Aquaculture 495 (2018) 675–681

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Comparative pharmacokinetics of oxytetracycline in tilapia (*Oreochromis spp.)* maintained at three different salinities

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ARTICLE INFO

Keywords:
Tilapia
Oxytetracycline
Pharmacokinetics
Fresh water
Brackish water
Salt water

ABSTRACT

Environmental factors, such as temperature, pH, and salinity of water may affect the pharmacokinetics (PK) of a drug in aquatic animals and in most instances water salinity is ignored in PK studies. This study compared PK profiles of oxytetracycline (OTC) following a single oral dosage of 50 mg/kg in tilapia (*Oreochromis spp.*) maintained in three aquatic environments: freshwater (FW: 0 ppt salinity), brackish water (BW: 15 ppt salinity) and salt water (SW: 30 ppt salinity). Water quality parameters between the three systems were similar except salinity. Following OTC administration, blood samples were collected at 23 time points: 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24 h, and 2, 4, 6, 8, 10, 12, 14, 18, 22, 26, 30, 34, 38 and 42 days. At each sampling time, six fish from each group were netted, sedated with buffered MS-222, bled and then euthanized. The OTC was extracted from plasma by Solid Phase Extraction (SPE) and analyzed by Ultra High Pressure Liquid Chromatography coupled by as tandem quadrupole mass spectrometer. The plasma concentration versus time data of OTC for the FW, BW and SW tilapia were subjected to PK analysis using non-compartment methods. Pharmacokinetics of OTC was characterized by rapid absorption and slow excretion in the FW and BW tilapia. Compared to the FW and BW groups, absorption and elimination of OTC was faster in the SW tilapia. The $AUC_{0-\infty}$ of OTC was in order of FW (165 h.μg/mL) > BW (145 h.μg/mL) > SW (55.5 h.μg/mL) group. In SW tilapia, terminal half-life (69 h) of OTC was > 2 times shorter than FW (177 h) and BW (155 h) groups. However, AUCs and terminal half-lives of the FW and BW groups were not significantly different. The study indicated that rise in water salinity level increases clearance of OTC in tilapia. It is suggested that OTC residues in tissues will not be the same in tilapia maintained at different water salinity levels. The results confirmed that infectious diseases associated with bacteria having a MIC of 0.5–1.0 μg/mL can be treated with the 50 mg/kg dosage of OTC in the FW and BW group, but the same dosage in the SW tilapia may lead to therapeutic failure and increased risk of resistance emergence.

1. Introduction

Aquaculture is one of the largest growing sectors around the world to supply food, with an average annual growth rate of 6.3% since 2000 (average 8.8% per year between 1980 and 2010) and currently accounts for about 47% of the world's fish supply (FAO, 2012). Fish living in the wild as well as reared in the aquaculture facilities are susceptible to infectious diseases. Bacterial diseases are a major limitation to productivity leading to significant economic losses by causing mortality up to 100% in the aquaculture industry (Tran et al., 2013). Threat of infectious diseases is on the rise, occurrence of infectious disease outbreaks wiping out entire stocks have been reported in farmed fish (Pulkkinen et al., 2010; Leung and Bates, 2013). For instance, bacterial infections in the United States caused 60% mortalities in catfish, while 30% of mortalities were a result of parasitic infestations, 9% of mortalities from fungal infections, and 1% of mortalities from viral etiologies (MSU, 2010). Bacteria causing infections in fish include; Gram-negative: *Aeromonas hydrophila, Aeromonas salmonicida, Flavobacterium columnare*, *Vibrio spp.*, and *Pseudomonas* spp.; and Gram-positive: *Streptococcus* spp. and *Mycobacterium* spp. (Austin and Austin, 1999). Treating bacterial infections in fish is one of the toughest challenges due to limited choices of antimicrobials available for aquaculture use

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https://doi.org/10.1016/j.aquaculture.2018.06.044
Received 16 May 2018; Received in revised form 11 June 2018; Accepted 17 June 2018
Available online 19 June 2018
0044-8486/

and no chemotherapeutic agent is approved specifically for tilapia. Antibacterial drugs approved by the US Food and Drug Administration for use in food fish include oxytetracycline, sulfadimethpoxine/ormetoprim, sulfamerazine and florfenicol (Smith et al., 2008).

Oxytetracycline (OTC) is one of the most widely used antimicrobials in the livestock industry due to its broad-spectrum activity, good penetration into body fluids and tissues, low cost and low toxicity risk. It binds to the 30S ribosomal subunit of susceptible microbes to produce antimicrobial activity. Upon binding, the OTC interferes with transfer RNA's ability to bind with messenger RNA, thereby preventing bacterial protein synthesis (Rivere and Spoo, 1995). In the US, OTC has been approved to treat bacterial hemorrhagic septicemia (*Aeromonas liquefaciens*) and pseudomonas (*Pseudomonas* spp.) infection in catfish, ulcer disease (*Hemophilus piscium*), furunculosis (*Aeromonas salmonicida*), bacterial hemorrhagic septicemia (*A. liquefaciens*), and pseudomonas disease *(Pseudomonas* spp.) in salmonids, cold-water disease (*Flavobacterium psychrophilum)* in freshwater reared salmonids, columnaris disease (*F. columnare*) in rainbow trout, and gaffkemia (*Aerococcus viridians*) in lobsters (FDA Approved Aquaculture Drugs, 2018). Limited approval of OTC is partly due to the lack of data pertaining to elimination kinetics, target animal efficacy and safety in species other than salmonids and catfish. Off-label use of OTC in feed has historically been used to treat infections such as flavobacteriosis in common carp (*Cypinus carpio*) and grass carp (*Ctenopharyngodon idella*); furunculosis in coho salmon (*Oncorhynchus kisutch*); and columnaris and streptococcosis in rainbow trout (*Oncorhynchus mykiss*) (Treves-Brown, 2000). Despite this widespread use, information on the pharmacokinetics (PK) and pharmacodynamics (PD) of OTC is very limited for farmed fish species. Pharmacokinetics of OTC have been studied in African catfish (Grondel et al., 1989), Atlantic salmon (Elema et al., 1996), chinook salmon (Abedini et al., 1998; Namdari et al., 1998), carp (Grondel et al., 1987), rainbow trout (Black et al., 1991; Bjorklund and Bylund, Björklund and Bylund, 1991; Abedini et al., 1998), red pacu (Dio et al., 1998), summer flounder (Hughes, 2003), tench (Reja et al., 1996) and yellow perch (Bowden, 2001).

Tilapia is a popular farmed fish species in the US and > 75% of the annual production is supplied by recirculating systems (Zajdband, 2012). Despite this, no studies on the PK of OTC have been conducted in tilapia (*Oreochromis spp.*) except tissue depletion studies in which residues of OTC at a limited number of time points were determined in serum of tilapia following in feed drug administration (Chen et al., 2004; Paschoal et al., 2012). Thus, for off-label usage in tilapia dosing regimens are often extrapolated from other species that may lead to therapeutic failure and antibiotic resistance. In fish, extrapolation is not advised because of excessive PK variability between species, route of administration and formulation of drugs. Moreover, in aquatic animals, the PK of a drug can be affected by environmental factors, such as temperature, pH, and salinity of water in which the animals are raised (Rigos and Smith, 2013). The tilapia can be and are grown in freshwater, brackish water and seawater around the world, but therapeutic compounds are currently approved for a particular species of fish, regardless of the salinity in which they are maintained. Our hypothesis is that a single therapeutic recommended dose of OTC may not be appropriate for the different environmental salinities and no study comparing the PK of OTC at different water salinity levels in the same species of fish has been found in literature. Therefore, the purpose of this study was to compare PK profile of OTC following oral administration in tilapia (*Oreochromis spp.*) maintained in water with different salinity levels to determine the effect of salinity on the metabolism of the drug.

2. Material and methods

2.1. Animals

The experiments were conducted according to the guidelines of Institutional Animal Care and Use Committee of Virginia Tech. Four hundred and fifty juvenile tilapia (*Oreochromis spp.)* were obtained from a commercial aquaculture facility (Blue Ridge Aquaculture, Martinsville, VA, USA) and maintained at the Conservation Aquaculture Facility at Center Woods, Virginia Tech. The fish were placed in a 500-gal recirculation system and acclimated for 2 weeks. The system was fitted with a mechanical filter for solids removal, a bio-filter for nitrification, and air stones for aeration and carbon dioxide removal. During this time, water quality parameters monitored on a daily basis were dissolved oxygen and temperature; while ammonia, nitrite, nitrate, alkalinity and pH were monitored three times a week using a commercial aquaculture kit (HACH Company, Loveland, CO, USA). A standard pelleted tilapia diet containing 36% protein and 6% fat (Zeigler Brothers, Inc., Gardners, PA, USA) was fed to fish at a rate of 3% body weight per day. The feed was delivered continuously over a period of 12 h using an automated 24 h belt feeder (Pentair AES, Apopka, FL, USA). After 2 weeks of acclimation, fish were arbitrarily divided into three groups of 138 fish and placed into three separate recirculation systems each of which was fitted with a mechanical filter for solids removal, a bio-filter for nitrification, and air stones for aeration and carbon dioxide removal (Table 1). As demonstrated in the past, activated carbon filter material was also installed in the filter of each system to bind any free OTC or OTC metabolites excreted into the water column by the fish (Hughes, 2003). Total fish acquired were 450 (138 × 3groups = 414 + 36 = 450) because an additional 36 fish were included to account for any transportation mortality and/or unacceptable small sized fish.

2.2. Treatment groups

The three separate recirculation systems were arbitrarily assigned to one of the three experimental aquatic environments: freshwater (FW: 0 ppt salinity), brackish water (BW: 15 ppt salinity) and salt water (SW: 30 ppt salinity) (Table 1). The fish in the BW and SW systems initially started in freshwater (0 ppt) followed by a slow increase in water salinity adjusting to a final salinity of their respective group (15 ppt or 30 ppt) over 2 weeks by adding synthetic marine salts (Instant Ocean, Blacksburg, VA, USA) to each system. The BW fish arrived at their final salinity in 5 days, while 11 days elapsed for the SW fish to arrive at salinity of 30 ppt as salinities were not increased > 3 ppt over a 24 h period. In each of the three systems fish were allowed to acclimate to their respective final salinities for an additional 3 weeks prior to initiation of the PK study.

Table 1
Experimental design of oxytetracycline pharmacokinetic study in FW, BW and SW tilapia.

	Treatment groups		
	Freshwater (FW)	Brackish water (BW)	Salt water (SW)
Water salinity (ppt)	0	15	30
Water temperature (°F) - mean (SD)	78.8 (1.2)	78.9 (1.1)	78.3 (1.3)
Number of tilapia per group (n)	23 × 6 = 138	23 × 6 = 138	23 × 6 = 138
Number of sample times collected	23	23	23
Weight (g) of tilapia (n = 138)	Mean = 123.6 Min = 86 Max = 171	Mean = 123.7 Min = 91 Max = 168	Mean = 118.7 Min = 87 Max = 178
Dose of OTC by oral gavage	50 mg/kg	50 mg/kg	50 mg/kg
Mortality during experiment	0	0	0

Mean = Mean weight; Min = minimum weight; Max = maximum weight.