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BIOCHEMICAL RESPONSE IN INTESTINE AND GILLS OF *CARASSIUS AURATUS GIBELIO* TO ACUTE MANGANESE(II) INTOXICATION

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We report the effects of manganese (II) acute intoxication on antioxidant defense system in *Carassius auratus gibelio* intestine and gills. The fish were divided in five groups: group 1-control group, groups 2,3,4 and 5-exposed to 0.5mg Mn^{2+}/L and were sacrificed after one, 2,3 and 7 days, respectively. Our research revealed that intestine and gills catalase activity increased significantly after the second day of manganese exposure. The gluthatione reductase showed a different response during the manganese exposure. In the intestine, a decrease in this activity was noticed after 3 and 7 days, while, in the case of the gills, the gluthatione reductase activity was activated, including an increase by more than 5-fold after 3 days. The gluthatione peroxidase activity was inhibited in the intestine, and remained unchanged in the gills, during manganese exposure. Our results showed specific changes in intestine and gills in the antioxidant system as a consequence of acute manganese intoxication of *Carassius auratus gibelio.*

INTRODUCTION

Manganese occurs in soil, sediments and water both naturally and as a result of environmental contamination. It is one of the widely used metals in the industry. Manganese exposure is associated with many occupations including mining, dry cell battery manufacturing, welding, and agricultural application of manganese containing pesticides.[1] Manganese in surface waters is an essential micronutrient for plants and animals in aquatic systems,[2] but elevated concentrations are toxic to fish and impair the quality of drinking water. Conifer afforestation is associated with enhanced manganese in runoff. This metal is leached from conifer foliage and litter, the mature conifers enhance acid deposition and loss of manganese from acidified catchment soils.[3]

High levels of this metal are toxic, generating a series of pathologies, based on the reactive oxygen species (ROS). In almost all aerobic cells, the primary free radical is superoxide (O_2^-), a major source being the "leakage" of electrons onto O_2 from various components of the cellular electron transport chains, such as those of mitochondria and the endoplasmic reticulum. Despite the moderate chemical reactivity of O_2^- in aqueous solution, the biological systems convert it in more reactive species such as hydroperoxyl, hydroxyl, etc.[4] Most of the hydroxyl generated *in vivo*, except during excessive exposure to ionizing radiation, comes from the metal-dependent breakdown of H_2O_2, according to the general equation:

$$Me^{n+} + H_2O_2 \rightarrow Me^{(n+1)+} + \cdot OH + OH^- \quad (1)$$

in which Me^{n+} can be: Fe^{2+}, Cu^{2+}, Mn^{2+}, etc.

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Due to the highly reactive oxygen species, most components of cellular structure and function are likely to be targets of oxidative damage: nucleic acids, unsaturated lipids, proteins and carbohydrates.[5]

Cells have two primary lines of defence against oxidative stress. The first involves cellular molecules directly involved in preventing oxidative damage to the cell. These include antioxidant enzymes such as the superoxide dismutases (SOD), glutathione peroxidases (GPX), catalase (CAT) and glutathione reductase (GR) and low molecular weight antioxidant molecules such as glutathione and ascorbate. The second line of defence consists of repair enzymes. These repair systems remove and/or repair oxidatively damaged macromolecules.

In modern ecotoxicology, fish have become the major vertebrate model. When aquatic ecosystems are polluted with organic or inorganic contaminants, fish will almost inevitably be contaminated. These contaminants are retained by fish to a substantial extent and finally lead to considerably higher biotic than abiotic concentrations.[6]

The aim of this paper is to evaluate some biochemical effects of the *in vivo* manganese induced acute intoxication in intestine and gills of *Carassius auratus gibelio.*

RESULTS

Figure 1a) shows that the specific activity of CAT in the intestine increased significantly after the second day of acute manganese exposure. Thus, after 2 days of Mn^{2+} treatment, the CAT activity increased by 73.2%, while, after 3 days of exposure, the activity was almost double.

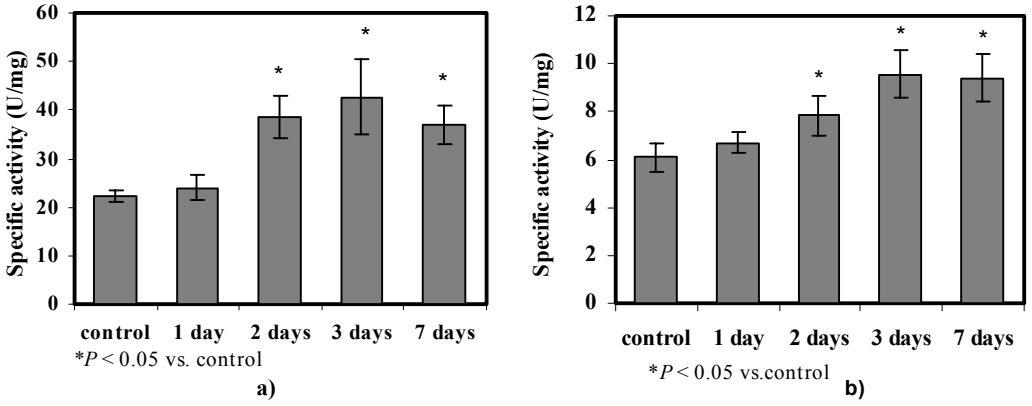

Fig. 1 – The CAT activity in intestine (a) and gills (b) of *Carassius auratus gibelio* exposed to 0.5mg Mn^{2+}/L.

Similar results were noticed for CAT in the gills, Figure 1 b, but in this organ the increases in activities were smaller than in the intestine. The gills CAT has increased by 28.8% after 2 days of manganese exposure, and, by 54%, after 7 days, respectively.

The intestine glutathione reductase specific activity decreased significantly during the period of experiment (Figure 2 a). Thus, the activity level decreased by 22.4% after 3 days, and by 65% after 7 days of manganese intoxication. A different GR enzymatic profile was observed in the gills of *Carassius auratus gibelio* exposed to manganese (Figure 2 b). Manganese seems to activate this antioxidant enzyme. The level of GR increased by 56.5% after one day of exposure, by 99.7% after 2 days, while, after 3 days the GR activity has increased by more than 5-fold.

GPX is the key enzyme of the antioxidant system under normal and oxidative stress conditions. We noticed a significant decrease in the intestine GPX after 2, 3 and respectively 7 days of exposure (Figure 3 a). GPX was diminished by 48.4% after 2 days, by 58.7% after 3 days and by 44.8% after 7 days, respectively. The GPX activity remained unaltered in the gills after manganese exposure compared to control (P>0.05) as it can be seen in Figure 3 b.

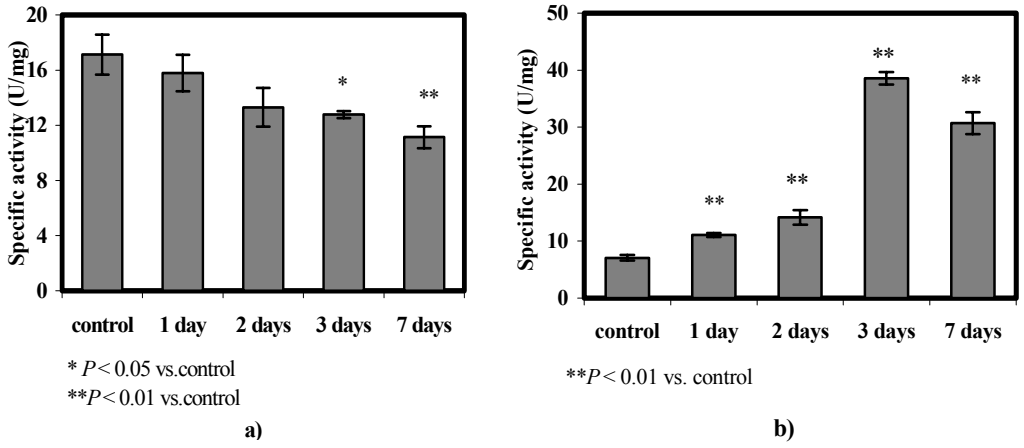

Fig. 2 – The GR activity in the intestine (a) and gills (b) of Carassius auratus gibelio exposed to 0.5mg Mn^{2+}/L.

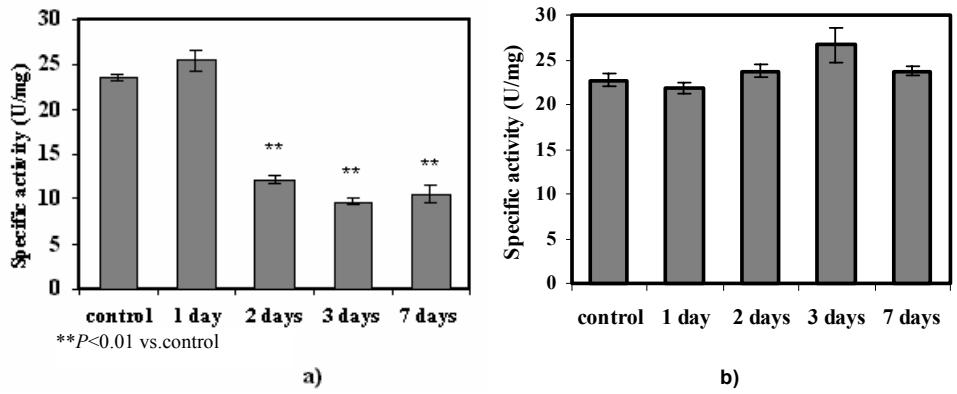

Fig. 3 – The GPX activity in the intestine (a) and gills (b) of *Carassius auratus gibelio* exposed to 0.5mg Mn^{2+}/L.

DISCUSSION

The 5 mg/L manganese acute exposure for one, 2, 3 and 7 days generates a pollutant-induced adaptative response in fish intestine and gill. Once enters the cell, manganese crosses the mitochondrial inner membrane via mechanisms that transport Ca^{2+}, is concentrated in this organelle and is bound to the inner membrane or to matrix proteins.[7] Mn^{2+} could take place of Fe^{2+} in cytochromes of cellular respiratory chain that have a structure close to haemoglobin. This can lead to incomplete reduction of O_2 with the formation of free radicals and possibly oxygenated compounds such as O_2^- and H_2O_2. In tissues where Fe/Mn ratios are generally low (the intestine and gill can be considered as such in comparison with blood) this replacement could generate cellular damages. As a consequence, the synthesis and/or release of intestine and gills CAT is stimulated after more than two days of exposure (Figure 1 a and b). According to Missy and coworkers,[8] the standard potential of the Fe^{3+}/ Fe^{2+} couple (0.77 V in the absence of complexation) varies according to the environment of Fe, from 0.07 V (cytochrome b) to 0.55 V (cytochrome a_3) which means a decrease of 0.22 V (0.77 – 0.55) in the case of a_3. Taking in account that the potential of the O_2/H_2O couple is 0.82 V at pH 7, the cytochrome a_3 can thus reduce O_2 to H_2O. In the case of Mn^{3+}/Mn^{2+} couple (standard potential of 1.50 V in the absence of complexation) the decrease in its redox potential is 1.28 V (1.5 – 0.22). As a consequence, the reduction of O_2 to H_2O is no longer possible, because the last cytochrome of the respiratory chain (cytochrome a_3) has a higher redox potential (1.28 V) than the O_2/H_2O couple (0.82 V).[8]

The significant decrease of the intestine GR after more than 2 days of exposure (Figure 2 a) could appear because Mn^{2+} can substitute Mg^{2+} in cells. At high concentration of Mg^{2+}, and also Mn^{2+}, inhibit the Na^+, K^+-ATPase,[9] which modifies the Na^+ gradient in the intestinal epithelial cells. As a consequence, the

glucose/Na^+ symport in the apical domain of the epithelial cell can be impaired and a decrease of the intracellular quantity of this monosaccharide could occur. This decrease of the glucose level can generate less NADPH (reduced nicotinamide adenine dinucleotide phosphate) in the oxidative branch of the pentose phosphate pathway and as a result of the fact that the activity of the GR decreased. The increase of the gills GR activity (Figure 2 b) generates, possibly, a higher level of reduced glutathione, which is an immediate agent against the reactive oxygen species.

In the intestine, the decrease of GPX activity (Figure 3 a) is correlated with the decrease of glutathione reductase one, which entails a smaller quantity of glutathione. Because this tripeptide is one of the substrates of the glutathione peroxidase, its low level generates a decrease of the enzymatic activity. Low activity of glutathione peroxidase in the intestine of intoxicated fish, as noticed in the present study, demonstrates inefficiency of this organ in neutralizing the impact of peroxides and thus resulting in increased lipid peroxidation. In the case of gills, this activity is almost unchanged (Figure 3 b), possibly because the increase of the glutathione reductase activity generates higher quantity of reduced glutathione which prevents lipid peroxidation.

Our results obtained during manganese experimental intoxication are according with those obtained by Staicu and co-workers[10] who had evidenced structural modifications in the intestine and gills of *Carassius auratus gibelio* intoxicated with 0.5 mg Mn^{2+}/L. It seems that mechanisms of antioxidant protection against acute manganese intoxication present peculiarities linked to the organ type.[11] The changes observed in the investigated enzymes activities, could be considered as indicators of stress status induced in animals by changes in the environmental conditions.

EXPERIMENTAL PART

Animals and tissue preparation

Specimens of *Carassius auratus gibelio* weighing 20-30 g were obtained from Nucet Fishery Research Station, Romania. Before the experiments, fish were kept in glass aquaria containing aerated dechlorinated tap water at 25 ^oC. After 3 days of adaptation, the fish were randomly divided into five groups of ten each and were not fed. Group 1, the control group, was kept in tap water. Groups 2, 3, 4 and 5 were exposed for one, 2, 3 and 7 days, respectively, to 0.5 mg Mn^{2+} per L water. Metal was added only in the first day of experiment as $MnCl_2$. After the mentioned periods of time, the fish of each group were sacrificed, and intestine and grills were excised and frozen at –80 ^oC.

The frozen tissues were homogenized with a Potter-Elvejhem homogenizer in 0.1 M Tris-EDTA buffer, pH 7.4, and then centrifuged at 8, 000 g for 30 min at 4 ^oC. Aliquots of the supernatants were used for analysis.

Enzyme assays

The catalase (EC 1.11.1.6) activity was assayed by monitoring the disappearance of H_2O_2 at 240 nm, according to the method of Aebi.[12] One unit (U) of catalase activity represents the decrease of a 1 μmol of H_2O_2 per minute.

Gluthatione reductase (EC 1.6.4.2) activity was recorded by Golberg and Spooner method,[13] using GSSG and NADPH as substrates. The convertion of NADPH to $NADP^+$ was followed by recording the change in absorbance intensity at 340 nm. One unit of GR activity was calculated as 1 nmol of NADPH consumed per minute, using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione peroxidase (EC 1.11.1.9) activity was assayed according to the method described by Beutler [14] and one unit was expressed as 1 nmol of NADPH consumed per minute.

All the enzymatic activities were expressed as specific ones, namely in units per mg of protein. The protein content was determinated using the method of Lowry[15] with bovine serum albumin (BSA) as standard.

Statistical Analysis

All values were expressed as means ± SEM. The differences between control and manganese-treated groups were compared by Student's *t*-test using standard statistical packages. The results were considered significant if the *P* value was less than 0.05.

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