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Integrated toxic evaluation of sulfamethazine on zebrafish: Including two lifespan stages (embryo-larval and adult) and three exposure periods (exposure, post-exposure and re-exposure)

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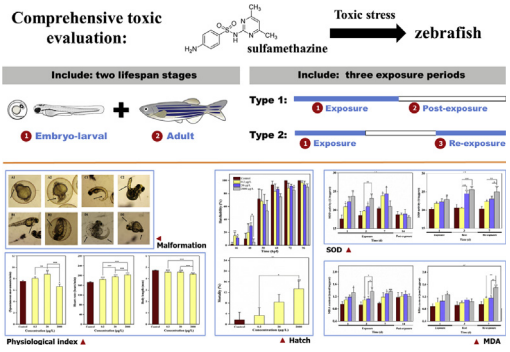
HIGHLIGHTS

- Two lifespan stages and three exposure periods were considered.
- Embryos were more sensitive to SMZ than adult zebrafish.
- SMZ induced high incidences of spinal curvature and edema in embryos.
- Embryos exposed to SMZ exhibited increased heartbeats and reduced body length.
- SOD and MDA in adult zebrafish markedly increased in re-exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Persistence of antibiotics in aquatic environment may pose a risk to the non-target aquatic organisms. This study provided an integrated evaluation to analyze the toxic stress of sulfamethazine (SMZ) on zebrafish in two lifespan stages (embryo-larval and adult) and three exposure periods (exposure, post-exposure and re-exposure). Zebrafish embryos and adult zebrafish were exposed to SMZ at 0.2, 20 and 2000 μg/L, respectively. The results showed that SMZ at any given concentration inhibited the hatching of embryos at 58–96 hpf (hours post-fertilization). Our result also indicated that two major kinds of the malformation, which was induced by the antibiotic, were edema and spinal curvature. Additionally, the antibiotic stimulated the heartbeat while reduced the body length of the embryo at 72 hpf. Superoxide dismutase (SOD) activities and malondialdehyde (MDA) contents significantly increased at 120 hpf when the embryos were exposed to the lowest concentration (0.2 μg/L) of the antibiotic. On the other hand, the antibiotic induced SOD activities and MDA contents in adult zebrafish in the exposure and re-exposure periods. The MDA contents could recover while SOD activities still increased in 2 d after the exposure. Both SOD activities and MDA contents could recover in 7 d after the exposure. Levels of SOD and MDA in the re-exposure were higher than those in the first exposure. Our results suggested that

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SMZ had toxic effects on both embryos and adult zebrafish, and provided an integrated evaluation of the toxic effects of SMZ on zebrafish at a new perspective.

1. Introduction

Antibiotics have been widely used as medicines for the treatment and prevention of diseases among human and animals, as well as growth promoters for promoting animal growth in aquaculture and livestock industries. Due to the mass production, long-term use and continuous discharge, various antibiotics are frequently detected in the environment, particularly in the aquatic environment, with concentrations ranging from ng/L to μg/L (Kümmerer, 2009; Guan et al., 2017). Sulfonamides (SAs), as a class of antibiotics, are extensively used in healthcare and veterinary applications. In 2013, consumption of SAs for humans and animals was up to 7, 890 tons, accounting for 8.51% of the total use of the 36 kinds antibiotics (Zhang et al., 2015b). SAs are not completely metabolized and removed, therefore the compounds have been frequently detected in the aquatic environment, including sulfamethoxazole (2.2–764.9 ng/L), sulfadiazine (4.9–112.5 ng/L) and sulfamethazine (19.9–389.4 ng/L) (Chen and Zhou, 2014). Sulfamethazine (SMZ), as one of the broad-spectrum SAs, is widely used in China. In 2013, 677 tons of SMZ were used in humans and animals (Zhang et al., 2015b). It was confirmed that SMZ and its N^4-acetylated metabolites in the human body were not readily hydrolyzable and biodegradable, which means that SMZ could exist in the aquatic environment in a long time (Biošić et al., 2017). SMZ was detected in the surface water of the Pearl River in South China (40–1390 ng/L) (Bu et al., 2013) and the groundwater water at Jianghan Plain in Central China (1.21–15.9 ng/L) (Yao et al., 2017). SMZ, as a commonly used veterinary antibiotic, was also present in animal wastewaters around farms in Jiangsu Province of China (2290–211000 ng/L) (Wei et al., 2011).

The occurrence of antibiotics in aquatic environments may pose a potential risk to ecosystems. Increasing evidences have demonstrated that antibiotics could cause adverse effects on non-target aquatic organisms (e.g., microalgae, cladocera and fish), including developmental deformation, fecundity function disorder and changes in levels of biomarkers (De Liguoro et al., 2009; Borecka et al., 2016; Yan et al., 2016). For instance, antibiotics could induce biochemical disturbance in fish, reflecting as the induction or inhibition of biomarkers (e.g., acetylcholinesterase (AchE), 7-ethoxyresorufin O-deethylase (EROD), superoxides dismutase (SOD), catalase (CAT) and malondialdehyde (MDA)) associated with physiological functions, drug metabolism or oxidative stress (Lin et al., 2014; Liu et al., 2014; Yan et al., 2016). When considering the potential toxicity of SAs, SMZ is of particular interest owing to its extensive use and toxic effects on organisms (De Liguoro et al., 2009). A previous study had showed that SMZ can cause thyroid tumors in rats (Poirier et al., 1999). It was also noted that SMZ can inhibit the growth and reproduction of *Daphnia magna* (De Liguoro et al., 2009). In addition, this compound can result in changes in physiological parameters (such as heart rate) and induce oxidative stress response in zebrafish even at a low exposure concentration (1 μg/L) (Lin et al., 2013, 2014).

Fish has an extensive interaction with water and sediment, and occupies different habitats and trophic levels. Due to the important role in aquatic environment, numerous studies have investigated the toxic effects of antibiotic contaminants on fish embryos (Wang et al., 2014; Zhang et al., 2015a) or adult fish (Liu et al., 2014; Zhao et al., 2016), respectively. Because of the rapid growth, continuous reproduction and embryonic transparency, zebrafish is widely used in toxicology studies as a typical vertebrate model organism (Dai et al., 2014). However, traditional toxicity tests on zebrafish usually performed several methodological limitations that need to be addressed. First of all, the fish embryo or adult fish was selected independently in a given testing, while an integral study which focusing on the above two life stages of the fish as together is still rare. In some cases, fish embryos may not be sufficient to replace adult fish in the toxicity test, owing to the barrier effect of embryonic chorion to reduce the toxicity of contaminants towards embryos (Embry et al., 2010). On the other hand, only the adult stage could not represent the entire lifespan of the fish either. Thus, with integrally assess the impacts of contaminants on fish, both embryonic and adult fish should be considered. Secondly, traditional toxicity test was processed at a given exposure period, while neglected the possible delayed effects after the exposure. The previous study also indicated that the feeding depression on the rotifer also persisted even if the exposure was ended (Yan et al., 2017). Finally, in traditional toxicological studies, the test organism was exposed to the target compound at a given time, thus frequently failed to consider the influence of the exposure background for the first time on the subsequent exposure. Actually, due to surface runoff and accidental spillage from factories or aquafarm, the occurrence of contaminants in the aquatic environment is often intermittent rather than continuous (Alonso and Camargo, 2009).

Thus, the impact assessment of the target compound should consider the above methodological flaws. The present work aimed to gain insights into the impact stress of SMZ, one of the widely used SAs in China, on zebrafish. The present study has provided an integrated assessment including two lifespan stages (embryo-larval and adult) and three periods (exposure, post-exposure and re-exposure). The exposure concentrations were ranging from 0.2 to 2000 μg/L, covering the environmental relevant concentration.

2. Materials and methods

2.1. Chemical and analytical method

SMZ (purity > 99%) was purchased from Shandong Yakang Pharmaceutical Co., Ltd. (Shandong, China). The physical-chemical properties of SMZ were presented in Table S1. The actual concentration of SMZ in configured stock solution was determined by the HPLC method. The analyses were conducted using the Agilent 1200 Series equipped with an Agilent C18 column (150 mm × 4.6 mm, 5 μm) and UV detector. The detection wavelength was 270 nm. Isocratic elution was performed using the mobile phase (a mixture of acetonitrile, methanol, water and acetic acid (2: 2: 9: 0.2, (v/v)) at a flow rate of 1.0 mL/min. The temperature of the column was maintained at 35 °C and the injection volume was 20 μL. The analytical results demonstrated that there was no significant difference between the measured and the nominal concentrations of SMZ. Therefore, the nominal concentrations could be used to represent SMZ actual concentrations in this study. The total protein, SOD and MDA assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The 20× PBS solution (pH 7.4–7.6) was obtained from Sangon Biotech Co., Ltd. (Shanghai,