

## Pharmacokinetics of Enrofloxacin in Koi Carp (*Cyprinus carpio*) after Various Routes of Administration

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### ABSTRACT

The pharmacokinetics of enrofloxacin was studied in koi carp (*Cyprinus carpio*) after single administration by intraperitoneal (IP) and intramuscular (IM) injection, oral gavage (PO) at a dose of 10 mg/kg body weight and by 5 mg/liter bath for 5 hr at room temperature (27°C). Blood samples were obtained from caudal vein at 0, 15, 30, 60 min and 2, 8, 24, 48, 72 and 96 hr following administration. Serum antimicrobial concentrations were determined by microbioassay using *Bacillus subtilis* as an indicator organism where maximal concentrations (C<sub>max</sub>) of drug in plasma were 36.6 ± 21.3, 4.6 ± 2.4, 14.4 ± 7.7 and 0.9 ± 0.3 mg/L after IP, IM, PO and bath at 60, 60, 5 and 5 min, respectively. According to one-compartment pharmacokinetic model, elimination half-life (T<sub>1/2</sub>) were 16.1, 17.9, 16.6 and 42.1 hr, volumes of distribution (V<sub>d</sub>) were 0.3, 3.1, 1.5 and 10.4 L/kg, and area under the curve (AUC) were 797.8, 82.7, 156.4 and 29.1 mg-hr/L after IP, IM, PO and bath, respectively. It was found that those 4 routes of enrofloxacin administration to koi were effective since the concentrations of drug in plasma were greater than minimal inhibitory concentration (MIC) of most fish bacterial pathogens.

**Key words:** enrofloxacin, antibiotic, pharmacokinetic, carp

### INTRODUCTION

Ornamental fish has been known to be popular pet throughout the world. One of the important problems in fish health is an infectious disease which is caused by bacteria, fungi, virus and external parasite (Plumb,1999). Then drugs or chemicals are necessary for prevention or treatment. U.S. Food and Drug Administration (US FDA) permits only 3 kinds of antimicrobials in food fish under the information from Food Animal Residue Avoidance Database (FARAD<sup>®</sup>). Those 3 antibiotics are oxytetracycline (Terramycin for fish<sup>®</sup>), ormetoprim and

sulfadimethoxine (Romet-30<sup>®</sup>) and sulfamerazine (Burka *et al.*,1997). Nevertheless, ornamental fish or non-food fish can use any antibiotics as extralabel use. Food and drug authority of Thailand permits the use of oxytetracycline, sulfadimethoxine/or metroprim, enrofloxacin, sulfamethoxazole/ trimetroprim and neomycin for aquatic animal.

Enrofloxacin is a synthetic antimicrobial agent of the fluoroquinolone group which is extensively used in veterinary medicine. It inhibits prokaryotic topoisomerase II (DNA gyrase) which is an important enzyme for bacterial replication (Vancutsem *et al.*,1990). It has broad spectrum

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activity, especially against gram negative bacteria, such as *Aeromonas salmonicida* (causing furunculosis), *Renibacterium salmoninarum* (causing bacterial kidney disease), *Vibrio anguillarum* (causing enteric red mouth disease), and intracellular organism, such as rickettsia, chlamydia and mycoplasma. Enrofloxacin is widely used in ornamental fish although there is a few pharmacokinetic information (Stoffregen *et al.*,1997).

The objective of this study was to determine the pharmacokinetic parameters of enrofloxacin in koi carp following a single intraperitoneal (IP) and intramuscular (IM) injection, oral gavage (PO) and bath administration at room temperature (27°C). Results of this research would provide a proper therapeutic regimen of enrofloxacin for this ornamental fish.

## MATERIALS AND METHODS

### Animals

Three hundred healthy koi (78.3 ± 5.3 g weight and 16.2 ± 3.5 cm total length) were maintained in 10 glass tanks (92 × 50 × 46 cm) at least 3 weeks for acclimation. Fish were fed a commercial feed ad lib daily and maintained on a 12-h light/12-h dark photoperiod. Water quality during all experiments was: pH 7.0, unionized ammonia <0.001 mg/l and nitrite <0.10 mg/l, temperature 27-28°C (room temperature). Food was withheld 24 hours before administration of enrofloxacin in order to reduce stress (Horberg, 1994).

### Drug administration and sample collection

Commercial enrofloxacin (Baytril 5% Injectable Solution and Baytril 10% Oral Solution; Bayer AG, Leverkusen, Germany) were used in this experiment. Each group of 50 fish received 10 mg/kg enrofloxacin IP, IM and PO (via gavage tube), and 5 mg/L of water for 5 hr bath.

For IP, IM and PO, fish were sedated with

low concentration of clove oil (10 mg/L) before handling and drug administration. After drug administration, fish were kept in clean, unmedicated water at room temperature and fed ad lib daily. Blood was taken from the caudal vein from 5 fish in each group at the following times: 0, 15, 30, 60 min, 2, 8, 24, 48, 72 and 96 h. All fish were collected blood once. Plasma was immediately separated from blood and stored at -20°C until sample analysis was performed.

### Antimicrobial assay and pharmacokinetic analysis

Plasma samples were assayed for antimicrobial activity using the agar-well diffusion microbiological assay. *Bacillus subtilis* (ATCC 6633) was used as an indicator organism (Stoffregen *et al.*,1997). Mueller-Hilton agar (MHA) was moltened and inoculated with *B. subtilis* spores at the concentration of  $0.5 \times 10^5$  cfu/ml of agar. After cooling, 8-mm wells were cut into the solidified MHA. Standard enrofloxacin (Sigma) were prepared at concentrations of 0.1-100 µg/ml which were diluted with 50 mM sodium bicarbonate buffer, pH 10.5. Standard enrofloxacin and plasma samples were pipetted into the wells. Plates were incubated at 37°C for 24 h and diameters of inhibition zones were recorded. All samples including controls were replicated. The control drug concentration versus inhibition zone were plotted and regression line was generated on a semi-logarithmic graph. The lower limit of sensitivity of the assay was 0.2 µg/ml. The pharmacokinetic parameters of enrofloxacin in plasma were performed using PK solutions 2.0TM, Noncompartment Pharmacokinetics Data Analysis, Summit Research Services, CO, USA.

## RESULTS

After a single IP, IM, oral gavage administration of enrofloxacin 10 mg/kg and bath at dose 5 mg/l for 5 h to koi carp at 27°C, plasma

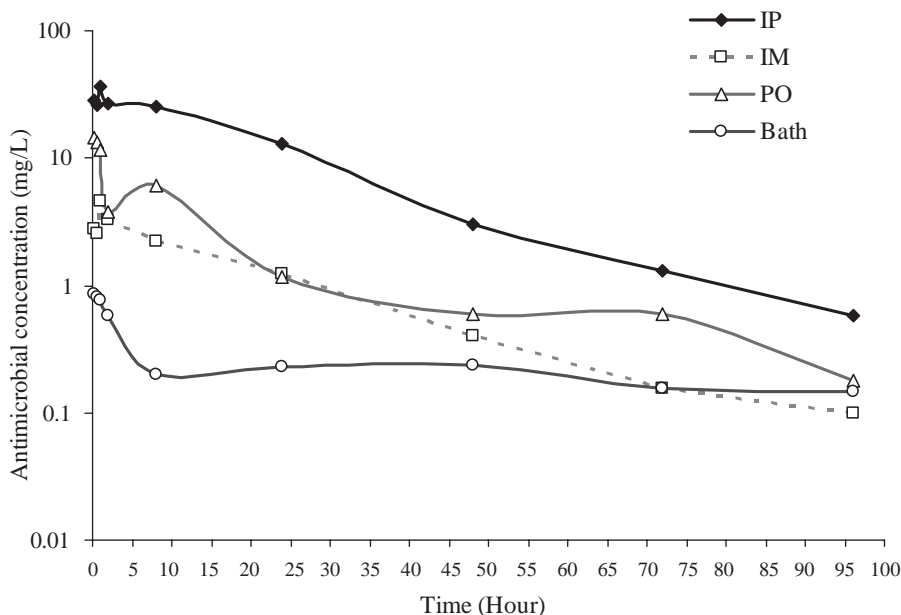
drug concentrations were detected by using a microbioassay (Figure 1, Table 1). Intraperitoneal injection caused higher plasma drug concentrations than PO and IM samples. The lowest plasma drug concentrations were shown in bath administration. The maximum drug concentrations ( $C_{max}$ ) were  $36.64 \pm 21.30$  and  $4.59 \pm 2.43$  mg/L at 60 min after IP and IM injection, respectively, and  $14.36 \pm 7.66$  and  $0.86 \pm 0.26$  mg/L at 15 min after oral gavage and bath administration.

The pharmacokinetic parameters of enrofloxacin were described by an one-compartment pharmacokinetic model (Table 2). Elimination half-life ( $t_{1/2}$ ) was 42.1, 17.86, 16.6 and 16.1 hr following bath, IM, PO and IP, respectively. The AUC values were 797.8, 156.4, 82.7 and 29.1 mg-hr/L after IP, PO, IM and bath administration, respectively. Then relative bioavailability (F) of enrofloxacin was calculated by comparing AUC of the other routes of drug

administration to AUC of IP. It presented F value to be 19.6, 10.4 and 3.6 % for PO, IM and bath administration, respectively.

## DISCUSSION

Enrofloxacin, one of fluoroquinolones, is lipophilic drug and is distributed to organs and fat tissues, e.g. kidney, liver, muscle, skin (Stoffregen *et al.*, 1997). It is supported that longer half-life following bath administration than the other 3 routes in this study was related with high volume of distribution values (10.4 L/kg) after enrofloxacin enters into blood circulation via absorption through gill and skin (Treves-Brown, 2000). However, dose of enrofloxacin used in the study was higher than the other studies. The AUC of 156.4 mg-hr/L for PO administration is similar in rainbow trout where the results showed that AUC was 154.77 mg-hr/L after PO administration of enrofloxacin to rainbow trout at



**Figure 1** Semilogarithmic plot of antimicrobial concentration (mg/L) (enrofloxacin and its metabolites) in plasma versus time following intraperitoneal (IP), intramuscular injection (IM) and oral gavage (PO) administration of enrofloxacin at a dose 10 mg/kg body weight and bath administration of enrofloxacin 5 mg/l for a 5 hour duration.

15°C (Bowser *et al.*,1992). Normally, IP administration in fish gives the maximal drug concentration in blood similar to intravenous administration (Kleinow *et al.*,1992). C<sub>max</sub> was 0.17 mg/L at 2 hr after bath red pacu (*Colossoma branchyomum*) with 2.5 mg/l enrofloxacin for 5 hr (Lewbart *et al.*,1997). In addition, using enrofloxacin for bath treatment to seabass (*Dicentrarchus labrax*) at doses of 5 or 10 mg/L for 24 hr and 50 mg/L for 4 hr caused plasma drug concentration above MIC (Intorre *et al.*,2000). Fish muscle is not well vascularized that may affect poor drug absorption. When the skin does not retract over the injection site, then the administered

**Table 1** Mean enrofloxacin concentration (mg/L) in plasma after a single bolus intraperitoneal (IP), intramuscular (IM) injection and oral gavage (PO) of 10 mg/kg body weight and bath 5 mg/liter water for 5 hr to koi (*Cyprinus carpio*) at 27°C. n=5 fish at each sampling period.

Time (hr)	Concentration (mg/L ± SD)			
	IP	IM	PO	Bath
0	0.00	0.00	0.00	0.00*
0.25	28.51 ± 6.72	2.74 ± 1.47	14.36 ± 7.66	0.86 ± 0.26
0.5	25.90 ± 5.40	2.53 ± 0.85	13.19 ± 5.66	0.80 ± 0.36
1	36.64 ± 21.30	4.59 ± 2.43	11.45 ± 21.39	0.77 ± 0.28
2	26.93 ± 7.07	3.28 ± 1.79	3.76 ± 2.83	0.58 ± 0.21
8	25.02 ± 11.66	2.23 ± 1.35	6.15 ± 6.24	0.20 ± 0.09
24	12.96 ± 5.88	1.23 ± 0.54	1.16 ± 1.00	0.23 ± 0.05
48	3.06 ± 1.77	0.40 ± 0.31	0.60 ± 0.23	0.24 ± 0.10
72	1.30 ± 0.78	0.15 ± 0.05	0.60 ± 0.28	0.15 ± 0.04
96	0.57 ± 0.31	0.10 ± 0.06	0.18 ± 0.08	0.15 ± 0.03

\* For bath treatment, time 0 means time before bath administration.

**Table 2** Pharmacokinetic parameters (units) of enrofloxacin in koi carp after a single intraperitoneal (IP), intramuscular (IM) injection and oral gavage (oral) at dose 10 mg/kg, and bath treatment at dose 5 mg/L water for 5 hr at room temperature (27°C).

Pharmacokinetic parameters	Units	Route of enrofloxacin administration			
		IP	IM	PO	Bath
t <sub>1/2</sub>	Hr	16.1	17.9	16.6	42.1
C <sub>max</sub>	mg/L	36.64 ± 21.30	4.59 ± 2.43	14.36 ± 7.66	0.86 ± 0.26
T <sub>max</sub>	Hr	1.0	1.0	0.25	0.25
AUC <sub>∞</sub> (area)	mg-hr/L	797.8	82.7	156.4	29.1
MRT (area)	Hr	20.9	23.8	23.0	75.3
Vd (area) / kg	L/kg	0.3	3.1	1.5	10.4
CL (area) / kg	L/hr/kg	0.013	0.121	0.064	0.172

Note: t<sub>1/2</sub> = half-life from Vd and CL

C<sub>max</sub> = Maximum observed concentration

T<sub>max</sub> = Time at maximum observed concentration

AUC<sub>∞</sub> (area) = Area under the curve

MRT (area) = Mean Residence Time (time for 63.2% of administered dose to be eliminated)

Vd (area) / kg = Volume of distribution based on AUC<sub>∞</sub> divided by body weight in kg

CL (area) / kg = Clearance based on AUC<sub>∞</sub> divided by body weight in kg

drug may leak out (Horberg,1994). This might explain why the drug plasma concentrations by IM were lower compared to oral gavage administration.

The pharmacokinetic parameters of enrofloxacin administration in fish are different depending on many factors, e.g. fish species, size and age of fish, drug dosage, frequency of drug administration and water temperature during study (Kleinow *et al.*,1992; Hayton,1998). For example, low water temperature could cause lower bioavailability of enrofloxacin in rainbow trout in which F values were 48.8 and 24.0 % after 10 mg/kg PO administration at 15° and 10°C, respectively (Bowser *et al.*,1992).

The plasma drug concentration of these 4 routes of administration were above MIC (minimal inhibitory concentration) values for most fish bacterial pathogens. For example MIC ranges of enrofloxacin against *Flavobacterium psychrophilum* were 0.00098-0.25 µg/ml (Rangdale *et al.*,1997), *Renibacterium salmoniarum* were 0.0064-0.032 µg/ml (Bowser and House,1990), *Aeromonas salmonicida* was 0.16 µg/ml (Stoffregen *et al.*,1993), *A. salmonicida* subsp. *Salmonicida* and atypical *A. salmonicida* were 0.005-0.80 µg/ml, *Vibrio anguillarum* were 0.001-0.08 µg/ml, *V. salmonicida* were 0.005-0.10 µg/ml, and *Yersinia ruckeri* were 0.01-0.03 µg/ml (Martinsen *et al.*,1992). Also, MIC of enrofloxacin against susceptible *A. hydrophila* from Thailand and Philippines were lower than 0.5 µg/ml (Maluping *et al.*,2005). This study indicated that it was possible to obtain therapeutic blood concentration of enrofloxacin in carp using IP, IM, PO and bath administration to koi in which the concentrations of drug in plasma were greater than MIC of most fish bacterial pathogens.

Those 4 routes of drug administration were practical to treat the ornamental fish (pet fish) in the animal clinics or hospitals. The choosing of drug administration is depended on how

practical it is. For example, IP and IM administration is appropriate when fish present an acute bacterial infection, in which need rapidly high drug concentration in the circulation. However, individual injection is not practical for small fish and time-consuming when high number of fish are subjected.

For oral gavage administration, it needs gavage tubes inserted into the stomach for completed administration without regurgitation. Handling fish during PO administration may cause stress to fish, even skin or mouth trauma. However, the drug concentration increased to maximal at 15 min after administration and continued above MIC through the experimental period. Nevertheless, drug can be used to mix with fish food, and then air dried or coated with vegetable oil for oral administration (Treves-Brown,2000). This method is limited since sick fish are normally anorexia and drug mixed food may be not palatable to eat.

Bath immersion treatment caused lower plasma drug concentration in plasma compared with the others, but it was above MIC. This method is not practical to add fluoroquinolone drugs into hard water or water with high levels of divalent cations, eg, magnesium, in which enrofloxacin will bind to divalent cations and causes non-effective therapy (Treves-Brown, 2000). For addition, enrofloxacin may kill bacterial in tank or pond, such as *Nitrosomonas* and *Nitrobacter* which are essential bacterial in filter system. Then, fish should be bathed in other container and biofilters should be moved out of the treatment tank temporarily. In addition, enrofloxacin treatment in saltwater fish should increase dose and duration of bath immersion for therapeutic efficiency.

## CONCLUSION

Enrofloxacin administrations at 10 mg/kg for IP, IM and PO and 5 mg/L for 5 hr bath

everyday were sufficient to treat the bacterial infection. Those administration methods produced adequate plasma levels for treatment in which the concentrations of drug in plasma were greater than minimal inhibitory concentration (MIC) of most fish bacterial pathogens.

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