

Tissue Distribution and Elimination of Florfenicol in Topmouth Culter (*Culter alburnus*) after Oral Administration

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Abstract

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The serum concentration and muscle residue of florfenicol following oral administration were evaluated in topmouth culter (*Culter alburnus*) under field conditions. Fishes were cultured in tanks and fed a commercial medicated diet containing 2000 mg/kg florfenicol in a single dose. The sampling was conducted at different intervals (0, 0.5, 1, 1.5, 2, 3, 4, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168 h) after the cessation of medication. Drug analysis was carried out by HPLC. The maximum concentration (C_{\max}) of 10.8 µg/g in serum and 4.2 µg/g in muscle were reached at the same time (t_{\max}) of 8 hours. If the EU MRL value of 1.0 µg/g is quoted for the topmouth culter tissue, the extrapolation of the data indicated that it will be passed after a 40.6 h withdrawal period for florfenicol in topmouth culter muscle.

Keywords: serum; elimination; florfenicol; topmouth culter (*Culter alburnus*)

Florfenicol is a fluorinated analogue of thiamphenicol and its structure also resembles that of chloramphenicol. Thiamphenicol and chloramphenicol have been used as broad-spectrum veterinary antibiotics (ZHANG *et al.* 2008). Florfenicol is a synthetic broad-spectrum antibiotic potentially effective in controlling a number of bacterial infections in the fish in which resistant bacteria are becoming increasingly prevalent. Studies have revealed its effectiveness against a wide range of pathogens such as *Pasteurella piscicida* (YASUNAGA *et al.* 1988), *Aeromonas salmonicida* (INGLIS *et al.* 1991; NORDMO *et al.* 1994), *Vibrio anguillarum*, and *Edwardsiella tarda* (FUKUI *et al.* 1987). POWERS *et al.* (1988) predicted that florfenicol, because of its high potency and great safety for humans as compared to related com-

pounds, could become a major drug in veterinary medicine.

The topmouth culter, *Culter alburnus* (Basilewsky), belongs to *Cypriniformes*, *Cyprinidae*, *Culter*, and is widely distributed in large rivers, reservoirs, and lake areas of China (CHEN 1998). The cultured production of the topmouth culter has greatly increased over the past decades, amounting to for about 1500 tons annually. Topmouth culter has already become one of the most important commercial freshwater fishes in China. However, the research into this species has been very sporadic both at home and abroad.

In aquaculture, florfenicol is mainly administered through medicated feeds. This practice may result in the antibiotics entering the environment by leaching from uneaten feeds or aquatic animals

excrements (ROBINSON *et al.* 2007). This may result in adverse ecological effects, including the development of resistant bacterial populations, direct toxicity to microflora and microfauna, and/or possible risks in the transfer of antibiotics resistance to human pathogenic microbes (HEKTOEN *et al.* 1995; RIGOS *et al.* 2004; CABELLO 2006).

The purpose of the present study was to obtain information on florfenicol distribution in muscle and serum, and to determine florfenicol withdrawal period for the topmouth culter (*Culter alburnus*).

MATERIALS AND METHODS

Chemicals and apparatus. Florfenicol (purity 99.7%) was obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products. Ethyl acetate and hexane were of HPLC grade and were purchased from Fisher Scientific (Fair Lawn, USA). PBS was prepared and kept at 4°C.

The HPLC system (AgilentTM, HP1100, USA) consisted of a pump (HP1100 quaternary pump), a manual injection valve with a 20 µl loop, a column (Agilent ZORBAX SBC18 5 µm 25 mm/4.6 mm), and a UV detector (G1314 A VWD).

Fish. One hundred healthy topmouth culters, *Culter alburnus*, were obtained from a certified farm in Xiaoshan Economic and Technological Development Zone in Hangzhou, Zhejiang province, and were reared in a pond. The average weight and length of fish was 50 g and 8 cm, respectively. A similar pond with the same number of fish was used for the non-medicated feed controls. The temperature was maintained at 23–25°C. The topmouth culters were acclimatised for 2 weeks and were starved for 2 days before the start of the experiment.

Dosing and sampling. Florfenicol for veterinary use was evenly mixed with the normal fish ration at the theoretical concentration of 2000 mg of drug/kg. The medicated as well as the non-medicated feeds were kept at 4°C.

The topmouth culters were treated orally with a single dose, followed by non-medicated feed administration for the remaining 7 days.

Blood (2 ml) from the caudal vein and muscle (5 g) of the fish were immediately taken at 0, 0.5, 1, 1.5, 2, 3, 4, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168 h after the cessation of medication. At each sampling time, six fish were randomly selected from the treated and the control specimens. Blood

was stored on ice. All samples were kept frozen at –20°C until analysed.

Florfenicol analysis protocol. The extraction and analysis of florfenicol in serum and muscle were carried out according to the protocol of (VUE *et al.* 2002) with some modifications. 5 g of the ground muscle sample (plasma) was weighed into a 40 ml centrifuge tube. 5 ml PBS and 20 ml ethyl acetate (1 ml PBS and 4 ml ethyl acetate for plasma) were added, and the mixture was homogenised in a homogeniser (Model 985-370, Biospec, USA) and then centrifuged at 1500 g for 20 minutes. The supernatant was transferred to a clean centrifugal tube. The extraction step was repeated. The extracts were combined and evaporated to dryness at 60°C under a gentle stream of nitrogen. The residue was dissolved in 3 ml of mobile phase consisting of acetonitrile/water (27/73, v/v) solution and 2 ml hexane, and then it was mixed. For the plasma samples, 0.6 ml mobile phase solution and 0.4 ml hexane were added. After centrifugation at 4000 g for 10 min, the hexane layer was discharged. The water-base phase was filtered through filter paper (0.45 µm × 25 mm). The resulting solution was injected (20 µl) into the HPLC system. The flow rate was 1 ml/min at a pressure of approximately 100 bar. Florfenicol was detected at 223 nm. Under these conditions, the retention time was 6.0 minutes.

Standard calibration curve. Acetonitrile stock solution of florfenicol was prepared at a concentration of 100.0 µg/ml and stored at 4°C. The working standard calibration curve was drawn by plotting the known florfenicol concentrations (0, 0.2, 1.0, 2.0, 2.5, 5.0, 10.0, 20.0 µg/ml) prepared from the stock solution by dilution against the average peak area. The florfenicol concentrations in unknown samples were read from the standard curve.

Detection limit and recovery percentage. The detection limit is defined as the concentration of florfenicol in topmouth culter tissue samples which can produce an HPLC-UV signal that is three times greater than the signal-to-noise ratio.

The recoveries were calculated by adding known quantities of florfenicol (range 0.5–20 µg/g) to the homogenised florfenicol-free fish tissues followed by extraction and detection.

Pharmacokinetic analysis. The mean florfenicol concentrations in the topmouth culter muscle were plotted against time semi-logarithmically. The elimination of the drug was followed by linear regression analysis (CAMPBELL *et al.* 2001). The elimination half-life ($t_{1/2}$) was then calculated

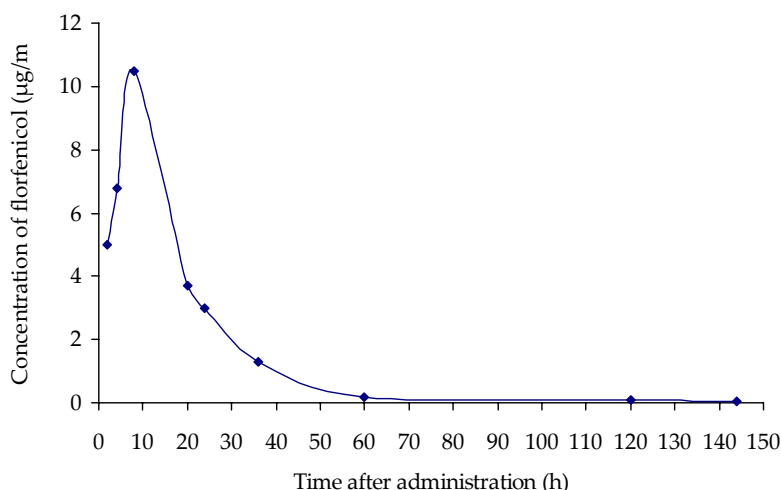


Figure 1. Serum concentrations of florfenicol following oral administration

using the equation: $t_{1/2} = \ln 2/\beta$, where β – slope (elimination rate coefficient) of the logarithmic function: drug concentration against time.

The data were fitted to a simple linear model. After ensuring that the data were normally distributed (Anderson Darling Test-A2), regression analysis was performed to determine the functional relationship between the dependent variables of each treatment and time.

RESULTS

Detection limit and recovery percentage

The detection limit was 0.05 µg/ml while as much as 72.5–113% of the florfenicol amount added to the tissue homogenates of topmouth culter could be recovered. The recovery percentage was higher in serum than in muscle.

Absorption of florfenicol

The mean concentrations in serum after the administration are shown in Figure 1. The maximum concentrations (C_{\max}) of 10.8 µg/g in serum and 4.2 µg/g in muscle were reached at the same time (t_{\max}) of 8 hours. After 144 h, the concentrations of florfenicol in serum and in muscle were not detectable (less than 0.03 µg/g). At most sampling times, especially in the later period (from 8 h to 144 h), the concentrations of florfenicol in serum were higher than those in muscle.

Persistence of florfenicol in the muscle of fish

The concentration of florfenicol in fish muscle exhibited mono-exponential elimination. The muscle concentration–time curve following the oral dose of florfenicol is illustrated in Figure 2,

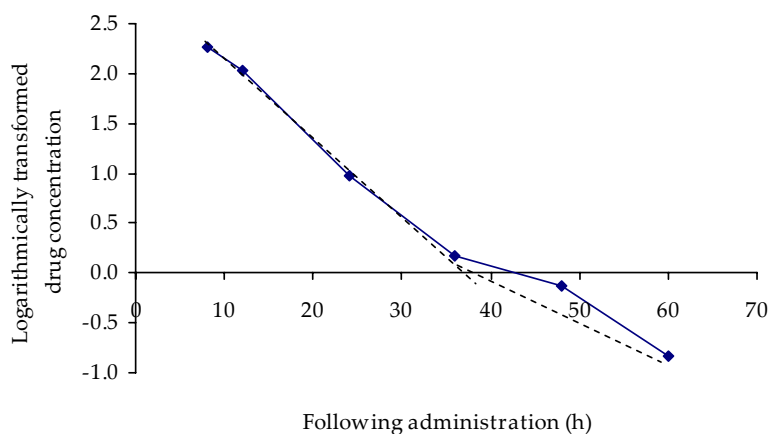


Figure 2. The depletion curve on logarithmically transformed mean muscle concentration versus time

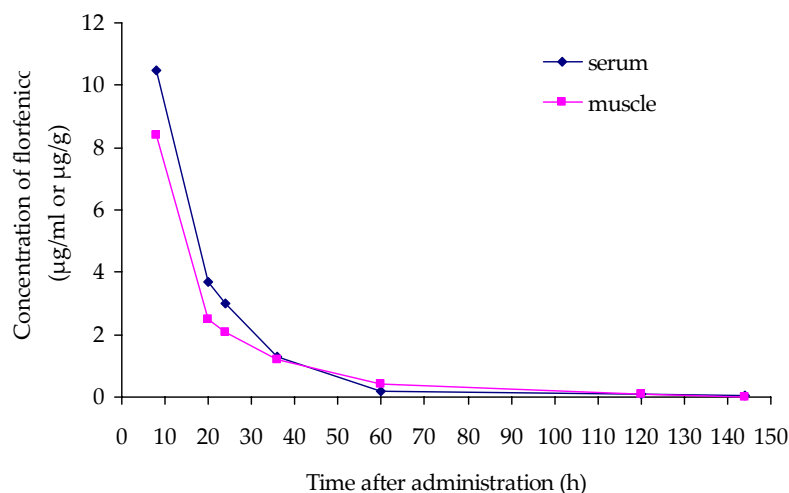


Figure 3. Florfenicol concentrations in serum and muscle of topmouth culter after oral administration

which shows two elimination phases (a rapid initial elimination phase and a slow secondary elimination phase) in the muscle tissue. The model equations describing the depletion of this drug as a semi-logarithmic function of time (t) are:

$$\ln C = -0.0443t + 1.7976$$

$$\ln C = -0.0348t + 1.4380$$

where:

C – concentration ($\mu\text{g/g}$)

t – time (h)

In the rapid initial elimination phase (major elimination periods), the elimination half-life ($t_{1/2}$) was 15.64 h. The European Agency for the Evaluation of Medicinal Products has established a microbiological ADI of 3 $\mu\text{g/kg}$ bw, a toxicological ADI of 10 $\mu\text{g/kg}$ bw and according to these data, the maximum residue limit (MRL) set for florfenicol was 1.0 $\mu\text{g/g}$ in fish muscle and skin. If the MRL value is extended to topmouth culter tissue, the extrapolation of the data indicates that it will be passed after a 40.6 h withdrawal period for florfenicol in topmouth culter muscle.

DISCUSSION

This is one of the few studies on florfenicol elimination in topmouth culter (*Culter alburnus*). The results are in general agreement with those of the previous studies on fishes (MARTINSEN *et al.* 1993; FENG *et al.* 2008). The depletion half-life of florfenicol in our research was 15.64 h, while the value in freshwater tilapia muscle was 8.03 h

(FENG *et al.* 2008). The discrepancy between the depletion profiles in two freshwater fish species could be reasonably interpreted. One main possible factor influencing the elimination speed of drugs is the water temperature. Previous literature stressed that an increased water temperature seemed to affect the kinetic profile of drugs (BJORKLUND & BYLUND 1990; KLEINOW *et al.* 1994). BJORKLUND and BYLUND (1990) administered OTC to rainbow trout (*O. mykiss*) as a single oral dose of 75 mg/kg body weight at water temperature of 5°C and 10°C. Maximum OTC concentrations in the fillet tissues were acquired after 9 (5°C) and 4 (10°C) days after the treatment. RIGOS *et al.* (2004) demonstrated that the elimination half-life of OTC from sea bass muscle was calculated to be 0.6 day and 1 day at 22°C and 13.5°C, respectively, following the intravascular administration (40 mg/kg). In our experiment, the water temperature remained at 23–25°C, while tilapia accustomed to higher water temperature were cultured at 28°C. Though the two depletion half-life values made a difference, it is worth noting that the peak concentration of florfenicol in topmouth culter muscle was reached 8 h after a single dose. In a previous work, the maximum concentration of florfenicol was reached after 12 h in freshwater tilapia muscle (FENG *et al.* 2008). This aspect may be associated with species differences. It has been indicated that pharmacokinetic parameters among species may be due to physiological specificities. Topmouth culter is a fierce species which feeds on live fish. However, how much this factor accounts for the data given above is unknown.

Our results agreed with the common phenomena that after administration high levels of veterinary

drugs appear usually in the highly vascularised tissues, whereas low levels of drugs are generally found in the poorly perfused tissues, such as muscle (Figure 3) (NAMDARI *et al.* 1999). Furthermore, florfenicol concentrations of topmouth culter in serum decreased rapidly with the post-dose time (Figure 3), whereas in the poorly perfused tissue (muscle) the lower florfenicol concentrations decreased slowly with time (Figure 3). The reason is most likely related to the blood perfusion rate and redistribution of the drug in the tissues.

These results are not comparable to those given in the literature consulted on other finfish because of different drug concentrations in the diet and doses intakes or the environmental factors such as temperature. Our choice of an experimental protocol reflecting the real conditions of fish farming made the interpretation of the results and data processing very difficult. This issue was strictly related to the fact that the variation in the feed intake could not be controlled in this kind of experiment. More data obtained under the field conditions would help to discuss these issues and to address the introduction of a proper legislation setting both MRLs and experimental protocols for the research into drug residues in aquatic species.

Based on the toxicological and environmental effects, the future use of florfenicol in the treatment of diseases in farmed aquaculture species may be taken into consideration. The research on the tissue distribution of drugs in aquaculture should combine the results of the toxicological studies so as to choose an antibacterial agent with a higher bioavailability, satisfactory distribution, low toxicity, and a short withdrawal time.

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