Environmental Pollution 235 (2018) 245–254

Contents lists available at ScienceDirect

Environmental Pollution

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

Environmental concentrations of antibiotics impair zebrafish gut health☆

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ARTICLE INFO

Article history:
Received 12 August 2017
Received in revised form
12 December 2017
Accepted 20 December 2017

Keywords:
Antibiotic
Intestinal microbiota
Gut health
Zebrafish

ABSTRACT

Antibiotics have been widely used in human and veterinary medicine to both treat and prevent disease. Due to their high water solubility and low bioavailability, many antibiotic residues have been found in aquatic environments. Fish are an indispensable link between the environmental pollution and human health. However, the chronic effects of environmental concentrations of antibiotics in fish have not been thoroughly investigated. Sulfamethoxazole (SMX) and oxytetracycline (OTC) are frequently detected in aquatic environments. In this study, zebrafish were exposed to SMX (260 ng/L) and OTC (420 ng/L) for a six-week period. Results indicated that exposure to antibiotics did not influence weight gain of fish but increased the metabolic rate and caused higher mortality when treated fish were challenged with *Aeromonas hydrophila*. Furthermore, exposure to antibiotics in water resulted in a significant decrease in intestinal goblet cell numbers, alkaline phosphatase (AKP), acid phosphatase (ACP) activities, and the anti-oxidant response while there was a significant increase in expression of inflammatory factors. Antibiotic exposure also disturbed the intestinal microbiota in the OTC-exposed group. Our results indicated that environmental antibiotic concentrations can impair the gut health of zebrafish. The potential health risk of antibiotic residues in water should be evaluated in the future.

1. Introduction

Antibiotics are natural or synthetic drugs with the capacity to kill or inhibit the growth of bacteria. In addition to treating infectious diseases, some antibiotics are used as feed supplements to promote growth of livestock (Carvalho and Santos, 2016). In the United States of America and Europe, the annual usage of antibiotics has been estimated to be in the range of tens of thousands of tons (Food and Drug Administration, 2009; European Medicines Agency, 2011). In China, approximately 162,000 tons of antibiotics were used in 2013 (Zhang et al., 2015). Most antibiotics are poorly absorbed by humans or animals after intake, with approximately 25–75% of antibiotics discharged into aquatic environment as unchanged or modified as metabolites via feces or urine (Karthikeyan and Meyer, 2006). Due to their low bioavailability, high water solubility, and widespread misuse, various antibiotic residues have been detected in many aquatic environments (Chang et al., 2010; Chen et al., 2015a; Godeaux, 2015; Leung et al., 2012; Ma et al., 2015; Wei et al., 2011).

Antibiotic residuals in water have been reported to cause adverse effects on aquatic organisms. However, information on ecological toxicity is generally limited to acute lethal effects, which occurs at concentrations in the mg/L range (Ji et al., 2012). Generally, the environmental concentrations of antibiotic residues in water range from ng/L to μg/L, several orders of magnitude lower than concentrations resulting in acute effects. More recently, studies have been conducted to evaluate the influence of antibiotics using concentrations relevant to those in the environment *in situ* but they are mainly limited to short-term tests (Carlson et al., 2017;

☆ This paper has been recommended for acceptance by Dr. Harmon Sarah Michele.

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https://doi.org/10.1016/j.envpol.2017.12.073
0269-7491/

Elizalde-Velazquez et al., 2017; Oliveira et al., 2013). As many aquatic species are continuously exposed to water over long periods of time or perhaps over their entire life cycle, it is more apposite to measure the chronic influence of antibiotics using environmental concentrations on aquatic animals (Fent et al., 2006). Fish are particularly important targets for evaluating environmental risks, as they provide an indispensable link between antibiotics released into aqueous environments and human health (Zhao et al., 2015). Furthermore, they are good models for chronic toxicity analyses (Nannou et al., 2015).

A functional and stable gut microbiota is important to host health. Antibiotics can deplete commensal microbiota or directly affect host tissues, causing adverse effects on animal physiology (Morgun et al., 2015). Intact intestinal structures and normal intestinal function help animal hosts prevent many diseases. For example, it has been shown that an intact intestine affords a barrier to exogenous toxins through oxidative and reductive processes which are the key components of metabolic pathways of xenobiotics, including drugs (Rodrigues et al., 2017). Although the intestine is the main target of antibiotic absorption, previous studies have mainly focused on the functions of liver, kidney or gills when the risks of antibiotics were evaluated (Madureira et al., 2012; Rodrigues et al., 2017; Yonar et al., 2011). Recent studies have suggested involvement of the gut microbiota in the development of a wide range of disorders, including metabolic dysfunctions and inflammatory disease (Fei and Zhao, 2013; Xiao et al., 2014). Furthermore, strong evidence also showed the critical role of intestinal microbiota in drug metabolism and toxicity (Choi et al., 2013).

Sulfamethoxazole (SMX) and oxytetracycline (OTC) are two commonly used antibiotics in human and veterinary medicine (Rigos and Troisi, 2005; Trovó et al., 2009). Consequently, SMX and OTC have been frequently detected in surface water, groundwater and seawater (Nie et al., 2013). The concentrations of these antibiotics vary in different places or seasons. It has been observed that the concentrations of SMX range from 2 to 11600 ng/L in natural or waste water (Gust et al., 2012; Terzic et al., 2008). In Shanghai for example, a mean concentration of 259.6 ng/L SMX was found in Huangpu River (range of 2.2 ng/L to 764.9 ng/L) (Chen and Zhou, 2014). The geometric mean concentration of OTC in Tha Chin River was 220 ng/L in the dry season and 500 ng/L in the wet season (Rico et al., 2014). The mean concentration of OTC in water from the Hailing Bay Region was found to be 417.76 ng/L with a maximum concentration of 15.16 μg/L (Chen et al., 2015b) (Table S1).

Similar to other antibiotics, the risk assessment for these two antibiotics and their effect on aquatic animals is mainly obtained from acute toxicological research studies. For example, a combination of SMX and trimethoprim at a concentration of 40 ppm increased plasma cortisol and glucose and resulted in a decreased *C*-reactive protein concentration in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), after one hour of treatment (Yildiz and Altunay, 2011). Acute exposure (96 h) of rainbow trout (*Oncorhynchus mykiss*) to OTC at a concentration ranging from 5 μg/L to 50 mg/L did not change catalase (CAT) activity in gill tissues of rainbow trout. However, chronic exposure (28 days) to OTC at a concentration ranging from 0.3125 μg/L to 5 μg/L decreased CAT activity in gills (Rodrigues et al., 2017), indicating that tissues function differently when exposed to chronic and acute antibiotic exposure. However, there is very limited information relating to environmental concentrations of these two widely distributed antibiotics especially at ng/L level, and how they can adversely influence fish physiology.

Considering that the gut is the main area of antibiotic absorption and that intestinal health is very important to fish, the aim of this study was to identify the chronic influence of environmental concentrations of antibiotics on the intestinal health of fish. Zebrafish were exposed to SMX and OTC at ng/L levels for a six-week period. Gut histological morphology, oxidative stress status, inflammatory reactions, and microbiota composition were investigated to explore the influence of antibiotic residues in water at ng/L concentrations on gut health of zebrafish.

2. Materials and methods

2.1. Ethical considerations

All experiments were conducted under the Guidance of the Care and Use of Laboratory Animals in China. This research was approved by the Committee on the Ethics of Animal Experiments of East China Normal University.

2.2. Experimental animals and antibiotic exposure

Six hundred zebrafish (0.1 g each) were purchased from Yuyi tropical fish farm (Shanghai, China). Before the experiment, fish were allowed to acclimatize for two weeks in 100 cm × 45 cm × 45 cm tanks with aerated dechlorinated tap water and a natural photoperiod of 12 h light and 12 h dark. The water temperature and pH were kept at 26 ± 2 °C and pH 6.8–7.5, respectively. During acclimatization, fish were hand-fed a commercial diet (Shengsuo Co., Shandong, China) twice daily.

Sulfamethoxazole (SMX, CAS:723-46-6) and oxytetracycline (OTC, CAS: 615 3-64-6) were purchased from Yuanmu Biotechnology Co., Ltd, Shanghai, China. SMX is dissolved in water with a concentration at 260 mg/L as a stock solution while OTC is dissolved in water with a concentration at 210 mg/L as a stock solution. Based on the available literature on the environmental concentrations of the two antibiotics, the concentrations of SMX and OTC used in the present study were set at 260 ng/L and 420 ng/L, respectively (Chen and Zhou, 2014; Chen et al., 2015b). After two weeks of acclimatization, 600 zebrafish were randomly divided into three triplicate groups: control, SMX-bath and OTC-bath groups. During the experiment, fish were hand-fed a basic diet at a ration of 2% of body weight, twice daily (Supplemental Table S2). The weight of the fish in each tank was recorded during the experiment, and the quantity of feed was adjusted correspondingly. All treatments were performed in clean tanks, and half of the water from each tank was replaced every two days. On each occasion when the water was changed, the water in the antibiotic-treated tanks was replaced by an equal amount of water containing the initial concentration of antibiotics in order to maintain the antibiotic concentrations within the test range throughout the experiment. Water in the control tank was equally replaced with clean and fresh dechlorinated tap water. The duration of the experiment lasted for six weeks. At the end of the experiment, fish were captured and their final weight was determined.

2.3. Determination of oxygen consumption rate

The metabolic rate was evaluated by examining the oxygen consumption rate (OCR). The OCR of zebrafish was measured at the end of the experiment using a Strathkelvin Instrument 782 Oxygen Meter (Strathkelvin Instruments Limited, North Lanarkshire, Scotland, UK). Zebrafish were fasted for 24 h to exclude interference with postprandial metabolism. Before the measurement, ten fish were placed in one chamber with 650 ml air-saturated water at 24 °C until the baseline on the instrument shows a smooth curve. To minimize the effect of low oxygen concentration on the metabolic rate and oxygen consumption, the time allowed for detecting the oxygen consumption was limited for 5 min and the oxygen