Aquatic Toxicolog 120–121 (2012) 119–125

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Responses of acid/alkaline phosphatase, l so me, and catalase activities and lipid peroxidation to mercur exposure during the embr onic development of goldfish *Carassius auratus*

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ARTICLE INFO

Article history:
Received 17 March 2012
Received in revised form 9 Ma 2012
Accepted 12 Ma 2012

Keywords:
Phosphatase
L so me
Catalase
Lipid peroxidation
Mercur
Fish embr o

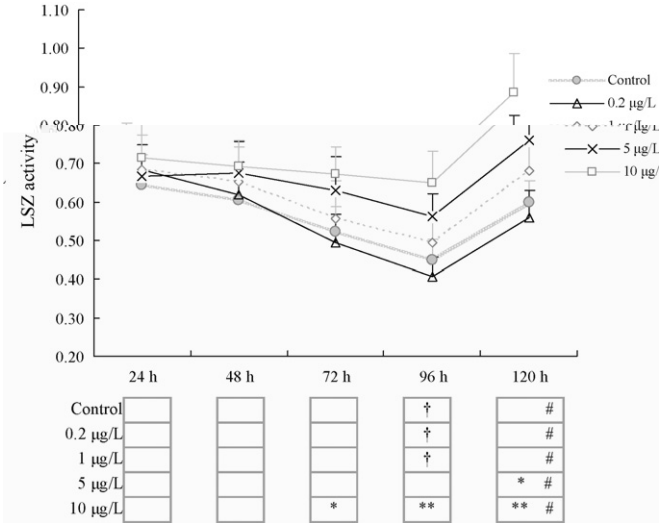
ABSTRACT

This stud assessed the impact of mercur exposure on goldfish (*Carassius auratus*) embr os based on the d namic characteristics of chemical parameters. Da old embr os were exposed to different Hg^{2+} concen trations (0, 0.2, 1, 5, and 10 μg/L). Subsequentl , the embr os were sampled ever 24 h during embr onic development to measure acid phosphatase (ACP), alkaline phosphatase (AKP), l so me (LSZ), and cata lase (CAT) activities, as well as malondialdeh de (MDA) content. The results revealed that the responses of ACP and AKP to mercurd475ult/F11Tf7.1731007.1731511.1Tmf.000200 .0002484.9944499.6006Tm()Tj8.1280

batter of biomarkers is more effective to assess the influence of environmental pollutants (Cajaraville et al., 2000; Chevre et al., 2003; Dondero et al., 2006).

The increasing heav metals in water can lead to serious effects on fish embr os that are particularl sensitive to intoxication dur ing embr onic development (Je ierska et al., 2009). Waterborne mercur can directl affect the hatching process of embr os and larvae qualit (Huang et al., 2010a,b). Therefore, high qualit water without mercur disturbance pla s a significant role in maintain ing the health of the embr os during embr onic development. Although mercur can penetrate the egg membrane and exert an adverse effect on fish embr os (Devlin, 2006; Huang et al., 2010b), toxic effects on larvae, fr , and juvenile fish have been the main focus of most previous studies (Berntssen et al., 2003; Huang et al., 2010a; Monteiro et al., 2010; Sastr and Gupta, 1978; Sastr and Sharma, 1980). However, the responses of biochemical indices, par ticularl phosphatase, l so me, and lipid peroxidation (LPO), to mercur exposure in fish embr os have not et been full eluci dated; the biochemical mechanism used in coping with mercur stress remains unclear. On the otheavetheunclear.the mercur

CAT, as well



. 3. Changes of LSZ activities in developmental embr os of *C. auratus* exposed to different mercur concentrations. All data are presented as means + standard deviation (M + SD). En me activit unit is U/mg Pr. Compared with the control, * represents significant difference ($p < 0.05$) and ** represents extremel signifi cant difference ($p < 0.01$). Compared with the 24 h exposure, † refers to significant difference ($p < 0.05$) and †† refers to extremel significant difference ($p < 0.01$). For comparison between the 96 and 120 h exposure, # stands for significant difference ($p < 0.05$) and ## stands for extremel significant difference ($p < 0.01$).

3.3. LSZ activity responses to mercury exposure in embryos

The changes in LSZ activit in *C. auratus* embr os at various mer cur concentrations indicated similar correlations with embr onic development to some extent (Fig. 3). LSZ activities at different con centrations during the same exposure time showed no significant effects on fish embr os at 24 and 48 h compared with the con trol ($p > 0.05$). A significant increase in LSZ activit was observed at 72 and 96 h exposures onl at 10 μg/L ($p < 0.01$ or $p < 0.05$). On the other hand, LSZ activities significantl increased at 120 h at 5 and 10 μg/L ($p < 0.05$). LSZ activities exhibited graduall decreas ing trend with embr onic development during the time dependent effects at 0, 0.2, and 1 μg/L up until 96 h. At 96 h, LSZ activit reached the minimum; afterward, it increased. LSZ activities significantl decreased at 96 h ($p < 0.05$) at 0, 0.2, and 1 μg/L compared with 24 h exposure. However, LSZ activities showed no significant dif ference at 5 and 10 μg/L ($p > 0.05$). Moreover, LSZ activities at 113Tm()Tj/F11TTj/F584369Tm(until)T428345Tm(at)Tj/F2hTf7.9701317.9701311.78we9

continuousl increased at 5 and 10 μg/L at 120 h exposure, indicat ing a significantl higher amount ($p < 0.01$); however, no significant difference occurred at 0.2 and 1 μg/L ($p > 0.05$). MDA content in the control significantl increased onl at 120 h compared with the 24 h exposure ($p < 0.01$). MDA content in the exposure groups was remarkabl higher at 96 and 120 h at

96 h, suggesting that the increased CAT activit can minimi e MDA production and reduce the degree of oxidative stress that resulted from ROS. On the other hand, the enhanced s nthesis of metabolic en mes in fish embr o can improve the abilit to maintain ph si ological homeostasis and ensure normal embr onic development. However, MDA accumulation was not reduced even after 96 h when mercur concentration was be ond the adjust critical value, partic ularl at 5 and 10 μg/L. Therefore, fish embr os cannot cope with ox gen stress caused b exposure to higher mercur concentration, thereb resulting in their abortion. Thus, a higher number of dead embr os were observed at higher Hg^{2+} concentrations, as described b Wang (2011).

4.3. Responses of LSZ activities to mercury exposure in embryos

The innate immunit in fish pla s an important role in main taining the immune defense s stem to prevent bacterial infections. The corresponding immune levels of fish are modulated to cope with adverse effects of pollution when the are subjected to heav metal contaminants (Zelikoff, 1993). Therefore, investigating fish immunotoxicit under mercur exposure is important. One of the important innate immunit factors in fish is LSZ, which cov ers a wide antibacterial spectrum and destro s the peptidogl can la er of the cell wall of predominant Gram positive bacteria and some Gram negative bacteria (Skouras et al., 2003). LSZ activit is regulated to improve the immune defense when the increasing pathogenic bacteria and other various stress factors attack the fish.

In this stud , LSZ activities in fish embr os significantl increased at 72, 96, and 120 h at 10 μg/L compared with the control. LSZ activities increased onl at 120 h at 5 μg/L. It was indicated that LSZ activit can be induced onl at higher Hg^{2+} concentrations with longer exposure time. Therefore, the responses of LSZ are not sensi tive to mercur exposure in fish embr os, which ma be attributed to the weak abilit to s nthesi e LSZ in the embr os. However, LSZ can also be stimulated to adjust en me activit when mer cur concentration further increases. Wang (2011) has addressed that LSZ activit can be induced to improve the weakened immu nit defense under a certain mercur stress, which agrees with the previous proposal that LSZ activit can be induced b mer cur exposure (Low and Sin, 1998). For example, LSZ activit can be induced in the kidne of blue gourami (*Trichogaster trichopterus*) with mercur exposure at 90 μg/L for 2 weeks (Low and Sin, 1998). Moreover, LSZ activit is also enhanced in fish treated b a rel ativel low dosage of mercur (Low and Sin, 1998). In addition, LSZ activit can be significantl induced in the serum and kidne of tilapia (*Oreochromis aureus*) exposed to 0.6 mg/L mercur solu tion (Low and Sin, 1995a,b). Thus, LSZ activit can be induced b exposure to mercur at specific concentration. LSZ, as an impor tant immunologic factor, pla s an essential role in immune defense; the antibiotic activit can be modulated b self adjustment under specific mercur exposure.

In the present stud , the graduall decreased LSZ activities were observed up to 96 h (similarl observed in the control) as the exposure time was extended. Therefore, the weakening abil it to s nthesi e LSZ is not sufficient to complement the graduall consumed LSZ, as described b Kong et al. (2011). However, LSZ activities in fish embr os are obviousl higher at 120 h than at 96 h, impl ing that the s nthesi ing abilit of LSZ can be enhanced after a specific period of embr onic development.

5. C

The activities of metabolic en mes in fish embr os were affected b exposure to mercur in concentration dependent and time dependent manners despite the var ing response patterns of different metabolic en mes to mercur . The activities of ACP and AKP were sensitivel induced under mercur exposure, which is mainl used in enhancing the reactions of dephosphor lation. LSZ activit showed minimal responses to mercur exposure; however, LSZ activit can be modulated at higher concentration and longer time. The declined CAT activit induced b mercur damage can result in MDA accumulation, thereb causing LPO. At the same time, the strong oxidative stress occurred at higher mercur concentra tion. At the higher mercur concentrations, the activities of ACP, AKP, and CAT, as well as MDA content can be used as biomark ers in evaluating the impact of mercur exposure on *C. auratus* embr onic development and potential ecological risk on larval health. Moreover, the activities of ACP, AKP, and LSZ in fish embr os were enhanced after a specific period of embr onic development. The biological effects of mercur exposure on developmental fish embr o are complicated and ma var in different fish from var ious niches. Therefore, further studies are encouraged to obtain additional evidence that would support the proposed ideas in this stud and to better understand the ph siological and biochemi cal regulator mechanism under mercur exposure. Stud ing gene regulation of metabolic en mes is also necessar to illustrate the biological effects of mercur exposure on fish embr o at a molec ular level.

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This work was funded b the Program for Science and Technolog Innovation Talents in the universities of Henan Province (Project No. 2011HASTIT012). We thank Professor Wa ne Carmichael from Wright State Universit for his technical assis tance in revising and polishing the grammatical construction of the manuscript. We also extend our gratitude to our colleagues for their valuable suggestions in the experimental design and in the overall manuscript preparation.

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