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**Biochemical alterations in juvenile carp (*Cyprinus carpio*)
exposed to zinc: Glutathione reductase as a target**

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Abstract

The aim of this study was to investigate biochemical changes in juvenile carp (*Cyprinus carpio*) exposed to zinc chloride (10, 30 and 100 μ M) for a period of 48 hours. Zinc exposure caused a concentration-dependent reduction in glutathione reductase (GR) activity in gills, liver and brain. Gill glutathione S-transferase (GST) was reduced when animals were exposed to the highest concentration of 100 μ M zinc. The phosphorylation of p38^{MAPK} increased in the brain of fish exposed to zinc 100 μ M, while phosphorylation of the extracellular signal-regulated protein kinase 1/2 (ERK1/2) and c-

Jun N-terminal protein kinase 1/2 (JNK1/2) remained unchanged. Expression of proteins HSP60 and HSP70 were not affected by zinc exposure. Considering the significant concentration-dependent inhibition of GR in all tissues analyzed, this enzyme could be a potential biomarker of exposure to zinc, which has to be confirmed.

Keywords: Zinc; *Cyprinus carpio*; Glutathione reductase inhibition; MAPK; p38; HSP60; HSP70

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Environmental pollutants such as metals pose serious risks to many aquatic organisms by changing genetic, physiological, biochemical and behavioral parameters (Scott and Sloman, 2004).

Zinc is an essential nutrient to living organisms, being a structural component of more than 300 enzymes (Sandstead, 2000), but in higher exposure concentrations zinc causes cytotoxicity to both vertebrates (Bossy-Wetzel et al., 2004) and invertebrates (Lock and Janssen, 2003). Environmental contamination by zinc usually occurs due to industrial activities and the use of organo-zinc pesticides, which can be hazardous to both human and wildlife. The aim of this study was to investigate mechanisms of zinc toxicity based on some biochemical alterations in juvenile carp exposed to zinc chloride.

Juvenile carp specimens, weighing 2-5 g, were adapted for 7 days to dechlorinated water under aeration at 16 ± 1 °C, at a density lower than 2 g.L⁻¹ with complete water renewal every day. Three groups of 8 carp specimens each were exposed to 10, 30 and 100 µM zinc chloride, respectively. A fourth group was kept as a non-exposed group. Animals were killed after 48 h of exposure and tissues were immediately excised for biochemical analysis. The activity of the antioxidant and auxiliary enzymes glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferase (GST) was examined in the brain, gill and liver of each animal. The expression of the heat shock proteins HSP60, HSP70 and the phosphorylation of mitogen activated protein kinases (MAPKs) were also analyzed in the brain. Methodology details can be found in Franco et al. (2006) and in Leal et al. (2006). A behavioral parameter based on fish locomotion was quantified. The number of squares crossed by individual fish was measured during 5 min after 48 hours of zinc

exposure. One way analysis of variance was applied to all data, and the Duncan *post hoc* test was applied when appropriate.

Zinc exposure caused a significant concentration-dependent decrease in GR activity in gills, liver and brain (Fig. 1A, B and C). GST activity was significantly reduced in gills when the animals were exposed to 100 μM zinc, while GPx activity remained unchanged (data not shown). Glutathione levels, protein thiols and lipid peroxidation (TBARS) in gills and liver were not affected by zinc treatment (data not shown). Phosphorylation of p38^{MAPK} in brain slices was significantly increased in the fish exposed to 100 μM zinc (Fig. 2). ERK1/2 and JNK1/2 phosphorylation was unaffected, as well as the expression of heat shock proteins HSP60 and HSP70 (data not shown). Zinc exposure did not affect the motor activity of fishes (data not shown).

The results indicate that carp exposure to zinc leads to inactivation of important antioxidant enzymes, which can be taken as a pro-oxidative condition. It is well documented that metals produce oxidative stress and the ability of protein-bound zinc to become redox active is relevant to redox signaling (Maret, 2005). The marked inhibition of GR in all carp tissues studied points out this enzyme as a potential biomarker of exposure to zinc. However, special consideration must be given to the fact that zinc is usually present in a mixture of pollutants that can modulate the antioxidant defense system. A pioneer study from Mize and Langdon (1962) reported the inhibition of purified GR enzyme from rat liver by zinc, possibly at the enzyme active site. Based on *in vitro* studies, GR displayed an $\text{IC}_{50} \sim 7 \mu\text{M}$ (unpublished results) for zinc chloride. Assuming that the *in vivo* effect is similar, we can consider that intracellular zinc concentration fell within the range of 1-10 μM .

The slight reduction in GST activity by zinc could represent an impaired capacity to detoxify toxic compounds. However, oxidative markers, such as lipid

peroxidation products, glutathione and protein thiol levels did not increase in our study (data not shown). In another study, we showed that the brown mussel, *Perna perna*, exposed to similar zinc concentrations for 48 hours developed a more pronounced oxidative stress condition (Franco et al., 2006), suggesting that *Cyprinus carpio* is more resistant to short term exposure to zinc than *P. perna*.

ERK1/2, JNK and p38^{MAPK} have been identified in fish and play pivotal roles in the physiology of these organisms (Kultz and Avila, 2001). Activation of p38^{MAPK} is associated to environmental stress such as heat shock, osmotic and oxidative stress or toxic insults (Cowan and Storey, 2003). ERK1/2 and p38^{MAPK} are activated in fish cerebellum by lead exposure (Leal et al., 2006). In the present study carp-exposure to zinc increased brain p38^{MAPK} phosphorylation, suggesting that activation of this pathway is more sensitive to zinc when compared to ERK and JNK. Taken the scarce literature on aquatic organisms, our data adds to the understanding of zinc modulation of cell signaling proteins under environmentally relevant metal concentrations.

In conclusion, our results demonstrate an important mechanism of zinc toxicity in fish related to GR inhibition in gills, liver and brain and p38^{MAPK} activation in the brain. We propose that monitoring GR activity could be a potential biomarker of exposure to zinc but, chronic experiments involving mixtures with other contaminants are recommended to confirm this potential.

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Figure Captions

Fig. 1. Effects of 48 hour-exposure to zinc chloride on glutathione reductase (GR) activity in liver, gills and brain of juvenile carp. GR activity in liver (A), gills (B), and brain (C) are expressed as mean $\pm$ SEM in mU/mg protein (n = 8 animals per group).

*p < 0.01 - different than control group.

Fig. 2. Effects of 48 hour-exposure to zinc on the phosphorylation/expression of mitogen activated protein kinase p38^{MAPK} in carp brain. Densitometric analysis of p38^{MAPK} is presented as mean $\pm$ SEM (n = 8 animals per group). * p < 0.01 - different than control group.

Figure 1.

Figure 2.