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Impact on growth, oxidative stress, and apoptosis-related gene transcription of zebrafish after exposure to low concentration of arsenite

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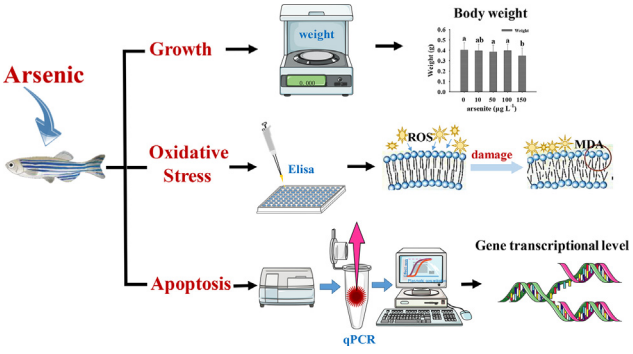
HIGHLIGHTS

- Our study demonstrated the toxic effect of low concentrations of arsenite on zebrafish.
- Exposure to low concentration arsenite can decrease the growth of zebrafish.
- Exposure to low concentration arsenite can cause oxidative stress and damage on zebrafish.
- Exposure to low concentration arsenite can disrupt the transcriptional levels of apoptosis-related genes (*nrf2*, *p53* and *c-jun*).

GRAPHICAL ABSTRACT



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ABSTRACT

Low concentrations of arsenic (As) contamination in aquatic environment is a worldwide issue, which is of great concern. To evaluate the impact of low concentrations of As on zebrafish, we measured the growth, antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT), oxidative damage (malondialdehyde, MDA) and apoptosis-related genes (*nrf2*, *p53* and *c-jun*) of adult zebrafish after exposing to different AsIII concentrations (0, 10, 50, 100 or 150 μg L^{-1}) for 28 d. Results indicated that exposure to low AsIII concentrations decreased the zebrafish weight by 14%, increased the activities of SOD and CAT by 23–41% and 31–59%, decreased the contents of MDA by 29–54%, and modulated transcription of apoptosis related genes. Our study showed that chronic exposure to AsIII concentrations <150 μg L^{-1} generated oxidative stress and damage on zebrafish, and altered apoptosis-related genes in zebrafish.

1. Introduction

As a ubiquitous metalloid, arsenic (As) is widely distributed in the environment (Patlolla et al., 2012). Various anthropogenic

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activities result in As contamination in aquatic environment (Roccaro et al., 2005). Arsenic pollution in aquatic ecosystem and its impact on aquatic organism are of environmental issues (Sharma and Sohn, 2009; Ventura-Lima et al., 2011). In aquatic environment, both arsenite (AsIII) and arsenate (AsV) are present. Although AsV is the major arsenic species in well-oxygenated water, it is easier eliminated by conventional As removal technologies (Fan et al., 2016; Wu et al., 2018). However, different from AsV, AsIII is more toxic, more soluble and more difficult to be removed from waters. Moreover, the conversion of AsIII to AsV in oxygenated water may also take a long time depending on specific conditions (Dixit and Hering, 2003; Zhang et al., 2007). So the presence of AsIII in water is a considerable threat for aquatic organisms.

Fish, as the top predators in aquatic environment, are exposed to more external contamination than other species, thus the toxic effect of AsIII on fish has attracted more attentions. Hitherto many studies reported the impacts of AsIII on fish (Ahmed et al., 2011; Lavanya et al., 2011; Guardiola et al., 2013), most focused on As concentrations far above typical levels (21–150 $\mu g\,L^{-1}$) in aquatic environment (Wu et al., 2009; Jing, 2011). However, low As concentrations in aquatic environment also adversely impacts fish, which has been also studied (Datta et al., 2009a, b; Sarkar et al., 2014, 2017). For example, Datta et al. (2009a, b) reported AsIII concentration at 100 $\mu g\,L^{-1}$ induced macrophage apoptosis and reduced the innate immune system in *Clarias batrachus* after 30-d exposure. Sarkar et al. (2014, 2017) manifested that 50 $\mu g\,L^{-1}$ AsIII affected the antioxidant system and disturbed the transcriptional levels of antioxidant related genes (*nrf2*, *Cu–Zn SOD*, *GPx* and *CAT*). However, these studies focused on a single AsIII concentration, therefore we explored the effects of AsIII at different concentrations.

Oxidative stress has been considered the mechanisms responsible for pollutant-induced detrimental effect on fish. It is known that antioxidant defense plays a crucial role in dealing with oxidative stress, with superoxide dismutases (SOD) and catalase (CAT), being used to assess the oxidative stress. When oxidative stress exceeds their capability, an increase in lipid peroxidation occurs. Malondialdehyde (MDA), an end product of tissue lipid peroxidation, has been used to indicate oxidative damage (Sun et al., 2008). Excessive oxidative stress not only induced lipid peroxidation in cell membrane, but also involved in cell apoptosis (Franco et al., 2009; Matés et al., 2012). Cell apoptosis, is a process of programmed cell death, which can protect the organism by removing potentially damaged cells (Yamashita, 2003; Kang et al., 2005). Of the process, nuclear factor erythroid 2-related factor 2 (*nrf2*, play a crucial role in maintaining cell homeostasis), *p53* (a tumor suppressor protein) and *c-jun* (an important members of proto-oncogenes) play a central role in the cellular response to xenobiotic stimulus, which can transiently and rapidly trigger apoptosis after receiving the stress via multiple pathways (Sheikh and Fornace, 2000; Jung et al., 2012; Wu et al., 2017), as they are typical indicators of apoptosis to elucidate the relationship between apoptosis and oxidative stress caused by AsIII.

Due to its fast reproductive capacity, well-defined development processes, and known sequenced genome, Zebrafish (*Danio rerio*) has been a popular model to conduct toxicology research (Hill et al., 2005; Mcgrath and Li, 2008). In this study, zebrafish was employed as the test organism to determine the effect of low AsIII concentrations (0, 10, 50, 100, or 150 $\mu g\,L^{-1}$) on: (1), the growth performance, (2) the activities of antioxidant enzymes (SOD and CAT), (3) the contents of MDA, and (4) the transcriptional levels of apoptosis related genes (*nrf2, p53* and *c-jun*). The results should shed light on the adverse impact of low AsIII concentrations on fish.

2. Materials and methods

2.1. Fish species and test conditions

Adult zebrafish (*Danio rerio*) (weight: 345 ± 55 mg; length: 3.44 ± 0.19 cm) were obtained from China Zebrafish Resource Center (Wuhan, China). Fish were acclimated in large glass tanks containing de-chlorinated tap water under laboratory conditions for 7 d prior to experiments. They were fed with fairy shrimp food three times a day during acclimation and experiment. The experiments were conducted with a water temperature of 28 ± 0.5 °C, a dissolved oxygen concentration above 6.0 mg L^{-1} attained by continuous aeration, and a 12 h light/dark photoperiod. Temperature, and dissolved oxygen were monitored and recorded daily.

2.2. Experimental design

Arsenic test solutions were prepared by dissolving arsenic trioxide ($NaAsO_2$, Sigma-Aldrich, ≥90%) in Milli-Q water to make AsIII solutions. Based on As levels in aquatic environment, the AsIII concentrations were 0, 10, 50, 100, or 150 $\mu g\,L^{-1}$. Twenty individuals were placed per glass tank (25 cm × 25 cm × 25 cm), and each one filled with 10 L solution. Each concentration was run in duplicate, and the test solutions in each glass tank were replaced with fresh test solutions every 2 d. The experiment was conducted for 28 d. During the whole experimental process, all animal care and experimental procedures were in accordance with the principles and guidelines for the care and use of Laboratory Animals (Zhejiang Academy of Medical Sciences).

At the end of experiment, 18–20 fishes in each tank were selected, anaesthetized for 5 min in MS222, and then weighed. After weighting, all fish were dissected to remove the livers and stored in a −80 °C refrigerator.

2.3. Oxidative stress analysis

To determine the oxidative stress and damage of zebrafish, CAT, SOD, MDA and protein were extracted from zebrafish livers according to (Sun et al., 2013). Briefly, twelve zebrafish from each treatment were over-anaesthetized with MS-222 and sacrificed immediately. The liver tissues were removed and rinsed with ice-cold physiological saline (0.68%, w/v). The liver tissue was grinded after addition of physiological saline solution (liver/total was 10%), and the homogenate was centrifuged at 4, 000 g for 10 min at 4 °C to remove cellular debris and cartilage fragments. Samples were kept on ice during the entire procedure. Subsequently, the supernatant was divided into different aliquots and stored at −80 °C refrigerator before biochemical analysis. The activities of CAT and SOD, and the contents of MDA and total protein in supernatant were measured using commercial chemical assay kits (Nanjing Jiancheng Bioengineering Institute, China).

2.4. RNA isolation and cDNA synthesis

RNA isolation and cDNA synthesis were determined according to Sun et al. (2017). The total RNA was isolated from zebrafish livers using a homogenizer with TRIzol reagent according to the manufacturer's protocols (TaKaRa, Japan). Briefly, the frozen tissue was homogenized in 1 mL of TRIzol using a homogenizer on ice. Homogenates were transferred into sterile centrifuge tubes with 0.2 mL of chloroform. After vortexing for 15 s, mixtures were kept at room temperature for 5 min and centrifuged at 12,000 g for 15 min at 4 °C. Subsequently, Aqueous supernatant was carefully removed into a new tube and 0.5 mL of isopropanol was added and mixed. After mixing, the mixtures were kept at room temperature