin i.v. (Bowser *et al.*, 1992), the elimination half-life determined by a bioassay technique was 24.4 h, a value close to that obtained in the i.m. pacu study.

In many species of animals, enrofloxacin is de-ethylated to ciprofloxacin, the primary metabolite of enrofloxacin (Tyczkowska *et al.*, 1989). Both of these quinolone compounds work similarly and are active against many gram-negative organisms raising the possibility that the presence of both drugs supplies some benefit to the patient (Flammer *et al.*, 1991).

While this study does not address a specific bacterial pathogen, the existence of *in vitro* MIC data supports the statement that effective plasma concentrations of enrofloxacin can be obtained in the red pacu after a single 5 mg/kg i.m. injection. The 4 h C_{max} of 1.64 μg/mL easily exceeds the *in vitro* MIC for 20 bacterial organisms known to infect fish (Bowser & House, 1990). For the majority of these organisms, the red pacu C_{max} was more than 10 times greater than the MIC. These MIC results support data presented in other studies (Vancutsem *et al.*, 1990) on gram-negative pathogens of reptiles and higher vertebrates.

At 48 h post i.m. enrofloxacin administration, the mean plasma enrofloxacin concentration was 0.33 μg/mL. This concentration is well above the MIC for most gram-negative fish pathogens. Therefore, a dosing regimen of 5 mg/kg i.m. every 48 h is recommended in the red pacu.

The gavage method of oral enrofloxacin administration produced a much lower mean C_{max} than the i.m. route and three distinct plasma peaks were noted (8, 24, and 36 h) and are evident in Fig. 2. These results show some similarities with oral enrofloxacin dosing via feed in rainbow trout. In the trout (Bowser *et al.*, 1992) a C_{max} of 0.94 μg/mL at 6–8 h was observed and the authors noted that the 24- and 36-h serum levels were only slightly lower that the 6–8-h value. It is possible that there is entero-hepatic recirculation of enrofloxacin resulting in delayed peaks or that there is intraspecies variability in rate of absorption.

The C_{max} value for the oral enrofloxacin study was well above the previously reported *in vitro* MIC values.

Inherent problems exist when bath immersion is elected as a route of antibiotic administration in fish. A broad spectrum quinolone such as enrofloxacin will likely compromise the biological filter by killing or reducing numbers of the nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*. Antibiotics in the water are also difficult to dispose of and can become an environmental contaminant. A third consideration, largely dependent on the volume of water used in the bath, is the expense of this type of treatment due to the large amounts of antibiotic required. However, when fish are too small for parenteral drug administration or are anorexic, bath immersion may be the only treatment option.

Reimlinger *et al.* (1990) reports the effects of enrofloxacin in the water on five species of ornamental fishes. The results of their study led them to recommend a 30 μg/L 5-h bath. Their study included only subjective observations of how fish responded to bath treatments ranging in concentration from 10 μg/L to 600 μg/L. No blood or tissue drug concentration data were reported.

We empirically selected a bath immersion concentration of 2.5 mg/L for 5 h in this study. Our results support reports in the pet fish industry that enrofloxacin is effective when administered as a bath and is absorbed into the bloodstream from the water. Since freshwater fish do not drink, the most likely point of absorption during a bath treatment is across the high surface area gill epithelium. The C_{max} of 0.17 μg/mL was noted on the 2-h post-treatment plasma sample. Plasma levels of enrofloxacin exceeded published *in vitro* MIC's for most fish bacterial pathogens 72 h after treatment was concluded. A 5-h bath of

2.5 mg/L enrofloxacin administered every 24–48 h would be sufficient to produce adequate blood levels of enrofloxacin in the red pacu.

This study indicates that it is possible to obtain therapeutic blood concentrations of enrofloxacin in the red pacu using p.o., i.m., and bath immersion administration. The i.m. route is the most predictable and results in the highest plasma concentrations of the drug. The variability in the p.o. gavage method is most likely due to inaccurate dosing due to regurgitation which likely occurred to some extent. We were unable to quantify this finding. Bath immersion, the dosing method used most frequently by ornamental fish enthusiasts, does produce adequate plasma levels when dosed at 2.5 mg/L for 5 h.

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