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Oxytetracycline Residues in Muscle of Red Tilapia Medicated Orally and Cultured in Brackishwater

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Abstract

Oxytetracycline (OTC) was fed at a rate of 80 mg·kg^{-1} for 10 d to healthy red tilapia (182.1±51.3 g) cultured in brackishwater of 20 ppt salinity, at an average water temperature of 28.4±1.0°C and pH 7.5±0.2. After treatment, the muscle of the fish (25 g of muscle taken along the lateral line) was analyzed by high performance liquid chromatography for OTC residues. The initial OTC concentration in the muscle was 0.48±0.12 μg·g^{-1}. Due to this low absorption, the use of OTC for treatment of brackishwater/marine fish should be discouraged, and be replaced by more effective drugs.

Introduction

Oxytetracycline HCl (OTC) is an antibiotic commonly used for the treatment of fish diseases such as furunculosis, vibriosis, ichthyiotherius, enteric redmouth disease and columnaris disease. Nusbaum and Shotts (1981) reported that OTC is very useful in controlling *Aeromonas hydrophila* complex, *A. salmonicida, Flexibacter columnaris*-like organisms and *Pseudomonas fluorescens*. OTC, however, has been reported to be poorly absorbed by fish (Cravedi et al.1987; Björklund and Bylund 1990; Rogstad et al. 1991; Ueno et al. 1995), especially in seawater (Lunestad and Goksøyr 1990; Lunestad 1991). Even though the efficacy of OTC has been known to be poor in seawater, fish farmers continue to use it to treat fish and prawns cultured in brackish- and seawater. More studies, especially under tropical conditions, should be carried out to determine the efficacy of OTC, and farmers duly advised on whether this drug should be used for treating brackish- or seawater fish.

This study attempts to measure the OTC residues in muscles of market-size red tilapia medicated orally and cultured in brackishwater (20 ppt) in the hatchery of the Fisheries Research Institute in Glugor, Penang. The efficacy of OTC under brackishwater/marine condition is also discussed.

Materials and Methods

Experimental Conditions

Healthy red tilapia (182.1±51.3 g) were obtained from the Freshwater Fish Culture Station in Jitra, Kedah. The fish were kept in 2-tonne rectangular fiberglass tanks (300 x 100 x 80 cm) in the hatchery of the Fisheries Research Institute in Glugor, Penang, under natural lighting of approximately 12 h light and 12 h darkness. The fish were gradually acclimated from fresh- to brackishwater (20 ppt) over a period of two weeks. The temperature of the water was recorded twice daily (morning and afternoon) using an LCD display Ama-digit ad 16th thermometer (Germany). The salinity of the water was measured daily with an Atago refractometer (Japan), and the pH of the water was measured with a Corning pH meter 220 (Suffolk, England).

Drug Administration

The fish were acclimated for a week in brackishwater and were starved for 2 d before the start of the experiment. The type of feed (CPP-TPP^{-1}, Central Pangan Pertiwi, Indonesia) used, the preparation and feeding schedules were similar to that described by Choo (1995). The OTC (oxytetracycline dihydrate - Sigma Chemical Co., St. Louis, USA) was incorporated by mixing a ratio of 100 g pellet feed to 100 ml distilled water to which OTC was dissolved. The dough was extruded through a meat mincer, cut into pellets and fed moist. The OTC dosage of 80 mg·kg^{-1} fish was incorporated into 1% body weight of feed. The tanks were cleaned daily with approximately 80% water change. After the 10-d medication, three fish were used each time for OTC analyses at 0, 3, 6, 9, 12 and 15 d after treatment. OTC analyses were carried out immediately after sampling. The samples taken at 0 d were collected 2 h after the last medicated feed, and immediately analyzed after sampling.

Chemical Analysis

CHEMICALS

Methanol, dichloromethane, acetonitrile (all of HPLC grade), petroleum benzine extra pure DAB (b.p. 40-60°C) and hydrochloric acid fuming 37% GR were obtained from Merck (Darmstadt, Germany), oxalic acid (Analar Grade) and sodium hydroxide pellets (Analar Grade, minimum assay 99%) from BDH (Poole, England). OTC was obtained from Sigma Chemical Co. (St. Louis, USA). Alpha-Q Water (Millipore) was used for the preparation of aqueous solutions.

APPARATUS

Apparatus used included a homogenizer, separatory funnels, 15-ml conical centrifuge tubes graduated to 0.1 ml, pippetes, conical flasks, filter

funnels and glasswools. A High Performance Liquid Chromatography (HPLC) Pump Model Waters 600E (Millipore Corporation, USA) connected to an autosampler and injector Model Waters 715 Ultra WISPS, a UV detector Model Waters 486 and a Waters Maxima 825 Chromatography Software and Workstation were used. The column used was a Novapak C_{18} steel column (3.9 x 150 mm with spherical packing material of 4 µm) which was connected to a Guard-Pak™ µ Bondapak™ C_{18} HPLC precolumn insert.

SAMPLE PREPARATION

Sample preparation was carried out as described by Choo (1995). OTC extraction and recovery were adapted from the method described by Moats (1986). A 25-g sample of muscle tissue taken from the lateral line region behind the operculum of the fish was homogenized for 10 minutes with 75 ml 1N hydrochloric acid. Eight ml of the homogenate was deproteinized with 32 ml acetonitrile. After standing for 5 minutes, the supernatant was decanted by passing through a plug of glass wool sitting in a filtration funnel. A liquid-liquid extraction using 20 ml dichloromethane and 20 ml petroleum benzine was carried out on the filtrate, and the OTC portion was partitioned in the aqueous layer. The detection limit and recovery were determined using untreated fish, with tissues spiked with 2 $mg \cdot l^{-1}$ OTC and allowed to stand for 30 minutes before analyses.

CHROMATOGRAPHY

The chromatography conditions and procedures were similar to that described by Choo (1995). The mobile phase (pH 4.01) used comprised of methanol, acetonitrile and 0.01 M aqueous oxalic acid (pH 3.5 adjusted with 6N NaOH) in the following ratio, 1:1.5:7.5. The mobile phase was filtered using an FH 0.5-mm millipore filter, and further degassed with a stream of helium (He) at 100 $ml \cdot min^{-1}$ for 15 minutes. A small stream of He (20 $ml \cdot min^{-1}$) was passed through the mobile phase throughout the analyses. The detection of OTC was made at a wavelength of 360 nm, a temperature of 30°C, a flow rate of 1.0 $ml \cdot min^{-1}$, a pressure of 1,200-1,400 psi, and a detector sensitivity of 0.01 AUF. Fifty µl of the aqueous extract was injected into the column using the autoinjector.

The standard curves were prepared using OTC hydrochloride (88.7% purity). The stock (100 ppm) was prepared by dissolving OTC in methanol, and the working solution was prepared by diluting the stock solution with the mobile phase.

The peak integration and concentrations (peak areas) were calculated using the Waters Maxima 825 chromatography software and workstation. The half-life (t ½) of OTC in the muscle was calculated as ln $2/\beta$, where β, the apparent elimination rate constant, is the slope of the terminal portion of the ln concentration OTC plotted against time (d) after withdrawal from medication (Björklund and Bylund 1990; Xu and Rogers 1993). The terminal portion of the concentration curve was taken from day 9 to 18.

Results

Culture Conditions

The average water temperature of the culture tank was 28.4±1.0°C, with pH at 7.46±0.18. At the feeding rate of 1% body weight of feed, about 95% of the feed given during the course of the experiment was consumed.

Detection Limit and Recovery

The detection limit (with a signal-to-noise ratio of 3) of OTC was 0.07 $\mu g \cdot g^{-1}$, and the recovery was 79±4% (coefficient of variation, n = 4). The HPLC response for OTC over the range of 0.01-2 ppm OTC standard solution was linear with a coefficient of determination (r^2) = 0.9997±0.0002. The retention time of OTC was 2.67±0.03 minutes.

OTC Level in Muscle

Table 1 shows the OTC concentration in the muscle of red tilapia following oral medication. The level of OTC in red tilapia analyzed 2 h after the last medicated feed was 0.48±0.12 $\mu g \cdot g^{-1}$. The plot of ln concentration against time (0-18 d) gave a linear regression of Y = -0.90-0.12X with a coefficient of determination (r^2) of 0.866. Assuming that 95% of the drug was consumed by the fish, the amount of OTC available in the muscle was 0.6% of total feed intake. The β value was 0.124±0.027, and t 1/2 of OTC in muscle tissue was 2.8±0.2 d.

Table 1. OTC concentration in muscle of red tilapia cultured in brackishwater following oral medication (80 mg· kg^{-1} fish for 10 d).

Days after treatment	No. of fish analyzed	Average size (g ± SD)	OTC (μg·g-1)
0[1]	3	154.4 ± 33.9	0.48 ± 0.12
3	3	168.4 ± 31.8	0.27 ± 0.08
6	3	236.2 ± 79.5	0.17 ± 0.02
9	3	224.6 ± 56.1	0.16 ± 0.03
12[2]	3	276.0 ± 101	0.08
15	3	246.4 ± 61.6	0.09 ± 0.02
18[3]	3	176.6 ± 63.1	0.04 ± 0

[1] 2 h after last feeding with medicated pellets
[2] Two fish had OTC levels below detection
[3] One fish had OTC levels below detection

Discussion

The initial OTC concentration (0.48±0.12 $\mu g \cdot g^{-1}$) in the muscle of red tilapia cultured in brackishwater was approximately 14% of the level found in red tilapia medicated orally, and of the same magnitude with red tilapia medicated through a bath and cultured in freshwater in a previous experiment conducted in the same hatchery (Choo 1995). Fish from both experiments were obtained from the same Freshwater Fish Culture Station in Jitra, and were possibly of the same stock.

The low absorption of OTC in fish medicated through bath has been well documented (Herman 1972; Strasdine and McBride 1979; Smith 1991; Choo 1995). Herman (1972) mentioned that bathing fish in chemotherapeutic agents rarely produces therapeutic tissue levels; O'Grady (1988) (quoted from Smith 1991) reported that the bath administration of the tetracycline is of little value. Since OTC concentration in muscle of red tilapia cultured in brackishwater and medicated orally is of the same magnitude in those cultured in freshwater and medicated through bath, the therapeutic effect of OTC in red tilapia under oral medication and cultured in brackishwater is questionable.

The minimum inhibition concentrations (MIC) of OTC have been reported to vary with various fish pathogens. MIC values are 0.25-1.76 $\mu g \cdot ml^{-1}$ for *Aeromonas* and *Vibrio* species, 5.0-25 $\mu g \cdot ml^{-1}$ for *Pseudomonas* sp. and 8 $\mu g \cdot ml^{-1}$ for *Flexibacter* sp. (Herman 1969 quoted from Grondel et al. 1987; Björklund et al. 1990). Bruno (1989), however, reported lower MIC values of 0.09-0.19 $\mu g \cdot ml^{-1}$ for *A. salmonicida*. For effective treatment, Stokes (1980) recommended a therapeutic concentration of at least 2-4 times the MIC.

OTC levels in muscles of fish medicated with OTC were reported by several authors and ranged from 0.56 to 11.74 $\mu g \cdot g^{-1}$ muscle. Rainbow trout (123±45 g) suffering from vibriosis and cultured in water temperature of 17-20°C and salinity of 0.5% had a muscle OTC level of 0.6±0.1 $\mu g \cdot g^{-1}$ (analysis done on last day of treatment) when they were fed 100 mg $OTC \cdot kg^{-1}$ $fish \cdot d^{-1}$; for salmon (52±14 g) also suffering from vibriosis and medicated with the same OTC dosage, an OTC level of 0.9±0.2 and 1.5±0.5 $\mu g \cdot g^{-1}$ (analysis done one day after treatment) were obtained (Björklund and Bylund 1990). Rainbow trout (ca 150 g) cultured in water temperature of 12°C recorded an OTC level of 0.56 $mg \cdot kg^{-1}$ in muscle after an oral medication of 50 mg $OTC \cdot kg^{-1}$ fish for 8 d (Jacobsen 1989). Hybrid bass cultured in water temperature of 32°C at 5 ppt salinity, and injected with 25 mg $OTC \cdot kg^{-1}$ fish and 50 mg $OTC \cdot kg^{-1}$ fish had OTC levels of 5.93 and 11.74 $\mu g \cdot g^{-1}$ muscle, respectively (Xu and Rogers 1993). Choo (1995) reported OTC values of 3.47±1.12 $\mu g \cdot g^{-1}$ in muscle of red tilapia reared in freshwater at a temperature of 27.3±0.7°C, and medicated orally at 80 $mg \cdot kg^{-1}$ $fish \cdot d^{-1}$ for 10 d.

Cravedi et al. (1987) reported that rainbow trout fed OTC at two different concentrations (0.1 and 0.5%) in a dry diet had an apparent digestibility of 7-9% for OTC. Grondel et al. (1987) reported a bioavailability for OTC of only 0.6% in the carp (*Cyprinus carpio* L.) medicated orally.

Rogstad et al. (1991) found that only 2.6% of the administered dose of 150 $mg \cdot kg^{-1}$ fish was absorbed by rainbow trout cultured in freshwater.

The efficacy of OTC has been reported to be reduced by di- and trivalent ions (Björklund and Bylund 1990; Lunestad and Goksøyr 1990; Lunestad 1991; Petersen et al. 1993). Cravedi et al. (1987) suggested that the poor digestibility of OTC could be improved by incorporating a proteolytic enzyme into the medicated diet or by reducing the calcium level of the diet. Lunestad and Goksøyr (1990) showed that magnesium formed a more avid ligand with OTC than with calcium. A diet low in magnesium will therefore also improve the bioavailability of OTC. However, when marine fish are medicated, OTC will unavoidably come into contact with Mg^{2-} and Ca^{2-} ions when the feed pellets are in seawater or in the gastrointestinal tract (Lunestad and Goksøyr 1990).

To increase the therapeutic levels of OTC in brackishwater/marine fish, the standard dosage of 50-100 $mg \cdot kg^{-1}$ $fish \cdot d^{-1}$ for 3-14 d could be increased. However, the standard dosage for fish is already 5-10 times higher than doses usually recommended for humans (Lunestad 1991). Any increase to this dosage would risk an increase in OTC contamination of the environment, especially in the accumulation of OTC in marine sediments (Jacobsen and Berglind 1988; Samuelsen 1989; Björklund et al. 1990, 1991), and an increased risk of building up antibiotic-resistance in bacteria (Aoki et al. 1985, Lewis and Plumb 1985; Björklund et al. 1991; McPhearson et al. 1991, Smith 1991; Nygaard et al. 1992).

Unless the digestibility of OTC can be substantially improved, its use in the brackishwater/marine environment should be discouraged. Farmers should be advised to replace the use of OTC in the treatment of brackishwater/marine fish with other more effective drugs.

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