

Pharmacokinetics of oxytetracycline in the red pacu (*Colossoma brachypomum*) following different routes of administration

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Oxytetracycline (OTC) pharmacokinetics were studied in the red pacu (*Colossoma brachypomum*) following intravenous (i.v.) and intramuscular (i.m.) administration at a dose of 5 mg/kg body weight. OTC plasma concentrations were determined by high-performance-liquid-chromatography (HPLC). A non-compartmental model was used to describe plasma drug disposition after OTC administration. Following i.m. administration, the elimination half-life ($t_{1/2}$) was 62.65 ± 1.25 h and the bioavailability was $49.80 \pm 0.01\%$. After i.v. administration the $t_{1/2}$ was 50.97 ± 2.99 h, the V_d was 534.11 ± 38.58 mL/kg, and Cl_b was 0.121 ± 0.003 mL/min.kg. The 5 mg/kg i.v. dose used in this experiment resulted in up to 48 h plasma concentrations of OTC above the reported MIC values for some strains of fish pathogens such as *Aeromonas hydrophila*, *A. liquefaciens*, *A. salmonicida*, *Cytophaga columnaris*, *Edwardsiella ictaluri*, *Vibrio anguillarum*, *V. ordalii*, *V. salmonicida* and *Yeersinia ruckeri*. These MIC values are below the susceptible range (4 µg/mL) listed by the National Committee for Clinical Laboratory Standards (NCCLS) as determined by the NCCLS susceptibility interpretive criteria.

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INTRODUCTION

Oxytetracycline (OTC) is widely used for the treatment of bacterial infections in aquaculture fish species, not only because it is one of the few approved antibacterial drugs, but also because of its broad-spectrum of activity, low toxicity and capacity for diffusion into most body fluids and tissues (Ory, 1980; Barragy, 1994; Kapusnik-Uner *et al.*, 1996). OTC has also found wide application in the treatment of ornamental fish, but dosing regimens are extrapolated from other species (Stoskopf, 1993). Extrapolation is not easy because fish species are physiologically diverse. There is a wide variability in pharmacokinetics of drugs among fish, depending on the species, environmental conditions, route of administration and formulation administered (Endo, 1992; Lafont, 1992). There have been several pharmacokinetics studies of tetracyclines in food fishes (Grondel *et al.*, 1987; Plakas *et al.*, 1988; Grondel *et al.*, 1989; Jacobsen, 1989; Black *et al.*, 1991), however, little attention has been paid to tropical species. The red pacu (*Colossoma brachypomum*), is a very popular aquarium fish and is related to important ornamental species, including the well-known tetras.

The purpose of this study was to determine the pharmacokinetics of OTC in the red pacu following intramuscular (i.m.) and

bolus intravenous (i.v.) administration. Appropriate dosage regimens of OTC in the red pacu were calculated based on the pharmacokinetic parameters obtained.

MATERIALS AND METHODS

Animals

The red pacu (*Colossoma brachypomum*) were obtained from a commercial tropical fish farm (EkkWill Tropical Fish, Gibsonton, FL, USA) and weighed approximately 200g at the time of the study. They were maintained in a research fish colony at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA. Fish were kept individually in 75 L glass aquariums. All glass aquariums shared a common freshwater supply maintained at 23°C via a recirculating water system. All fish were fed pelleted dry food (Big Strike® Floating Fish Food, Southern States Cooperative Inc., Richmond, VA, USA), at a rate of 1% body wt per day. All animals were held and treated in accordance with the Institutional Animal Care and Use Committee guidelines.

Antibiotic administration and sampling design

Seventy-two red pacu were divided into two groups of 36 fish (groups A and B), again divided into nine subgroups of four fish. One day prior to dosing, all fish were netted, placed in a tarred container filled with water, and weighed (198 ± 45 g). Three fish in each subgroup were netted, manually restrained and received 5 mg/kg of OTC hydrochloride (Sigma Chemical Co., St Louis, MO, USA) aqueous solution i.m. in the dorsal musculature, under the dorsal fin (group A), or i.v. in the caudal vein (group B). The remaining fish in each subgroup served as a negative control and were injected with an equal volume of 0.9% saline solution per kg body weight.

All fish were sampled at 0 h, immediately prior to drug administration. At each of the following time points, one subgroup was sampled (three treated fish (replicates) and one control). All sampling was done by manually restraining the fish, and no fish was sampled more than three times per experiment. The maximum amount of total blood volume taken from an individual fish in an experiment was 1.8 mL. Samples were taken at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 96 h by taking approximately 0.6 mL of blood from the caudal vein. Blood was stored on ice after collection and centrifuged for 10 min at 600 g within 10 min of phlebotomy. Plasma was then stored at -70°C until sample analyses were done.

Sample preparation

Plasma samples were extracted using modifications from previously published methods (Tyczkowska & Aronson, 1986; Riond *et al.*, 1989). Plasma (100 μL) was added to 100 μL of a releasing substance composed of 20% acetonitrile, 2% phosphoric acid and 78% deionized water and vortexed for 15 s. The mixture of the plasma and releasing substance was then transferred to a centrifuge filter (nominal molecular weight cutoff, NMWC 30 000, with a polysulfone membrane, Micron Separations Inc., Westboro, MA, USA) and centrifuged at 2,000 g for 30 min. Twenty μL of the filtrate obtained after centrifugation was injected into the high performance liquid chromatography system (HPLC).

Chromatographic conditions

The analytical column used was a Nucleosil C8, 150 mm \times 4.6 mm ID (Supelco-Sigma-Aldrich, Supelco Inc., Bellefonte, PA, USA) attached to a Nucleosil C8, 10 mm \times 4.6 mm ID (Supelco-Sigma-Aldrich, Supelco Inc.) guard column. The HPLC consisted of a 600 E Waters multi solvent delivery system (Waters, Millipore Corp, Waters Chromatography Div, Milford, MA, USA), SIL-9A Automatic sample injector (Shimadzu Co., Kyoto, Japan) and a Spectro monitor 3100 variable wavelength detector (LDC Analytical, Riviera Beach, FL, USA). Detection wavelength was 355 nm. The SMAD Data System[®] for Apple Macintosh Computers (SMADChrom Version 2.2, Morgan Kennedy Research, College Station, TX, USA) was used to record the data. The separation used modifications from a previously published

method (Oka *et al.*, 1984), and was isocratic (1 mL/min), at 25°C with a mobile phase composed of 5% methanol, 10% acetonitrile and 85% aqueous 0.01 M oxalic acid.

Data analyses

OTC was used as the external standard. Standard calibration curves in the range 0.0–10.0 $\mu\text{g/mL}$ were done in three replicates by plotting the known OTC concentrations vs. peak area. Regression analyses were then performed (Data Analysis, Microsoft Excel 5.0, Microsoft Corporation, Redmond, WA, USA) and the equation for the regression line obtained was $Y = 0.11381 + 0.0000546X$, with a correlation coefficient of $R^2 = 0.99$. The equation for the regression line was used to calculate the unknown OTC concentrations in the experimental samples.

The precision of the method was determined by analysing a sample three times in the same run. The relative coefficient of variation for the OTC peak area was 1.0%. The limit of detection, defined as a peak of at least three times the height of the baseline noise, was approximately 0.05 $\mu\text{g OTC/mL}$ of red pacu plasma. Detection limit was determined by injecting pacu plasma with known added concentrations of OTC (spiked) into the HPLC and determining the minimum detectable concentration. OTC recovery from red pacu plasma was $76.16 \pm 0.14\%$ at 1 $\mu\text{g/mL}$ ($n = 6$). Extraction recovery of OTC was calculated by comparing peak areas from serum spiked at 1 $\mu\text{g/mL}$ with peak areas from direct injection of standard diluted in mobile phase.

Quality control samples (1 $\mu\text{g/mL}$ of OTC diluted in mobile phase) were injected into the HPLC once a day to verify the chromatography conditions. Peak area variations of 15% or less were considered normal.

Mathematical methods

The data was fitted using the WinNonlin program (Scientific Consulting Inc., version 1.5, Cary, NC, USA), which estimates elimination half-life ($t_{1/2}$), slope of the elimination phase of the distribution curve (λ) and area under the curve (AUC). Other parameters were calculated from the following pharmacokinetic formulas (Baggot, 1977; Rowland & Tozer, 1989):

Dose = Clearance \cdot AUC where AUC is the area under the plasma concentration-time curve.

$V_{d_{\text{area}}} = \frac{\text{Dose}}{\text{AUC} \cdot \lambda}$ where $V_{d_{\text{area}}}$ is the volume of distribution calculated by the area method, and λ is the slope of the elimination phase of the disposition curve.

$F = \frac{(\text{AUC}_{\text{i.m.}})}{(\text{AUC}_{\text{i.v.}})} \cdot \frac{(\text{Dose}_{\text{i.v.}})}{(\text{Dose}_{\text{i.m.}})}$ where F is the bioavailability, i.m. is intramuscular and i.v. is intravenous.

Analyses of variance were used to detect differences between treatments (i.m. and i.v. administration) (SAS Analyses, The SAS System for Windows, Version 6.1, SAS Institute Inc, Cary, NC, USA).

Appropriate dosage regimens of OTC in the red pacu were calculated using the following formula (Rowland & Tozer, 1989):

$$C_{\min} = \frac{\text{Dose} \cdot e^{-\lambda \cdot td}}{Vd}$$

where C_{\min} is the minimum plasma concentration, λ is the slope of the elimination phase of the disposition curve, td is the duration of the effect and Vd is the volume of distribution.

RESULTS

The plasma concentration–time profiles of OTC after i.m. and i.v. administration to the red pacu are shown in Fig. 1 and pharmacokinetic parameters summarized in Table 1. The plasma concentrations obtained after i.m. and i.v. administrations were significantly different ($P < 0.05$) at all time points, but plasma concentrations within replicates were not significantly different ($P > 0.05$).

The area under the concentration–time curve (AUC) after i.v. administration of OTC to the red pacu ($688.89 \pm 18.52 \mu\text{g}\cdot\text{h}/\text{kg}$) was larger than after i.m. administration ($343.00 \pm 5.03 \mu\text{g}\cdot\text{h}/\text{kg}$). Calculated bioavailability was $49.80 \pm 0.01\%$ after i.m. administration of OTC.

DISCUSSION

The mean elimination half-lives of OTC after i.m. and i.v. administration to the red pacu (62.65 h and 50.97 h, respectively), as well as in other fish species (Grondel *et al.*,

Table 1. Pharmacokinetic parameters of oxytetracycline in the red pacu (*Colossoma brachyomum*). Oxytetracycline was dosed at 5 mg/kg and water temperature maintained at 23°C

Parameters*	Units	Values ^f	
		i.m.	i.v.
Weight of fish	g	227 ± 30	170 ± 38
λ	h^{-1}	0.0111 ± 0.0002	0.0136 ± 0.0008
$t_{1/2}$	h	62.65 ± 1.25	50.97 ± 2.99
$AUC_{0 \rightarrow \infty}$	$\mu\text{g}\cdot\text{h}/\text{mL}$	343.00 ± 5.03	688.89 ± 18.52
Cl_b	$\text{mL}/\text{min}\cdot\text{kg}$	N/A ^g	0.121 ± 0.003
Vd_{area}	mL/kg	N/A ^g	543.11 ± 38.58
F	%	49.80 ± 0.01	

*Pharmacokinetic parameters abbreviations; λ : Value of the slope of the elimination phase of the disposition curve; $t_{1/2}$: Elimination half-life of the drug; $AUC_{0 \rightarrow \infty}$: Area under the plasma concentration–time curve after a single dose administration; Cl_b : Body clearance of the drug, representing total body clearance; Vd_{area} : Apparent volume of distribution of the drug, calculated by the area method; F : Bioavailability; ^fValues represent the mean value and standard deviation of parameters obtained from concentration–time curves of replicates 1, 2 and 3; ^gValues cannot be appropriately calculated because absorption after i.m. administration is incomplete.

1987; Grondel *et al.*, 1989; Black *et al.*, 1991) were much longer than those reported for homeothermic species (Toutain & Raynaud, 1983; McElroy *et al.*, 1987; Dyer, 1989). Longer elimination half-lives of OTC in fish compared to mammals could be explained primarily because of the marked physiological differences in vascular perfusion, membrane permeability and muscular composition. Fish, as well as other poikilotherms, are reported to have lower heart rates and smaller cardiac outputs, with their tissues receiving much smaller amounts of blood in a given time when compared to mammals (Itazawa, 1970). In addition, the glomerular filtration rate is low in freshwater fish species (Hickman & Trump, 1969), an important issue when the elimination process of drugs occurs mainly by passive diffusion (Grondel *et al.*, 1989).

When compared to other fish species, the elimination half-lives of OTC in the red pacu were shorter than in species such as African catfish and carp (Grondel *et al.*, 1987; Grondel *et al.*, 1989), but longer than elimination half-lives of OTC in trench (Reja *et al.*, 1996). Data reporting elimination half-lives of OTC in rainbow trout showed a wide range of elimination half-lives, from 60.3 (Björklund & Bylund, 1991) to 89.5 h (Grondel *et al.*, 1989). These differences among and within species could be explained in part by environmental factors such as acclimatization temperature, which is known to play an important role in drug absorption, elimination and metabolism in fish (Jacobsen, 1989; Björklund & Bylund, 1990). However, there is not always a direct correlation between acclimatization temperature and elimination half-life of OTC in various fish species. Other factors such as muscle composition and associated vascular perfusion may also affect the elimination of OTC in fish. Salmonids are strong swimmers with approximately 50–60% of their musculature composed of white muscle fibres (Webb, 1993). On the other hand, species such as catfish, carp and freshwater tropical fish have predominantly white musculature (Beleau, 1993;

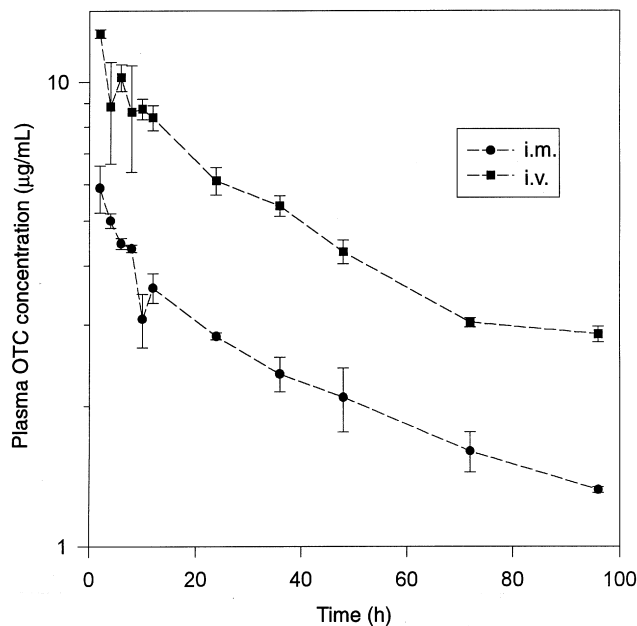


Fig. 1. Concentration–time profile of oxytetracycline after intramuscular (i.m.) and intravenous (i.v.) administration to the red pacu (*Colossoma brachyomum*).

Bolon, 1993a; Bolon, 1993b), which is considered by many to be less perfused than red musculature (Duff *et al.*, 1987). The mean volume of distribution of OTC in the red pacu after i.v. administration was relatively high (0.534 L/kg), but lower than in homeothermic endotherms studied (Toutain & Raynaud, 1983; McElroy *et al.*, 1987; Dyer, 1989). In this study there was no attempt to evaluate the pattern of OTC distribution into different tissues and organs of the red pacu, but studies in other fishes have shown that OTC accumulates in pronephros, bone tissue and scales (Grondel *et al.*, 1987).

The results of the pharmacokinetics of OTC in the red pacu after administration of 5 mg/kg needs to be examined in relation to the MIC for most bacteria pathogenic to fish. Seventy-two hours following i.m. and i.v. administration, the plasma concentration is still above reported MIC values for some strains of *Aeromonas hydrophila*, *A. liquefaciens*, *A. salmonicida*, *Cytophaga columnaris*, *Edwardsiella ictaluri*, *Vibrio anguillarum*, *V. ordalii*, *V. salmonicida* and *Yersinia ruckeri* (Grondel *et al.*, 1987; Stamm, 1989; Tsoumas *et al.*, 1989; Hawke & Thune, 1992; Martinsen *et al.*, 1992). After 5 mg/kg i.v., the plasma OTC concentration in pacu serum is above the reported MIC values for *Flexibacter columnaris*, *Pseudomonas fluorescens* and *Vibrio anguillarum*, 12 h after treatment (Grondel *et al.*, 1987; Martinsen *et al.*, 1992).

The National Committee for Clinical Laboratory Standards (NCCLS) lists the susceptible range of OTC as 4 µg/mL or less, as determined by the NCCLS susceptibility interpretive criteria. Thus, effective therapy should be obtained when plasma concentrations are above 4 µg/mL, provided that antimicrobial susceptibility tests determined that the target bacteria are not resistant to OTC. Appropriate OTC concentrations for treatment of bacterial infections in the red pacu can be theoretically obtained with i.v. doses of 3.0 mg/kg or i.m. doses of 7.0 mg/kg at 24 h dosing intervals, but multidose studies should be conducted to confirm these calculations.

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