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Tissue distribution and elimination of florfenicol in tilapia (*Oreochromis niloticus* × *O. caureus*) after a single oral administration in freshwater and seawater at 28 °C

Jing-Bin Feng [a,*], Xiao-Ping Jia [b], Liu-Dong Li [b]

[a] *The Key Laboratory of Applied Marine Biology of Guangdong Province and Chinese Academy of Sciences, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China*

[b] *Key Lab. of Marine Fishery Ecology Environment and Pollution Monitoring & Control Techniques, Chinese Academy of Fishery Sciences, South China Fisheries Institute, Guangzhou, China*

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Abstract

Tissue distribution and elimination of florfenicol in seawater-acclimated tilapia were compared with those in freshwater tilapia at 28 °C after they were given a single oral dose of 10 mg/kg body weight (b.w.). In most sampling time points, the concentrations of florfenicol in the tissues (muscle, liver, kidney, gill and bile) in freshwater tilapia were higher than those in seawater tilapia. In the major elimination phase the elimination half-lives ($T_{1/2\beta}$) in all the tissues (except for bile) of freshwater tilapia were longer than those in seawater tilapia. These findings indicated that the distribution level of florfenicol in freshwater tilapia was higher than that in seawater tilapia and the elimination of florfenicol in seawater tilapia was more rapid than that in freshwater tilapia. The main excretion pathway (gill excretion) of the drug in seawater-acclimated tilapia was different from that (bile excretion) in freshwater tilapia, which should be responsible for the considerable difference in pharmacokinetics from freshwater tilapia.

Keywords: Tilapia; Florfenicol; Distribution; Elimination

1. Introduction

Florfenicol is a monofluorinated derivative of thiamphenicol, a chloramphenicol analogue in which the *p*-nitro group on the aromatic ring is substituted with a sulphonylmethyl group (Cannon et al., 1990). Florfenicol has activity against a wide range of fish pathogens *in vitro* and *in vivo*, including *Aeromonas salmonicida* and *Vibrio salmonicida* in salmon. In aquaculture, florfenicol is usually added to fish feed (either incorporated prior to pelleting or coated onto pellets), and is administered at a dose rate of 10 mg/kg body weight for 10 consecutive days for treatment of bacterial disease in fish.

Tilapia is one of the most important warm-water cultured fish in the world, both in fresh and salt water. In order to guide the use of florfenicol for the treatment of fish disease in the farming of tilapia, information was needed on the pharmacokinetics in tilapia. Nevertheless, information concerning the pharmacokinetics of florfenicol in tilapia is not available. Studies on florfenicol pharmacokinetics and residues reported are performed usually with seawater fish such as salmon (Martinsen et al., 1993; Horsberg et al., 1994, 1996) and cod (Samuelsen et al., 2003). It is not reasonable that these pharmacokinetic data were used directly for tilapia because of the differences between species, temperature and salinity.

Under these conditions, this study was undertaken to investigate tissue distribution and elimination of florfenicol in both seawater-acclimated tilapia and freshwater tilapia and clarify the difference in the change of florfenicol levels between seawater-acclimated tilapia and freshwater tilapia.

* Corresponding author. Fax: +86 20 84451672.
E-mail address: fengjb0618@126.com (J.-B. Feng).

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2. Materials and methods

2.1. Chemicals

Florfenicol standard was obtained from Sigma Chemical, USA. Chloramphenicol standard was obtained from the National Institute for the Control of Pharmaceutical and Biological Products, P.R. China. Acetonitrile and methanol (HPLC grade) were from Dikma Technologies, P.R. China. All the other chemicals were of analytical grade.

Stock standard solutions of 1 mg/ml florfenicol and 0.5 mg/ml chloramphenicol were prepared by dissolving each substance in methanol and stored at −20 °C. Working standards were prepared by diluting the stock standards with water.

2.2. Chromatographic conditions

The analyses were performed on a LC-6200 HPLC system (Hitachi, Japan), consisting of a 655-a reaction pump, L-6200 intelligent pump, D-2500 chromato-Integrator, LC-Organizer and L-4200 UV–VIS detector at 223 nm. The chromatographic column was a 250 mm × 4.6 mm i.d. stainless steel column packed with 5 μm particle size reverse-phase chromatography medium (Nucleodur C18, Macherey-Nagel, Germany). The mobile phase of acetonitrile–water (25:75, v/v) was filtered through a 0.45 μm Millipore filter and degassed using sonication (5 min). The flow rate was 1.0 ml/min. The column was operated at 20 °C.

2.3. Experimental fish

Healthy tilapia (*Oreochromis niloticus* × *O. aureus*) weighing about 115 g obtained from a tilapia breeding plant in Guangdong province were reared in fresh water and were divided into a fresh water group and a seawater group. The fish in the seawater group were gradually acclimated to seawater with 16.5‰ salinity within 5 days and then were held for another 2 days.

And then both groups of fish were brought into the laboratory in fibre glass tanks containing flowing, dechlorinated water. The fish were fed with pellet dry feed in amounts of 1% of the body weight per day in 28 °C water. The photoperiod was adjusted to 16 h light and 8 h dark. The fish were allowed to adapt to these conditions for 3 days and were starved for another 3 days before administration of the drug. The temperature fluctuations during the experiment were less than 1 °C.

2.4. Drug and drug administration

The therapeutic florfenicol was donated by SCAU Technological Development Company (Guangzhou, P.R. China).

A suspension of florfenicol for oral administration (5 mg/mL) was prepared by dissolving florfenicol in distilled water.

The anterior part of each of a number of 1-ml disposable tuberculin syringes was cut off using an emery cloth, and thereafter polished. The syringes were then loaded with 0.2 ~ 0.3 g of unmedicated feed, pellet size 3 mm.

Individual fish was netted from the tank and weighed in a small tank of water on a T2000 electronic balance. Individual doses of 2 μl/g bodyweight, corresponding to 10 mg/kg bodyweight, the recommended daily dose of florfenicol, were pipetted into the syringe, and allowed to soak into the feed for one night. Each fish was then manually restrained, and dosed by inserting the syringe down the oesophagus into the stomach before depressing the plunger. The administration was performed without the use of anaesthesia. Following administration, each fish was transferred to an individual basin to check for possible regurgitation over a period of approximately 5 min. Fish that regurgitated feed pellets were excluded from the study and replaced.

2.5. Sampling

The samples were taken before drug administration and from 2 h to 168 h after oral administration. At every sampling point, five fish were killed by destroying their brains and samples of muscle, liver, kidney, gill and bile were obtained. All samples were immediately frozen and stored at −20 °C until analysed.

2.6. Sample preparation

Florfenicol extraction and analysis was carried out according to the procedure of Feng et al. (2005).

The tissue sample was sheared, and thereafter 1 g of ground tissue (muscle, liver, kidney, gill and bile) was weighed into a 40 ml centrifuge tube. Volumes of 20 μl of chloramphenicol (500 μg/ml) and 500 μl of water were added. Ethyl acetate (4 ml) was added, and the mixture was homogenized with an FJ-200 Disperser (Shanghai Specimen & Models Factory, China) for 10 s at 16,000 r/min. After centrifugation for 15 min at 4000 r/min, the supernatant was removed and transferred to a 15 ml glass-stoppered centrifuge tube. The extraction step was repeated. The combined ethyl acetate extract was then evaporated to dryness at 60 °C under a gentle stream of nitrogen. The residue was dissolved in 1 ml of mobile phase solution and 0.5 ml hexane, and then was whirlimixed. After centrifugation for 20 min at 16,000 r/min, the hexane layer was discharged. The water-based phase was filtered through a nylon centrifuge filter (0.2 μm). Aliquots of 20 μl were injected on the HPLC column. For quantitation, the peak-height measurements and the internal standard were used. A linear relationship existed in the calibration curve over the range of 0.03 μg/ml to 16 μg/ml, which always yielded a correlation coefficient exceeding 0.998. The within-run and inter-run precision and recovery rates for florfenicol were 2.21%~6.55%, 2.11%~7.71% and 89.16%~96.13%, respectively. The limits of quantitation were, based on the standard curves, set to 0.03 μg/g for florfenicol in tissues.

2.7. Pharmacokinetic analysis

The excretion of florfenicol was studied by plotting the logarithm (ln) of the drug concentrations against time after drug administration. The elimination half-life ($t_{1/2\beta}$) was calculated with the equation $T_{1/2\beta}=0.693/\beta$, where β was the elimination rate constant calculated by linear regression from the linear terminal portion of the elimination curve.

3. Results

3.1. Absorption and distribution of florfenicol

The mean concentrations (±s.d.) of florfenicol in the tissues in both freshwater and seawater tilapia after administration were shown in Figs. 1 and 2, respectively. We did not obtain these data on the kidney samples in seawater tilapia at 72 h, 96 h and 168 h since these samples were lost. The dramatical fluctuation of bile drug concentrations versus time in freshwater tilapia indicated the intermittent excretion of bile in freshwater tilapia. At 168 h, the concentration of florfenicol in gill of freshwater tilapia was not detectable (less than 0.03 μg/g).

The maximum concentrations of florfenicol in the tissues in both freshwater and seawater tilapia after administration were shown in Table 1. The absorption of florfenicol in tilapia was as fast in freshwater as in seawater. The maximum concentrations (C_{max}) in muscle, liver or kidney of freshwater tilapia were reached at the same time (T_{max}) as those of seawater tilapia after administration.

In most sampling time points, especially in the later period of sampling time (from 12 h to 168 h), the concentrations of florfenicol in the tissues in freshwater tilapia were higher than those in seawater tilapia. These findings indicated that the distribution level of florfenicol in freshwater tilapia was higher than that in seawater tilapia.

With freshwater tilapia, maximum concentrations in the liver and gill, 5.21 μg/g and 5.27 μg/g, respectively, were measured 2 h after administration; in muscle and kidney, 4.59 μg/g and 5.50 μg/g, respectively, were measured 12 h after administration (Table 1). The concentrations in the muscle, liver, kidney, bile and gill at 24 h after administration were 1.60 μg/g, 1.69 μg/g, 1.78 μg/g, 8.57 μg/g (maximum concentration) and 0.97 μg/g, respectively (Fig. 1).