Oxytetracycline Residue in the Muscle of Nile Tilapia

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Abstract

Oxytetracycline (OTC) residue in Nile tilapia *Tilapia niloticus* muscle was assayed after OTC injection and oral administration. The OTC concentration in fed fish was the greatest after 10 d of feeding medicated feed, and was 0.46 and 0.14 µg·g⁻¹ in the 75 and 50 mg OTC·kg⁻¹ treatments, respectively. The OTC residue was below a detectable level in fish fed 50 mg·kg⁻¹ 10 d after treatment, and in fish fed 75 mg·kg⁻¹ 15 d after cessation of medicated feed. The B-value and half-life (t₁/₂) were 0.1642 d⁻¹ and 4.2 d, respectively, for 50 mg·kg⁻¹ fed fish; and 0.1759 d⁻¹ and 4.0 d, respectively for 75 mg·kg⁻¹ fed fish. Muscle OTC concentration was higher in fish receiving an intraperitoneal (IP) injection than in fish fed OTC. The OTC residue decreased from 7.56 µg·g⁻¹ on the first day to 0.58 µg·g⁻¹ on day 10 after injection for 163-mm fish, and from 5.83 to 0.27 µg·g⁻¹ for 229-mm fish. Muscle OTC concentration was not detected in IP-injected fish 20 d after treatment in the samples of both size fish at 23.4°C. The B-value and t₁/₂ were 0.2687 d⁻¹ and 2.6 d for OTC-injected 163-mm fish, and 0.2554 d⁻¹ and 2.7 d for 229-mm fish. There was no significant difference in B-value and t₁/₂ between different sizes of fish injected with OTC. The OTC residue in muscle and liver of fish fed at rate of 1.5 and 3.0% body weight was significantly lower than fish receiving no feed.

Introduction

Nile tilapia *Tilapia niloticus* is a commonly cultured species in many tropical and subtropical regions and results in high yields in a short period on relatively low nutritional inputs (Hepher and Pruginin 1982). Production of tilapia in many regions will continue to expand in future since farmers are eager to take advantage of its rapid growth rate (Brown 1990). Bacterial infections associated with high mortality have been reported in the culture of tilapias (Roberts and Sommerville 1982; Okaeme 1989). Infections were more severe when fish were in poor water quality, injured by handling, or stressed due to temperature shock and transportation (Okaeme 1989). Fish cannot avoid contact with pathogenic bacteria, so there is a need to prevent and control bacterial diseases with antibiotics.

Oxytetracycline (OTC) is an antibiotic used frequently in fish culture, and its effectiveness against bacterial diseases has been extensively studied (Sniezko 1952; Irwin 1959; Bullock and Collis 1969; Curran and Herman 1969). OTC residue may remain in fish flesh posing a potential health hazard to humans, so it
is necessary to monitor the presence of antibiotics in fish tissues. Most studies on OTC residue have been conducted with salmonids (Herman et al. 1969; McCracken et al. 1976; Nordlander et al. 1987; Black et al. 1991), catfish (Fribourgh et al. 1969; Long et al. 1990) and carp (Grondel et al. 1987).

Microbial assay and the chromatographic method are two common approaches to measure OTC in biological fluids and tissues. The microbial assay is methodologically simple and its technical fundamentals have been known for more than 40 years (Platt 1986). It has high sample capacity with acceptable sensitivity, precision and accuracy. However, chromatographic methods are generally preferred for their greater selectivity and sensitivity for antibiotic analysis (Aoyama et al. 1991). Several recent papers described the high performance liquid chromatography (HPLC) method for determination of OTC concentration in fish tissues (Nordlander et al. 1987; Aoyama et al. 1991; Rogstad and Weng 1993). The microbial assay was used in this study due to the lack of HPLC facilities in our laboratory to conduct sample analysis.

The purpose of this study was to determine OTC residue in the muscle of Nile tilapia after OTC administration by intraperitoneal (IP) injection and by feeding. The effect of food consumption on OTC residue depletion in the muscle and liver tissues was also investigated.

Materials and Methods

Test vessels were 16 cylindrical-shaped metal cages, 90 cm in diameter and 75 cm in height. The cages were held in six concrete tanks, 7.32 x 2.75 m in size, filled with water to 0.5 m depth. Two methods for OTC introduction were employed: intraperitoneal (IP) injection and oral administration.

Two sizes of fish were used in the injection treatment; large fish averaged 229.3±10.2 mm in length and 241.3±34.6 g in weight; and small fish averaged 163.3±7.4 mm in length and 78.5±9.3 g in weight. After being anesthetized in 100 mg·l⁻¹ tricaine methane sulfonate (Argent Chemical Laboratories, Inc., Redmond, Washington, USA), fish were individually weighed and injected anterior to the pelvic fin with OTC (Sigma Chemical Company, St. Louis, Missouri, USA) at a dosage of 50 mg·kg⁻¹ fish. Control fish were injected with an equal amount of 0.85% sterile saline. Eight cages were used in this experiment, with three for OTC-injected small fish, three for OTC-injected large fish and two as control. Six cages with injected fish were kept in two concrete tanks (three cages per tank) and control cages were kept in another tank. Twenty-one fish were put in each cage and three fish were sampled from each cage 5 h after injection and then at 5-d intervals.

In the feeding test, eight cages and three concrete tanks were used. Twenty-one fish, averaging 168.8±13.8 mm in total length and 81.2±14.4 g in body weight, were stocked to each cage. Fish of three cages in a tank were fed OTC-medicated feed at a rate of 50 mg·kg⁻¹, and fish of three cages in another tank received OTC at a rate of 75 mg·kg⁻¹. The remaining cages served as control and fish were fed feed without OTC. Fish were fed unmedicated sinking feed for one week during the acclimation period but were not fed for 2 d prior to initial application of medicated feed. Fish were fed medicated feed at 2%
body weight daily for 10 d. A circle pan with a 40-cm diameter was suspended from the cage cover to 40 cm below the water surface to serve as a food platform in each cage. Feeds were provided to fish at about 1600 h daily and the feeds were usually consumed by the fish within 3-5 minutes. Three fish from each cage were randomly sampled after 10 d of feed treatment and then at 5-d intervals. The muscle OTC concentrations following IP injection or feeding were followed until they fell below the detectable limit (0.05 µg OTC·g⁻¹ of fish muscle).

To prepare the medicated feed, 2.63 g of OTC for 50 mg·kg⁻¹ rate and 3.94 g OTC for 75 mg·kg⁻¹ rate were weighed and dissolved in 250 ml distilled water (including a 5% additional amount of OTC to compensate for the loss of medicated feed processing). Commercial catfish feed was ground and the OTC solution was mixed with 1 kg feed powder in a twin-shell blender for 15 minutes. The moist mixture was extruded through a 3 mm-diameter die in a food grinder and broken into 3-4 mm lengths. The feed was air dried at room temperature and stored at 4°C until used.

Six 200-l troughs were used to determine the effect of feeding rate on OTC depletion in tilapia tissues. Troughs were provided flowing water at the rate of 1-1.5 l·minute⁻¹ and compressed air was used to increase dissolved oxygen through airstones. Water temperature was controlled at 22±2°C. Sixty fish were injected intraperitoneally at a rate of 50 mg OTC·kg⁻¹ fish. The OTC concentration in muscle and liver was analyzed 2 d after injection. The fish were then divided into six groups with 10 fish in each trough. Duplicate groups of three treatments were used: no feed, feeding 1.5%, and 3% body weight of commercial floating catfish feed (MFC Services, Inc., Madison, Mississippi, USA). Four samples of two fish from each trough were collected 4, 8 and 12 d after OTC injection to determine the effect of feeding rate on OTC depletion in tilapia tissues.

After both feeding and injection of OTC, samples were collected and tissues were frozen at -60°C immediately after sampling and analyzed individually within two weeks of storage. Ten grams of fish muscle from lateral sides of the body or 1-2 g liver were homogenized with four volumes of methanol/HCl (98:2) as an extractant (Billman and Clark 1968; Salte and Liestøl 1983) and reextracted with 20 ml methanol·HCl⁻¹. The fluid phase was concentrated under a stream of ultra high purity grade nitrogen to 5-10 ml with a Meyer nitrogen evaporator at 40°C (Nordlander et al. 1987). The extract was assayed employing a microbial method (Grove and Randall 1955; Salte and Liestøl 1983).

*Bacillus cereus* var. *mycoider* (ATCC 11778) was used as the test organism. Assay agar was seeded with the spore suspension. Six stainless steel cylinders were set on each plate about 60 degrees apart on a 2.8-cm radius. Three alternate cylinders on each plate were filled with a reference solution and the remaining three with a sample or standard solution. The extract was inoculated into cylinders (8±0.1 mm in diameter, 10±0.1 mm in height) onto a single layer of seeded agar (antibiotic medium #8, Difco 667), preincubated at 4°C for 1 h and subsequently incubated at 30°C for 18 h. Inhibition zones were measured with calipers. The procedure sensitivity was 0.05 of µg·g⁻¹ OTC.

Standard curves consisted of OTC concentrations of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µg·g⁻¹ with 0.2 µg·g⁻¹ serving as a reference concentration. The curves
were generated by plotting OTC concentration against inhibition zone on the B. cereus-seeded plates.

The OTC elimination rate constant (k) was obtained from the slope of the linear terminal portion of the excretion curve using linear regression analysis (Baggot 1977; Bjorklund and Bylund 1990). The excretion curve was generated by semilogarithmically plotting OTC concentration against time after injection. The half-life was obtained from t₁/₂ = In 2/k (Baggot 1977; Bjorklund and Bylund 1990).

Water quality in concrete tanks was measured during the experiments. Temperature was 25.2±4.1°C, varied from 15 to 31°C. Dissolved oxygen ranged from 5.8 to 8.4 mg l⁻¹, monitored with a Model 57 oxygen meter (Yellow Spring Instruments, Yellow Springs, Ohio). The pH was 7.6±0.17, measured with a Corning pH meter (Corning Medical, Medfield, Massachusetts, USA). Total alkalinity and hardness, determined with a Hoch water quality test kit (Hoch Chemicals, Loveland, Colorado, USA), were 36.1±3.3 and 20.4±3.2 mg l⁻¹, respectively. The OTC excretion data were analyzed by analysis of variance procedures (SAS Institute 1982). Probabilities of 0.05 or less were considered statistically significant.

Fig. 1. Orphenidine (OTC) residue in the muscle of Nile tilapia fed OTC at a rate of 50 or 75 mg kg⁻¹ of fish for 10 d. The excretion curves were generated by semilogarithmically plotting OTC concentration against time. The curves were not plotted at the points where OTC in muscle was below the detection limit.

Results and Discussion

No OTC was detected in control fish for either feeding or injection treatment. Five hours after cessation of medicated feeding, fish samples showed 0.46 and 0.54 μg OTC·g⁻¹ muscle from fish fed medicated feed at the rates of 75 and 50 mg kg⁻¹, respectively. OTC residue was below the detectable limit in fish fed at a rate of 50 mg kg⁻¹ 10 d after cessation of medicated feed (Fig. 1).
Muscle OTC residue in 75 mg·kg⁻¹ fed fish was higher than 50 mg·kg⁻¹ fed fish (P<0.05), and OTC residue was below the detectable limit 15 d after cessation of medicated feed. The β-value and half-life (t₁/₂) were 0.1642 d⁻¹ and 4.2 d, respectively, for 50 mg·kg⁻¹ fed fish; and 0.1759 d⁻¹ and 4.0 d, respectively, for 75 mg·kg⁻¹ fed fish.

In contrast to feeding, the OTC concentration in muscle after IP injection was much higher and elimination time was longer. The OTC residue decreased from 7.56 µg·g⁻¹ on the first day to 0.58 µg·g⁻¹ 10 d after injection for 163-mm fish, and from 5.83 to 0.27 µg·g⁻¹ for 229-mm fish (Fig. 2). OTC concentration in muscle by IP injection was below the detectable limit in samples of both size fish 20 d after treatment at 23.4°C. The β-value and t₁/₂ were 0.2687 d⁻¹ and 2.6 d, respectively, for 163-mm fish; and 0.2554 d⁻¹ and 2.7 d, respectively, for 229-mm fish. Muscle OTC residues were slightly higher in small fish than large fish, but no statistical difference was noted in residue between the two sizes of fish (Fig. 2). There was no significant difference in elimination rate constants and t₁/₂ between different sizes of fish. The result in this experiment was in agreement with that found by Salte and Liestøl (1983). They reported that the weight of rainbow trout had only a minor effect on the OTC elimination rate constant.

The accumulation and maximum concentration of OTC in fish depends upon the dose administered and a high dose leads to a more rapid excretion than a low dose (Jacobsen 1989). In this study, 0.46 µg·g⁻¹ of OTC was found in the first sample of 75 mg·kg⁻¹ fed fish and 0.14 µg·g⁻¹ in 50 mg·kg⁻¹ fed fish (Fig. 1). Muscle OTC residue was reduced to 0.10 µg·g⁻¹ in 75 mg·kg⁻¹ fed fish or less than 0.05 µg·g⁻¹ in 50 mg·kg⁻¹ fed fish after 10 d cessation of medicated feed. The muscle OTC residues were below the detectable limit in fish fed 50 or 75 mg·kg⁻¹ OTC 15 d after cessation of the treatment.
The amount of feed provided for fish has an effect on OTC residue depletion in tilapia. Muscle OTC residue in fed fish was significantly lower than fish receiving no feed (P<0.05). The fish fed 3% body weight of feed had the lowest OTC residue in muscle (Table 1). OTC residue in liver of tilapia without feeding was significantly higher than those fed fish (P<0.05). The fish fed at a rate of 1.5% body weight had higher OTC in liver than fish fed at a rate of 3% body weight (P<0.05). The result shows that an increase in the amount of feed provided to fish affects fish metabolism and hence enhances the elimination of OTC residue from fish tissues. In practice, it is important to provide a sufficient amount of feed to the treated fish after the antibiotic treatment. It is suggested from this study that feeding can help to reduce OTC residue from fish before they are harvested.

Many feed factors, such as macronutrients and some trace substances, can interact with each other to influence drug disposition, including drug absorption, distribution, metabolism and excretion (Vesell 1984). Effects of feed on drug metabolism depend on the type of feed, amount of feed consumed, characteristics of drug, and physical conditions of host (Roe 1989). Changes in the protein, carbohydrate and fat in feed can have significant effects on the rate of metabolism of therapeutic drugs. High-protein intake usually increases, while low-protein intake decreases the rate of drug metabolism (Vesell 1984; Roe 1989).

Feeding and injection are two common approaches to provide antibiotics to fish for treatment of bacterial diseases. With valuable fish or small numbers of fish, injection is preferable since it can obtain equal dose distribution among all fish; however, it is time consuming. Medicated feed is a more common way to administer OTC to a large fish population. Compared to injection, feeding needs less time, requires less manpower and reduces stress and handling injury to fish. Feeding medicated feeds is suitable for tilapia culture in ponds, raceways and cages.

The high level of drug residues in edible products of OTC-treated fish can constitute a hazard to the health of consumers. In the USA, OTC was approved by the Food and Drug Administration for use in salmonids and catfish at the beginning of feeding experiment (2 da after OTC injection). Each value is the mean of eight samples from four fish.
rate of 5.5-8.3 g·100 kg⁻¹ of fish per day for 10 d in feed. There is a 21-d pre-slaughter withdrawal period for OTC-treated fish before harvesting. The tolerance limit is 0.1 µg OTC·g⁻¹ edible tissues of fish. To ensure that levels of antibiotics in fish tissues are within acceptable limits, monitoring of drug residues is an essential aspect of aquaculture. Since no information has been found on OTC residue in Nile tilapia, the result of this study can be used as a reference for tilapia. This study shows that OTC residue is below the detectable limit 15 d after cessation of medicated feed in fish fed at a rate of 75 µg OTC·kg⁻¹ fish. The 21-d withdrawal period used in catfish and salmonids can also be used for tilapia.

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References


