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Sex-dependent effects of subacute mercuric chloride exposure on histology, antioxidant status and immune-related gene expression in the liver of adult zebrafish (*Danio rerio*)

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HIGHLIGHTS

- $HgCl_2$ exposure causes histological damage and oxidative stress in the liver of zebrafish.
- $HgCl_2$ exposure alters immune-related gene expression in the liver of zebrafish.
- Sex-dependent effects of $HgCl_2$ are observed.
- Male zebrafish are more vulnerable to $HgCl_2$ exposure than females.

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ABSTRACT

Mercury (Hg) is a global pollutant that causes negative health effects. In order to assess Hg-induced hepatotoxicity in fish and examine whether gender differences existed in response to Hg exposure, adult zebrafish were exposed to 0, 15 and 30 μg L^{-1} Hg^{2+} for 30 days, and histology, antioxidant status and the transcription levels of several immune-related genes were examined in the liver. Hg^{2+} exposure caused a dose-dependent increase in histopathological lesions of the liver, including vacuolization, parenchyma disorganization and pyknotic nucleus, and these lesions were more severe in males than in females. In females, Hg^{2+} exposure decreased CAT activity and its mRNA levels, while increased GSH content and the expressions of *sod1*, *gpx1a*, *gstr* and *keap1*. In males, the decrease in *cat1* expression and the increase in GST activity, GSH and MDA contents as well as *gpx1a*, *gstr*, *nrf2* and *keap1* mRNA levels were observed in Hg^{2+}-exposed groups, but the activities of CAT, SOD and GPX were only stimulated in the 15 μg L^{-1} Hg^{2+} group. Moreover, both in females and males, Hg^{2+} exposure down-regulated *il-8* expression while up-regulated *il-10* and *lyz* mRNAs. However, the down-regulation of *il-1β* and *tnfα* was detected only in males under Hg^{2+} treatments. Thus, our results indicated that $HgCl_2$ exposure induced histopathological damage, oxidative stress and immunotoxicity in the liver of zebrafish. Different response patterns of histology, antioxidant status and immune defenses to Hg^{2+} between females and males suggested sex-dependent effects of Hg, and males showed more vulnerable to Hg^{2+} exposure than females.

1. Introduction

Mercury (Hg) is ubiquitously distributed in the global environment and has been recognized as a precedence-controlled pollutant for its persistence, bioaccumulation and toxicity (Chen et al., 2010; Monteiro et al., 2013). In the aquatic ecosystem, Hg exists as element, inorganic compounds, and organic compounds, and inorganic compounds (such as Hg^{2+}) are the major form of Hg released by industries (Oliveira Ribeiro et al., 1996; Black et al., 2007). Generally, total Hg concentrations in worldwide background rivers and lakes ranged from 0.1 to 3.5 ng L^{-1} (Nriagu, 1990). However, due to frequent human activities such as coal combustion, waste incineration and metal mining, Hg has been widely diffused in aquatic environments, resulting in increased Hg concentrations in the water. For example, the concentrations of total Hg in stream water collected from Tongguan gold mining area

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(Shaanxi, China) varied from 0.4 to 880 μg L^{-1} with a geometric mean of 6.9 μg L^{-1} (Feng et al., 2006). In the Valdeazogues River (Almadén, Spain, a historic Hg mining district), the highest total Hg levels detected was 20.3 μg L^{-1} (Berzas Nevado et al., 2003). Elevated waterborne Hg concentrations can lead to an increase in Hg accumulation in fish body, thereby threatening its growth, development, reproduction and metabolism (Liao et al., 2006; Mieiro et al., 2011; Zhang et al., 2016a,b).

As in other vertebrates, the fish liver is not only a key organ for systemic regulation, but also a primary tissue for detoxification processes, acting in the elimination of pathogen, toxic substances and metabolic wastes, and maintaining normal physiological functions of other organs (Desantis et al., 2005; de Andrade et al., 2015). Existing studies have demonstrated that the liver is the major site for Hg accumulation and elimination (Mieiro et al., 2011). When excessive Hg accumulated in the liver, it can damage the hepatic structure and function (Giari et al., 2008). At present, although the liver has been considered as a critical target of Hg in fish, the mechanisms underlying Hg-induced hepatotoxicity remain largely unknown. Moreover, previous studies have often examined the response of a single sex (male or female) to Hg exposure, while the comparative study on females and males for evaluation of the gender-specific regulatory effect by Hg is still scarce.

Antioxidant defense and immune systems are known to protect the body from toxins. The antioxidant defense system includes antioxidant enzymes (such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST)) and non-enzymatic antioxidants (such as reduced glutathione (GSH) and vitamins E), playing a crucial role in combating reactive oxygen species (ROS) and protecting cell against oxidative stress (Zhang et al., 2016a). To date, several studies have demonstrated that the activities of these antioxidant enzymes can be activated or suppressed by Hg exposure and therefore, the antioxidant defense system may serve as a biomarker of Hg pollution (Guilherme et al., 2008; Zhang et al., 2016a). Unfortunately, the transcriptional response of the antioxidant defense system to subacute Hg exposure is poorly understood. On the other hand, the immune system is to identify and eliminate foreign materials, which can be activated to deal with tissue damage (Moalem et al., 1999). Studies have shown that Hg has the potential to induce immunotoxicity in fish, including increased the number of white blood cells (Maheswaran et al., 2008), decreased phagocytic activity of hemocytes (Fournier et al., 2001), and caused lympocytosis, neutrophillia, monocytosis, eosinophilia and thrombocytopenia (Kumar et al., 2004). In addition, our recent study conducted in zebrafish embryos-larvae indicated that the immune system could be mobilized to compensate for Hg-induced decreased immunity by up-regulating the expression of genes encoding lysozyme (LYZ) and cytokines (Zhang et al., 2016b). To the best of our knowledge, however, no data are available concerning the effects of Hg^{2+} exposure on the transcription levels of immune-related genes in the liver of fish.

Zebrafish has emerged as a model organism for toxicology studies (Zhou et al., 2010). The aim of this study is to investigate the effects of waterborne inorganic Hg exposure on histology, antioxidant defenses and immune responses in the liver of fish, and also observe whether there is a gender-specific regulatory effect following Hg exposure. To that end, zebrafish adults were exposed to 0 (control), 15, and 30 μg L^{-1} Hg^{2+} for 30 days, and the effects on histological structure, antioxidant status, as well as the mRNA expression of some representative immune-related genes, including interleukin-1β (*il-1β*), interleukin-8 (*il-8*), tumor necrosis factor α (*tnfα*), interleukin-10 (*il-10*) and lysozyme (*lyz*) in the liver were examined. Our results will help to clarify underlying molecular mechanisms for Hg-induced hepatotoxicity in fish.

2. Materials and methods

2.1. Chemicals

Mercury chloride ($HgCl_2$, purity ≥ 99.5%), used as a test substance, was purchased from Shanghai Sinopharm Group Corporation (Shanghai, China). Kits for the biochemical test and mRNA expression analysis were acquired from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and TaKaRa Biotechnology Co., Ltd (Dalian, China), respectively. MS-222 (3-aminobenzoic acid-ethyl ester methanesulfonate) was obtained from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Ethics statement

This study was carried out in strict accordance with the recommendation of the Guide for the Care and Use of Laboratory Animals and was approved by the Committee of Laboratory Animal Experimentation of Chongqing Normal University. All efforts were made to minimize suffering in animals.

2.3. Zebrafish maintenance and $HgCl_2$ exposure protocols

Zebrafish maintenance and $HgCl_2$ exposure protocols have been described in details in our recent publication (Zhang et al., 2016a). Briefly, adult wild type zebrafish (AB strain, 4 months old) were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences and acclimated in a flow-through system with filtered tap water for 2 weeks. Afterwards, males (0.51 ± 0.04 g) and females (0.72 ± 0.05 g) were separately exposed to 0 (control), 15 and 30 μg L^{-1} of Hg^{2+} (added as $HgCl_2$) for 30 days. For each Hg^{2+} concentrations, 3 replicated tanks were used and each tank contained 50 fish and 40 L of dechlorinated water. The exposure concentrations were designed with consideration of the environmental relevance (Berzas Nevado et al., 2003; Feng et al., 2006) and other reference reports (Senger et al., 2010; Cruz et al., 2013). During the exposure period, half of the water in each tank was replaced daily with fresh dechlorinated water containing corresponding Hg concentration. The fish were fed three times a day with freshly hatched *Artemia nauplii* and commercial flake diet at a rate of 3% of body weight. Exposure process was carried out at ambient temperature (28 °C) with a 14 h light/10 h dark cycle. Meanwhile, Hg concentrations in the test tanks were monitored once every week by using cold vapor atomic fluorescence spectrometry, and the measured values for three treatments were 0.3 ± 0.2, 13.2 ± 0.6 and 27.8 ± 1.5 μg L^{-1} Hg (mean ± SD, $n = 4$), respectively. Besides, Water quality parameters were measured weekly and each indicators were as follows: temperature, 27.6–28.8 °C; pH, 7.6–7.8; dissolved oxygen, 6.7–7.4 mg L^{-1}; hardness, 132.6–140.2 mg L^{-1} as $CaCO_3$; alkalinity: 68.3–72.5 mg L^{-1} as $CaCO_3$.

2.4. Sampling

After 30 days of exposure, fish were euthanized with MS-222 (100 mg L^{-1}), and livers were excised and divided into three aliquots. The first aliquot was immediately frozen in liquid nitrogen and stored at −80 °C for determination of enzyme activities and gene expressions. The second aliquot was fixed in Bouin's fluid for histological observation, and the third aliquot was stored at −80 °C for total Hg quantification.