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 Mn²⁺/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 glutathione 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 glutathione reductase activity was activated, including an increase by more than 5-fold after 3 days. The glutathione 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 (O₂⁻), a major source being the “leakage” of electrons onto O₂ from various components of the cellular electron transport chains, such as those of mitochondria and the endoplasmic reticulum. Despite the moderate chemical reactivity of O₂⁻ 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 H₂O₂, according to the general equation:

$$\text{Me}^{n+} + \text{H}_2\text{O}_2 \rightarrow \text{Me}^{(n+1)+} + \cdot\text{OH} + \text{OH}^-$$  \hspace{1cm} (1)

in which Meⁿ⁺ can be: Fe²⁺, Cu²⁺, Mn²⁺, 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.\textsuperscript{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.\textsuperscript{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.

![Graph](image1.png)

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 Mn²⁺/L.

Fig. 3 – The GPX activity in the intestine (a) and gills (b) of Carassius auratus gibelio exposed to 0.5mg Mn²⁺/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 Ca²⁺, is concentrated in this organelle and is bound to the inner membrane or to matrix proteins. Mn²⁺ could take place of Fe²⁺ in cytochromes of cellular respiratory chain that have a structure close to haemoglobin. This can lead to incomplete reduction of O₂ with the formation of free radicals and possibly oxygenated compounds such as O₂⁻ and H₂O₂. 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 Fe³⁺/Fe²⁺ 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 a₁) which means a decrease of 0.22 V (0.77 – 0.55) in the case of a₁. Taking in account that the potential of the O₂/H₂O couple is 0.82 V at pH 7, the cytochrome a₁ can thus reduce O₂ to H₂O. In the case of Mn⁷⁺/Mn²⁺ 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 O₂ to H₂O is no longer possible, because the last cytochrome of the respiratory chain (cytochrome a₃) has a higher redox potential (1.28 V) than the O₂/H₂O 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 Mn⁷⁺ can substitute Mg²⁺ in cells. At high concentration of Mg²⁺, and also Mn³⁺, 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-workers10 who had evidenced structural modifications in the intestine and gills of Carassius auratus gibelio intoxicated with 0.5 mg Mn²⁺/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 °C. 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 Mn²⁺/L water. Metal was added only in the first day of experiment as MnCl₂. After the mentioned periods of time, the fish of each group were sacrificed, and intestine and gills were excised and frozen at –80 °C. 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 °C. 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 H₂O₂ at 240 nm, according to the method of Aebi.12 One unit (U) of catalase activity represents the decrease of a 1 µmol of H₂O₂ per minute.

Glutathione reductase (EC 1.6.4.2) activity was recorded by Golberg and Spooner method,13 using GSSG and NADPH as substrates. The conversion 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 x 10³ M⁻¹ cm⁻¹.

Glutathione peroxidase (EC 1.11.1.9) activity was assayed according to the method described by Beutler14 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 determined using the method of Lowry15 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.

**REFERENCES**