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Integrated biomarker responses in zebrafish exposed to sulfonamides

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ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form
21 July 2014
Accepted 25 July 2014
Available online 4 August 2014

Keywords:
Sulfonamide
Zebrafish
Enzyme
Glutathione S-transferase
Malondialdehyde

ABSTRACT

Dispersed pharmaceuticals such as sulfonamides pose a threat to aquatic ecosystems. We evaluated potential biomarkers of sulfonamide exposure using an extended zebrafish (*Danio rerio*) toxicity test. The tested sulfonamides induced obvious effects on spontaneous swimming activity and heartbeat rate in zebrafish. Glutathione S-transferase (GST) and malondialdehyde (MDA) were examined to reflect the biomarker response of zebrafish exposed to three sulfonamides (sulfamethoxazole, sulfadiazine (SDZ) and sulfadimidine). Both GST and MDA showed time-dependent responses to sulfonamide exposure. GST activity was significantly increased after exposure to sulfonamides for 3 days, while MDA concentration reached a maximum during the first day and then declined. These results suggest that MDA may be a more sensitive biomarker of sulfonamide toxicity than GST. These investigations demonstrated that SDZ was a typical inducer of metabolic enzymes, suggesting that it poses a potential ecotoxicological risk to aquatic ecosystems.

1. Introduction

Antibiotics are widely used in medical treatment, aquaculture and livestock husbandry. Antibiotic residue become part of the water environment through municipal and industrial, hospital, and aquaculture wastewater, and from refuse landfill (Karthikeyan and Meyer, 2006; Petrović et al., 2005). Nowadays antibiotic residual in environmental water body is a hot issue in the field of environmental research. Antibiotics, currently mostly sulfonamides and quinolines, can be detected in the inflow and outflow of underground water, surface water and sewage treatment plants, as well as in drinking water, in concentrations ranging from nanograms to micrograms per liter (Michael et al., 2013). Among antibiotics, the sulfonamides are present in the highest ratio in drinking water and sewage, according to research conducted by United States Geological Survey (Kolpin et al., 2002). In the UK, surface water and effluent from sewage treatment plants have shown levels of sulfamethoxazole (SMZ) of 0.05 μg/L (Hilton and Thomas, 2003). The sulfonamide concentrations in sludge obtained from 58 water treatment facilities in China were around 20.1–117 μg/kg (Cheng et al., 2014). The high detection rate of sulfonamides in rivers is due not only to their extensive use, but also their strong hydrophilicity, which mean that they can easily enter into environmental water via drainage and rain wash (Buchberger, 2007).

Ecotoxicology of sulfonamides can be classified as the influence of soil organisms, aquatic organism, insects as well as its transition in the environment (Yu et al., 2004). The residual sulfonamides in the environment, accumulated by biodegradation and non-biological degradation, facilitate the

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http://dx.doi.org/10.1016/j.etap.2014.07.020

evolution of resistant strain, thus affecting the growth of flora and fauna. After being affected by the sulfonamides for a long time, human beings would suffer from the side effect of urination and hematopoietic disorders (Xu et al., 2004). These antibiotics have high carcinogenic potential and severe consequences for human health (Raich-Montiu et al., 2007). Researches on sulfonamides in the US, Canada as well as the EU countries now mainly concentrate on testing the trace analysis (Arroyo-Manzanares et al., 2014). However, there has been no comprehensive research on the environmental concentrations and toxicological consequences of sulfonamides. More information on the biochemical effects of sulfonamides on aquatic organisms is urgently needed.

A crucial aspect of detoxification systems in organisms is the induction of detoxifying enzymes by exposure to toxic pollutants. Detection of these enzymes, which reflects the impact of contaminant exposure on the organisms, is thus a significant method in toxicology research. Entry of exogenous contaminants, such as sulfonamides, into organisms can cause the production of high levels of reactive oxygen species (ROS), which represents the main mechanism of oxidative damage due to exogenous contaminants. ROS can cause cell damage in two ways: through peroxidation of unsaturated fatty acids in biological membranes, and through the decomposition products of lipidhydroperoxide (Fang and Zheng, 2002). The antioxidant defense system in organisms can eliminate free radicals and protect the cells from damage by oxidative stress. Research has shown that the liver is not only an important detoxifying organ but also the major site of drug metabolism in aquatic organisms. Therefore, the liver can also be the site of drug accumulation (Wang et al., 2004). The sulfanilamide content in livers is much higher than in other tissues. The level of oxidative stress caused by the contaminants can thus be reflected through induction or suppression of enzyme activities in liver tissues in aquatic organism. As a lipid peroxide, malondialdehyde (MDA) levels can reflect the severity of free radical attack indirectly, while glutathione S-transferase (GST) activity can reflect the ability to eliminate free radicals. Zebrafish have several merits as model organisms, including a short growth period and the availability of comprehensive genetic information (Ali et al., 2011; Nassef et al., 2010). Previous experiments have also demonstrated various biochemical effects of exposure to low concentrations of sulfonamides in zebrafish (Lin et al., 2013).

In this study, we investigated the effects of environmental concentrations of sulfonamides on spontaneous swimming activity (SSA) and heartbeat rate in zebrafish, and measured the effects on GST and MDA activities in zebrafish microsomes. We applied the dose-response relationship between antibiotics and enzymes to evaluate the ecotoxicity of residual sulfonamides in the water body.

2. Materials and methods

2.1. Materials

Wild-type zebrafish (Tubingen strain, Germany) were provided by the Model Animal Research Center of Nanjing University. The breeding method refers to Westerfield's method (Westerfield, 1995). Water temperature was maintained at 28.5 ± 1 °C in test chambers. All the fish were bred under a natural dark/light cycle of 10/14 h in the zebrafish aquarium facility. The zebrafish were fed daily for 3–5 min with commercial flakes (Sera Vipan, Petco, USA) as a staple feed, supplemented with freeze-dried chironomids (Nutrafin) and Artemia nauplii (Akvarieteknik). The pH in the aquarium ranged from 7.02 to 7.25 and the degree of water hardness was between 8.02 and 8.15° dH. The dissolved oxygen level in the water was maintained above 5 mg/L. The zebrafish used in the experiments were raised in the laboratory for 1 week before drug exposure.

Sulfamethoxazole (SMZ), sulfadiazine (SDZ) and sulfadimidine (SM2) were purchased from National Institute of Control of Pharmaceutical and Biological Products in Wuhan, China. There sulfonamides met the national standard (Chinese National Pharmacopoeia Committee, 2012). Culture solution for zebrafish embryos was in line with Zebrafish Book with the co-solvent DMSO (dimethyl sulfoxide, final concentration < 0.01%) (Hallare et al., 2006). The protein and GST determination kits were purchased from Nanjing Jiancheng Bioengineering Institute.

2.2. The experimental design

The concentration of sulfonamides used in research usually varies from micrograms to milligrams per liter. Based on the detectable concentrations of sulfonamides in the environment (Santos et al., 2010), we used five concentrations: 1 μg/L, 10 μg/L, 100 μg/L, 1 mg/L and 10 mg/L. Three parallel samples and a blank control were used in experiments.

2.3. SSA measurements

The test of spontaneous swimming activity was based on experiments made by Airhart et al. (2007). Control and experimental zebrafish, about 7 days post-fertilization (dpf), were tested daily for 7 days. Tests of spontaneous movement began at 7 dpf when zebrafish had an inflated swim bladder and exhibited a beat-and-glide swimming style. Each larva, ranging from 4.0 to 4.5 mm in length, was placed in a small glass Petri dish (30 mm × 10 mm). After acclimation for 5 min, the time interval between two spontaneous swimming movements was recorded. Experiments were performed in triplicate using eight randomly selected zebrafish per day.

2.4. Heartbeat rate measurements

Heartbeat rate was assessed daily in zebrafish for 7 days. The results at 7th day, having the most significance, were used to show effect of three sulfonamides on heartbeat rate of zebrafish. Control and experimental zebrafish were transferred individually to a depression slide and placed under an Olympus SZ stereo microscope (Shinjuku-ku, Tokyo, Japan) at 40× magnification. Heartbeat rate could be recorded easily without the need for restraint because of the transparency of the zebrafish. All heartbeat rate counts were performed at room temperature. Heartbeat rate was determined by manually counting the number of beats within 6 s using a Fisherbrand digital counter (Fisher Scientific, Pittsburg, PA,