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Activities of Superoxide Dismutase and Catalase in Erythrocytes and Plasma Transaminases of Goldfish (*Carassius auratus gibelio* Bloch.) Exposed to Cadmium

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Summary

Four groups of goldfish were exposed to cadmium in a concentration of 20 mg Cd/l water under aquarium conditions. The duration of exposure was 1, 4, 7 and 15 days. It was shown that the activity of superoxide dismutase (SOD) in the red blood cells (RBC) significantly decreased after the first day of cadmium exposure. However, the SOD activity increased after 7 and 15 days of cadmium treatment. Elevated activity of catalase (CAT) was found in erythrocytes of cadmium-treated fishes after 15 days, whereas plasma GOT levels was increased after 7 and 15 days and GPT levels after 1, 4, 7 and 15 days of cadmium treatment. This was accompanied by a significant decrease of blood hemoglobin concentrations (after 15 days) and hematocrit values (after 7 and 15 days). However, the concentration of blood glucose significantly increased after 1, 4, 7 and 15 days of cadmium exposure. These results indicate that cadmium causes oxidative stress and tissue damage in the exposed fishes.

Key words
Cadmium • Carassius • Superoxide dismutase • Catalase • Transaminases

Introduction

Cadmium causes significant metabolic alterations and injuries of biological systems at different levels (Pratap and Bonga 1990, Brown *et al.* 1984). After entering into the organism of freshwater fishes through the gills, cadmium binds to albumins and erythrocytes in the blood and then is transferred into tissues and organs where it is bound to proteins of low molecular mass producing metallothioneins by the induction of metallothionein mRNA synthesis (Gould and Karolus 1974, George *et al.* 1996). About 75 % of the total accumulated cadmium in the organism is deposited in the liver and kidneys (Marafante 1976, Kraal *et al*. 1995), but it can also be deposited in the heart, gills and other tissues (Vigh *et al.* 1996, Melgar *et al.* 1997). The synthesis of metalothioneins in certain organs decreases to some extent the toxicity of non-bound cadmium (Thornalley and Vasák 1985, Wormser *et al.* 1990, Olsson and Kille 1997). In fish, cadmium causes the destruction of erythrocytes, decreases the hematocrit value and hemoglobin concentration and leads to anemia. Cadmium

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also influences the differential blood count (Johansson-Sjöbeck and Larsson 1978, Gill and Epple 1993).

The activity of transaminases in fishes may be significantly changed under the influence of different toxic agents. Some metals, such as zinc, copper and cadmium significantly increase the activity of serum transaminases in some freshwater fishes (Nemcsók *et al.* 1981, Žikić *et al.* 1997). Oxidative stress caused by different metals may damage certain tissues and liberate various transaminases into the plasma.

Cadmium increases the production of reactive oxygen species (ROS) in tissues and inhibits the activity of some enzymes of the antioxidative defense system (Jackim *et al.* 1970, Pruell and Engelhardt 1980, Žikić *et al*. 1996). There are only scarce data available on the influence of cadmium on the activity of enzymes of the antioxidative defense system in erythrocytes of fishes (Thomas and Wofford 1993, Palace *et al.* 1993, Žikić *et al*. 1997).

In the present report, we studied the influence of cadmium on the activity of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) in erythrocytes of goldfish after exposure lasting 1, 4, 7 and 15 days, as well as on the activities of plasma glutamic acid-oxalacetic acid-transaminase (GOT, EC 2.6.1.1) and glutamic acid-pyruvic acid-transaminase (GPT, EC 2.6.1.2). Hematocrit (Ht), hemoglobin (Hb) and glucose concentrations in the blood were also measured.

Methods

Our experimental goldfish (*Carassius auratus gibelio* Bloch.) weighing 280 ± 30 g were adapted for 30 days to the aquarium conditions with water temperature of 13.0 ± 0.5 °C, pH 7.2 and concentration of dissolved oxygen of 4.0 ± 0.2 mg O_2 . l^{-1} dechlorinated and aerated water. The fishes were fed chow pellets. After the period of adaptation, four experimental groups of goldfish were exposed to cadmium in a concentration of 20 mg Cd . l^{-1} water by adding a solution of $CdCl_2$. Control fishes resided in non-polluted water. The fishes were sacrificed in groups after exposure to cadmium for 1, 4, 7 or 15 days, each group consisting of 7 fishes.

The concentration of oxygen in water was determined by using HI 9143 Microprocessor auto cal dissolved oxygen meter (Hanna instruments). The concentration of cadmium in water was determined by atomic absorption spectrophotometry (Perkin Elmer, Model 3300). All chemicals were Sigma products (St Louis, MO, USA).

After sacrificing the fishes freshly heparinized blood samples were collected immediately and prepared for further processing as recommended by Mazeaud *et al.* (1979) and Wdzieczak *et al.* (1982). The activity of superoxide dismutase (SOD) was determined spectrophotometrically at 480 nm by the epinephrine method (Misra and Fridovich 1972) and it was expressed in units of enzyme activity per gram of hemoglobin (U/g Hb), per ml of red blood cells (U/ml RBC) and per ml of blood (U/ml blood). The activity of catalase (CAT) was determined spectrophotometrically at 570 nm (Sinha 1972) and it was expressed in mmoles of decomposed hydrogen peroxide per second per g of Hb (mmol H_2O_2/s/g Hb), per ml RBC (mmol H_2O_2/s/ml RBC) and per ml blood (mmol H_2O_2/s/ml blood). The plasma transaminase activities were determined spectrophotometrically (Wootton *et al.* 1964) and were expressed in μmol/min/l. The hemoglobin (Hb) concentration in erythrocytes was determined by the cyanmethemoglobin method (Drabkin and Austin 1935) and was expressed in g/l. The hematocrit (Ht) value was determined by the standard microhematocrite method, and was expressed in %. The concentration of glucose was measured by Hultman (1959) colorimetric method, and was expressed in mmol/l. Proteins were determined according to Lowry *et al.* (1951). Data were analyzed using the non-parametric Mann-Whitney two-tailed test and differences at $p<0.05$ were considered as significant.

Results

The activity of SOD in erythrocytes of control fishes and fishes of four experimental groups are presented in Figure 1. Cadmium causes a significant decrease of SOD activity ($p<0.05$) after the first day of exposure (Figs 1A, 1B, and 1C). No significant differences were found after the fourth day of exposure compared to the control values, whereas after 7 and 15 days of exposure values were significantly increased ($p<0.05$) when expressed in U/ml RBC (Fig. 1B), and after 15 days ($p<0.05$) when expressed in U/ml blood (Fig. 1C).

CAT activity is presented in Figure 2. The activity of CAT expressed in U/g Hb was not significantly increased ($p<0.05$) until after 15 days of cadmium exposure (Fig. 2A). The obtained data show

that cadmium did not change the activity of CAT expressed in mmol H_2O_2/s/ml RBC (Fig. 2B) and mmol H_2O_2/s/ml blood (Fig. 2C).

The concentration of hemoglobin, hematocrit values and concentration of glucose are presented in Table 1. Cadmium causes a significant decrease of hemoglobin concentration after 15 days of exposure ($p<0.05$) and of the hematocrit values after 7 and 15 days ($p<0.05$). A stress effect of cadmium is expressed in the appearance of hyperglycemia ($p<0.05$) 1, 4, 7 and 15 days after cadmium exposure (Table 1).

Data on plasma transaminase activities (GOT and GPT) are presented in Table 2. The activity of GOT in goldfish exposed to cadmium was increased after 7 and 15 days ($p<0.05$), and the activity of GPT was increased after 1, 4, 7 and 15 days of cadmium exposure ($p<0.05$).

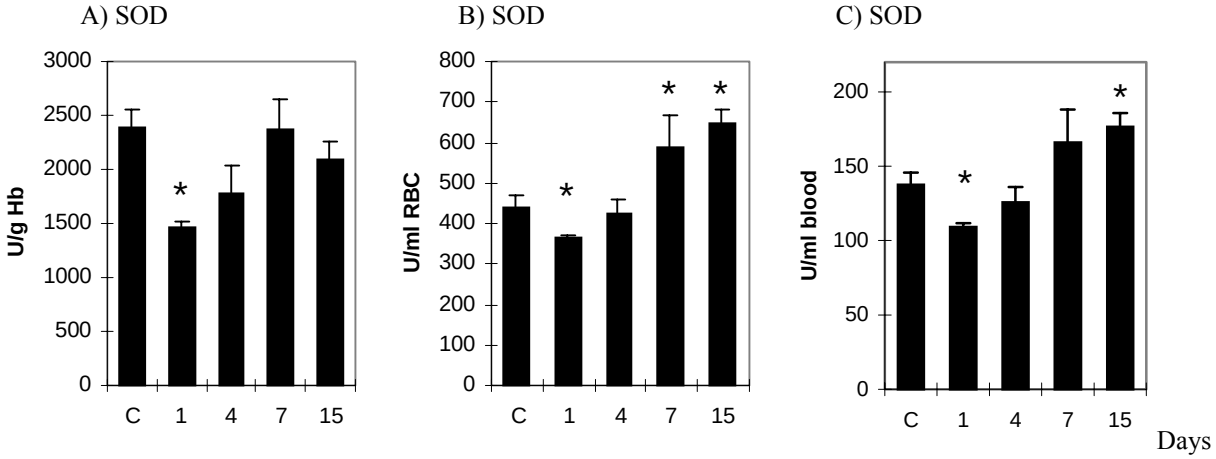

Fig. 1. *The activity of superoxide dismutase (SOD) in erythrocytes of control goldfishes (C) and goldfishes exposed to 20 mg/l cadmium for 1, 4, 7, and 15 days. The values are expressed in units of enzyme activities per g of hemoglobin (A, U/g Hb), per ml of red blood cells (B, U/ml RBC) and per ml of blood (C, U/ml blood). Means ± S.E.M. from 7 fishes in each group. Significantly different from controls, * $p<0.05$.*

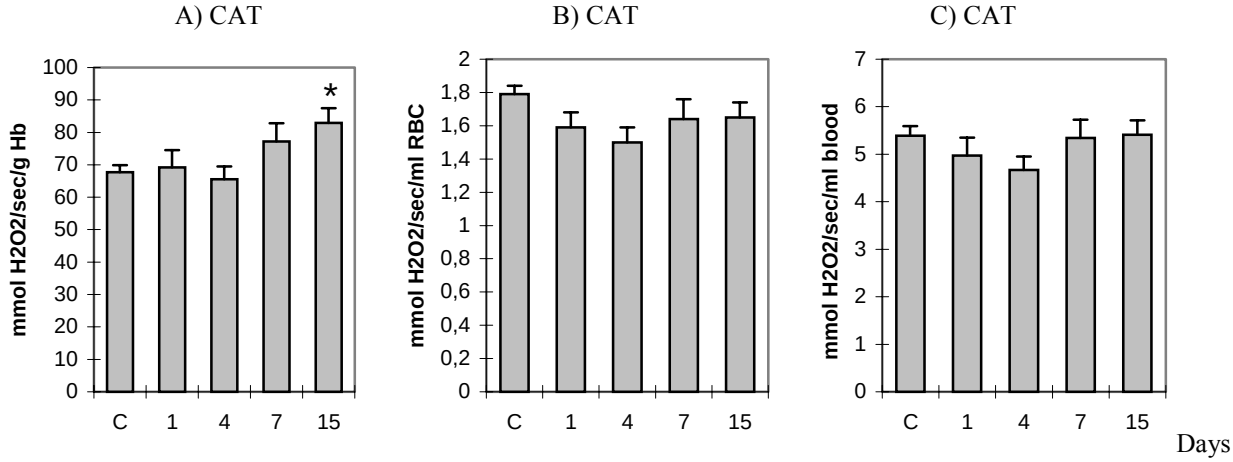

Fig. 2. *The activity of catalase (CAT) in erythrocytes of control goldfishes (C) and goldfishes exposed to 20 mg/L cadmium for 1, 4, 7, and 15 days. The values are expressed in mmol H_2O_2 per second per g of hemoglobin (A, mmol H_2O_2/s/g Hb), per ml of red blood cells (B, mmol H_2O_2/s/ml RBC) and per ml of blood (C, mmol H_2O_2/s/ml blood). Means ± S.E.M. from 7 fishes in each group. Significantly different from controls, * $p<0.05$.*

Discussion

Our data show that the activity of SOD is significantly decreased after one day of exposure to cadmium in comparison to control values (Figs 1A, 1B and 1C) ($p<0.05$). Similar results were obtained in previous investigations (Žikić *et al*. 1997) on carp during acute cadmium exposure (12, 18, and 24 h). However,

after the fourth day of exposure to cadmium the activity of this enzyme in goldfish was similar to the control values. During prolonged exposure to cadmium, the activity of total SOD was significantly increased after 7 (U/ml RBC) and 15 days (U/ml RBC and U/ml blood) (Figs 1B and 1C). These results indicate the activation of protective mechanisms necessary for scavenging of produced O_2^- radicals in erythrocytes. Previous results have shown that prolonged exposure to cadmium may enhance the activity of this enzyme in some mammalian tissues, such as interscapular brown adipose tissue (Kostić *et al*. 1993).

Table 1. Concentration of hemoglobin (Hb), haematocrit value (Ht) and concentration of glucose in the blood of control goldfishes (C) and goldfishes exposed to cadmium (20 mg/l) for 1, 4, 7 and 15 days.

Days	Hb (g/l)	Ht (%)	Glucose (mmol/l)
C	79.62 ± 5.54	31.20 ± 0.37	2.86 ± 0.18
1	71.81 ± 2.52	30.00 ± 1.05	5.25 ± 0.40*
4	71.23 ± 2.52	29.75 ± 0.48	5.59 ± 0.44*
7	69.24 ± 5.84	28.20 ± 0.80*	8.40 ± 0.65*
15	65.24 ± 2.84 *	27.25 ± 1.11*	8.27 ± 0.25*

*Data are means ± S.E.M.; n=7 fish in each group. * Significantly different from controls (p<0.05).*

Table 2. The activities of plasma glutamic acid-oxalacetic acid-transaminase (GOT) and glutamic acid-pyruvic acid-transaminase (GPT) in control goldfishes (C) and goldfishes exposed to cadmium (20 mg/l) for 1, 4, 7 and 15 days.

Days	GOT (μmol/min/l)	GPT (μmol/min/l)
C	377.95 ± 41.19	7.73 ± 0.38
1	393.37 ± 19.04	10.58 ± 0.83*
4	378.41 ± 26.65	12.75 ± 0.66*
7	683.25 ± 82.64*	12.17 ± 0.97*
15	759.33 ± 76.54*	12.86 ± 0.94*

*Data are means ± S.E.M.; n=7 fish in each group. * Significantly different from controls (p<0.05).*

Cadmium did not exhibit any effects on the activity of CAT when expressed per ml RBC and per ml of blood in all experimental groups. After 15 days of exposure, cadmium caused a significant increase of CAT activity in erythrocytes of goldfish when expressed per g of Hb (Fig. 2A). Our results show that during acute exposure (1 day), cadmium decreases total SOD activity. Similar results were obtained in our previous experiments on carps (Žikić *et al*. 1997). Results obtained in previous experiments on rats showed that cadmium caused significant alterations of energy metabolism in red blood cells (Petronijević *et al*. 1995). It also increased concentration of nonenzymatic components of the antioxidant defense system in plasma (ascorbic acid-AsA, vitamin E) (Žikić *et al.* 1995) of rats and enhanced activity of enzymes of antioxidant defense system: SOD, CAT, glutathione peroxidase and glutathione reductase in RBC of rats (Kostić *et al*. 1993).

Transaminases play an important role in protein and amino acid metabolism. Our results show that cadmium in plasma of goldfish (Table 2) significantly increased the activity of GOT after 7 and 15 days and increased the activity of GPT in all experimental groups as compared to the control values. These results are in accordance with the results of previous investigators on freshwater fishes (Wieser *et al*. 1980, Benson *et al.* 1987, Žikić *et al*. 1997). Under the influence of different heavy metals or in the state of stress, damage of the liver, kidneys, heart and other tissues and organs may occur with concomitant liberation of transaminases into the circulation. If the water is polluted with different metals, such as zinc, copper and cadmium (Nemcsók *et al*. 1981, Nemcsók and Boross 1981, Žikić *et al*. 1997), the activity of plasma transaminases in freshwater fishes are significantly altered.

The present data (Table 1) show that cadmium causes significant decrease of hemoglobin concentration in blood after 15 days and of the hematocrit values after 7 and 15 days of exposure. These results are similar to those obtained in experiments with mammalian blood (Kostić *et al.*1993), as well as in experiments on carps

(Žikić *et al.* 1997). It is well known that the presence of cadmium in the organism causes a decrease of blood iron level (Thomas *et al.* 1982) which may be the cause of decreased concentration of hemoglobin. Hemolyzed plasma of goldfish exposed to cadmium, which was observed in our experiments, indicates destruction of erythrocytes. The appearance of anemia is one of the most sensitive indications of cadmium intoxication. These data are in accordance with results of other investigators (Prigge *et al.* 1977, Palace *et al*. 1993, Žikić *et al*. 1997), who showed that cadmium as well as other metals, such as copper and zinc, cause destruction of erythrocyte membranes and hemolysis.

Previous investigations showed that cadmium altered the metabolism of carbohydrates, causing hyperglycemia in some marine (Thomas *et al.* 1982) and freshwater fish species (glyconeogenesis), (Larsson and Haux 1982, Žikić *et al*. 1997). These results are in accordance with our data and show that hyperglycemia appears in goldfish throughout the experiment (Table 1). Stress caused by cadmium increases the glucose content in blood, because of intensive glycogenolysis, and the synthesis of glucose from extrahepatic tissue proteins and amino acids (Larson and Haux 1982, Gill *et al*. 1993). Glucose may also be released into the circulation in cadmium-induced hypoxia, which enhances the mobilization of catecholamines and processes of glycogenolysis.

In cadmium-induced anemia (Table 2), however, the antioxidant status of goldfish erythrocytes is improved during the prolonged exposure (7-15 days). Our results have also shown a higher resistance of goldfish erythrocytes to cadmium in comparison to other freshwater cyprinides (Žikić *et al*. 1997).

It can be concluded that cadmium induces the appearance of anemia and alters the metabolism of carbohydrates and proteins in goldfish. Our results also show the decreased activity of SOD in erythrocytes of goldfishes during acute exposure to cadmium, which indicates the presence of ROS-induced peroxidation, which leads to the destruction of RBC membranes. The time course specificity of antioxidative defense was also demonstrated.

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