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Effect of waterborne zinc exposure on metal accumulation, enzymatic activities and histology of *Synechogobius hasta*

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ABSTRACT

The present study was conducted to determine the metal accumulation, antioxidant enzymatic response, hepatic intermediary metabolism and histological changes in *Synechogobius hasta* exposed to 0.35 (control), 9.7 and 19.2 mg/L Zn, respectively, on the 0, 4th, 8th and 12th day. Waterborne Zn exposure significantly reduced hepatosomatic index, hepatic lipid contents and fatty liver occurrence rate, increased Zn, Fe and Mn contents and reduced the contents of Cu and Ca in liver, and increased muscle Zn content. Waterborne Zn exposure also significantly influenced enzymatic activities involved in antioxidant responses (superoxide dismutase, catalase, glutathione-S-transferase, malondialdehyde) in liver and spleen, and changed hepatic intermediary enzymatic activities (succinate dehydrogenase, malic dehydrogenase, lactate dehydrogenase, lipoprotein lipase, hepatic lipase), impaired the histological structure of the gill and spleen, and reduced vacuolated hepatocytes. Thus, our study demonstrated for the first time that waterborne Zn exposure could reduce fatty liver syndrome in *S. hasta*.

1. Introduction

During the last decades, a dramatic increase in environmental poisoning by metals occurs as a consequence of industrial, agricultural and anthropogenic activities, thus aquatic organisms are exposed to a significant amount of these pollutants (Heath, 1987). Among the metals, essential metals are an interesting subject of research because they are required for the function of various enzymes and other cellular protein, but become toxic at increased waterborne levels. Zn is an essential micronutrient required for the various functions in biological development such as cell structure, enzyme activities, protein and carbohydrate metabolism of fish (Watanabe et al., 1997). However, excessive zinc (Zn) in the aquatic environments can be toxic, which has a devasting effect on fish species, affecting the gills morphological alterations and liver damage (van Dyk et al., 2007; Giardina et al., 2009). Compared to other metals such as Cu (Liu et al., 2010), the nutritional and toxic effects of Zn in fishes have been less investigated (Clearwater et al., 2002), and its toxicity mechanisms are still unclear or in dispute. Therefore, there is a substantial need to understand the effect of waterborne Zn exposure on fish in order to provide critical information for the environmental risk assessment of Zn in aquatic environments.

Synechogobius hasta (Perciformes, Gobiidae) are widely distributed over the southern coast of Liaoning Province, China, where there are fair amounts of metals such as Cd, Cr, Cu and Zn. However, in the recent years, commercial farming of this fish has become an increasing interest in northern China because of its euryhalinity, rapid growth, good taste and high market value. At present, in our laboratory, Luo et al. (2008, 2009) determined the characteristics of nutrient physiology for the fish species, and Liu et al. (2010, 2011) evaluated that effects of waterborne Cu and Cd exposure on *S. hasta*, and pointed out that Cu and Cd exposure could induce the fatty liver occurrence for the fish species. As a continuation, the objective of this study was to investigate the enzymatic responses and histological alterations in several tissues of *S. hasta* exposed to Zn at different concentrations while several metal element contents in fish body were also determined.

2. Materials and methods

The research was conducted in Panjin Guanghe Fisheries Co., Ltd. Two experiments with waterborne Zn exposure were conducted. The first experiment was involved in the acute Zn toxicity for *S. hasta*, determining the 96 h median lethal concentration (LC_{50}). The second experiment evaluated sublethal effects of waterborne Zn exposure on metal element composition, hepatic enzymatic activities histology of *S. hasta*. For the two experiments, Zn was added as Zn sulfate in distilled water for stock concentration. Individual test solutions in the first and second experiment were obtained by adding the appropriate volume of the primary stock to the dilution water. The Zn concentration in the test tanks was measured using the flame of an atomic absorption spectrophotometer. Two blanks were digested simultaneously during each run.

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We assure that the experiments performed on animals, animal care and all protocols followed the ethical guidelines of Huazhong Agricultural University for the care and use of laboratory animals.

2.1. Experiment 1: acute toxic experiment

S. hasta were obtained from a local pond in Panjin Guanghe Fisheries Co. Ltd, Liaoning Province, China. They were maintained in 300 l circular fiberglass tanks in static aquarium system for the 2-wk acclimatization. When beginning the experiment, uniform-sized fish (initial body weight: 35.83 ± 3.79 g, means $\pm$ SEM) were exposed to six concentration levels (0.35 control, 54.2, 71.1, 88.4, 97.4 and 113.2 mg Zn/l, respectively) to determine the 96-h LC_{50}, three replicates per level, with 28 fish in each replicate. During the 96-h experiment, water was aerated continuously. Each test solution in each tank was renewed 100% every day. Water temperature was maintained at 25.9 ± 2.7 °C and normal photoperiod at 14L:10D. The hardness of the water was 169.68 mg/l as $CaCO_3$, and dissolved oxygen, pH and salinity (‰) were 6.13 ± 0.26 mg/l, 7.79 ± 0.47 and 16.63 ± 0.54, respectively.

During the exposures, mortality was monitored each hour for the initial 12 hours and then at each 12-h interval to the end of the test. The criteria for death were no gill movement and no reaction to gentle prodding. Dead fish were removed and discarded once observed. The results of the LC_{50} at 96 h were computed using the probit analysis computer program (Finney, 1971) and was 72.0 mg/l.

2.2. Experiment 2: sublethal experiments

In the second experiment, *S. hasta* were exposed to three Zn concentrations of 0.35 (control), 9.7 and 19.2 mg Zn/l (corresponding to 0.5%, 13.5% and 26.7% of 96-h LC_{50}), respectively. Prior to the experiment, the fish from a local pond were kept in 300-l circular fiberglass tanks for 3-wk acclimatization. At the beginning of the trial, uniform-sized fish (initial body weight: 36.67 ± 2.34 g, means $\pm$ SEM) were transferred into 9 fiberglass tanks with 28 fish each, three replicate tank for each treatment. The experiment conditions (including water temperature, salinity, photoperiod, pH and water hardness) were the same as the above acute toxic experiment. Fish were fed trash fish twice daily at the rate of 8–10% of the average body weight (Liu et al., 2010). Water was renewed 100% every 12 h using sand-filtered sea water and Zn concentrations were monitored during the experimental period by the methods of flame atomic spectrometry. Dead juveniles and uneaten feed were removed every day in the morning (9:00 h) and afternoon (16:30 h) when the water was renewed. The experiment continued for 12 days.

2.3. Sampling and samples analysis

During the 12-day sublethal experiment, sampling occurred on the 0, 4th, 8th and 12th day. After each sampling day, all fishes were counted to determine survival. Then three fish per tank were randomly selected, weighed, measured for body lengths and dissected in ice to obtain gill, muscle, liver and spleen samples. For morphometrical parameters, whole body, liver and viscera were weighed to calculate condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI). The spleen, the left lobe of liver (sliced into 3-mm-thick slabs) and the second gill arch of the right side of each fish were collected for histological analysis. For enzymatic activities and MDA content, liver and spleen samples were removed immediately in ice using sterile forceps, and quickly stored at -80 °C (not longer than 2 weeks), until subsequent assay. For metal element analysis and hepatic lipid measurements, remaining samples were sub-dissected into livers and muscles. The metal element contents in the tissues were measured using the flame atomic absorption spectrophotometer. These analyses were conducted in duplicate. Hepatic lipid content was determined by the ether extraction (Luo et al., 2008).

For histological observation, samples of liver, gills and spleen were fixed for 24 h in 10% neutral buffered formalin. After dehydrated in graded ethanol concentrations and embedded in paraffin wax, sagittal sections (5 μm thick) were stained with hematoxylin/eosin (H & E), and then prepared for light microscopy (Liu et al., 2010, 2011).

For enzymatic activities analysis and MDA content determinations, samples were homogenized in physiological saline at 0 °C, to make 10% (W/V) homogenate and then the homogenate was centrifuged at 9500*g* for 15 min at 4 °C. The supernatant was kept under 4 °C until being measured for biochemical analysis. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by an indirect method involving the inhibition of cytochrome *c* reduction and spectrophotometric reading at 550 nm (McCord and Fridovich, 1969). Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically by measuring the disappearance rate of hydrogen peroxide (H_2O_2) at 240 nm according to the method of Aebi (1984). The activity of glutathione-S-transferase (GST, EC. 2.5.1.18) was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate at 340 nm, according to Habig et al. (1974). Lipoprotein lipase (LPL, EC 3.1.1.34) activity was measured using labeled triolein-^{3}H as a substrate, according to the modified methods by Ballart et al. (2003), succinate dehydrogenase (SDH, EC 1.3.99.1) activity as described by Slater and Borner (1952), lactate dehydrogenase (LDH, EC 1.1.1.27) according to Jones and Sidell (1982), malic dehydrogenase (MDH, EC 1.1.1.40) according to Ochoa (1955), hepatic lipase (HL, EC3.1.1.3) following Ehnholm et al. (1975) and modified by Burgaya et al. (1989). Soluble protein content of liver and spleen homogenates were determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. The assays were run in triplicate. All enzyme activities were expressed as U (units) per mg of soluble protein.

Lipid peroxidation was also determined by the thiobarbituric reactive species (TBARS) assay, which measured the production of malondialdehyde (MDA) that reacted with thiobarbituric acid, according to the method described by Livingstone et al. (1990). Absorbance was measured at 532 nm and MDA level was expressed as nanomoles of MDA per mg of soluble protein. All assays were run in triplicate.

2.4. Statistical analysis

Results are presented as mean $\pm$ standard errors. Prior to the statistical analysis, all data were tested for normality of distribution using the Kolmogornov–Smirnov test. Differences between control and individual Zn-treated group (9.7 and 19.2 mg Zn/l, respectively) were analyzed by Student's *T*-test for independent samples within the same day. Analysis was performed using the SPSS 10.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA), and the minimum significant level was set at 0.05.

3. Results

The gill from the control showed normal structure with periodic distributions of pillar cells, chloride cells and occasional hemocytes within the gill lamellae (Fig. 1A). However, histopathology observed in the gills from fish exposed to 19.2 mg Zn/l for 12 days included accentuated lifting of the lamellar epithelium, epithelium vascular congestion or lamellar aneurisms, evident edema in the filamentar epithelium, hypertrophic lamellae tips, hyperplasia of primary lamellar epithelium and hypertropy of secondary lamellar epithelium (Fig. 1B). The livers from control group showed congestion of the central vein and converging blood sinusoids by red blood cells, and an increase of cytoplasmatic vacuolation (Fig. 1C). However, fish exposed to 19.2 mg Zn/l showed normal central vein and converging blood sinusoids, and significantly decreased vacuoles (Fig. 1D). According to the histological analysis, during the 12-day exposure, only 40.91% of the livers from 19.2 mg Zn/l-exposed fish showed fatty liver syndrome with hepatocytes occupied by large lipid droplets. However, 74.51% of the liver from control fish had vacuolated hepatocytes. The spleens from Zn-exposed fish showed increased lymphoid cells and macrophages compared to the control. In control fish, lymphocytes maintained their normal morphology (Fig. 1E, F).

VSI and CF showed no significant differences among the treatments ($P > 0.05$) (Fig. 2A, B). Compared to control, during the 12-d exposure, HSI and hepatic lipid contents for the two tested groups significantly decreased ($P < 0.05$) (Fig. 2C). Survival significantly declined with increasing waterborne Zn levels ($P < 0.05$) (Fig. 2D). Waterborne Zn exposure also significantly reduced hepatic crude lipid content (Fig. 2E) and the fatty liver occurrence rate of *S. hasta* ($P < 0.05$) (Fig. 2F). Waterborne Zn exposure significantly increased Zn (Fig. 3A), Fe (Fig. 3E) and Mn (Fig. 3C) contents, and reduced the contents of Cu (Fig. 3B) and Ca (Fig. 3D) in liver ($P < 0.05$). In muscle, Zn content also increased with waterborne Zn concentrations (Fig. 4A), whereas Cu (Fig. 4B), Mn (Fig. 4C), Ca (Fig. 4D) and Fe (Fig. 4E) contents were variable, related to waterborne Zn concentration and experimental duration.

Effects of Zn exposure on enzymatic activities involved in antioxidant responses in liver and spleen are shown in Figs. 5 and 6, respectively. SOD activity within the 4-day exposure showed no significant differences between the tested groups and the control (Fig. 5A). However, within the 8-day exposure, SOD activity for the group of 9.7 mg Zn/l was significantly higher than that in the control ($P < 0.05$). Until the 12-day exposure, SOD activity of 19.2 mg/l was significantly higher than the control ($P < 0.05$). Waterborne Zn