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Disposition kinetics of orbifloxacin in tissues of crucian carp (*Carassius auratus*) following a single intramuscular administration

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Summary

Background: Orbifloxacin is being widely used in China to treat fish infections in an extra-label manner, which may cause its potential residues in edible tissues. **Aims:** The purpose of this study was to determine the disposition kinetics of orbifloxacin in crucian carp (*Carassius auratus*) following intramuscular administration for its further safe application in aquaculture industry. **Methods:** Tissue samples of skin, muscle, kidney, and liver were collected from six crucian carps reared at 25°C at 5, 10, 15, 30, 45 min, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h following a single intramuscular injection at 7.5 mg/kg body weight (BW). The orbifloxacin concentrations in tissues were determined using a high-performance liquid chromatography (HPLC) method with a fluorescence detector, then average concentrations *versus* time data were subjected to non-compartmental analysis to obtain the kinetic parameters. **Results:** The peak concentration of 5.68 ± 0.03 μg/g was calculated in kidney at 2 h, followed by muscle (5.51 ± 0.01 μg/g) at 4 h, liver (4.84 ± 0.20 μg/g) at 2 h, and skin (4.27 ± 0.08 μg/g) at 4 h. Area under concentration-time curve was calculated as 79.22, 94.72, 118.65, and 129.02 h·μg/g in kidney, liver, skin, and muscle, respectively. And the elimination half-lives were determined as 18.17, 18.41, 18.77, and 19.11 h in skin, kidney, muscle, and liver, respectively. **Conclusion:** It was shown that orbifloxacin was well distributed into tissues while relatively slowly eliminated in crucian carp reared at 25°C following a single intramuscular injection.

Key words: Crucian carp, Disposition kinetics, Intramuscular injection, Orbifloxacin, Tissue depletion

Introduction

Fluoroquinolones are being widely used to treat infections in food production animals including aquaculture because of their bactericidal effects against a broad range of pathogens (Yang *et al*., 2014; Munawar *et al*., 2017). These drugs can inhibit bacterial DNA gyrase to prevent DNA supercoiling (Yang *et al.*, 2015; Tavakoli and Pourtaghi, 2017; Yang *et al*., 2017). The tissue depletion studies have been reported for some fluoroquinolones in fish species, including flumequine in salmon (Elema *et al*., 1994), norfloxacin in flounder (Park *et al*., 1996), sarafloxacin in salmon (Martinsen *et al*., 1994), marbofloxacin (Zhu *et al*., 2009) and difloxacin (Ding *et al*., 2006) in crucian carp, danofloxacin in tilapia (Fan *et al*., 2015) and seabass (Vardali *et al*., 2017), and enrofloxacin in seabass (Intorre *et al*., 2000), rainbow trout (Lucchetti *et al*., 2004), and pacu (Paschoal *et al*., 2013).

Orbifloxacin, one of the third generation fluoroquinolones, shows enhanced antibacterial activities against Gram-positive and Gram-negative bacteria, mycobacteria, and anaerobes (Martinez *et al*., 2006). In the US, orbifloxacin was approved to treat urinary tract, soft tissue, and skin infections in cats and dogs (Watson *et al*., 2015), and the available dosage forms include tablet and oral suspension. While in Japan, orbifloxacin was approved to be intramuscularly injected to treat the respiratory and gastrointestinal infections in pigs and cattle (Engberg *et al*., 2001). In China, orbifloxacin is not licensed in fish species. In spite of this, an extra-label use of orbifloxacin in fish is common in China, and it is often intramuscularly administered to treat the bacterial infections in aquaculture. The clinical experiences in China have confirmed the safety and effectiveness of the orbifloxacin application in fish (Li *et al*., 2008). But this may cause the potential residues in edible tissues.

Orbifloxacin has a lower toxicity including gastrointestinal disturbances and photosensitivity. In addition, the adverse reactions proved to be mild and reversible after discontinuation (Cazedey and Salgado, 2013). The biggest public concern about its residue in aquaculture product is the potential of its low level exposure to alter human microflora, leading to disease and the possible development of resistant strains. Thus, the extra-label application of orbifloxacin in fish should be monitored, and the disposition kinetics in edible tissues needs to be clarified.

Crucian carp (*Carassius auratus*) is one of the fish species most commonly cultured in China. It also occurs widely in northern European regions. The purpose of this study was to determine the disposition kinetics of

orbifloxacin in crucian carp for its further safe application in Chinese or global aquaculture industry. The disposition kinetics of orbifloxacin in tissues was determined in crucian carp reared at water temperature of 25°C following a single intramuscular injection at 7.5 mg/kg body weight (BW).

Materials and Methods

Chemicals

Raw material of orbifloxacin was obtained from Hubei United Medical Co., Ltd. (Wuhan, Hubei, China), and its analytical standard (99.8%) was purchased from Sigma-Aldrich Co. Ltd. (Shanghai, China). Unless otherwise noted, all chemicals employed to quantitate orbifloxacin were of high-performance liquid chromatography (HPLC) grade, and obtained from Sinopharm Chemical Reagent Co. Ltd. (Xi'an, Shaanxi, China). Ultrapure water was produced by a Milli-Q ultrapure water system (Millipore Corp., Bedford, MA, USA).

Animals

A total of 94 clinically healthy crucian carp weighting from 137 to 168 g were purchased from an aquaculture plant in Henan Province. After acclimatization for 7 days, 84 fish were randomly selected and equally divided into 14 groups (n=6). Each group was cultured in fiberglass tank with an approximate volume of 9.4 L under continuous aeration. The remaining fish (n=10) served as control and were reared in other tanks. They just provided blank samples for quantitation of orbifloxacin. The water quality was monitored daily, and its pH was kept at about 7.4, while the dissolved ammonia and oxygen concentrations were maintained at <0.1 mg/L and >8 mg/L, respectively. The water temperature was kept at 25 ± 1°C by air conditioning equipment. All fish were fed daily with drug-free feed (formula for crucian carp, Henan Tongwei Feed Co., Ltd., Xinxiang, Henan, China). This investigation (animal study protocol no. 201704006) was approved by the IACUC in Henan University of Science and Technology.

Disposition kinetics study

For each fish, orbifloxacin was injected at a dose of 7.5 mg/kg BW in the epaxial muscles just caudal to the dorsal fin. This dose was extrapolated directly from dogs (Hnot *et al*., 2015) and cats (Tynan *et al*., 2016). The orbifloxacin solution was prepared by dissolving 1 g of orbifloxacin raw material (calculated as pure orbifloxacin) in 100 ml of 2% lactic acid solution. A magnetic stirrer was used for full agitation and complete dissolution. Then the solution was filtered using a 0.22 µm filter to prevent bacterial contamination.

After intramuscular dosing, fish of one group were randomly sacrificed at each sampling time point including 5, 10, 15, 30, 45 min, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h. Each fish was euthanized by concussion, and the control fish were sacrificed at the last time point. Then the tissues of muscle, skin, kidney, and liver were collected from each fish. All samples were frozen at -20°C until further analysis.

Determination of orbifloxacin concentration

High-performance liquid chromatography with a fluorescence detector was used to determine the orbifloxacin concentration according to a previously published method (Kay-Mugford *et al*., 2002). Briefly, 0.5 g of tissue was added to 4 ml of acetonitrile and 100 µL of phosphate buffer (pH=7.4; 0.1 M). After vortexing for 3 min and centrifugation at 12000 × g for 15 min, the supernatant was collected and further partitioned with 3 ml of hexane, followed by sonication for 10 min and centrifugation at 12000 × g for 10 min. The lower layer was collected, evaporated to dryness with a nitrogen stream at 30°C, reconstituted in 0.5 ml of mobile phase and then centrifuged for 10 min at 12000 × g. A total of 20 µL of the supernatant was injected into a Hypersil ODS-C18 column (4.6 × 250 mm, 5 µm) purchased from Elite Analytical Instruments Co., Ltd. (Dalian, Liaoning, China), and the column was kept at 25°C.

The mobile phase consisted of 0.02% trifluoroacetic acid, 25% acetonitrile, and 75% water. And the flow rate was set as 1.0 ml/min. An Agilent 1100 Series HPLC system was used to detect orbifloxacin concentration using a fluorescence detector, which was performed at the excitation and emission wavelengths of 338 and 425 nm, respectively.

Analysis of disposition kinetics

Average orbifloxacin concentration at each time point in each tissue was firstly calculated. Then average concentration-time data were subjected to non-compartmental analysis using the software of WinNonlin (version 5.2; Pharsight Corporation, Mountain View, CA, USA). Trapezoidal method was used to calculate the areas under the area under the concentration-time curve from the time of dosing to infinity ($AUC_{0-\infty}$) and the area under the moment curve from the time of dosing to infinity ($AUMC_{0-\infty}$). The maximum observed concentration (C_{max}) and time to reach peak concentration (T_{max}) were read directly from the observed concentrations *versus* time data. While the terminal phase constant of first order rate constant associated with the terminal phase (λz) was calculated by the linear regression analysis of average concentrations vs. time data.

Results

Analytical method

The analytical method presented here was selective for orbifloxacin, and no endogenous interferences were observed on the chromatograms. It was shown to be reproducible and linear in the range of 0.05-10 µg/g. In order to determine its accuracy and precision, three replicates at the concentrations of 0.05, 1, and 10 µg/g were determined for three days to assess recoveries and coefficients of variation. It was found that average recovery in tissues was 84.56%, and the inter- and

intra-day coefficients of variation were below 5.14% and 4.26%, respectively. The limits of quantitation (LOQ) and limits of detection (LOD) were determined in all tissues based on a signal-to-noise ratio >10 and >3 as 0.05 and 0.02 µg/g, respectively. No orbifloxacin was detected in all control samples based on the present LOQ and LOD.

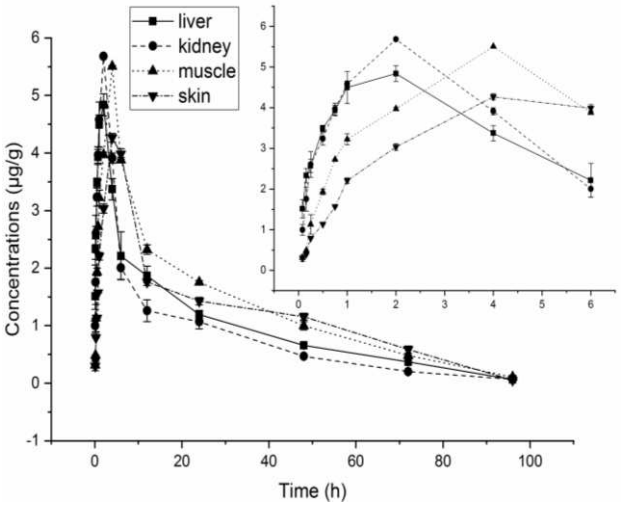

Fig. 1: Tissue concentrations (µg/g) of orbifloxacin in crucian carp at 25°C after a single intramuscular injection at 7.5 mg/kg BW

Disposition kinetics

The profiles of orbifloxacin concentrations in different tissues are presented in Fig. 1. Orbifloxacin was detected in liver, kidney, muscle, and skin up to 96 h. Its peak concentration (5.68 ± 0.03 µg/g) was measured in kidney at 2 h, followed by muscle (5.51 ± 0.01 µg/g) at 4 h, liver (4.84 ± 0.20 µg/g) at 2 h, and skin (4.27 ± 0.08 µg/g) at 4 h. The kinetic parameters are listed in Table 1. After a single intramuscular injection, the terminal half-lives ($t_{1/2\lambda z}$s) of orbifloxacin were 18.17, 18.41, 18.77, and 19.11 h in skin, kidney, muscle, and liver, respectively, which were numerically close to each other. The $AUC_{0-\infty}$s were 79.22, 94.72, 118.65, and 129.02 h·µg/g in kidney, liver, skin, and muscle, respectively, while the corresponding values of $AUMC_{0-\infty}$s were 1909, 2633, 3745, and 3742 h^2·µg/g, respectively.

Discussion

To our knowledge, the disposition kinetics of orbifloxacin in crucian carp was reported here for the first time. No abnormalities were observed during the experiment, and no systemic or local adverse reactions were found in crucian carp after intramuscular administration.

There are few studies on the pharmacokinetics or tissue kinetics of orbifloxacin in fish species. In our previous study (Yang *et al*., 2018), the plasma pharmacokinetic was reported for orbifloxacin in crucian carp following a single intravenous or intramuscular dose. We observed that an orbifloxacin dosage of 7.5 mg/kg BW administered intravenously or intramuscularly would be expected to successfully treat crucian carp infected by strains with minimum inhibitory concentration of ≤0.5 µg/ml. Orbifloxacin tissue kinetics has been reported in mammals. Its wide distribution was observed in dogs and cats following a single subcutaneous injection, and its concentrations in most tissues including muscle, lung, kidney, liver, and intestine at 2 h after the injection were all higher than that in plasma (Matsumoto *et al*., 1997). Similar results were found in pigs and cattle (Matsumoto *et al*., 1998), and the peak concentration was measured in kidney, followed by liver, muscle, and skin, which is similar to the present results in fish. However, in the previous reports in cats, dogs, pigs, and cattle (Matsumoto *et al*., 1997; Matsumoto *et al*., 1998), tissue concentrations were only reported at a single time point with no kinetic parameters. The present results proved that orbifloxacin was well distributed into the sampled tissues in crucian carp.

The orbifloxacin concentrations in skin were also reported in dogs with normal skin and those with pyoderma (Kay-Mugford *et al*., 2002). Compared with healthy skin, significantly higher concentrations were found in the infected skin. This indicates that infection may affect the distribution and/or disposition of orbifloxacin. Therefore, evaluation of disposition of orbifloxacin in infected fish needs to be further carried out. In another research (Haines *et al*., 2001), it was proved that orbifloxacin was well distributed into mares'

Table 1: Kinetic parameters of orbifloxacin in crucian carp cultured at water temperature of 25°C after a single intramuscular injection at 7.5 mg/kg BW

Parameters [a]	Units	Kidney	Liver	Muscle	Skin
λz	1/h	0.0376	0.0363	0.0369	0.0382
$t_{1/2\lambda z}$	h	18.41	19.11	18.77	18.17
T_{max}	h	2	2	4	4
C_{max}	µg/g	5.68 ± 0.03	4.84 ± 0.20	5.51 ± 0.01	4.27 ± 0.08
$AUC_{0-\infty}$	h·µg/g	79.22	94.72	129.02	118.65
$AUMC_{0-\infty}$	h^2·µg/g	1909	2633	3742	3745
MRT	h	24.11	27.80	29.01	31.57

BW: Body weight. [a] The disposition parameters were abbreviated as the following symbols: λz: First order rate constant associated with the terminal phase, $t_{1/2\lambda z}$: Terminal half-life, T_{max}: Time to reach peak concentration, C_{max}: Maximum observed concentration, $AUC_{0-\infty}$: Area under the concentration-time curve from the time of dosing to infinity, $AUMC_{0-\infty}$: Area under the moment curve from the time of dosing to infinity, MRT: Mean residence time, and h: Hour

body fluids and endometrial tissue. Our main concern in this study was the disposition kinetics in edible tissues, so the distribution of orbifloxacin into body fluids was not evaluated.

To our knowledge, the researches about orbifloxacin residues are focused mainly on milk penetration. Some research showed its good distribution to the udder and extensive penetration into milk after parenteral administration in ewes (Goudah *et al*., 2009), goats (Marin *et al*., 2007), camels (Goudah and Abo-El-Sooud, 2008), and does (Abd El-Aty *et al*., 2009). These studies indicated that orbifloxacin had success against susceptible mastitic pathogens; however, it might bring the potential residues in milk. In Japan, orbifloxacin was approved to be intramuscularly administered to cattle, including lactating dairy cattle, at multiple doses of 2.5-5 mg/kg/day for 3-5 continuous days, and a withdrawal time of 21 days was established in tissues; however the milk discard time was only 72 h (Nakamura, 1995). Orbifloxacin was also approved in pigs under the same dosage regimen with cattle, however, with a relatively short withdrawal time (14 days; Nakamura, 1995). To our knowledge, the information about the maximum residue limit (MRL) and withdrawal time is only available in Japan. Its marker residue is the parent compound, and the target tissues are kidney, liver, skin, and muscle in both pigs and cattle. All MRLs in these tissues were 0.2 µg/kg. For lactating dairy cattle, the MRL in milk is also 0.2 µg/L. Because of the paucity of MRL information in fish species, the withdrawal time was not determined in the present study.

Orbifloxacin was relatively slowly eliminated from crucian carp with $t_{1/2\lambda z}$s of 18.17, 18.41, 18.77, and 19.11 h in skin, kidney, muscle, and liver, respectively. The $t_{1/2\lambda z}$s determined here were much shorter than those of difloxacin from crucian carp cultured at 10°C, which were 231, 187.3, 169, and 157.5 h in skin, kidney, liver, and muscle, respectively (Ding *et al*., 2006). While at 20°C, the corresponding values of difloxacin were 86.6, 66.7, 87.7, and 66.6 h, respectively (Ding *et al*., 2006). These differences between difloxacin and orbifloxacin might be due to the variation of drug characteristics, dosage regimens, different water temperature, etc. The results of difloxacin revealed that its elimination from crucian carp became slower at lower temperature. After oral administration of marbofloxacin in crucian carp (Zhu *et al*., 2009), it was shown that the $t_{1/2\lambda z}$s from all tissues at 15°C were longer than those at 25°C. In a way that $t_{1/2\lambda z}$s at 15°C were 59.02, 97.10, 115.77, and 200.67 h in liver, muscle, kidney, and skin, respectively, while the corresponding values at 25°C were 34.60, 51.90, 60.20, and 94.06 h, respectively (Zhu *et al*., 2009). Therefore, the disposition of orbifloxacin needs to be further characterized at lower water temperatures to validate its potentially slower elimination in crucian carp.

In conclusion, it was demonstrated that orbifloxacin was well distributed into tissues while relatively slowly eliminated in crucian carp after a single intramuscular injection, which might bring the risk of residues in edible tissues. Further studies should be carried out, including determination of the MRLs of orbifloxacin in crucian carp, determination of the withdrawal time after multiple intramuscular and/or oral doses, and estimation the rate constants of elimination at different water temperatures.

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Conflict of interest

All authors have no conflict of interest.

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