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Activities of Superoxide Dismutase and Catalase in Erythrocytes and Transaminases in the Plasma of Carps (Cyprinus carpio L.) Exposed to Cadmium

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Summary

Total superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) activities in erythrocytes and the glutamic acid-oxalacetic acid-transaminase (GOT, EC 2.6.1.1) and glutamic acid-pyruvic acid-transaminase (GPT, EC 2.6.1.2) activities in the plasma were measured in experimental groups of carps (*Cyprinus carpio L.*) exposed to cadmium in a concentration of 20 mg Cd/l water under aquarium conditions for 6, 12, 18 and 24 hours and in control fishes. It was shown that the total activity of SOD in the erythrocytes is significantly decreased after 12, 18 and 24 hours of cadmium exposure. Increased activities of CAT (after 24 hours) in the erythrocytes and GOT and GPT in the plasma were found in cadmium-treated fishes. At the same time the concentration of blood haemoglobin and haematocrit values were significantly diminished. These results indicate that cadmium causes oxidative stress and tissue damage in the exposed fishes.

Key words
Cadmium – Carps – Superoxide dismutase – Catalase – Transaminases

Introduction

Cadmium is a heavy metal commonly used in ecotoxicological studies because its concentration is raised in the environment due to industrial and domestic sewage waste in streams. In fish, cadmium has deleterious effects on growth and reproduction and causes osmoregulatory stress, and it was shown to alter the structure and function of various organs and tissues (Soengas *et al.* 1996). After being taken into the organism of freshwater fishes through gills, cadmium enters the blood binds to albumins and erythrocytes. From there it is transferred into tissues and organs where it binds to proteins of low molecular mass producing metallothioneins (MT) (Gould and Karolus 1974). From the total accumulated cadmium in the organism about 75 % are deposited in the liver and kidneys (Marafante 1976) but it can also be deposited in the heart, gills and other tissues (Vigh *et al.* 1996). Cadmium causes metallothionein synthesis *via* production of MT mRNA (George *et al.* 1996). The production of metallothioneins in certain organs decreases the toxicity of non-bound cadmium to some degree in different organisms (Thornalley and Vašak 1985, Wormser and Ben Zakine 1990).

In fishes, cadmium causes the destruction of erythrocytes, decreases haematocrit (Htc) values and haemoglobin (Hb) concentration, and induces anaemia. Cadmium also influences the differential blood count (Johansson-Sjöbeck and Larsson 1978).

The activity of transaminases in fishes may be significantly changed under the influence of various toxic substances. Oxidative stress caused by the effect of different metals produces damage of certain tissues and liberation of transaminases into the plasma. Some metals, such as zinc, copper and cadmium, significantly increase the activity of serum transaminases in fishes (Nemcsók and Boross 1982).

Sparse information is available about the influence of cadmium on the activity of enzymes of the

antioxidative defence system in erythrocytes and in tissues of fishes (Pruell and Engelhardt 1980, Palace *et al.* 1993, Žikic *et al.* 1996).

In the present experiments, the influence of cadmium on the activities of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) in erythrocytes of carps was studied after exposures lasting 6, 12, 18 and 24 hours. The activities of glutamic acid-oxalacetic acid transaminase (GOT, EC 2.6.1.1) and glutamic acid-pyruvic acid transaminase (GPT, EC 2.6.1.2) in the plasma, as well as the haematocrit value, haemoglobin concentration and glucose concentrations in the blood were also estimated.

Material and Methods

The carps (*Cyprinus carpio L.*) weighing 280±30 g were adapted to 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 in dechlorinated and aerated water. After a period of adaptation, four experimental groups of carp were exposed to cadmium in a concentration of 20 mg Cd/l water by adding a solution of $CdCl_2$, while the control fishes were not thus exposed. The fishes were sacrificed in groups after exposure to cadmium lasting 6, 12, 18 and 24 hours. Each experimental group consisted of 6 fishes.

The concentration of oxygen in the water was determined by using HI 9143 Microprocessor auto cal dissolved oxygen meter. The concentration of cadmium in the water was determined by atomic absorption spectrophotometry.

After sacrifice, fresh heparinized blood samples were collected and were prepared for procedure as recommended by Mazeaud *et al.* (1979) and Wdzieczak *et al.* (1982). The haemoglobin concentration in erythrocytes was estimated by the cyanmethaemoglobin method (Drabkin and Austin 1935). The haematocrit values were obtained by a standard microhaematocrit method using microhaematocrit tubes, and the concentration of glucose was measured by Hultman's (1959) colorimetric method. The plasma transaminase activities (GOT and GPT) were assessed spectrophotometrically (Wootton *et al.* 1964). The activity of superoxide dismutase was determined spectrophotometrically at 480 nm by the epinephrine method (Misra and Fridovich 1972) and expressed in units of enzyme activity per gram of haemoglobin (Units/g Hb), per ml of red blood cells (Units/ml RBC) and per ml of blood (Units/ml blood). The activity of catalase was determined spectrophotometrically at 570 nm (Sinha 1972) and 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). Proteins were assayed by the method of Lowry *et al.* (1951). Data were analysed using the non-parametric Mann-Whitney two tailed test and values $p<0.05$ were considered as significant.

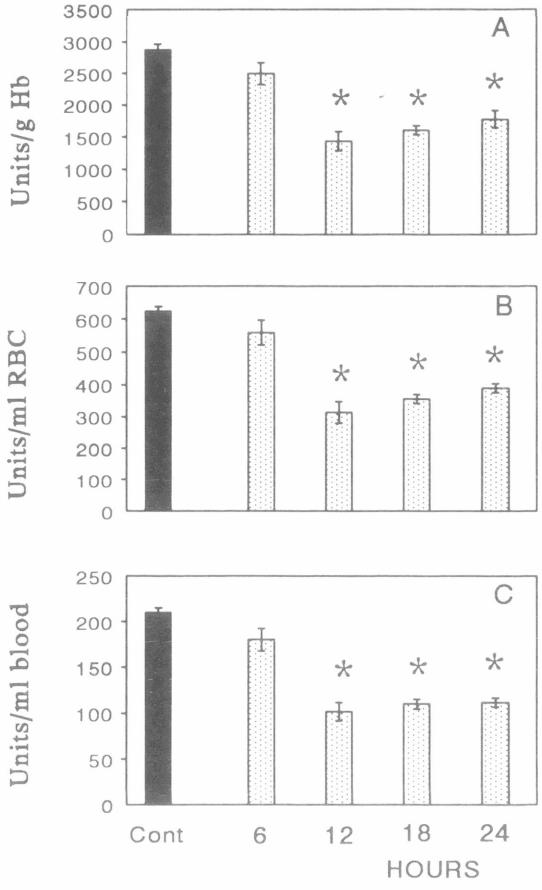

Fig. 1. *The activity of superoxide dismutase (SOD) in erythrocytes of control carps (Cont) and carps exposed for 6, 12, 18, and 24 hours to Cd (20 mg/l). The values are expressed A) in Units per g of haemoglobin (Units/g Hb), B) per ml of red blood cells (Units/ml RBC) and C) per ml of blood (Units/ml blood). Means±S.E.M. from 6 animals in each group. Significantly different from controls. * $p<0.05$.*

Results

During the exposure of carps to a high concentration of cadmium (20 mg Cd/l), the accelerated and irregular swimming, increased defecation and cessation of food intake were observed in all the exposed groups. After a period of 24 to 30 hours of exposure most fishes died.

Exposure of carps to cadmium lasting 18 and 24 hours (Table 1) resulted in a significant decrease of

haematocrit and blood haemoglobin concentration ($p<0.05$). The glucose concentration (Table 1) was significantly increased ($p<0.05$) after 6, 12, 18 and 24 hours of cadmium exposure. The activities of plasma transaminase GOT an GPT in carps exposed to cadmium were increased after 6, 12, 18 and 24 hours of exposure ($p<0.05$) (Table 2).

Table 1. Haemoglobin (Hb), haematocrit (Htc) and concentration of glucose in the blood of control carps (C) and carps exposed for 6, 12, 18 and 24 hours to Cd (20 mg/l)

Hours	Hb (g/l)	Htc (%)	Glucose (mmol/l)
C	73.16±0.60	33.70±0.60	2.63±0.07
6	72.66±1.52	32.00±0.78	3.08±0.14*
12	70.11±0.82	32.10±0.43	5.28±0.34*
18	68.47±1.24*	30.70±0.89*	7.22±0.40*
24	63.04±3.33*	28.50±0.65*	7.77±0.34*

*Means ± S.E.M. from 6 animals in each group. Significantly different from the controls, * $p<0.05$.*

SOD activity is presented in Figure 1. Under the influence of cadmium, the activity of SOD in carp erythrocytes was significantly decreased after 12, 18 and 24 hours ($p<0.05$) when the activity was expressed either per Units/g Hb (Fig. 1A), per Units/ml red blood cells (Fig. 1B) or per Units/ml of blood (Fig. 1C).

Table 2. The activities of glutamic acid-oxalacetic acid-transaminase (GOT) and glutamic acid-pyruvic acid-transaminase (GPT) in the plasma of control carps (C) and carps exposed for 6, 12, 18 and 24 hours to Cd (20 mg/l)

Hours	GOT (μmol/min/l)	GPT (μmol/min/l)
C	81.48±4.22	7.61±0.49
6	138.82±13.89*	12.14±0.62*
12	207.70±13.90*	14.33±0.74*
18	234.61±19.87*	16.46±0.81*
24	296.75±20.71*	18.03±1.50*

*Means ± S.E.M. from 6 animals from each group. Significantly different from the controls, * $p<0.05$.*

The changes of catalase activity are shown in Fig. 2. The data obtained show that cadmium did not change the activity of CAT expressed in Units/g Hb and in Units/ml RBC (Fig. 2A and 2B). However, the activity of CAT (Fig. 2C) (expressed in Units/ml blood) was significantly decreased ($p<0.05$) in all the experimental groups exposed to cadmium.

Discussion

Previous investigations showed that cadmium influences the metabolism of carbohydrates, causing hyperglycaemia in some marine (Bally and Neff 1982) and freshwater fish species (Larson and Haux 1982). However, long-term exposure of some marine fishes

(e.g. *Anguilla rostrata*) to cadmium significantly lowered the plasma glucose concentration (Gill *et al.* 1993). The results obtained in our study are in accordance with these data and show that hyperglycaemia appears in carps after 6 h exposure to cadmium (Table 1). The stress caused by cadmium increases glucose concentrations in the blood, because of intensive glycogenolysis on one hand, and because of glucose synthesis from tissue (extrahepatic) proteins and amino acids on the other (Larson and Haux 1982). In addition, cadmium causes hypoxia which enhances the mobilization of catecholamines and glycogenolytic processes. In this way, glucose may also be liberated into the circulation.

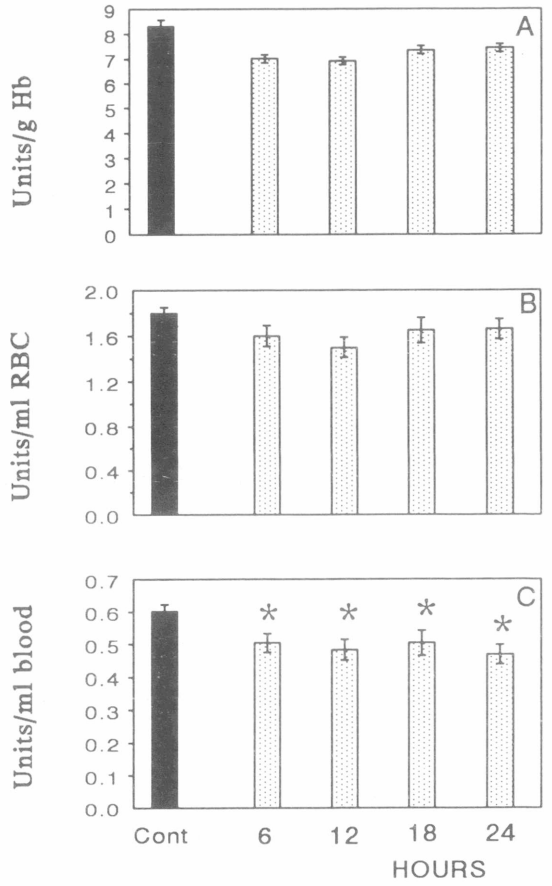

Fig. 2. *The activity of catalase (CAT) in erythrocytes of control carps (Cont) and carps exposed for 6, 12, 18, and 24 hours to Cd (20 mg/l). The values are expressed A) in Units per g of haemoglobin (Units/g Hb), B) per ml of red blood cells (Units/ml RBC) and C) per ml of blood (Units/ml blood). Means ± S.E.M. from 6 animals in each group. Significantly different from controls.* $^{*}p<0.05$.

Our results (Table 1) show that cadmium causes a significant decrease of haematocrit values and haemoglobin concentration in the blood of carps after 18 h exposure. These results are similar to those obtained by Gardner and Yerich (1970) in experiments on tissues, as well as with those obtained by analysis of mammalian blood (Kostic *et al.* 1993). It is well known that the presence of cadmium in the organism decreases iron levels in the blood (Bally and Neff 1982) which may be the cause of decreased concentrations of haemoglobin. Haemolysed plasma of carps exposed to cadmium, which was observed in our experiment, indicates that the decreased haematocrit value is the consequence of increased destruction of erythrocytes. These data are in accordance with the results of other investigators (Prigge *et al.* 1977, Palace *et al.* 1993) who showed that cadmium, as well as other metals such as copper and zinc, damages the erythrocyte membrane and leads to haemolysis.

Transaminases (GOT and GPT) play an important role in metabolism of proteins and amino acids. Our results show that cadmium significantly enhances the activity of these enzymes in the plasma of carps (Table 1). This is in accordance with the results of previous investigations (Wieser *et al.* 1980). During stress or under the influence of different heavy metals, the damage of liver, kidneys, heart and other organs may be associated with concomitant liberation of transaminases into the plasma. Investigations on freshwater fishes have shown that the activity of plasma transaminases is significantly altered if the water is polluted by different metals, such as zinc and copper (Nemcsók and Boross 1982), or by other pollutants, such as ammonia or paraquat (Wieser *et al.* 1980).

Our results show (Fig. 1) that the activity of SOD significantly decreased after 12 hours of exposure to cadmium in comparison to control values. The decrease of the activity of this enzyme was observed when expressed in relation to the concentration of haemoglobin, RBC volume as well as to blood volume.

The decreased catalase activity in erythrocytes of carps is the consequence of decreased concentration of haemoglobin and lowered haematocrit (Fig. 2, Table 1). These data indicate that cadmium did not exert an inhibitory effect on the activity of catalase when expressed per ml RBC or per g of haemoglobin. Our previous investigations (Žikic *et al.* 1988) showed that manganese, similarly as cadmium, depressed SOD activity expressed per ml of blood and also decreased CAT activity expressed per ml RBC.

According to the present data, it can be concluded that acute exposure of carps to high concentrations of cadmium seriously disturbs carbohydrate and protein metabolism. This work suggests that the activity of SOD and CAT against reactive oxygen species in red blood cells can be compromised by cadmium ions, and that this

disbalance can cause increased membrane lipid peroxidation. The inhibitory effect of the administered doses of cadmium is more effective against superoxide dismutase.

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