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Pharmacokinetics of Florfenicol in the Orange-spotted Grouper, *Epinephelus coioides*, Following Oral Administration in Warm Seawater

Jing-Bin Feng[1]

Key Laboratory of Tropical Marine Bio-resources and Ecology (LMB), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
Key Laboratory of Applied Marine Biology of Guangdong Province (LAMB), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
Tropical Marine Biological Research Station in Hainan, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Sanya 572000, China

Hui-Ting Ruan

Department of Aquaculture, College of Animal Science, South China Agricultural University, Guangzhou 510640, China

Hai-Gang Chen

Guangdong Provincial Key Laboratory of Fishery Ecology and Environment, South China Fisheries Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China

Jun-Zhi Luo

Department of Aquaculture, College of Animal Science, South China Agricultural University, Guangzhou 510640, China

Jun-De Dong

Key Laboratory of Tropical Marine Bio-resources and Ecology (LMB), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
Key Laboratory of Applied Marine Biology of Guangdong Province (LAMB), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
Tropical Marine Biological Research Station in Hainan, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Sanya 572000, China

Abstract

Pharmacokinetics of florfenicol were studied in the orange-spotted grouper, *Epinephelus coioides*, at 29.0 ± 1.0 C following oral administration of 5 or 10 mg/kg. Rapid absorption occurred, with a T_{max} (time to reach the maximum concentration) of 2–12 h in plasma, muscle, skin, liver, bile, intestine, kidney, and gill. The absorption rate appeared to decrease with increasing dosage, according to the observed T_{max} in plasma, muscle, skin, liver, and kidney. The maximum concentration (C_{max}) in plasma was 6.04 ± 1.11 and 13.01 ± 6.18 μg/mL at 5 and 10 mg/kg, respectively, and was lower than that in the intestine but higher than in the other six tissues. A similar absorption and availability was found between the two doses by comparison of the ratios of area under the concentration–time curve (AUC) and dose among the tissues. A relatively low distribution level occurred in extravascular tissues based on the AUC values, with the exception of bile and intestine. The shortest and longest elimination half-life ($T_{1/2\beta}$) occurred in plasma (8.51 and 9.34 h at 5 and 10 mg/kg) and bile (18.13 and 17.63 h at 5 and 10 mg/kg), respectively. The elimination rate from plasma, muscle, and kidney was more rapid at 5 than at 10 mg/kg, according to the $T_{1/2\beta}$; however, the opposite situation was observed in the remaining five tissues.

KEYWORDS

absorption, distribution, elimination, florfenicol, grouper, pharmacokinetics

[1] Correspondence to: fengjb0618@126.com

The orange-spotted grouper, *Epinephelus coioides*, is one of the most economically important maricultured fish species in China. The grouper farming industry in China has undergone severe bacterial disease problems, mainly caused by *Vibrio* spp. (Feng et al. 2010), which still require improvements in medication.

Florfenicol is a synthetic, broad spectrum amphenicol antibiotic that was specifically developed for veterinary use (Lim et al. 2010; Feng et al. 2016). Florfenicol is usually administered in pellet form for treatment in fish, at a dose of 10 mg/kg for 10 consecutive days (EMEA 2000). Florfenicol has been extensively used in the global aquaculture industry and combines highly effective control of susceptible bacterial diseases, such as furunculosis and vibriosis in salmon and columnaris associated with *Flavobacterium columnare* in freshwater-reared finfish, and is safe for fish (Carty et al. 2007). To guide the use of florfenicol in the treatment of bacterial disease, further information regarding its pharmacokinetics in fish is required.

Pharmacokinetic profiles of florfenicol have been depicted in Atlantic salmon (Martinsen et al. 1993; Horsberg et al. 1996), cod (Samuelsen et al. 2003), koi carp (Yanong et al. 2005), Korean catfish (Park et al. 2006), tilapia (Feng and Jia 2009), olive flounder (Lim et al. 2010), crucian carp (Zhao et al. 2011), channel catfish (Gaunt et al. 2011), rice field eel (Xie et al. 2012), and yellow catfish (Yang et al. 2013). The results showed that the drug was absorbed rapidly and distributed extensively, with a high bioavailability ($100 \pm 9\%$) in Atlantic salmon, cod, Korean catfish, and channel catfish and a low bioavailability ($43.00 \pm 5.71\%$) in olive flounder.

Recently, Feng et al. (2016) investigated the pharmacokinetics of florfenicol and the behavior of its metabolite, florfenicol amine, in eight tissues of the orange-spotted grouper following a single per oral (p.o.) administration at 24 mg/kg held in seawater at 23.3 ± 0.8 C. A high drug distribution was found following administration at such a high dose and relatively low temperature. It is well known that the kinetic properties change with oral dose and environmental temperature (Feng et al. 2008, 2016; Lim et al. 2010). Here, in order to investigate whether the high drug distribution in grouper is maintained following administration at a relatively low dose and high seawater temperature, we examined the pharmacokinetics, tissue distribution, and elimination of florfenicol in the orange-spotted grouper following oral administration at 5 and 10 mg/kg and an experimental temperature of 29.0 ± 1.0 C.

Materials and Methods

Chemicals and Reagents

Florfenicol (99.5% standard) was obtained from the China Institute of Veterinary Drugs Control (Beijing, China). Chloramphenicol (99.6% standard) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Dikma Technologies (Beijing, China). All other chemicals were analytical grade.

Chromatographic Conditions

Analyses were performed using an Agilent 1260 HPLC system (Agilent Technologies Inc, Santa Clara, CA, USA). The chromatographic column was a 250×4.6 mm i.d. stainless steel column packed with 5-μm particle size reversed-phase chromatography medium (Spursil C18, Dikma Technologies). The mobile phase, acetonitrile : water (25:75, v/v), was filtered through a 0.45-μm Millipore filter and degassed by sonication for 15 min. The analysis was carried out at a flow rate of 1.0 mL/min and with ultraviolet detection at a wavelength of 220 nm. The column was operated at room\break temperature.

Experimental Fish

One hundred sixty healthy orange-spotted grouper (120 fish were used for pharmacokinetic experiments and the remaining fish were used to establish the HPLC method) weighing 103–301 g (204.84 ± 34.97 g) were obtained from a grouper breeding plant (Shenzhen,

Guangdong Province, China). The fish were brought into the laboratory at the Shenzhen Base of South China Sea Fisheries Research Institute and reared in fiberglass tanks of 0.5 m^3 capacity, supplied with flowing seawater at a salinity of approximately 33‰ and divided into two groups (60 fish per group) for oral doses of 5 and 10 mg/kg body weight, respectively. The natural seawater was pumped from Daya Bay, cleaned by sand filter, and used as cultural seawater. The fish were fed daily with pelleted dry feed in amounts of 1% body weight. The photoperiod was adjusted to 16 h light and 8 h dark. The fish were allowed to acclimate to these conditions for 3 d and were subsequently starved for additional 3 d prior to administration of the drug. The water temperature was 29.0 ± 1.0 C during the experiment.

Drug Administration

The therapeutic florfenicol premix (20%) was purchased from SCAU Technological Development Company (Guangzhou, China). For the 5 mg/kg dose group, the moist medicated feed, with 10 mg florfenicol per g of feed was made from a mixture of 3 g florfenicol premix, 30 mL distilled water, and 27 g ground formulated pellet feed. For the 10 mg/kg dose group, a drug suspension was prepared by dissolving 3 g florfenicol premix in 15 mL of distilled water. Twelve grams of ground formulated pellet feed was added to the drug suspension. Following sufficient mixing, the moist medicated feed was made to contain a final concentration of 20 mg florfenicol per g of feed. For the two groups of fish, the medicated feed was orally administered with the amount of 0.5 mg/kg body weight and the equivalent drug doses were 5 and 10 mg/kg body weight, respectively.

Individual fish were netted from the tank and weighed in a small tank of water on a T2000 electronic balance (G & G Measurement Plant, Changshu, China). Each fish was subsequently manually restrained. The drug administration was performed without the use of anesthesia, as it was fast and gentle enough not to cause a strong physical reaction or harm the fish. Medicated feed was administered into the stomach using a silicon hose and a 1-mL disposable tuberculin syringe. Following administration, each fish was observed for possible regurgitation over a period of approximately 5 min. Fish that regurgitated feed were excluded from the study and replaced.

Sampling

Samples were taken prior to drug administration and at 2, 4, 6, 9, 12, 18, 24, 48, 72, 96, and 168 h following oral administration. At every sampling point, samples of blood, skin, muscle, liver, kidney, gill, bile, and intestine were collected from five fish. Blood samples were taken from the caudal vein using a heparinized 2.5-mL syringe. Plasma was isolated by centrifugation at approximately 1160 *g* for 10 min. For gut tissue samples, the contents and mucus were squeezed out and discarded. All samples were frozen immediately and stored at −20 C until analysis.

Sample Preparation

Florfenicol extraction was carried out using a modified version of a previously described procedure by Feng et al. (2005). A 0.5-mL plasma sample and 0.5 mL of 0.2 mol/L phosphate-buffered saline (PBS, a mixture of 0.2 mol/L Na_2HPO_4 solution and 0.2 mol/L NaH_2PO_4 solution at a ratio of 61:39, v/v, pH 7.0) was added to 10-mL graduated plastic-stoppered centrifuge tubes, along with 20 µL chloramphenicol (50 µg/mL) for use as the internal standard. For bile, 0.2 mL of sample was added. Four milliliters of ethyl acetate was added to each tube to precipitate the proteins, followed by removal of the supernatant, which was transferred to a fresh 10-mL plastic-stoppered centrifuge tube.

For tissue, each sample was sheared and 0.5 g of muscle, skin, liver, gill, kidney, or intestine was weighed into a 10-mL polypropylene centrifuge tube. Following the addition of 0.5 mL PBS and 20 µL chloramphenicol (50 µg/mL), the mixture was vortexed for 1 min. A total of 4 mL of ethyl acetate was subsequently added, and the mixture was homogenized using an FJ-200 Disperser (Shanghai Specimen Model Factory, Shanghai, China) for 15 sec at 16,000 r/min

(for skin samples, the mixture was shaken for 20 min and then extracted with ultrasound for a further 20 min). Following centrifugation for 10 min at 8200 *g*, the supernatant was removed and transferred to a 10-mL plastic-stoppered centrifuge tube.

Both the plasma and tissue sample extraction steps were repeated. The extracts were evaporated to dryness under a gentle nitrogen stream at 45 C. The residue was dissolved in 0.5 mL mobile phase solution and 5 mL hexane, and then whirl mixed. Following centrifugation for 10 min at 12,400 *g*, the hexane layer was decanted. This defatting step was repeated. The water-based phase was filtered through a nylon centrifuge filter (0.2 µm). Twenty microliters of aliquots were injected automatically onto the HPLC column.

Method Validation

Method validation was conducted using each set of tissue assays, with fortified sample concentrations of 0.5 and 1.0 µg/g (/mL). The intraday precision was obtained from five duplicate aliquots of fortified sample on the same day, and the interday precision was calculated from spiked sample data on different days. For quantitation, the peak height measurements and the internal standard were used (Hormazabal et al. 1993). The standard curves were determined using florfenicol standard solutions at concentrations ranging from 0.05 to 50 µg/mL. The limits of quantitation were confirmed, according to recommendation by Samuelsen et al. (2003).

Pharmacokinetic and Statistical Analyses

Determination of the pharmacokinetic parameters of florfenicol was carried out by the noncompartmental pharmacokinetic model based on the statistical moment theory, using the WinNonLin software program (Version 4.1; Pharsight Corporation, Mountain View, CA, USA), according to the method described by Gibaldi and Perrier (1982).

The differences in drug concentration in each tissue between the two dose groups were examined by ANOVA. The ratio of area under the concentration–time curve (AUC) and dose represents the drug absorption and availability degrees. The relationship of drug absorption to availability between doses was tested by Pearson correlation analysis. SPSS version 19.0 statistical software (International Business Machines Corporation, Armonk, NY, USA) was used for all statistical analyses.

Results

The chromatograms for the analytical standards revealed a retention time of 14.0 min for florfenicol and 16.3 min for chloramphenicol (Fig. 1). Calibration curves with a linear range of 0.05–50 µg/mL were calculated by the linear regression equation: $Y = 1.4104X - 0.0699$, where X is the peak height ratio of florfenicol and chloramphenicol and Y is the concentration of florfenicol (µg/mL). The calibration curves had an r^2 value of 0.9999. The intraday and interday precision and recovery rates were 2.16–7.27, 5.76–8.32, and 94.22–116.03%, respectively. The limits of quantitation were set to 0.05 µg/g (/mL) for florfenicol, based on the standard curves, and sample concentrations below this value were not used for pharmacokinetic analysis.

Overall, the profiles of the drug concentration–time curves between the two dose groups were quite similar (Fig. 2). However, the drug concentrations within the same tissue at the same time points were significantly higher at 10 mg/kg compared with 5 mg/kg, with P values <0.01 in the plasma, muscle, skin, liver, bile, kidney, and gill and <0.05 in the intestine.

The mean concentrations (±SD) of florfenicol in the plasma of the orange-spotted grouper following oral administration at two different doses in seawater at 29 C are shown in Figure 2A. The corresponding pharmacokinetic parameters are given in Table 1. At 5 mg/kg, the corresponding maximum concentration (C_{max}), time to reach the maximum concentration (T_{max}), and elimination half-life ($T_{1/2\beta}$) were 6.04 ± 1.11 µg/mL, 2 h, and 8.51 h, respectively. At 10 mg/kg, the corresponding C_{max}, T_{max}, and $T_{1/2\beta}$ were 13.01 ± 6.18 µg/mL, 6 h, and 9.34 h, respectively.

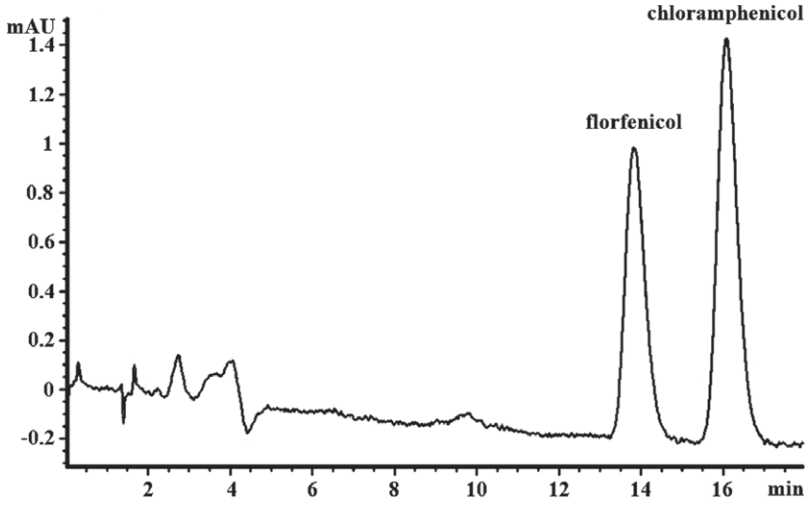

FIGURE 1. *High-performance liquid chromatography chromatograms of standard solutions containing florfenicol (1.0 μg/mL) and chloramphenicol (internal standard, 2.0 μg/mL).*

TABLE 1. *Pharmacokinetic parameters of florfenicol in grouper plasma following oral administration.*

Parameter	Unit	Value	
Dose	mg/kg	10	5
C_{max}	μg/mL	13.01 ± 6.18	6.04 ± 1.11
T_{max}	h	6	6
$T_{1/2\beta}$	h	9.34	8.51
AUC	h·μg/mL	335.65	173.75

AUC = area under the concentration–time curve; C_{max} = maximum concentration, values represent the mean ± SD; $T_{1/2\beta}$ = elimination half-life; T_{max} = time to reach maximum concentration.

The mean concentrations (±SD) of florfenicol in the tissues of the orange-spotted grouper following oral administration with two different doses in seawater at 29 C are shown in Figure 2B–H. At 5 mg/kg, the C_{max} (μg/mL or μg/g) were the intestine (11.61 ± 2.90) > plasma (6.04 ± 1.11) > bile (5.50 ± 1.93) > gill (5.07 ± 1.13) > muscle (4.92 ± 0.24) > kidney (4.60 ± 1.20) > skin (4.39 ± 0.71) > liver (2.87 ± 0.71). The observed T_{max} ranged from 2 to 12 h.

At 10 mg/kg, the T_{max} in tissues ranged from 2 to 9 h (Tables 1 and 2). The C_{max} (μg/mL or μg/g) were intestine (32.2 ± 6.76) > plasma (13.01 ± 6.18) > bile (12.69 ± 5.96) > gill (12.37 ± 1.46) > skin (10.35 ± 2.23) > muscle (10.18 ± 5.44) > kidney (8.67 ± 1.39) > liver (7.32 ± 2.57).

Drug absorption in the orange-spotted grouper was relatively more rapid at 5 mg/kg than at 10 mg/kg. The T_{max} (2–4 h) in the plasma, muscle, skin, liver, and kidney at 5 mg/kg were lower than those (4–9 h) at 10 mg/kg (Tables 1 and 2), which appeared to increase with oral dose. There existed the same T_{max} of 2 h in both the intestine and gill tissues between the two dose groups. An exception was that the T_{max} of 12 h for the bile tissue at 5 mg/kg was slower than (T_{max} = 6 h) at 10 mg/kg. Comparatively, the C_{max} in the same tissue, except for the kidney at 10 mg/kg, was twofold that at 5 mg/kg.

Based on the AUC values, the relative distribution and availability of florfenicol between the plasma and tissue in the orange-spotted grouper following administration at two different doses are given in Table 3. At 5 mg/kg, the ratio of the AUC between tissue and plasma ($AUC_{tissue/plasma}$) was highest for bile (1.43); high for intestine (1.16); moderate for skin (0.84), muscle (0.83), gill (0.79), and kidney

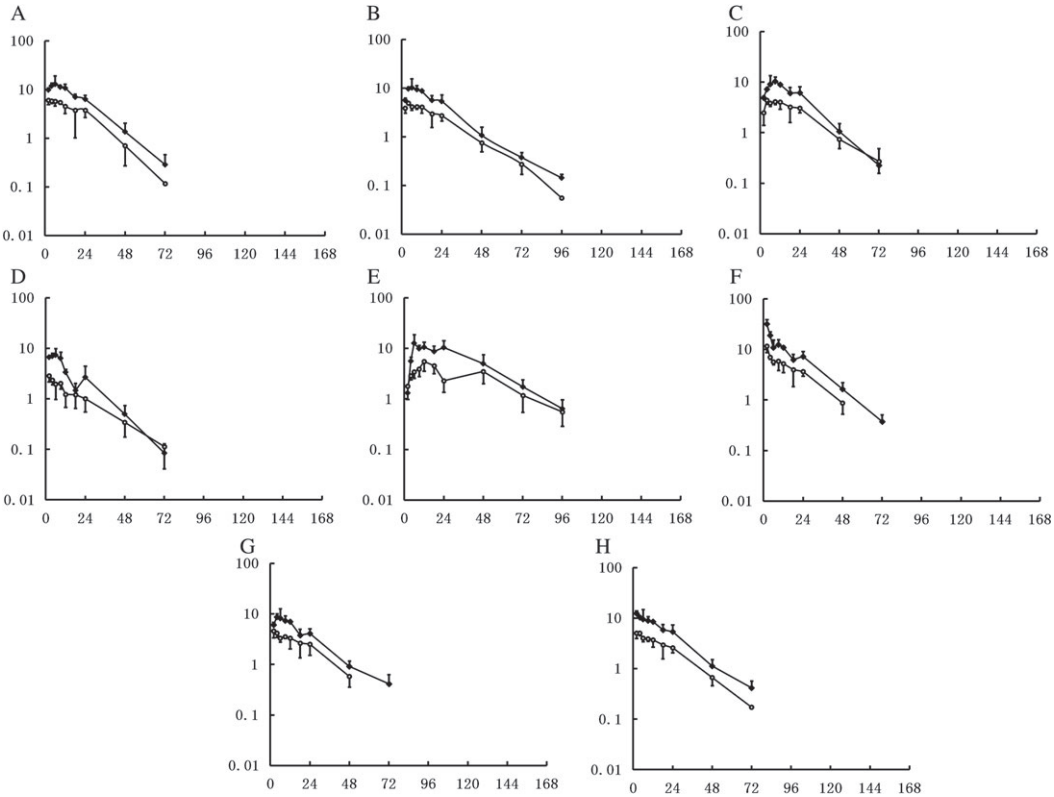

FIGURE 2. *Concentrations (mean ± SD,* n = *5) of florfenicol in grouper following a single oral administration of 10 mg/kg (◆) and 5 mg/kg (○), respectively. Abscissa: hours following administration; Ordinate: drug concentrations (μg/g or μg/ml); A: plasma; B: muscle; C: skin; D: liver; E: bile; F: intestine; G: kidney; H: gill.*

TABLE 2. T_{max} *(h) and* C_{max} *(μg/mL or μg/g) of florfenicol in grouper tissues following oral administration.*

Dose	Parameter	Muscle	Skin	Liver	Bile	Intestine	Kidney	Gill
10 mg/kg	T_{max}	6	9	6	6	2	4	2
	C_{max}	10.18 ± 5.44	10.35 ± 2.23	7.32 ± 2.57	12.69 ± 5.96	32.2 ± 6.76	8.67 ± 1.39	12.37 ± 1.46
5 mg/kg	T_{max}	4	4	2	12	2	2	2
	C_{max}	4.92 ± 0.24	4.39 ± 0.71	2.87 ± 0.71	5.50 ± 1.93	11.61 ± 2.90	4.60 ± 1.20	5.07 ± 1.13

C_{max} = maximum concentration, values represent the mean ± SD; T_{max} = time to reach maximum concentration.

(0.72); and the lowest for liver (0.35). Similarly, at 10 mg/kg, the $AUC_{tissue/plasma}$ was highest for bile (1.53); high for intestine (1.23); moderate for gill (0.86), muscle (0.83), skin (0.83), and kidney (0.67); and the lowest for liver (0.43).

The AUC value for the same tissue at 10 mg/kg was approximately twofold at 5 mg/kg (Tables 1 and 3), and the sum of AUC calculated from all eight tissues was exactly twice as much as that in the latter. There existed an extremely significant positive correlation between the ratios of AUC and dose in the eight tissues, between the two doses ($R = 0.99$, $P < 0.01$). These results indicate that there was almost identical drug absorption and availability in the orange-spotted grouper between doses at the same water temperature.

The elimination of florfenicol in the orange-spotted grouper was rapid. The drug concentrations declined to below the detection limit in muscle and bile at 168 h and in the remaining six tissues at 96 h at two doses, with the exception of the intestine and kidney at 5 mg/kg,

TABLE 3. *AUC (h·μg/mL or h·μg/g) and $AUC_{tissue/plasma}$ of florfenicol in grouper tissues following oral administration.*

Dose	Parameter	Muscle	Skin	Liver	Bile	Intestine	Kidney	Gill
10 mg/kg	AUC	277.28	279.00	143.40	515.03	411.83	223.65	289.61
	$AUC_{tissue/plasma}$	0.83	0.83	0.43	1.53	1.23	0.67	0.86
5 mg/kg	AUC	143.48	145.93	61.06	248.68	201.63	125.17	136.71
	$AUC_{tissue/plasma}$	0.83	0.84	0.35	1.43	1.16	0.72	0.79

AUC = area under the concentration–time curve; $AUC_{tissue/plasma}$ = ratio of the AUC between tissue and plasma.

TABLE 4. *The elimination half-life ($T_{1/2\beta}$, h) of florfenicol in grouper tissues following oral administration.*

Dose	Muscle	Skin	Liver	Bile	Intestine	Kidney	Gill
10 mg/kg	16.36	10.11	9.46	17.63	11.20	14.82	11.16
5 mg/kg	13.58	14.81	15.07	18.13	14.24	13.87	12.20

which were below the detection limit at 72 h. At 5 mg/kg, the $T_{1/2\beta}$ were bile (18.13 h) > liver (15.07 h) > skin (14.81 h) > intestine (14.24 h) > kidney (13.87 h) > muscle (13.58 h) > gill (12.20 h) > plasma (8.51 h) (Tables 1 and 4). At 10 mg/kg, the corresponding $T_{1/2\beta}$ in the tissues were: bile (17.63 h) > muscle (16.36 h) > kidney (14.82 h) > intestine (11.20 h) > gill (11.16 h) > skin (10.11 h) > liver (9.46 h) > plasma (9.34 h) (Tables 1 and 4).

On the basis of the comparison of the $T_{1/2\beta}$ values between the two dose groups (Tables 1 and 4), the drug elimination in plasma, muscle, and kidney was relatively more rapid at 5 mg/kg than at 10 mg/kg. However, the drug elimination in the five remaining tissues, skin, liver, bile, intestine, and gill, was relatively more rapid at 10 mg/kg than at 5 mg/kg.

Discussion

In the present study, the experimental water temperature was 29.0 ± 1.0 C, which is the typical water temperature for intensively cultured grouper, and is within the proper temperature range for its growth (Yu et al. 2004). Therefore, at such a relatively high water temperature, we examined the pharmacokinetics and tissue disposition of florfenicol in the orange-spotted grouper following a single oral administration of 5 and 10 mg/kg body weight.

Florfenicol was rapidly absorbed into the orange-spotted grouper following oral administration. The T_{max} was 2 h in the intestine and gill in both dose groups, and in plasma, liver, and kidney at 5 mg/kg. The T_{max} ranged from 4 to 12 h in the remaining evaluated tissues. In a recent study, rapid absorption was also observed in this grouper species following oral administration of 24 mg/kg at 23.3 ± 0.8 C, with a T_{max} of 2–6 h in examined tissues, with the exception of 24 h in bile (Feng et al. 2016). Similarly, a plasma T_{max} of 4 h was found in the cold-water olive flounder following oral administration of a solution form at 20 mg/kg and an experimental temperature of 18.5 ± 1.7 C (Lim et al. 2010). Horsberg et al. (1996) found a T_{max} of 6 h in Atlantic salmon following administration of a coated pellet at 10 mg/kg and an experimental temperature of 10 ± 0.5 C. In general, the oral absorption was rapid in seawater fish. This may be attributed to a beneficial gastrointestinal environment representing a high pH and cation content formed by active drinking for osmosis regulatory purposes, which may have facilitated the transfer of the drug across the mucosal barrier. It can be expected that florfenicol is unionized in the stomach because of its high pKa value (9.0). Thus, a large proportion of florfenicol may be rapidly absorbed as compared with freshwater fish species (Lim et al. 2010). Moreover, the solution form and coated pellets as compared with the pellet dosage together with a high water temperature could promote drug absorption.

In the present study, the rapid absorption of florfenicol led to a high distribution level in the plasma of the orange-spotted grouper. The C_{max} were 6.04 ± 1.11 and 13.01 ± 6.18 μg/mL in the plasma following oral dosing of florfenicol at 5 and 10 mg/kg, respectively. The corresponding plasma C_{max} was 28.28 μg/mL at 24 mg/kg and 23.3 ± 0.8 C (Feng et al. 2016).

Samuelsen et al. (2003) found the C_{max} to be 10.8 μg/mL in the plasma of cod following administration of florfenicol at 10 mg/kg at the same dosage but at a lower temperature of 8 C. Considering the change in C_{max} with the dose used, our results are consistent with those of Samuelsen et al. (2003). With the recommended dose of 10 mg/kg, a slightly lower C_{max} was found in Atlantic salmon (9.1 μg/mL) following administration of a coated pellet at 10.0 ± 0.3 C (Horsberg et al. 1996), and in Atlantic salmon at 10.8 ± 1.5 C (4.0 μg/mL^{-}; Martinsen et al. 1993) and freshwater tilapia at 22 C (4.46 μg/mL^{-}; Feng and Jia 2009) following administration of the pellet form.

The $AUC_{tissue/plasma}$ represents the relative distribution and availability of the drug between the plasma and tissues. In the present study, the $AUC_{tissue/plasma}$ value was higher than 1.0 for bile and intestine, lower than 0.5 for liver, and ranged from 0.67 to 0.86 in gill, muscle, skin, and kidney in both dose groups. A similar situation was found in the orange-spotted grouper following oral administration at 24 mg/kg and an experimental water temperature of 23.3 ± 0.8 C (Feng et al. 2016). These findings indicate a relatively low distribution and availability of florfenicol in the extravascular tissues of the orange-spotted grouper, with the exception of bile and intestine.

In the present study, the elimination of florfenicol was rapid following administration of two different doses. The $T_{1/2\beta}$ were 8.51 and 9.34 h in the plasma at 5 and 10 mg/kg, respectively. In a recent study, $T_{1/2\beta}$ of 11.57 h was found in the plasma of the orange-spotted grouper following oral administration at a dose of 24 mg/kg florfenicol at an experimental water temperature of 23.3 ± 0.8 C (Feng et al. 2016). When tilapia held in freshwater at 22 C were administrated a pellet form at 10 mg/kg, the $T_{1/2\beta}$ was 10.03 h in plasma (Feng and Jia 2009), which was similar to that in grouper. These findings are consistent with the fact that the drug elimination rate mainly depended on the water temperature. Slightly longer $T_{1/2\beta}$ of 12.2 and 14.7 h were found in cold-water Atlantic salmon following intravenous (i.v.) administration at 10 mg/kg as reported by Martinsen et al. (1993) and Horsberg et al. (1996), respectively, which is likely attributed to the relatively lower experimental water temperature. Moreover, in cod, the lack of a metabolic pathway further resulted in longer $T_{1/2\beta}$ of 43 and 39 h following i.v. and p.o. administration at 10 mg/kg, respectively (Samuelsen et al. 2003). In addition, Lim et al. (2010) reported $T_{1/2\beta}$ of 38.06 and 51.18 h in the olive flounder held in seawater at 18.5 ± 1.7 C following i.v. and p.o. administration at 5 and 20 mg/kg, respectively. The lack of a metabolic pathway, less perfusion than other fish species, and the low excretion capacity resulting from the extremely low gill surface area may be responsible for the slower drug elimination in this flat fish at such a high water temperature. Further, individual size could also affect the drug elimination rate. In terms of the $T_{1/2\beta}$ value among all the examined tissues, the slowest elimination of florfenicol occurred in bile in the orange-spotted grouper, which is consistent with that in tilapia (Feng and Jia 2009) and crucian carp following intramuscular or p.o. administration in a solution form at 40 mg/kg (Sun et al. 2010) and supports the opinion that bile behaves as a drug reservoir in fish.

Notably, according to the depletion curves of logarithmically transformed mean tissue concentration versus time (data not shown), other than the results of the noncompartmental pharmacokinetic model, two or three elimination phases were observed in the liver, bile, intestine, and kidney in the present study. A similar phenomenon was also found in another study on the orange-spotted grouper by Feng et al. (2016), indicating complicated pharmacokinetics of florfenicol in the orange-spotted grouper. This phenomenon is commonly considered to result from the enterohepatic circulation, fractionated gastric emptying, and separated "absorption windows" along the intestinal tract (Ding et al. 2012; Feng et al. 2016).

Florfenicol is a time-dependent antimicrobial drug; thus, its effective therapeutic concentration in tissues of the orange-spotted grouper must exceed the minimal inhibitory concentration (MIC) of the pathogen during the medication period (AliAbadi and Lees 2000). The *in vitro* MICs of *Vibrio anguillarum* isolates were investigated by Fukui et al. (1987) and Zhao

et al. (1992), who obtained values of 0.4–0.8 and 0.2–0.8 μg/mL, respectively. Samuelsen and Bergh (2004) found that the MIC value of florfenicol for a *V. anguillarum* isolate was 0.5 μg/mL. Results from the present study show that the drug concentrations in tissues were 1.00–11.61 and 0.92–32.20 μg/g (except for 0.5 at 48 h in the liver) within 48 and 24 h following oral administration at 5 and 10 mg/kg, respectively, exceeding the above-mentioned MIC values with a good margin. However, when taking into account the effects of different doses, dosage forms, diets, and individual size on pharmacokinetics, one should be very cautious when using florfenicol to treat *Vibrio* infections in fish farming. Moreover, to simulate actual breeding situations, the orange-spotted grouper should fully adapt to the aquaculture system, and thus, the acclimation time of experimental fish in pharmacokinetic studies should be sufficient (more than 3 wk). In addition, in order to obtain definite data regarding the clinical efficacy and to ensure correct usage of florfenicol in production, it is necessary to conduct field trials in the orange-spotted grouper prior to the use of this drug.

In summary, we investigated the pharmacokinetics, tissue distribution, and elimination of florfenicol in the orange-spotted grouper held in seawater at 29.0 ± 1.0 C following oral administration of 5 and 10 mg/kg body weight, respectively. Rapid drug absorption occurred in the orange-spotted grouper, which led to a high distribution. The absorption rate appeared to decrease with oral dose, according to the observed T_{max} in plasma, muscle, skin, liver, and kidney between two dose groups. An almost exact absorption and availability between the two doses was found by comparison of the ratios of AUC and dose in tissues. A relatively low distribution occurred in extravascular tissues based on the AUC values, with the exception of bile and intestine. In terms of $T_{1/2\beta}$, the fastest elimination occurred in the plasma. The slowest elimination and largest AUC value was observed in bile among all the tissues measured, indicating that bile behaves as a drug reservoir in fish. Drug elimination in plasma, muscle, and kidney was relatively more rapid at 5 mg/kg than at 10 mg/kg; however, the opposite situation was observed in the five remaining examined tissues.

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