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SHORT COMMUNICATION

The Activities of Superoxide Dismutase, Catalase and Ascorbic Acid Content in the Liver of Goldfish (Carassius auratus gibelio Bloch.) Exposed to Cadmium

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Summary

The goldfish (*Carassius auratus gibelio Bloch.*) were exposed to cadmium in the concentration of 20 mg Cd/l water under aquarium conditions for 1, 4, 7 and 15 days. After exposure to cadmium, the activities of superoxide dismutase (SOD) and catalase (CAT) were significantly decreased. At the same time, the liver ascorbic acid (AsA) content was increased.

Key words
Cadmium – Goldfish – Superoxide dismutase – Catalase – Ascorbic acid

Cadmium causes significant metabolic alterations and injuries of biological systems at different levels (Pratap and Wendelaar-Bonga 1990, Brown *et al.* 1994). Cadmium increases the production of reactive oxygen species in the organism and inhibits the activity of some enzymes of the antioxidative system (Pruell and Engelhardt 1980, Kostic *et al.* 1993, Ognjanovic *et al.* 1995).

Ascorbic acid (AsA) may represent the accessory protective mechanism in tissues, reacting with reactive oxygen species (Sato *et al.* 1990). In contrast to other teleosts (Yamamoto *et al.* 1978), the cyprinid fish have sufficient amounts of enzyme n-gulonolactone oxidase which catalyses the last step of AsA synthesis (Sato *et al.* 1978).

The results of investigations on marine fish show that high concentrations of cadmium significantly diminish the activity of catalase (CAT) in the liver (Jackim *et al.* 1970, Pruell and Engelhardt 1980).

Since there are no data available on the influence of cadmium on the antioxidative defense system in goldfish and few data with other cyprinids (Jackim *et al.* 1970, Pruell and Engelhardt 1980) in these investigations, we have studied the activities of SOD and CAT in the liver of goldfish under the influence of cadmium. The content of ascorbic acid was also assessed.

Goldfish *Carassius auratus gibelio (Bloch)* weighing 270–300g were adapted to aquarium condition with water temperature at 12.0±1.0 °C, pH 7.2 and concentration of dissolved oxygen of 4.0±0.2 mg O_2/l dechlorinated water. After a period of adaptation, four experimental groups of fish were exposed to cadmium in a concentration of 20 mg Cd/l water in the form of $CdCl_2$. Each experimental group consisted of seven animals. The fish were decapitated after exposure to cadmium for 1, 4, 7 and 15 days. The liver was dissected, minced and washed with saline. Homogenates were sonicated as described by Takada *et al.* (1982).

The concentration of cadmium in the liver was determined by atomic absorption spectrophotometry. The content of ascorbic acid in liver was determined by the dinitrophenyl hydrazine method (Roe 1957).

The activities of SOD (Misra and Fridovich 1972) and CAT (Sinha 1972) were determined spectrophotometrically. Total protein concentrations were determined by the method of Lowry *et al.* (1951). The data were analyzed using the non-parametric Mann-Whitney two-tailed test and $p<0.05$ was accepted as significant.

Table 1
The activities of superoxide dismutase (SOD) (units/mg protein) and catalase (CAT) (mM H_2O_2/s/mg protein) in the liver of goldfish exposed to cadmium (20 mg Cd/l water) during 1, 4, 7 and 15 days compared with the control.

Days of cadmium exposure	SOD	CAT
Control	41.2±4.1	22.9±0.7
1	39.7±1.3	20.9±1.1
4	31.3±2.3*	17.1±1.4*
7	22.5±1.5*	16.4±1.4*
15	22.2±2.5*	14.2±1.1*

Means ± S.E.M. from 7 animals in each group.
** $z>1.96$ for $p<0.05$ by the Mann-Whitney test.*

In the fish exposed to cadmium, liver SOD and CAT activities were significantly decreased after 4, 7 and 15 days ($p<0.05$) when compared to the controls (Table 1).

In all the exposed groups, the concentration of cadmium in the liver was increased in comparison to the control values ($p<0.05$). After the first day of exposure to cadmium, the concentration of ascorbic acid in the liver was decreased ($p<0.05$) while it increased significantly ($p<0.05$) after the 7th and the 15th day.

In all the exposed groups of fish significantly increased concentrations of cadmium were found in the liver in comparison to the control values (Table 2). The highest concentration of cadmium was found after the 15th day of exposure. These data are in accordance with the results of previous investigations in fish (Marafante 1976, Pratap and Wendelaar-Bonga 1990). Cadmium in the liver and kidneys of fish binds to low molecular proteins building the metallothionein complexes in which cadmium is being inactivated (Marafante 1976, Cosson 1994).

Our results (Table 1) show that the increased concentration of cadmium significantly decreases the activities of SOD and CAT in the liver. This may be due to the inhibitory effects of cadmium on the activities of some enzymes of the antioxidative defense system. Similar results were obtained in other cyprinodont marine fish species (Jackim *et al.* 1970, Pruell and Engelhardt 1980).

Table 2
Ascorbic acid (AsA) content (mg/100 mg wet mass), and cadmium concentration (mg/g wet mass) in the liver of goldfish exposed to cadmium (20 mg Cd/l water) during 1, 4, 7 and 15 days compared with the control.

Days of cadmium exposure	AsA	Cd
Control	8.16±0.77	0.53±0.02
1	6.53±0.62*	7.86±0.43*
4	9.40±0.84	4.84±0.19*
7	16.78±1.15*	5.33±0.74*
15	23.23±1.79*	8.97±0.56*

Means ± S.E.M. from 7 animals in each group.
** $z>1.96$ for $p<0.05$ by the Mann-Whitney test.*

Concomitantly with the increase of liver concentration cadmium, the concentration of ascorbic acid also increases significantly (Table 2), especially after the 7th and 15th day of exposure. Ascorbic acid, as a scavenger of O_2.-, singlet oxygen ($1O_2$), OH. and OH_2-, (Padh 1990) may act in synergy with vitamin E inhibiting the oxidation of lipoproteins by free radicals (Sato *et al.* 1990, Mukhopadhyay *et al.* 1993). Our results indicate that the increased concentration of AsA in the liver may play an important role in the removal of harmful effects of cadmium, as well as in the prevention of tissue damage, which is in accordance with previous investigations on fish (Sato *et al.* 1978, Yamamoto *et al.* 1978). It can be concluded that increased accumulation of cadmium decreases the activities of SOD and CAT and that AsA to some extent compensates these inhibitory effects of cadmium. Ascorbic acid may play a role in scavenging reactive oxygen species in the liver of goldfish diminishing thus the effects of lipid peroxidation caused by cadmium.

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