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Life-cycle exposure to cadmium induced compensatory responses towards oxidative stress in the liver of female zebrafish

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HIGHLIGHTS

- A life cycle toxic assessment of cadmium was examined in fish.
- Adaptive responses towards oxidative stress were found in zebrafish exposed to Cd.
- Compensatory responses towards metal homeostasis and antioxidant system were demonstrated.
- Several negative feedback loops were found to maintain ROS homeostasis.

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ABSTRACT

The current study examined effects of waterborne cadmium (Cd) at environmental relevant concentrations (0, 2.5 and 5 μg/L) on growth, survival, histology, ultrastructure, metal homeostasis, and antioxidant responses in female zebrafish from embryos to sexually maturity for 15 weeks. Growth and survival rate were not significantly affected by Cd exposure. There were no significant changes in ultrastructure of cellular organelles, reactive oxygen species (ROS) levels, lipid peroxidation (LPO) in liver. However, Cd exposure increased Cd and lipid accumulation, reduced contents of zinc, copper and reduced glutathione (GSH), and down-regulated activity of copper/zinc-superoxide dismutase (Cu/Zn-SOD) in liver of zebrafish. Contrarily, the mRNA and activity levels of catalase (CAT), the mRNA levels of Cu/Zn-SOD, and the mRNA and protein levels of metallothioneins (MTs) were up-regulated. The transcriptional regulation of Cu and Zn transporters might be a vital mechanism by which fish slow the Zn and Cu uptake. Taken together, our data demonstrated that long-term and low-dose Cd induced adaptive responses with interlinked compensatory mechanism, which may protect fish against oxidative stress.

1. Introduction

Cadmium (Cd) pollution has become a key issue in the field of aquatic toxicology as a result of increasing environmental concentrations by human activities. Cd concentrations fluctuated from 1.120 to 4.474 μg/L in freshwater system of China (Li and Liu, 2009), and has been shown to cause lethal effect in adult zebrafish (5 mg/L for 96-h LC50, Zheng et al., 2016a) and roho labeo fry (1 mg/L for 96-h LC50, Vadlamani et al., 2018). As a result, fish are living in an environment polluted by Cd. It has been suggested that Cd causes a wide variety of impacts to fish, including histopathological alterations (Thophon et al., 2003; Guo et al., 2018), behavioral deficits (Qi et al., 2017), DNA damage and methylation (Pavlaki et al., 2016; Pierron et al., 2014), deregulation of metal homeostasis (Komjarova and Bury, 2014), oxidative damage and immunotoxicity (Tang et al., 2016; Qu et al., 2016; Ling et al., 2017), hepatocyte apoptosis (Costa et al., 2010), and reproductive damage (Wang et al., 2014).

Investigating toxicity of Cd at an environmentally realistic dose may be challenged because traditional toxicological results such as growth, survival and biochemical parameters may be not sensitive to reflect the negative effect. The impact of metals could be significant by extending exposure time, even persisting exposure for a whole life cycle (Guo et al., 2017), or for generations (Xu et al., 2016), in order to find delayed toxicity (Beyger et al., 2012). However, most previous studies emphasize metal toxicity during sensitive developmental periods mainly focusing on embryonic and

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larval stages (Achiorno et al., 2010; Wang et al., 2018). Since fish are likely to be continuously exposed to metals from embryos to sexual maturity in natural water, the assessment of life cycle should be not neglected.

Cd is known to cause toxicity to fish through the induction of oxidative stress. Reactive oxygen species (ROS) are oxidative stress-induced mediators, and can serve as second messengers and function as the stabilization of many physiological processes including host anti-oxidative defenses (Leto et al., 2009). However, the overproduction of ROS can result in oxidative damage such as peroxidation of lipids and proteins, frequently cause mortality and pathological injury (Tang et al., 2013). Different defense systems help organisms cope with the threat. For example, superoxide dismutase (SOD) converts O^{2-} to O^2 and H_2O_2 that is converted to H_2O by catalase (CAT). Metallothioneins (MTs) play important roles in transport and detoxification of metals by binding, removal of their redox potential, and scavenging ROS (Hart et al., 2001). Zn and Cu transporters regulate metal uptake into cytoplasm and distribution in organelles, including copper transporter (CTR1), Cu-transporting ATPases (ATP7A and ATP7B), zinc transporter (ZnT1 and ZnT5), and Zir-, Irt-like proteins (ZIP7, ZIP8 and ZIP10). Furthermore, some studies have illustrated that oxidative stress that is arisen from Cd is controlled by nuclear transcription factor pathways including NF-E2-related factor 2 (Nrf2)/antioxidant responsive element (ARE) and metal-responsive element-binding transcription factor-1 (MTF-1)/metal-responsive element (MRE) in fish (Chen et al., 2007; Cheuk et al., 2008). Recently, Cd-induced antioxidant defense mechanisms have well been elucidated in fish in our lab, perhaps involved in Nrf2/ARE and MTF-1/MRE pathways (Yuan et al., 2017; Guo et al., 2017; Zheng et al., 2016a,b,c,d). There are also some reports about the effect of Cd on metal transport systems including MTs in fish (Cheuk et al., 2008; Chouchene et al., 2011; Komjarova and Bury, 2014; Tang et al., 2016). To date, however, the molecular mechanism of antioxidant response and metal homeostasis and associated signal pathways are largely unknown in fish when considering a life cycle exposure to Cd.

In the present study, zebrafish embryos at 24 h post-fertilization were subjected to environmental relevant levels of Cd (0, 2.5, and 5 μg/L) until sexual maturity. Growth and survival rate were evaluated after 15-week exposure. Because of an essential role in detoxification and accumulation of metals, liver was selected to investigate the toxic impacts of Cd on hepatic histology, ultrastructure, accumulation of metals (Cd, Zn and Cu), lipid peroxidation (LPO), ROS and reduced glutathione (GSH) in zebrafish. In particular, we examined changes in the protein and mRNA levels of MTs, the activity and mRNA levels of Cu/Zn-SOD and CAT, and the transcriptional levels of MTF-1, Nrf2, ZnT1, ZnT5, ATP7A, ATP7B, CTR1, ZIP7, ZIP8, and ZIP10.

2. Materials and methods

2.1. Cd exposure and sampling

Embryos were collected directly after fertilization and immediately transferred to plastic culture dishes in 0.5 × E2 medium with 0.5 mg/L Methylene Blue (7.5 mM NaCl, 0.25 mM KCl, 0.5 mM $MgSO_4$, 75 μM KH_2PO_4, 25 μM Na_2HPO_4, 0.5 mM $CaCl_2$, 0.35 mM $NaHCO_3$; pH 7.2). At 1dpf, fertilized and good quality eggs were selected under a stereomicroscope, and transferred to 12 culture dishes in pools of 50 embryos/well in the medium with 0, 2.5 and 5 μg/L Cd until 5 dpf. The environmental relevance concentrations of Cd were selected according to the studies from Faucher et al. (2008) and Sandhu et al. (2014). Larva fish were randomly transferred to 12 tanks supplied with charcoal-filtered and aerated tap water with different concentrations of Cd. Fish were fed commercial flake food (Tetra, Germany) and freshly hatched *Artemia nauplii* three times daily at a rate of 5.0% of body weight (Su et al., 2016). The experiment lasted for 15 weeks.

The water temperature was maintained at 27.5 ± 0.5 °C under a photoperiodic regime of 12-h light and 12-h dark with dissolved oxygen 7.37 ± 0.32 mg/L, pH 7.52 ± 0.34, and hardness 97.7 ± 0.7 as CaCO3. Cd concentrations in water for the control, 2.5, and 5 μg/L groups were 0, 2.47 ± 0.11, and 4.78 ± 0.19 μg/L, respectively. Water was renewed 100% ever day. Metal concentrations in water and liver tissue were assessed using flame atomic absorption spectroscopy (FAAS), according our previous study (Zheng et al., 2011) (detailed descriptions referred to Supporting Information).

Fish were starved for 24 h before the termination of the experiment. Fish were subjected to euthanasia with 0.02% tricaine methanesulfonate. Body weight and survival rate were investigated. Female liver tissues were collected on ice and stored in liquid nitrogen immediately. Liver tissue from one fish was used for RNA extraction (N = 4). Livers from 10 fish were pooled for biochemical analysis (N = 4). The other liver samples were prepared for observations of histology and ultrastructure.

2.2. Histology and ultrastructure

The observation of histology of liver samples referenced to the study from Zheng et al. (2016a). Generally, samples from 4 fish were sliced into 2 mm thick slabs and fixed in 10% neutral buffered formalin for 24 h. After fixation, the slices were dehydrated in ethanol and embedded in paraffin. Finally, 5 μm sections were stained with hematoxylin/eosin (H &E) and analyzed under an optical microscope. The observation of the hepatic ultrastructures followed the protocols described previously (Yuan et al., 2017). In brief, samples from 4 fish were prefixed and post-fixed by 2.5% glutaraldehyde and 1% aqueous osmium tetroxide for 1 h at 4 °C respectively, dehydrated through a graded ethanol series, and embedded in epon-araldite. Ultrathin sections (80 nm in thickness) were collected and then double stained with uranyl acetate and lead citrate, and examined through a HITACHI H-600 electron microscope.

2.3. Biochemical analysis

The supernatants for biochemical analysis were prepared according to our recent study (Guo et al., 2017). The measurements of MTs protein and LPO levels were conducted by a spectrophotometric assay, as suggested by Viarengo et al. (1997) and Viarengo et al. (1997) respectively. The DCFH-DA method was applied to determine ROS levels (LeBel et al., 1992). Cu/Zn-SOD and CAT activity levels were examined, according to the descriptions of Beauchamp and Fridovich (1971) and Beutler (1982) respectively. GSH assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was used to detect GSH content in accordance with the manufacturer's protocol. Soluble protein content was determined using the coomassie brilliant blue method (Bradford, 1976). Detailed procedures referenced to Supporting Information.

2.4. Real-time PCR

We used commercial kits to perform RNA extraction, cDNA synthesis, and quantitative real-time PCR, including RNAiso Plus Kit, PrimeScript® RT reagent Kit, and SYBR® Premix Ex Taq™ Kit. Melting curve and were analyzed to identify a single product with specific primers (Table 1). Amplification efficiencies of all genes remained approximately 100%. Abundances of transcripts of genes were calculated according to the $2^{-\Delta\Delta Ct}$ method, normalizing to the