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Responses of acid/alkaline phosphatase, lyso yme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic development of goldfish *Carassius auratus*

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ABSTRACT

This study assessed the impact of mercury exposure on goldfish (*Carassius auratus*) embryos based on the dynamic characteristics of chemical parameters. Day old embryos were exposed to different Hg^{2+} concentrations (0, 0.2, 1, 5, and 10 μg/L). Subsequently, the embryos were sampled every 24 h during embryonic development to measure acid phosphatase (ACP), alkaline phosphatase (AKP), lyso yme (LSZ), and catalase (CAT) activities, as well as malondialdehyde (MDA) content. The results revealed that the responses of ACP and AKP to mercurf./F201.29.0233490.0364Tm()Tj/F11Tmf.000200 .0002484.9944499.6006Tm()Tj8.1280

battery 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 heavy metals in water can lead to serious effects on fish embryos that are particularly sensitive to intoxication during embryonic development (Je ierska et al., 2009). Waterborne mercury can directly affect the hatching process of embryos and larvae quality (Huang et al., 2010a,b). Therefore, high quality water without mercury disturbance plays a significant role in maintaining the health of the embryos during embryonic development. Although mercury can penetrate the egg membrane and exert an adverse effect on fish embryos (Devlin, 2006; Huang et al., 2010b), toxic effects on larvae, fry, and juvenile fish have been the main focus of most previous studies (Berntssen et al., 2003; Huang et al., 2010a; Monteiro et al., 2010; Sastry and Gupta, 1978; Sastry and Sharma, 1980). However, the responses of biochemical indices, particularly phosphatase, lyso yme, and lipid peroxidation (LPO), to mercury exposure in fish embryos have not yet been fully elucidated; the biochemical mechanism used in coping with mercury stress remains unclear. On the

CAT, as well

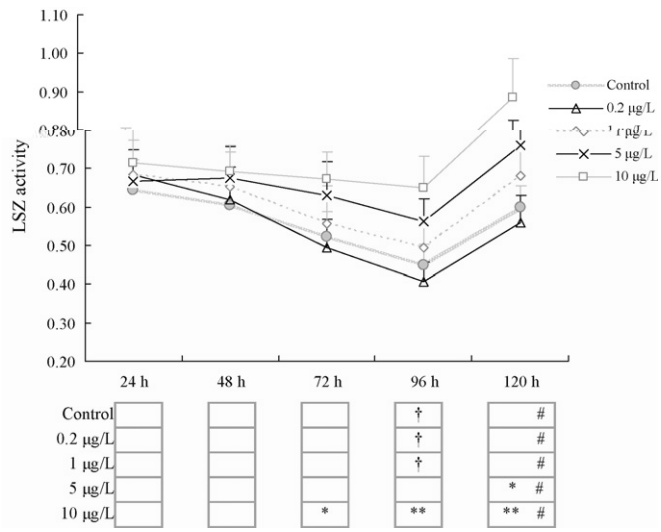

Fig. 3. Changes of LSZ activities in developmental embryos of *C. auratus* exposed to different mercury concentrations. All data are presented as means + standard deviation (M + SD). Enzyme activity unit is U/mg Pr. Compared with the control, * represents significant difference ($p < 0.05$) and ** represents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

3.3. LSZ activity responses to mercury exposure in embryos

The changes in LSZ activity in *C. auratus* embryos at various mercury concentrations indicated similar correlations with embryonic development to some extent (Fig. 3). LSZ activities at different concentrations during the same exposure time showed no significant effects on fish embryos at 24 and 48 h compared with the control ($p > 0.05$). A significant increase in LSZ activity was observed at 72 and 96 h exposures only at 10 μg/L ($p < 0.01$ or $p < 0.05$). On the other hand, LSZ activities significantly increased at 120 h at 5 and 10 μg/L ($p < 0.05$). LSZ activities exhibited gradually decreasing trend with embryonic development during the time dependent effects at 0, 0.2, and 1 μg/L up until 96 h. At 96 h, LSZ activity reached the minimum; afterward, it increased. LSZ activities significantly 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 difference at 5 and 10 μg/L ($p > 0.05$). Moreover, LSZ activities at

continuously increased at 5 and 10 μg/L at 120 h exposure, indicating a significantly 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 significantly increased only at 120 h compared with the 24 h exposure ($p < 0.01$). MDA content in the exposure groups was remarkably higher at 96 and 120 h at

96 h, suggesting that the increased CAT activity can minimi e MDA production and reduce the degree of oxidative stress that resulted from ROS. On the other hand, the enhanced synthesis of metabolic en ymes in fish embryo can improve the ability to maintain physi ological homeostasis and ensure normal embryonic development. However, MDA accumulation was not reduced even after 96 h when mercury concentration was beyond the adjust critical value, partic ularly at 5 and 10 μg/L. Therefore, fish embryos cannot cope with oxygen stress caused by exposure to higher mercury concentration, thereby resulting in their abortion. Thus, a higher number of dead embryos were observed at higher Hg^{2+} concentrations, as described by Wang (2011).

4.3. Responses of LSZ activities to mercury exposure in embryos

The innate immunity in fish plays an important role in main taining the immune defense system to prevent bacterial infections. The corresponding immune levels of fish are modulated to cope with adverse effects of pollution when they are subjected to heavy metal contaminants (Zelikoff, 1993). Therefore, investigating fish immunotoxicity under mercury exposure is important. One of the important innate immunity factors in fish is LSZ, which cov ers a wide antibacterial spectrum and destroys the peptidoglycan layer of the cell wall of predominant Gram positive bacteria and some Gram negative bacteria (Skouras et al., 2003). LSZ activity is regulated to improve the immune defense when the increasing pathogenic bacteria and other various stress factors attack the fish.

In this study, LSZ activities in fish embryos significantly increased at 72, 96, and 120 h at 10 μg/L compared with the control. LSZ activities increased only at 120 h at 5 μg/L. It was indicated that LSZ activity can be induced only at higher Hg^{2+} concentrations with longer exposure time. Therefore, the responses of LSZ are not sensi tive to mercury exposure in fish embryos, which may be attributed to the weak ability to synthesi e LSZ in the embryos. However, LSZ can also be stimulated to adjust en yme activity when mer cury concentration further increases. Wang (2011) has addressed that LSZ activity can be induced to improve the weakened immu nity defense under a certain mercury stress, which agrees with the previous proposal that LSZ activity can be induced by mer cury exposure (Low and Sin, 1998). For example, LSZ activity can be induced in the kidney of blue gourami (*Trichogaster trichopterus*) with mercury exposure at 90 μg/L for 2 weeks (Low and Sin, 1998). Moreover, LSZ activity is also enhanced in fish treated by a rel atively low dosage of mercury (Low and Sin, 1998). In addition, LSZ activity can be significantly induced in the serum and kidney of tilapia (*Oreochromis aureus*) exposed to 0.6 mg/L mercury solu tion (Low and Sin, 1995a,b). Thus, LSZ activity can be induced by exposure to mercury at specific concentration. LSZ, as an impor tant immunologic factor, plays an essential role in immune defense; the antibiotic activity can be modulated by self adjustment under specific mercury exposure.

In the present study, the gradually decreased LSZ activities were observed up to 96 h (similarly observed in the control) as the exposure time was extended. Therefore, the weakening abil ity to synthesi e LSZ is not sufficient to complement the gradually consumed LSZ, as described by Kong et al. (2011). However, LSZ activities in fish embryos are obviously higher at 120 h than at 96 h, implying that the synthesi ing ability of LSZ can be enhanced after a specific period of embryonic development.

5. C

The activities of metabolic en ymes in fish embryos were affected by exposure to mercury in concentration dependent and time dependent manners despite the varying response patterns of different metabolic en ymes to mercury. The activities of ACP and AKP were sensitively induced under mercury exposure, which is mainly used in enhancing the reactions of dephosphorylation. LSZ activity showed minimal responses to mercury exposure; however, LSZ activity can be modulated at higher concentration and longer time. The declined CAT activity induced by mercury damage can result in MDA accumulation, thereby causing LPO. At the same time, the strong oxidative stress occurred at higher mercury concentra tion. At the higher mercury concentrations, the activities of ACP, AKP, and CAT, as well as MDA content can be used as biomark ers in evaluating the impact of mercury exposure on *C. auratus* embryonic development and potential ecological risk on larval health. Moreover, the activities of ACP, AKP, and LSZ in fish embryos were enhanced after a specific period of embryonic development. The biological effects of mercury exposure on developmental fish embryo are complicated and may vary in different fish from var ious niches. Therefore, further studies are encouraged to obtain additional evidence that would support the proposed ideas in this study and to better understand the physiological and biochemi cal regulatory mechanism under mercury exposure. Studying gene regulation of metabolic en ymes is also necessary to illustrate the biological effects of mercury exposure on fish embryo at a molec ular level.

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