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[Article]

Pharmacokinetic Studies of Florfenicol in Koi Carp and Threespot Gourami *Trichogaster trichopterus* after Oral and Intramuscular Treatment

ROY P. E. YANONG* AND ERIC W. CURTIS[1]

Tropical Aquaculture Laboratory, Department of Fisheries and Aquatic Sciences, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, Florida 33570, USA

ROBERT SIMMONS

Schering-Plough Animal Health Corporation, Union, New Jersey 07083, USA

VENKATESH ATUL BHATTARAM, MATHANGI GOPALAKRISHNAN, NAHAL KETABI, NELAMANGALA V. NAGARAJA,[2] AND HARTMUT DERENDORF

Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Florida 32610, USA

Abstract.—The pharmacokinetics of florfenicol were studied in koi carp *Cyprinus carpio* (hereafter, koi) and threespot gourami *Trichogaster trichopterus* after oral (50 mg/kg) and intramuscular (25 mg/kg) administration of the drug in warm water conditions (24–25°C). The estimates of clearance, volume of distribution, and half-life were 0.05 $L \cdot h^{-1} \cdot kg^{-1}$, 1.0 L/kg, and 16 h, respectively, in koi. In threespot gourami, the corresponding estimates were 0.32 $L \cdot h^{-1} \cdot kg^{-1}$, 2.0 L/kg, and 4 h. In koi, minimal drug absorption was observed after bath treatment. Analysis of florfenicol leaching from fish feed indicated that about 50–80% of the coated drug is lost and is not available for therapeutic benefit for either species. The minimum inhibitory concentrations of florfenicol, determined for bacterial isolates from tropical fish, ranged from 0.5 to 2 μg/mL. For effective dosing regimens in koi and threespot gourami, the differences in pharmacokinetics should be considered in future studies.

Ornamental fish are one of the largest groups of pets, and the ornamental aquarium hobby is purported to be the second most popular one in the United States. In 2002 alone, overall fish and fish product retail sales were estimated to be US$1.2 billion. The farm-gate value (price paid to the producer) for U.S.-produced ornamental fish was estimated at $69 million.

Freshwater fish are more commonly kept by hobbyists because saltwater fish are more expensive to maintain. Koi carp *Cyprinus carpio* (hereafter, koi) and threespot gourami *Trichogaster trichopterus* are two economically important freshwater species of ornamental fish. Koi are commonly kept in indoor and outdoor water gardens and are both imported and domestically produced. In 1998, the U.S. farm-gate value of koi (i.e., the price paid to the U.S. producer) was estimated at approximately $3.9 million. Gouramis (family Osphronemidae) have labyrinth organs, an anatomical adaptation that permits these species to breathe atmospheric oxygen, which is especially useful when dissolved oxygen levels are low. Gouramis and related species are staples in the ornamental fish trade and make up an important crop raised domestically, primarily in the state of Florida.

Currently, no FDA-approved antibiotics or antibacterials are available for use in ornamental fish. Florfenicol, a broad-spectrum antibacterial (Aquafen, Aquaflor; Schering-Plough Animal Health Corp., Union, New Jersey), is currently being registered for use in food fish in the United States. Florfenicol, closely related to chloramphenicol, is effective against chloramphenicol-resistant bacterial isolates and also lacks the functional group associated with human toxicity.

The pharmacokinetics of florfenicol have been reported in Atlantic salmon *Salmo salar* at 11°C (Horsberg et al. 1994, 1996), rainbow trout *On-*

* Corresponding author: rpy@ifas.ufl.edu

[1] Present address: John G. Shedd Aquarium, Chicago, Illinois 60605, USA.

[2] Present address: Millennium Pharmaceuticals, Drug Safety and Disposition, Cambridge, Massachusetts 02139, USA.

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corhynchus mykiss at 10°C (Pinault et al. 1997), red pacu *Piaractus brachypomus* (cited in the literature as *Colossoma brachypomum*) at 25°C (Lewbart et al. 1999), and Atlantic cod *Gadus morhua* (Samuelsen et al. 2003). Other than in red pacu, no report has been published of the pharmacokinetics of florfenicol studied in tropical ornamental fish. The ornamental fish industry comprises thousands of species, with differing physiological characteristics that could influence the pharmacokinetics. Species-specific, or at least group-specific, pharmacokinetic information is needed to ensure more accurate dosing and avoid toxicity.

The main objective of the study was to determine single-dose pharmacokinetics of florfenicol in koi carp and threespot gourami after oral and intramuscular treatment at 25°C. Minimum inhibitory concentrations (MICs) of florfenicol against tropical fish pathogens isolated from clinical cases were determined to suggest appropriate dosing regimens for better therapeutic benefit.

Methods

Dosing Materials and Chemicals

The various sources for the substances used in the study are as follows: florfenicol (Schering-Plough); Zeigler's 38% Trout Grower Sinking Pellet (pellet size, 2.4 mm) and Zeigler's 55% Number 2 Fin Fish Starter Sinking Pellet (pellet size, 0.8–1.2 mm) from Zeigler Bros, Inc. (Gardners, Pennsylvania); polyethylene glycol (PEG-300; Sigma Chemical Co., St. Louis, Missouri); menhaden oil (Omega Protein, Reedville, Virginia); tricaine methanesulfonate (Tricaine-S; Western Chemical, Inc., Ferndale, Washington); thiamphenicol (Sigma); and hexane/methanol and acetonitrile (HPLC-grade; Fisher Scientific USA).

Fish Experiments

Koi (144 ± 44 g [mean ± SD]) and threespot gourami (19 ± 3 g) were purchased from local fish farms in Hillsborough County, Florida. The fish were housed in different 38 L-tanks on a recirculating system during the acclimation period for 14–21 d. Water-quality parameters were maintained at total ammonia nitrogen no more than 0.8 mg/L; nitrite nitrogen no more than 0.04 mg/L; temperature 23–25°C; pH 7.8–8.0; and dissolved oxygen 6–8 μL/L.

Oral administration to koi (N = 74;) and threespot gourami (N = 37).—To formulate a 50-mg/kg dose in koi and threespot gourami, we mixed 0.55 g or 1.64 g of florfenicol with 1.5 mL of fish oil and 100 g of feed (koi: Zeigler 38% Trout Grower Sinking Pellet; threespot gourami: Zeigler 55% Number 2 Fin Fish Starter), respectively, for at least 5 min until the florfenicol−oil mixture appeared to be evenly distributed on the pellets. The pellets were allowed to dry for at least an hour before being used in the experiment. The treated fish feed was analyzed for florfenicol content.

The koi presented an interesting problem in that they would eat the food only if another fish was in the tank. Therefore, the study was performed with two fish in each 38-L test tank. The koi were fed at 1% body weight and given 5 min to eat. Four replicates, each consisting of two fish in one tank, were run for each sampling time.

The threespot gourami were fed at 0.5% body weight and were given 7 min to eat. For the threespot gourami study, four replicates, consisting of one fish per 38-L tank, were run for each sampling time.

Water samples were taken during treatment and after removal of fish in oral experiments. After the specified time, any uneaten food was siphoned from the tank, collected into a fine-mesh net, dried at 60°C for 24 h, and weighed, and the weight measured was subtracted from the amount fed to determine the correct dose received by the fish. The loss of florfenicol into water from pellets was studied in vitro up to 10 min (before study). The amount of florfenicol remaining in the uneaten food (after study) was also determined.

Intramuscular administration to koi and threespot gourami (N = 37 each).—We mixed pure powdered florfenicol with PEG-300 at room temperature (approximately 25°C) using a stir bar in an Erlenmeyer flask. The desired concentration of the mixture was such that injection volume could be kept under 50 μL and still correspond to doses of 25 and 50 mg/kg for koi and threespot gourami, respectively.

Fish were removed from test tanks, anesthetized by immersion in a sodium bicarbonate–buffered solution of Tricaine-S (koi: 200 mg/L; threespot gourami: 400 mg/L) at pH = 7.4–7.6; monitored until sufficiently anesthetized (loss of buoyancy control, minimal response to touch) within 1–2 min; and then weighed. The injections were approximately 1–1.5 cm deep within muscle for koi and about 0.5–0.7 cm deep for threespot gourami. The fish were then returned to their original tanks and remained there until sampled. Their time to recovery (normal, upright swimming behavior, responsive) was 1–2 min. Four replicates, consisting of one fish per tank, were run for each sampling time.

Blood Sampling

Fish were removed from test tanks and anesthetized by immersion in a sodium bicarbonate–buffered, 200-mg/L (for koi) or 400-mg/L (for threespot gourami) solution of Tricaine-S. They were monitored until sufficiently anesthetized and then weighed. Approximately 0.8–1.0 mL (koi) or 0.3–0.5 mL (threespot gourami) of blood was drawn from the caudal vein into a heparin-coated 1-mL syringe with a 25-gauge needle. For oral and intramuscular administration, we sampled blood at 0.5, 1, 3, 5, 7, 9, 12, and 24 h postdose for threespot gourami and until 36 h postdose for koi. Each fish was sampled once and then removed to a separate recovery tank. The blood was immediately transferred to a siliconized microcentrifuge tube and centrifuged at 3,000 × gravity for 15 min at 6°C. The plasma was transferred to a clean siliconized microcentrifuge tube and stored at −70°C until analysis.

Minimum Inhibitory Concentrations

We tested 10 bacterial isolates cultured from clinical cases submitted to the University of Florida Tropical Aquaculture Laboratory's Fish Disease Diagnostic Laboratory (TAL; Ruskin, Florida). They were one isolate of *Aeromonas veronii*/*sobria*, two isolates of *A. caviae*, six isolates of *Streptococcus* spp. (five of *S. iniae*, one of *S. vestibularis*), and one isolate of *Vibrio metschnikovii*. Bacterial identification was determined with the Biolog Microbial ID System (Biolog, Hayward, California). Because the Biolog System was not designed specifically to identify fish pathogens, there may be some discrepancies among currently recognized bacterial fish pathogens and those identified by the system. Nonetheless, the Biolog System is used by a number of laboratories working with fish, and characteristics of some taxa determined with the Biolog System are cited in at least one important text in the literature, which mentions the potential adaptability and usefulness of the system (Austin and Austin 1999). In addition, new species of bacteria isolated from diseased fish are being reported each year. For the sake of consistency, we have identified the isolates in this article based on their Biolog identification. However, clinical signs, response to treatment, and the lead author's personal experience working with disease in ornamental fish species assure us that these isolates were a component of the disease in each case.

The MICs for the isolates tested were determined by one of two methods, used for availability and logistical reasons. The MICs of florfenicol for six of these tropical fish isolates—*A. caviae* (isolate 1), *S. iniae* (isolates #1–3), *S. vestibularis* (1 isolate), and *V. metschnikovii*—were determined with the Sensititre MIC System (AccuMed International, Inc., Chicago, Illinois). In addition, we determined the MICs of florfenicol for four of the tropical fish isolates submitted from the TAL—*A. veronii*/*sobria* (1 isolate), *S. iniae* (isolates #4, 5), and *A. caviae* (isolate 2)—using the standard agar dilution susceptibility test (NCCLS performance standard for disk and dilution susceptibility test for bacteria isolated from animals: approved standard NCCLS DOC M31-A, ISBN 1-56238-377-9, 1999).

Analysis of Plasma Samples and Fish Feed

The concentration of florfenicol in plasma was analyzed by a high-performance liquid chromatographic (HPLC) method as previously reported (Vue et al. 2002), with minor modifications.

Chromatography.—The chromatographic system consisted of a Perkin-Elmer series 200 LC pump, a Perkin-Elmer ISS 200 autosampler, an Applied Biosystems 785A variable-absorbance detector (λ = 224 nm), and a Perkin-Elmer Nelson 900 series interface with Turbochrom software (Ver 4.0). The chromatographic column was a 150 mm × 4.6 mm column packed with Zorbax (C_{18}, 5 μm particles); a 30 mm × 4.6 mm precolumn was filled with same material. The mobile phase, which consisted of 22% acetonitrile and 78% 0.05 M ammonium acetate buffer, was pumped at a flow rate of 1.0 mL/min. Chromatographic analyses were performed at room temperature (25°C).

Plasma sample cleanup.—The plasma samples were extracted by solid-phase extraction. To 0.25 mL of plasma (sample, standard, blank), 0.75 mL of doubly distilled water and 100 μL of thiamphenicol (internal standard, 8 μg/mL) were added and vortex-mixed well. The samples were then transferred onto a Supelco C_{18} solid-phase extraction column preconditioned before the transfer with 2 column volumes of ethanol and 3 column volumes of water (1 column volume = 6 mL). We washed the sample-containing columns with 2 mL of 15% acetonitrile in water and 3 mL of hexane and eluted the column with 3 mL of 100% acetonitrile. The acetonitrile eluent was evaporated to dryness, reconstituted in 100 μL of the mobile phase, and injected onto the HPLC. The plasma concentration of florfenicol was determined by comparison with standards prepared in florfenicol-free human plasma. We compared human plasma with fish plasma

to check for interferences in the assay and recovery. The absolute recovery of florfenicol from both human and fish plasma was about 90%. Calibration curves (0.1 μg/mL to 25 μg/mL) were prepared freshly by adding florfenicol to blank human plasma on the day of analysis. Triplicates of quality-control samples at low (0.25 μg/mL), medium (4.0 μg/mL), and high (12.5 μg/mL) florfenicol concentrations were used during sample analysis. The assay method was specific to florfenicol. Variations in intra- and interbatch accuracy and precision were within acceptable limits of less than ±20% at low (0.25 μg/mL) concentrations and less than ±15% at medium (4.0 μg/mL) and high (12.5 μg/mL) concentrations.

Fish feed analysis.—The fish feed samples were weighed and ground finely. To 0.2 g of feed was added 3 mL of acetonitrile. The samples were kept at room temperature for 20 min and centrifuged at 5,000 × gravity for 10 min. The supernatant was injected directly onto the HPLC. Control samples (5 mg/g) were used to check for analytical recovery.

Pharmacokinetic Data Analysis

Noncompartmental analysis.—The noncompartmental parameters were calculated from the relationship between mean concentration-time profiles by using KINETICA (version 4.1; Innaphase Corp.). Pharmacokinetic parameters—namely, maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), half-life of drug in plasma ($t_{1/2}$), and area under the concentration–time curve (AUC)—were obtained and compared between koi and threespot gourami.

Compartmental analysis.—The compartmental analysis was performed by population approach using NONMEM (version V, Level 1.1, Globomax LLC; Boeckman et al. 1994). The plasma concentration after oral administration in koi carp normalized for assumed dose was calculated with the following formula (for two fish per tank):

$$C = \frac{a \cdot W_a + b \cdot W_b}{W_a + W_b},$$

where C = plasma concentration normalized for standard dose; W_a = weight of fish A (kg); W_b = weight of fish B (kg); a = plasma concentration of florfenicol (g/mL; (fish A); and b = plasma concentration of florfenicol in (μg/mL; fish B)

Normalization was not necessary for the intramuscular studies in koi and threespot gourami and for the oral administration study in threespot gourami.

Preliminary examination of the data suggested a one-compartment model with elimination from the central compartment. The model was expressed in terms of absorption rate constant (k_a), clearance (CL) and volume of distribution (V), respectively. A lognormal distribution was assumed for the pharmacokinetic parameters, that is,

$$\theta_i = \theta_0 \cdot \exp(\eta_i),$$

where θ_0 denotes the vector of population mean pharmacokinetic parameters and η_i denotes a vector of interindividual random effects, which are multivariate normal with mean $E(\eta_i) = 0$ and variance $V(\eta_i) = \omega^2$. Residual intraindividual variability was identically distributed and was modeled by using the additive proportional error model. The additive error model is described by

$$C_{pij} = C_{pmij} + \varepsilon_{ij},$$

where C_{pij} is the ith observed plasma concentration for the jth individual, C_{pmij} is the ith concentration predicted by the model at the ith observation time for the jth individual, and ε_{ij} is a normally distributed parameter with a mean of zero and a variance of σ^2. With the fixed and random effects chosen, we subsequently obtained empirical Bayes estimates of the pharmacokinetic parameters, using the POSTHOC option in NONMEM program. The relative oral bioavailability (F_{rel}) was estimated by simultaneously fitting both oral and intramuscular ($F = 1$, assumption) concentration–time profiles.

Results

Fish Feed

The analyses of the fish feed samples are shown in Figures 1A and 1B for koi and threespot gourami, respectively. About 50% of the drug in the case of koi and 80% of the drug in the case of threespot gourami was lost from the medicated feed after 5 min in water.

Noncompartmental Analysis

The noncompartmental parameter estimates for florfenicol are given in Table 1. The estimates of AUC after oral and intramuscular administration were 263 and 444 $\mu g \cdot h^{-1} \cdot mL^{-1}$ for koi and 28 and 189 $\mu g \cdot h^{-1} \cdot mL^{-1}$ for threespot gourami, respectively. Florfenicol half-life ($t_{1/2}$) was estimated as 14.0 and 7.6 h in koi and 6.6 and 2.5 h in threespot gourami after intramuscular and oral ad-

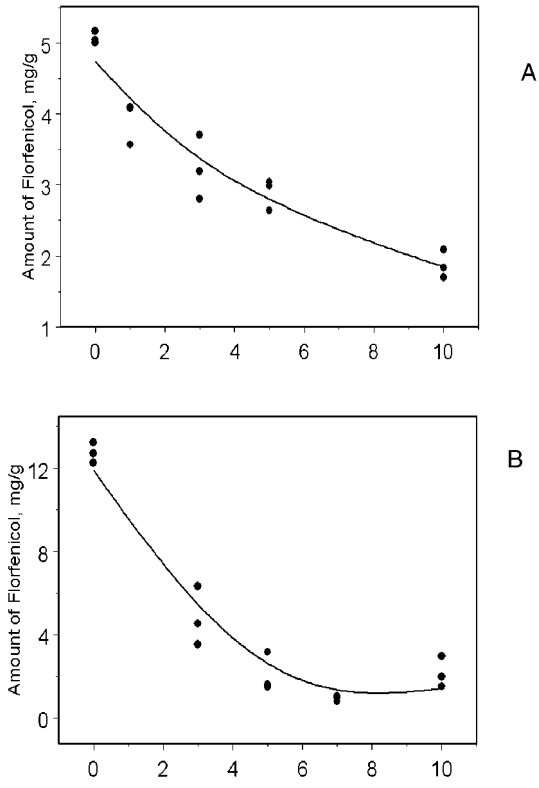

FIGURE 1.—(**A**) Florfenicol remaining in feed at various sampling points in koi carp; (**B**) florfenicol remaining in feed at various sampling points in threespot gourami.

ministration, respectively. A single oral dose (50 mg/kg) in koi carp maintains plasma levels in excess of 4 μg/mL for 24 h; the same dose in threespot gourami maintains plasma levels at 1–3 μg/mL for about 10–12 h.

Compartmental Analysis

The observed and predicted plasma concentration–time profile after oral and intramuscular administration in koi and threespot gourami are shown in Figures 2A and 2B, respectively. Applying a population-modeling approach, we determined that a simple one-compartment model with first-order absorption and elimination was adequate to characterize the pharmacokinetics of florfenicol simultaneously in koi and threespot gourami after oral and intramuscular administration. Two koi fish that had some hemorrhaging after intramuscular injection were excluded from data analysis.

The estimated absorption rate constant (k_a) for oral doses was 0.5/h for koi and 1.2/h for threespot gourami. After intramuscular administration, k_a was estimated as 0.2/h for koi and 6.0/h for threespot gourami. CL and V were estimated as 0.05 $L \cdot h^{-1} \cdot kg^{-1}$ and 1 L/kg for koi and 0.32 $L \cdot h^{-1} \cdot kg^{-1}$ and 2.0 L/kg for threespot gourami, respectively. The estimated relative oral bioavailability for koi was 29%, 13% for the threespot gourami (Table 2).

Minimum Inhibitory Concentrations

Table 3 lists the MICs determined by use of either the Sensititre System or standard agar dilution susceptibility test. The MICs of florfenicol for the tropical fish isolates were less than 2 μg/mL.

Discussion

Ornamental fish species are an economically important group among the approximately 24,000 living species of fish. Striking physiological and anatomical differences can be observed among different species of ornamental fish. Koi carp can, with proper acclimation, tolerate a relatively wide

TABLE 1.—Noncompartmental analysis of florfenicol in koi and threespot gourami after oral (50 mg/kg) and intramuscular (25 and 50 mg/kg) administration. Abbreviations are as follows: C_{max}, maximum plasma concentration; T_{max}, time at which C_{max} is observed; AUC, area under the curve; CL, clearance; *V*, volume of distribution; $t_{1/2}$, half-life; *F*, bioavailability; and MRT, mean residence time.

	Oral		Intramuscular	
Variable	Koi	Gourami	Koi	Gourami
C_{max} (μg/mL)	12.3	2.6	18.0	28.0
T_{max} (h)	3.0	5.0	24	3.0
AUC ($\mu g \cdot h^{-1} \cdot mL^{-1}$)	262.6	27.5	444.4	188.6
CL/*F* ($L \cdot h^{-1} \cdot kg^{-1}$)	0.2	1.8	0.04	0.3
V/*F* (L/kg)	2.9	23.3	1.2	1.3
$t_{1/2}$ (h)	7.6	6.6	13.9	2.5
MRT (h)	15.4	13.0	40.0	4.8

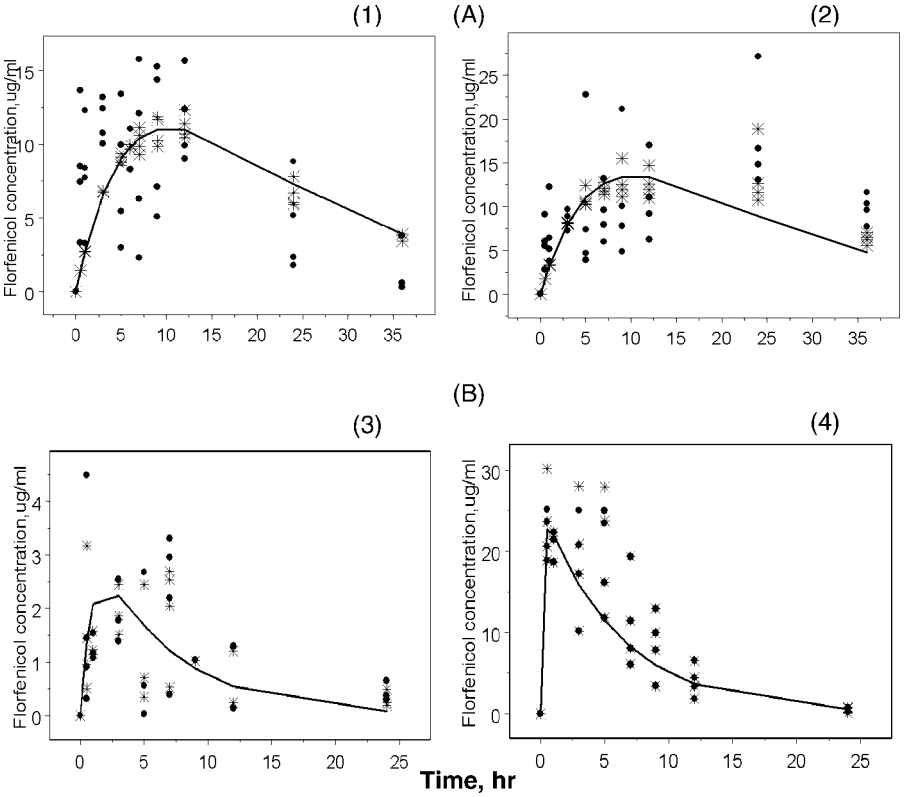

FIGURE 2.—Plasma concentration–time profiles of florfenicol. The circles represent observed data, the asterisks individual predicted concentrations, and the lines population predicted concentrations after oral (panels 1 and 3) and intramuscular (panels 2 and 4) administration in **(A)** koi carp and(**B**) threespot gourami.

range of temperatures (~1–32°C); threespot gourami, on the other hand, require much warmer temperatures (~22–32°C). Anatomically, koi lack true acid stomachs, whereas the threespot gourami have gizzard-like stomachs. These physiological and anatomical differences between species could translate into different pharmacokinetic behavior for a given drug. Florfenicol, a fluorinated derivative of thiamphenicol, is a potent, broad-spectrum antibacterial agent with bacteriostatic properties.

TABLE 2.—Population pharmacokinetic parameters (compartmental analysis) for florfenicol in koi and threespot gourami after oral (50 mg/kg) and intramuscular (25 and 50 mg/kg) administration. Interindividual variability (%) is shown in parentheses. The abbreviation k_a stands for the absorption rate constant, σ for residual variability; see Table 1 for other abbreviations.

Variable	Koi	Gourami
$k_{a,oral}$ (h^{-1})	0.5 (119)	1.2 (131)
$k_{a,intramuscular}$ (h^{-1})	0.2	6.0
CL ($L \cdot h^{-1} \cdot kg^{-1}$)	0.05 (47)	0.3 (76)
V (L/kg)	1.03[a]	2.0[a]
F (%)	29.5	13.1
$t_{1/2}$ (h)	15.8	4.2
σ	38%	0.4 μg/mL

[a] Interindividual variability not estimated.

TABLE 3.—Minimum inhibitory concentrations (MICs) of florfenicol for bacterial pathogens of tropical fish isolated from clinical cases submitted to the Tropical Aquaculture Laboratory. University of Florida, 2000–2002. Identification is based on the Biolog system.

Isolate	MIC (μg/mL)
Aeromonas caviae 1	≤1
Streptococcus iniae 1	≤1
S. iniae 2	≤1
S. iniae 3	≤1
S. vestibularis	≤1
Vibrio metschnikovii	≤1
Aeromonas caviae 2	2
A. veronii/sobria	0.5
S. iniae 4	2
S. iniae 5	2

In this study, we studied the pharmacokinetics of florfenicol in koi and threespot gourami at 24–25°C, which is representative of the temperature conditions in which ornamental fish are typically maintained. The main highlight of the experimental procedure is that florfenicol was administered orally, as would be the route of choice for treatment of systemic bacterial infections in commercial operations. Because attempts to have antibiotics premixed into feed by a manufacturer were unsuccessful (see comments below), we chose top-dressing to provide a more uniform concentration and distribution of drug upon replicate analysis. FDA methods would most likely require premixed antibiotic-medicated feeds, although in the past, top-dressing has been a common and effective practice (Horsberg et al. 1996). The first major problem arose when a small batch of experimental, manufactured, premedicated feed was used. This particular diet formulation was not stable in water (it disintegrated within a few minutes), was not uniform in antibiotic distribution, and was unpalatable to the fish. After trying numerous other methods—tube feeding with a catheter was ineffective, because samples that had food coloring dyes incorporated to test this method demonstrated significant loss out the mouth and gills (koi do not have a true stomach), as was incorporating antibiotic into small, live grubs (fish were not interested in consuming these grubs)—we obtained the best results by top-dressing the feed as used in this study.

Results from the bath treatment pilot study with koi fish indicated little to no absorption into the bloodstream at both concentrations tested (50 and 80 mg/L); therefore, we determined this to be an ineffective method of administration (data not reported). We also assumed that the drug loss from the pellets into the surrounding water in the oral pharmacokinetic study would not provide another source of input of drug to influence the plasma concentration of florfenicol in koi. Absorption of florfenicol through the bath treatment procedure was not evaluated from threespot gourami.

A population pharmacokinetic model for plasma florfenicol was developed using NONMEM after oral and intramuscular administration in koi and threespot gourami because the sampling was sparse (one sample from each fish). The data from both studies (oral and intramuscular) were simultaneously analyzed by a simple one-compartment model with first-order absorption and elimination. Because adequate data were not available to characterize the absorption phase, we tested the sensitivity of the model analysis by analyzing results for a range of values, from 0.02/h to 2/h. The value of k_a that resulted in successful convergence and overall model stability was chosen. The estimated elimination half-life in koi was 16 h as compared with 4 h in threespot gourami. The elimination half-life of florfenicol reported in Atlantic salmon is 12–15 h (Martinsen et al. 1993; Horsberg et al. 1994, 1996) and 43 h in cod *Gadus morhua* (Samuelsen et al. 2003). The differences in the T_{max} in koi after oral and intramuscular administration of florfenicol could be due to the high variability; in the threespot gourami T_{max} appears to be reached by 3–6 h after both routes.

The estimate of bioavailability was 29% in koi and 13% in threespot gourami. In other species however, the bioavailability of florfenicol was reported to be greater than 90% (Martinsen et al. 1993). The reason for the difference in bioavailability may not be attributable to species differences but rather to the loss of drug in solution after feed is placed in the fish tank as part of the natural feeding process. Analysis of the dissolution studies of feed samples in vitro showed that about 50% of the drug was lost at 0–5 min after administration to koi fish and 80% was lost to threespot gourami at 0–7 min after administration. The uneaten pellets collected from the fish tanks after approximately 5 and 7 min in the oral pharmacokinetic study that indicated about 70 and 80% of the drug was lost into water in the case of koi and threespot gourami, respectively. This might result from the coated drug not adhering properly to the feed or from batch-to-batch variations in manual feed preparation. However, the drug released into water would not influence the pharmacokinetics in koi, given that very low plasma concentrations were observed in these fish after bath treatment. The drug that was released by the pellets was removed immediately as the system water was continuously circulating through activated carbon. Because most of the fish consumed all of the feed presented, we could assume that for koi, of the 30% of the drug consumed through the feed, 29% is bioavailable, which could be translated to a relative oral bioavailability of approximately 99%. In the threespot gourami, the relative bioavailability calculated similarly would be 65% (13/20). However, the important finding in this study is that the procedure likely to be followed in real life, compared with individual oral gavage, can result in lower concentrations and high variability, which can affect the efficacy of florfenicol.

Our study has attempted to determine the phar-

macokinetics of florfenicol in a previously common, more standard approach—application of top-dressed feeds. Studies in the past have relied on artificial means for feed administration. The resulting pharmacokinetic data should thus be understood to be applicable only for the specific method of oral administration used in that specific trial. Leaching of drugs from medicated feeds, regardless of preparation, is a known phenomenon (Xu and Rogers 1994; Rigos et al. 1999), although many studies do not acknowledge this. Losses of oxolinic acid through leaching were shown to be as high as 55.5% and oxytetracycline were as high as 42.5% at 16°C in one study (Rigos et al. 1999). In another study, when oxytetracycline was sprayed onto catfish feed, 23.9–37.5% was lost to water after 5–25 min of immersion at 30°C (Xu and Rogers 1994). Although medicated feeds mixed in during feed preparation do have greater stability than top-dressed feeds, both methods do result in leaching. In one study, leaching of oxytetracycline from sprayed sinking feeds was four times more than from mixed sinking feeds after 25 min (Xu and Roger 1994). Most likely, however, governmental and industry standards will necessitate that drugs be mixed during feed preparation rather than top-dressed.

Although pharmacokinetic trials were run only on koi and threespot gourami (for economic and logistical reasons), interest in the effectiveness of florfenicol against bacteria isolated in other species is high in the industry. In Florida alone, hundreds of ornamental species (many with numerous varieties) are in production, and different opportunistic bacterial pathogens have been isolated from multiple species of ornamental tropicals. Consequently, we determined MICs for isolates taken from clinical cases of a variety of species tested. Routine workups include microbiological sampling. Microbiological samples from moribund fish showing typical clinical signs within a given diseased population are obtained with aseptic technique. In all species, microbiological samples collected included brain and kidney, although other internal organs are also routinely sampled. The isolates chosen appeared pure upon primary isolation and were subcultured before identification and sensitivity determination. Antibiotics chosen on the basis of sensitivities in these cases have resulted in significantly reduced mortality in the respective populations.

The MICs of florfenicol against 10 bacterial isolates were all 2 μg/mL or less, demonstrating that relatively low levels of florfenicol are effective against both gram-negative and gram-positive pathogens. The rapid clearance of florfenicol as observed in its short half-life in threespot gourami could necessitate repeated dose administration every 12 h with antibiotic properly incorporated into feed to maintain higher plasma concentration levels above the desired MIC of 1–2 μg/mL. The proposed dosing regimens can be tested in future studies.

Conclusion

The pharmacokinetics of florfenicol were studied in koi and in threespot gourami after oral and intramuscular administration. To the best of our knowledge, this is the first report of pharmacokinetic studies done in threespot gourami and the first comparing the pharmacokinetics of a drug in these two species of ornamental fish.

The choice of the feed and appropriate coating or incorporation procedure will help in attaining the desired effective concentrations in plasma. The pharmacokinetic information derived for koi and threespot gourami, which are representative and important species in the ornamental fish trade, could be used to design rational dosing regimens. However, pharmacokinetics of florfenicol should be evaluated with other groups of ornamental fish to see if there are any significant differences between them.

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