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Pharmacokinetics of oxytetracycline in hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) after intravascular and oral administrations

Amnart Poapolathep [1], Kednapat Sriphairoj [2], Sittichai Hatachote [2], Kannika Wongpanit [2], Duangkamol Saensawath [2], Narumol Klangkaew [1], Napasorn Phaochoosak [1], Mario Giorgi [3], Saranya Poapolathep [1,*]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[2]Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand
[3]Department of Veterinary Science, University of Pisa, San Piero a Grado, Pisa 56121, Italy

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***Corresponding author:**
Saranya Poapolathep
Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
Email: fvetsys@ku.ac.th
saranya.po@ku.th
https://orcid.org/0000-0002-5249-903X

ABSTRACT

Importance: Over the past decade, catfish farming has increased in Southeast Asia. However, there has been no existing for pharmacokinetic data in the hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*).
Objective: This study was designed to evaluate the pharmacokinetic characteristics of oxytetracycline (OTC) in the hybrid catfish, following single intravascular (IV) or oral (PO) administration at a single dosage of 50 mg/kg body weight (BW).
Methods: In total, 140 catfish (each about 100–120 g BW) were divided into two groups (n = 70). Blood samples (0.6–0.8 mL) were collected from ventral caudal vein at pre-assigned times up to 144 h (sparse samples design). OTC plasma concentrations were analyzed using high-performance liquid chromatography-photodiode array detector.
Results: The pharmacokinetic parameter of OTC was evaluated using a non-compartment model. OTC plasma concentrations were detectable for up to 144 and 120 h after IV and PO, respectively. The elimination half-life value of OTC was long with slow clearance after IV administration in hybrid catfish. The average maximum concentration value of OTC was 2.72 μg/mL with a time at the maximum concentration of 8 h. The absolute PO bioavailability was low (2.47%).
Conclusions and Relevance: These results showed that PO administration of OTC at a dosage of 50 mg/kg BW was unlikely to be effective for clinical use in catfish. The pharmacodynamic properties and clinical efficacy of OTC after multiple medicated feed are warranted.

Keywords: Oxytetracycline; pharmacokinetics; catfish; animal feed; oxytetracycline

INTRODUCTION

Over the past decade, catfish farming has increased in Southeast Asia, including in Thailand. This rapid expansion has been due to the production of post-larvae in hatcheries [1]. One of the most favored catfish for manufacturing practice is the hybrid catfish that is produced

ORCID iDs

Amnart Poapolathep
https://orcid.org/0000-0001-5322-3281
Kednapat Sriphairoj
https://orcid.org/0000-0001-8587-9541
Sittichai Hatachote
https://orcid.org/0000-0002-5201-2551
Kannika Wongpanit
https://orcid.org/0000-0003-4970-3033
Duangkamol Saensawath
https://orcid.org/0009-0000-8377-7357
Narumol Klangkaew
https://orcid.org/0009-0006-8106-009X
Napasorn Phaochoosak
https://orcid.org/0009-0008-1237-7215
Mario Giorgi
https://orcid.org/0000-0003-3657-4703
Saranya Poapolathep
https://orcid.org/0000-0002-5249-903X

Author Contributions

Conceptualization: Poapolathep S, Poapolathep A; Formal analysis: Poapolathep S; Fund acquisition: Poapolathep S; Investigation: Sriphairoj K, Hatachote S, Wongpanit K, Saensawath D, Klangkaew N, Phaochoosak N, Poapolathep A, Poapolathep S; Methodology: Sriphairoj K, Hatachote S, Wongpanit K, Saensawath D, Klangkaew N, Phaochoosak N, Poapolathep A, Poapolathep S; Project administration: Poapolathep S; Software: Giorgi M, Poapolathep A; Validation: Poapolathep S, Giorgi M, Poapolathep A; Writing - original draft: Poapolathep S, Poapolathep A; Writing - review & editing: Poapolathep S, Giorgi M, Poapolathep A.

Conflict of Interest

The authors declare no conflicts of interest.

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from crossbreeding between *Clarias macrocephalus* (female) and *C. gariepinus* (male). The F1 hybrid catfish has a rapid growth rate, greater infection tolerance, and favorable meat texture [2]. This hybrid has been cultured broadly with 93,600 tonnes harvested in 2022, which was the second-highest level of freshwater fish production in Thailand [3]. Generally, the hybrid catfish is an air breather that can tolerate water with a low oxygen level [4]. However, since it is usually cultured in an intensive system at an extremely high density, this fish is susceptible to a variety of bacterial diseases, when subjected to adverse environmental conditions [5]. Therefore, antibiotics are one of the methods used to improve disease control and treatment in fish farming [5]. However, such usage has raised problems due to evolving bacteria resistance to antimicrobials in connection with unreasonable treatment. The potential transmission of resistance poses a serious concern to the food chain consumers [6]. The surrogate of appropriate drug pharmacodynamics and pharmacokinetics in therapeutic plans are critical for treatment potency, animal welfare, human safety, and environmental care [7].

Oxytetracycline (OTC) is one of the antimicrobial agents that is most frequently used to be in charge of bacterial outbreaks in aquaculture worldwide, especially when there is no prophylactic option due to the lack of available vaccines. The U.S. Food and Drug Administration and the U.S. Environmental Protection Agency have approved three antibiotics, including OTC, for use with fish. The Codex Alimentarius Commission and European Commission have set an acceptable level of OTC at 0.1 mg/g in products from animal origin [8]. OTC is classified of the tetracycline group and a broad-spectrum antibiotic produced from *Streptomyces* spp., which has been used toward a board range of bacteria, mycoplasma, rickettsia, chamydiae, and even some protozoa [9]. However, OTC is known to have low bioavailability, generally less than 10% [10]. There have been reports regarding using OTC in fish species, including rainbow trout (*Oncorhynchus mykiss*) [11,12], channel catfish [13], Atlantic salmon (*Salmo salar*) [14], chinook salmon (*Oncorhynchus tshawytscha*) [15], yellow perch (*Perca flavescens*) [16], sea bass (*Dicentrarchus labrax*) [17], sea bream (*Sparus aurata*) [18], grass carp (*Ctenopharyngodon idellus*) [19], tilapia (*Oreochromis* spp.) [20], yellow catfish (*Pelteobagrus fulvidraco*) [21], Arctic char (*Salvelinus alpinus*) [22], Japanese eel [23], and black sea bream (*Sparus macrocephalus*) [24]. However, to date, there has been no report regarding pharmacokinetic characteristics in the hybrid catfish (*C. macrocephalus* x *C. gariepinus*) after single intravascular (IV) or oral (PO) administrations. Therefore, the current research aimed to evaluate the pharmacokinetic profiles of OTC in the hybrid catfish (*C. macrocephalus* x *C. gariepinus*) after single IV or PO administrations at dosages of 50 mg/kg body weight (BW).

METHODS

Animals

A sample was obtained of 140 healthy catfish (*C. macrocephalus* x *C. gariepinus*), with a body weight of 100–120 g. All 140 experimental catfish were housed in concrete tanks and acclimatized for 10 days before drug administration at a fish farming facility of the Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Thailand. The fish were free of OTC, as assessed using high-performance liquid chromatography (HPLC) in control fish (T0). The catfish were fed with commercial antibiotic-free pellets twice a day at 3% BW. All experimental approaches were carried out in agreement with the Guidelines for Animal Experiments and approved by the Animal Ethics Research Committee of the Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

Drugs and chemicals

Standard OTC hydrochloride (purity 99%) and chlortetracycline (CTC, internal standard [IS], purity 99%) were purchased from Sigma Chemical (USA). The other reagents and chemicals were analytical grade. Milli-Q water purification system from Millipore (USA) was operated to obtain the purified water.

Experimental design

The 140 catfish were separated into 2 groups (n = 70) using a parallel study design. Each 10 catfish were housed in a single 200 L fiberglass tank. The water temperature was in the range 26°C–28°C, while the ambient temperature was in the range 28°C–37°C. Each group was administered OTC IV or PO at a dosage of 50 mg/kg BW. The IV administrations of drug were performed using tuberculin syringes, into the midline just posterior caudal vein at the anal fin, while PO administration was performed by inserting a 1 mL injector with a feeding tube down the esophagus. Blood samples (0.6–0.8 mL) were collected according to a sparse samples study design (with one sample from a single fish because of size limitations) from the ventral caudal vein (at mid part of fish) of each fish using heparinized syringes at 0, 15, and 30 min and at 1, 4, 8, 10, 12, 24, 48, 72, 96, 120, and 144 h after administration. The plasma was separated using centrifugation (1,986 × *g*) for 15 min and immediately stored at −20°C for 2 weeks before analysis. The dose of OTC used in this study was according to the study of pharmacokinetics in the yellow catfish [21].

Drug extraction procedure and HPLC analysis

The extraction and the analytical method for OTC in plasma of catfish was performed as described elsewhere [25,26] with modifications and revalidation. Briefly, plasma samples were extracted using a protein precipitation procedure. A plasma sample (250 µL) was added with 25 µL of CTC as the IS and then vortex mixed. After that, 125 µL of cold acetonitrile with 15% trichloroacetic acid was added to precipitate the protein. Finally, the mixture was centrifuged at 15,200 × *g* for 15 min at −4°C. The supernatant was collected, filtered through a 0.22 µm syringe filter (Sartorius AG, Germany). Then, the OTC plasma concentrations were immediately analyzed using HPLC.

The HPLC system used was an Agilent 1260 series system consisting of a binary pump, an automatic sample injector, a column thermostat, and a diode array detector (Agilent Technologies, USA). A chromatographic separation of OTC was performed using a Nova-Pak column (4 µm, 3.9 × 150 mm) with a C18 guard column (4 µm, 3.9 × 20 mm) (Waters, USA). The column was kept at 35°C. Mobile phase was 0.01 M oxalic acid in a water-to-acetonitrile solution with a gradient condition. The injection volume was 50 µL at a flow rate of 1.0 mL/min. The diode array wavelength was set at 355 nm.

Method validation

Linearity was challenged by spiking the working standard solution into control catfish plasma to yield final concentrations of 0.1, 1, 5, 10, 50, 100, and 200 µg/mL. The coefficient of determination (R^2) value of the OTC calibration curves was 0.998, which was considered to confirm a good fit between the curve and the calibration data. The same instrument was used to analyse seven duplicates of low, medium and high concentrations at 0.1, 10, and 100 µg/mL, respectively. Quantification was calculated using the peak area ratio between the analyte and the IS. The analysis was carried out by the same operator on the same day and on five different days to evaluate the assay's recoveries, intra- and inter-day precision and accuracy.

Pharmacokinetic analysis

The average concentration of OTC in the experimental catfish with respect to time was pharmacokinetically analyzed using a non-compartment model (PK analix 2023, Lixoft Software, France). The PO bioavailability (F) was based on the area under the curve (AUC) and calculated using the equation:

$$(\%)\ F = (AUC_{PO})/(AUC_{IV}) \times 100$$

RESULTS

Animals

All animals maintained health and physical condition during both the acclimatization and study periods. No adverse reactions, whether local or general, were detected after IV and PO administrations of OTC.

Analytical method

The mean extraction recovery values ± SD were 92.19 ± 2.52, 97.12 ± 1.95, and 98.24 ± 3.11% for 0.1, 10, and 100 μg/mL, respectively. The limit of detection and limit of quantitation of OTC were 0.01 and 0.05 μg/mL whereas; the value of intra-day precision and accuracy was in the range 2.89%–4.66% and greater than 95%, respectively.

Pharmacokinetic analysis

The semi-logarithmic plots of the mean plasma concentration-time curves of OTC at a dose rate of 50 mg/kg BW after IV or PO administrations are reported in **Fig. 1**. The OTC plasma concentrations were detectable for up to 144 and 120 h after IV and PO, respectively, at a dosage of 50 mg/kg BW The long $t_{1/2\lambda}$ value of OTC (41 h) found in catfish after IV administration was in agreement with the quite slow clearance rate and somewhat similar to that after PO administration. The average maximum concentration (C_{max}) value of OTC was 2.72 μg/mL with a T_{max} of 8 h. The absolute PO bioavailability was low (2.47%). The pharmacokinetic parameters of OTC are showed in **Table 1**.

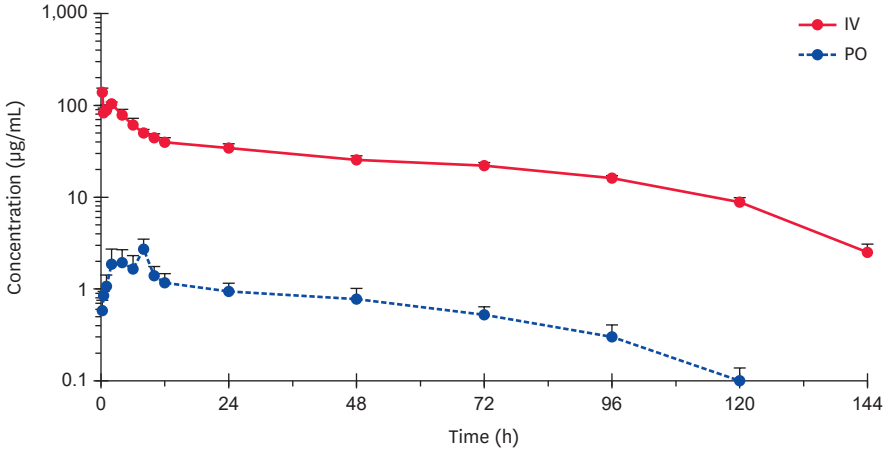

Fig. 1. Mean values (± SD) of oxytetracycline concentration in plasma of hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) after IV and PO administrations at dosage of 50 mg/kg body weight. Each fish was sampled once and each time point had 5 blood collections.
IV, intravascular; PO, oral.

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Table 1. Pharmacokinetic parameters of oxytetracycline following intravascular and oral administrations at a dosage of 50 mg/kg body weight, in hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*)

Pharmacokinetic parameter (unit)	50 mg/kg body weight	
	Intravascular	Oral
λ_z (h^{-1})	0.017	0.019
$T_{1/2\lambda z}$ (h)	41.38	35.87
T_{max} (h)[a]	-	8.0
C_{max} (μg/mL)	-	2.72
$AUC_{0\text{-}24h}$ (mg*h/L)	1,220.0	33.0
$AUC_{0\text{-}t}$ (mg*h/L)	3,371.76	82.49
$AUC_{0\text{-}inf}$ (mg*h/L)	3,770.11	93.09
CL (mL/g*h)	0.015	-
V_{dss} (mL/kg)	0.68	-
MRT $_{0\text{-}\alpha}$ (h)	62.75	54.04
F (%)	-	2.47

λ_z, elimination rate constant; $T_{1/2\lambda z}$, elimination half-life; T_{max}, time at maximum concentration; C_{max}, maximum concentration; $AUC_{0\text{-}24}$, area under curve from zero to 24 h; $AUC_{0\text{-}t}$, area under curve from zero to the last time point; $AUC_{0\text{-}inf}$, area under curve from zero to infinity; CL, clearance; V_{dss}, volume of distribution at steady state; MRT, mean residence time; F, oral bioavailability.
[a]Median value.

DISCUSSION

Although a number of pharmacokinetic reports of OTC have reported on various species of fish, no information exists on the pharmacokinetics of OTC in the hybrid catfish (*C. macrocephalus* x *C. gariepinus*). The present work was conducted to evaluate the pharmacokinetic properties of OTC after IV and PO administrations at a dosage of 50 mg/kg BW in the hybrid catfish.

OTC in the hybrid catfish had a higher value for C_{max} (2.72 μg/mL) than reported in yellow catfish (1.46 μg/mL) [21] and sea bream (2.5 μg/mL) [18], while it was lower than that of Arctic char (3.93 μg/mL) [22] and black sea bream (8.48 μg/mL) [24] after PO administration at a dosage of 50 mg/kg BW (**Table 2**). Additionally, the T_{max} value of OTC in hybrid catfish (8 h) was quite similar in yellow catfish (7.75 h) [21], while it was lower than in Japanese eel (24 h) [23] after PO administration. The value of OTC PO absolute bioavailability (F_{PO}) was very low in hybrid catfish (2.47%), in line with other fish species, such as common carp (0.4%) [27], Ayu (3.8%–9.3%) [28], Atlantic salmon (1.9%–6.9%) [14], Arctic char (3.2%–7.3%) [22], rainbow trout (5.6%) [11], and Japanese eel (0.69%) (**Table 2**) [23].

The $t_{1/2\lambda}$ values were high (35.87–41.38 h), indicating that the overall rate of elimination of OTC in hybrid catfish was slow. Additionally, the $t_{1/2\lambda}$ value obtained in the hybrid catfish

Table 2. The value of C_{max}, T_{max} and $T_{1/2\lambda z}$ of oxytetracycline in fish species

Fish species	Pharmacokinetic parameter				Reference
	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2\lambda z}$ (h)	F (%)	
Hybrid catfish	2.72	8.0	35.87	2.47	From this study
Yellow catfish	1.46	7.75	3.92	-	[21]
Seabream	2.5	-	-	-	[18]
Arctic char	3.93	-	-	3.73	[22]
Black seabream	8.48	-	-	-	[24]
Japanese eel	-	24.0	-	0.69	[23]
Common carp	-	-	50.0	0.4	[27]
Ayu	-	-	53.1–63.2	3.8–9.3	[28]
Atlantic salmon	-	-	-	1.9–6.9	[14]
Rainbow trout	-	-	-	5.6	[11]

C_{max}, maximum concentration; T_{max}, time at maximum concentration; $t_{1/2\lambda z}$, elimination half-life; F, oral bioavailability.

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was lower than those reported in other finfish species, such as common carp (50 h) [27] and Ayu (53.1–63.2 h) [28]; however, it was longer than in yellow catfish (3.92 h) (**Table 2**) [21]. These differences might have resulted from differences of species-specific, temperatures, or blood collection time [29]. Furthermore, Ellis et al. [30] reported that 10% change in the elimination rate of OTC could be occurred when the temperature was 1°C increasing in fish. In addition, differences in analytical method, age, size, health status, drug formulation and dosage might exert influence [5]. However, it can be generally assumed that species which vary in their taxonomy and ecological niches may have different excretory patterns and consequently pharmacokinetic of drugs. But there is no reports of differences on liver metabolisms/metabolomics have any impact on pharmacokinetic in fish species.

The minimum inhibitory concentration (MIC) value for OTC is about 0.125 and 0.78 μg/mL for most susceptible microorganisms that can cause high levels of disease in fish species [5]. The Clinical Laboratory Standard Institute suggests the susceptible breakpoint of OTC is less than 1 μg/mL [31]. However, the persistence of antibiotic concentrations in plasma and tissues above the MIC is the pharmacodynamic variable related to the clinical efficacy of drugs [32]. Nonetheless, due to inter- and intraspecies differences between test subjects, it is necessary to specify the dosage regimen for the prudent use of antibiotics on a species-by-species basis. The AUC/MIC, C_{max}/MIC and T > MIC can be used for antibacterial achievement evaluation in clinical use of OTC in animals [5,33,34]. Although, the T > MIC was used to evaluate as the pharmacokinetic/pharmacodynamic surrogate for this study, but the AUC/MIC for the unbound fraction of the OTC can also be used for tetracyclines [35]. Additionally, Bruno [36] reported that the injection of 20 mg/kg OTC successfully controlled an *Aeromonas salmonicida* infection (MIC of 0.09–0.19 μg/mL) in Atlantic salmon. Based on this value, the dose administered to catfish reached greater than the susceptible breakpoint value (< 1 μg/mL) from 1 h to 12 h for 50 mg/kg BW after PO administration. Therefore, these results suggested that PO administration of OTC at a dosage of 50 mg/kg BW would likely be effective 12 h for clinical use with catfish infected with sensitive bacteria. However, studies on clinically infected hybrid catfish warranted confirmation of this information. The MIC values of the critical bacterial pathogens that affect hybrid catfish should be evaluated for the prudent use of OTC in hybrid catfish.

In conclusion, OTC was quantified in the plasma of the hybrid catfish (*C. macrocephalus* x *C. gariepinus*) after IV and PO administrations at dosage of 50 mg/kg BW. Based on the pharmacokinetic information derived from the present study, a single PO administration of OTC at 50 mg/kg BW in the hybrid catfish is unlikely to be effective against several bacterial pathogens of MIC for 12 h. However, the experimental approach of this study (naïve averaged data approach) does not provide an estimate of the variability in the population making it hard to get insight the kinetic profile of OTC in a larger population from this design. Further studies carried out with a standard two stage or population pharmacokinetic design and pharmacodynamics are warranted to confirm the appropriate dosage and the wash out period of OTC in the hybrid catfish.

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