Aquatic Toxicology 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, lysozyme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic development of goldfish *Carassius auratus*

Xianghui Kong*, Shuping Wang, Hongxia Jiang, Guoxing Nie, Xuejun Li

College of Life Sciences, Henan Normal University, Xinxiang 453007, PR China

ARTICLE INFO

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

Keywords:
Phosphatase
Lysozyme
Catalase
Lipid peroxidation
Mercury
Fish embryo

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), lysozyme (LSZ), and catalase (CAT) activities, as well as malondialdehyde (MDA) content. The results revealed that the responses of ACP and AKP to time dependent manners. The enzyme activities were significantly induced with increased concentrations and extended exposure (at 5 μg/L after 72 h and 10 μg/L after 48 h; $p < 0.05$ or $p < 0.01$). LSZ was not sensitive to lower Hg^{2+} concentrations, whereas LSZ significantly increased at higher concentrations and longer exposure (at 5 μg/L at 120 h and 10 μg/L after 72 h; $p < 0.05$ or $p < 0.01$). CAT activities were significantly inhibited at different periods of embryonic development, particularly at 5 and 10 μg/L ($p < 0.05$ or $p < 0.01$). Reduced CAT activities were observed at 72, 96, and 120 h at 1 μg/L ($p < 0.05$ or $p < 0.01$), whereas a decline at 0.2 μg/L was evident at 96 h ($p < 0.01$). MDA content significantly increased at various stages of embryonic development, particularly at 10 μg/L ($p < 0.05$ or $p < 0.01$), and increased further at 72, 96, and 120 h at 5 μg/L ($p < 0.05$ or $p < 0.01$). At 96 h, MDA content was only increased by exposure to 0.2 and 1 μg/L ($p < 0.01$). The activities of ACP, AKP, and LSZ remarkably increased at 120 h in contrast to 96 h ($p < 0.05$ or $p < 0.01$). Therefore, 96 h is an important shifting period of embryonic development because the activity of enzyme has been enhanced at this time. Thus, the increased ACP, AKP, and LSZ activities revealed an enhanced ability of the embryos to synthesize more enzymes and attenuate mercury damage. CAT activity negatively correlates with MDA accumulation. The enhanced enzyme activities after specific embryonic stages are used to strengthen the ability to cope with mercury stress and attenuate mercury damage. The biochemical parameters, except LSZ, exhibited sensitivity to mercury suggesting that they may act as potential biomarkers in assessing the environmental mercury risk on *C. auratus* embryos.

1.

In recent years, increasing amounts of heavy metals have been detected in aquaculture as the effluent from industrial and agricultural manufacturing is discharged into the rivers. Mercury is one of the most hazardous heavy metals, which comes from natural phenomena (e.g., crust erosion) and anthropogenic activities (e.g., released chemicals and pesticides) (Das et al., 2001; Nanda, 1993). Increased mercury levels in rivers mainly result from anthropogenic activities and potentially lead to environmental contamination and human health risk

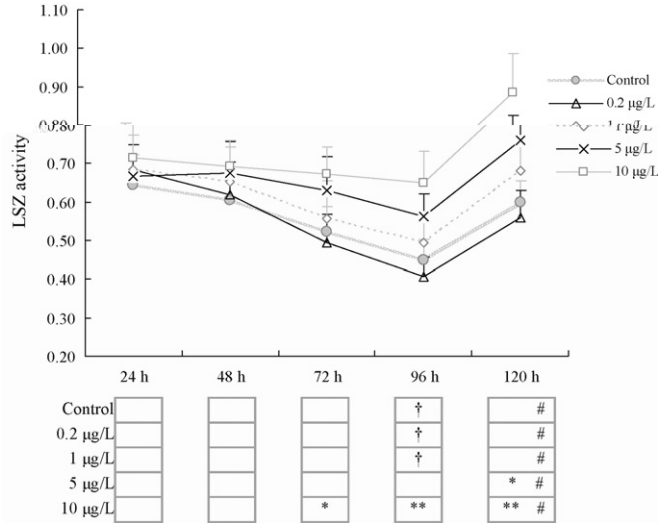
stability of aquatic ecosystems (Pelletier, 1995; St Amand et al., 1999). Therefore, Elia et al. (2007) suggested that fish can be considered as an early indicator of environmental risk due to their sensitive responses to environmental fluctuations. Biochemical indices in fish have been proven to show the corresponding phosphorylation, antioxidation, and immune responses to mercury exposure, some of which have been proposed as biomarkers to assess the mercury contaminants in aquatic ecosystems (Martinez et al., 2005). These biomarkers indicate sensitively the impact of mercury pollutants on fish, and a

0166-445X/$ see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquatox.2012.05.005

battery of biomarkers in more ... the influence of environmental pollutants (Ca... 0; Chevre et al., 2003; Dondero et al., 2006).

The increasing heavy metal ... to serious effects on fish embryos that are partic... ntoxication during embryonic development ... 9). Waterborne mercury can ... embryos and larvae quality (Hu... et ... ality water without ... tain ing the health of ... ment. 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, lysozyme, 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 otheTf.00020101007.9701188.2336579.03457701007.9701161.7396579.0345Tm(On)Tj/F2.0003f7./F1l7589.4953Tm(b

CAT, as well



F. . 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$concentrations indicated similar correlations with embr$nic 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$nic development during the time dependent effects at 0, 0.2, and 1 μg/L up until 96 h. At 96 h, LSZ activit$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

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 minimize MDA production and reduce the degree of oxidative stress that resulted from ROS. On the other hand, the enhanced synthesis of metabolic enzymes in fish embryos can improve the ability to maintain physiological 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, particularly 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 maintaining 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 covers 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 sensitive to mercury exposure in fish embryos, which may be attributed to the weak ability to synthesize LSZ in the embryos. However, LSZ can also be stimulated to adjust enzyme activity when mercury concentration further increases. Wang (2011) has addressed that LSZ activity can be induced to improve the weakened immunity defense under a certain mercury stress, which agrees with the previous proposal that LSZ activity can be induced by mercury 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 relatively 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 solution (Low and Sin, 1995a,b). Thus, LSZ activity can be induced by exposure to mercury at specific concentration. LSZ, as an important 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 ability to synthesize 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 synthesizing ability of LSZ can be enhanced after a specific period of embryonic development.

5. C

The activities of metabolic enzymes in fish embryos were affected by exposure to mercury in concentration dependent and time dependent manners despite the varying response patterns of different metabolic enzymes 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 concentration. At the higher mercury concentrations, the activities of ACP, AKP, and CAT, as well as MDA content can be used as biomarkers 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 embryos are complicated and may vary in different fish from various 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 biochemical regulatory mechanism under mercury exposure. Studying gene regulation of metabolic enzymes is also necessary to illustrate the biological effects of mercury exposure on fish embryos at a molecular level.

A

This work was funded by the Program for Science and Technology Innovation Talents in the universities of Henan Province (Project No. 2011HASTIT012). We thank Professor Wayne Carmichael from Wright State University for his technical assistance 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.

Arabi, M., 2004. Analyses of impact of metal ion contamination on carp (*Cyprinus carpio* L.) gill cell suspension. Biological Trace Element Research 100, 229 245.
Atli, G., Canli, M., 2010. Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotoxicology and Environment Safety 73, 1884 1889.
Bano, Y., Hasan, M., 1989. Mercury induced time dependent alterations in lipid profiles and lipid peroxidation in different body organs to catfish *Heteropneustes fossilis*. Pesticides, Food Contaminants, and Agricultural Wastes 24, 145 166.
Berntssen, M.H.G., Aatland, A., Handy, R.D., 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquatic Toxicology 65, 55 72.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein dye binding. Analytical Biochemistry 72, 248 254.
Broeg, K., 2003. Acid phosphatase activity in liver macrophage aggregates as a marker for pollution induced immunomodulation of the non specific immune response in fish. Helgoland Marine Research 57, 166 175.
Cajaraville, M.P., Bebianno, M.J., Blasco, J., Porte, C., Sarasquete, C., Viarengo, A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Science of the Total Environment 247, 295 311.
Chandran, R., Sivakumar, A.A., Mohandass, S., Aruchami, M., 2005. Effect of cadmium and inc on antioxidant enzyme activity in the gastropod *Achatina fulica*. Comparative Biochemistry and Physiology 140 (C), 422 426.
Cheng, L.J., Meng, Z., 1994. Molybdate colorimetric method for determination of catalase in serum. Chinese Journal of Clinical Laboratory Science 12, 6 8 (in Chinese).
Chevre, N., Gagne, F., Gagnon, P.P., Blaise, C., 2003. Application of rough sets analysis to identify polluted aquatic sites based on a battery of biomarkers: a comparison with classical methods. Chemosphere 51, 13 23.
Das, S., Patro, S.K., Sahu, B.K., 2001. Variation of residual mercury in penaeid prawns from Rushikulya Estuary East Coast of India. Indian Journal of Marine Sciences 30, 33 37.
Devlin, E.W., 2006. Acute toxicity uptake and histopathology of aqueous methyl mercury to fathead minnow embryos. Ecotoxicology 15, 97 110.
Dondero, F., Dagnino, A., Jonsson, H., Capr, I.F., Gastaldi, L., Viarengo, A., 2006. Assessing the occurrence of a stress syndrome in mussels (*Mytilus edulis*) using a combined biomarker/gene expression approach. Aquatic Toxicology 78, 13 24.

Elia, A.C., D rr, A.J.M., Galarini, R., 2007. Comparison of organochlorine pesticides, PCBs, and heav$ metal contamination and of detoxif$ing response in tissues of *Ameiurus melas* from corbara, alviano, and trasimeno lakes, Ital$. Bulletin of Environment Contamination and Toxicolog$ 78, 463 468.

Farina, O., Ramos, R., Bastidas, C., Garc a, E., 2008. Biochemical responses of cnidar ian larvae to mercur$ and ben o(a)p$ene exposure. Bulletin of Environment Contamination and Toxicolog$ 81, 553 557.

Franco, R., S$anche Olea, R., Re$es Re$es, E.M., Pana$iotidis, M.I., 2009. Environmen tal toxicit$, oxidative stress and apoptosis: menage trois. Mutation Research 674, 3 22.

Gilbertson, M., Carpenter, D.O., 2004. An ecos$tem approach to the health effects of mercur$ in the Great Lakes basin ecos$tem. Environmental Research 95, 240 246.

Goth, L., 1991. A simple method for determination of serum catalase activit$ and revision of reference range. Clinica Chimica Acta 196, 143 151.

Huang, W., Cao, L., Ye, Z., Yin, X.B., Dou, S.Z., 2010a. Antioxidative responses and bioaccumulation in Japanese flounder larvae and juveniles under chronic mer cur$