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Effects of Intra-peritoneal Injection of Inorganic Mercury on Blood Parameters and Hepatic Oxidative Stress Enzyme Activities in Common Carp (*Cyprinus carpio* L.)

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Abstract - The effects of intra-peritoneal injection of inorganic mercury on haematological parameters and hepatic oxidative stress enzyme activities were studied in common carp, *Cyprinus carpio*. The fish were injected thrice intra-peritoneally with mercuric chloride (1, 5, 10 mg Hg Kg b.w.$^{-1}$). After exposure of three different mercury concentrations a physiological stress response was exerted on *C. carpio* by causing changes in the blood status such as erythropenia in blood and oxidative stress in liver. Red blood cell counts, hemoglobin concentration and hematocrit level were reduced in most cases by inorganic mercury. Remarkable low level of serum chloride, calcium and osmolality were also observed in the mercury-exposed fish. However, serum magnesium and phosphate were not altered by exposure to mercury. An increased activity of hepatic glutathione peroxidase was observed in the lowest treatment group of carp (1 mg Hg Kg b.w.$^{-1}$), hence, hepatic catalase and glutathione peroxidase of carp exposed to higher concentration of mercury (5, 10 mg Hg Kg b.w.$^{-1}$) showed significant reduction in such activities.

Key words : antioxidant enzyme, *Cyprinus carpio*, haematological parameters, inorganic mercury

INTRODUCTION

Investigations of mercury in aquatic animals have been concerned with anthropogenic sources that result in high Hg levels in these organisms and subsequent human contamination through consumption (Kim 1995). Most of the mercury in water, soil, sediments, or biota (i.e., all environmental media except the atmosphere) is in the form of inorganic mercury salts and organic forms of mercury. Total mercury levels in lakes and streams generally are lower than mercury levels found in precipitation, with levels typically well under 20 ng L^{-1} (NJDEPE 1993). However, mercury (Hg) contamination of aquatic environments is an important ecological and human health concern (Nriagu and Pacyna 1988; Fitzgerald and Clarkson 1991).

Mercury has always been present at varying levels in environmental media and biota, and all mercury is, in a sense, naturally occurring; that is, mercury is not a substance of human origin. Anthropogenic activities are thought to redistribute mercury from its original matrix through the atmosphere to other environmental media. Numerous studies indicate that the amount of mercury being deposited from the atmosphere has increased since the onset of the industrial age (Johansson *et al.*

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1991; Nater and Grigal 1992; Swain *et al.* 1992). Some of the deposited mercury arises from natural sources and some from anthropogenic activities.

Absorption, distribution, metabolism, and excretion of mercury is dependent upon its form and oxidation state (ATSDR 1989; Goyer 1991). Organic mercurials are more readily absorbed than inorganic forms. An oxidation-reduction cycle is being involved in the metabolism of mercury and its compounds by both animals and humans (ATSDR 1989). Indeed, mercury poisoning results in necrosis of epithelial cells, epithelial hyperplasia and inhibition of Na-K-ATPase activity (Bouquegneau 1977; Lock *et al.* 1981). During the early embryonic life of zebrafish, elevated Hg levels causes reduced survival time and increased time to hatch by Dave and Xiu (1991).

Numerous studies have described the high toxicity of mercury to fishes after acute and chronic exposures (reviewed in Mance 1990). The disposition of inorganic mercury in fishes has been characterized after water, oral, and intraperitoneal administration, with the pattern of tissue distribution varying depending on the administrative route. Regardless of the exposure route, the liver and kidney tended to accumulate the highest quantities of these metals (Weisbart 1973; Sorenson 1991). However, the data on oxidative stress of mercury exposure are lacking in fish.

Therefore, the objective of this study were to evaluate the effects of intraperitoneal injection of inorganic mercury on blood parameters and hepatic antioxidant enzyme activities in common carp, *Cyprinus carpio*.

MATERIALS AND METHODS

1. Fish and rearing conditions

Healthy, cultured common carp (*Cyprinus carpio* L.) were obtained from a commercial farm in Miryang, Gyeongnam, Korea. Prior to exposure, fish were held three weeks for acclimatization and evaluation of over-all fish health under the laboratory condition in 12 : 12 h light: dark cycle for further studies. During acclimation fish were fed with basal diet twice daily. After acclimatization, fish (mean body weight 231.5±7.8 g) were selected for the experiments.

2. Exposure conditions

The exposure took place in 250 L glass aquaria containing 25 fish in each treatment groups under flow-through conditions. Each tank received a flow of 50 L h^{-1} with continuous aeration. Mercury (II) chloride (Sigma, USA) were dissolved in PBS immediately before the intra-peritoneal injection and the pH was adjusted to approximately 7.4. The fish were injected i.p. with 1, 5 and 10 mg kg^{-1} body weight (b.w.) Hg (II) as $HgCl_2$. The first booster injection was given 3 days after primary injection, and the second was given 4 days after the first treatment. The control group was subjected to the same regime injected an equal volume of PBS only.

3. Blood sample and hepatic tissue preparation

Blood and hepatic tissue samples were taken to determine their haematological disturbances and hepatic oxidative stress after 3, 7 and 15 days post injection. After each post-injection period fish were anesthetized with 3-aminobenzoic acid ethyl ester methanesulfonate. Blood samples were taken from each fish by puncture of the caudal vessel using heparinized syringes for haematological test. For serum analysis blood was collected by unheparinized syringe. Blood was allowed to coagulate at room temperature for 2 hr and serum was obtained by centrifugation of an amount of blood i.e. approximately 1.5 mL, at 3,000 × g for 8 min at 4°C (MIKRO 22R, Hettich, Germany) and then stored at -80°C until analyzed.

4. Haematological properties

Total red blood cell (RBC) counts were made according to Klontz (1979), using modified Yokoyama diluting fluid and a Spencer Bright-line hemocytometer. Blood hemoglobin (Hb) levels were determined using the Drabkin Austin cyanmethemoglobin technique (Kit 525, Sigma). Hematocrit (Ht) was determined by the microhematocrit centrifugation technique.

5. Serum inorganic components

Serum samples were analyzed for inorganic phosphorus (SIGMA Diagnostics kit 360, Ultraviolet method), calcium (SIGMA Diagnostics kit 588, Colorimetric method), magnesium (SIGMA Diagnostics kit 595, Colo-

rimetric method) and chloride (SIGMA Diagnostics kit 461, Colorimetric method). Plasma osmolality was measured directly on 20 μL samples using a Model 3300 advanced micro-osmometer (Advanced Instruments, Inc., USA).

6. Hepatic enzyme assay

Glutathione Peroxidase (GPx) Activity was assayed by an adaptation of the method of Paglia and Valentine (1967) with cumene hydroperoxide as substrate. Both sample and reference tubes contained 0.05 M phosphate buffer, pH 7.2, 4.3 mM EDTA, 0.28 mM NADPH, 0.5 U of glutathione reductase, 4 mM glutathione, and the appropriate amount of hepatic tissue supernatant. The oxidation of NADPH by cumene hydroperoxide (0.18 mM) added to the sample tube was followed spectrophotometrically at 340 nm at 30°C An additional blank, containing all components except the sample, was used to correct for nonenzymatic oxidation of GSH and NADPH by cumene hydroperoxide. One unit of GPx was defined as 1 mM substrate converted in 1 min. Catalase activity was measured as described by Johansson and Borg (1988). Catalase is able to decompose H_2O_2 by two types of reaction. Both reactions include a first step of formation of an intermediate consisting of the enzyme and H_2O_2. In a second step the catalase activity was measured by reaction of this intermediate with a hydrogen donor other than H_2O_2. Methanol was used as the hydrogen donor, and we measured the production of formaldehyde spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Sigma, USA) as a chromogen.

7. Statistical analysis

Statistical analysis was performed using SPSS/ PC+ statistical package. The level of significance was established at $P<0.05$.

RESULT AND DISCUSSION

1. Haematological properties

Red blood cell (RBC) count, hemoglobin (Hb) and hematocrit (Ht) of fish exposed to the different levels of inorganic mercury were summarized in Table 1. The predominant haematological finding was a significant decrease of the total number of RBC in fish exposed to 5 and 10 mg Hg Kg b.w.$^{-1}$ thereby indicating a severe anaemia (Table 1). These results, according to those of other studies carries out on *Dicentrarchus labrax* (Gwozdzinski *et al.* 1992), *Oreochromis aureus* (Allen 1994), and *Aphanius dispar* (Hilmy *et al.* 1980) exposed to mercury exhibited higher mercury concentration induced anaemia. Our study also show that erythrocyte count, Hb concentration and Ht level in fish exposed inorganic mercury were significantly decreased in comparison to control (Table 1). With regard to the Hb concentration, no significant differences between treatment and control groups could be observed at day 3. However, The haemoglobin content was significantly decreased in the mercury-treated group ($\geq$ 1 mg Hg Kg b.w.$^{-1}$) compared to control group. It has been observed that higher

Table 1. Red blood cell count, hemoglobin concentration and hematocrit level of common carp, *Cyprinus carpio* L. exposed to inorganic mercury

Parameter	Day	Mercury administration dose (mg Hg Kg b.w.$^{-1}$)			
		Control[1]	1	5	10
RBC (million mm^{-3})	3	168.4±9.4	172.4±11.9	154.9±8.5	146.4±7.3*
	7	172.6±10.5	161.4±9.4	145.6±6.9*	137.3±10.6**
	15	169.4±9.6	153.4±9.4	139.4±9.4**	135.6±8.6**
Hb (g dL^{-1})	3	7.5±0.5	7.3±0.3	7.1±0.5	6.8±0.4
	7	7.7±0.3	6.3±0.4*	5.3±0.3*	5.1±0.2**
	15	7.4±0.4	6.5±0.5	5.4±0.6*	4.9±0.3**
Ht (%)	3	29.5±1.9	28.2±3.1	28.3±2.6	27.2±1.1*
	7	30.1±0.9	27.3±1.2*	26.5±1.3*	24.1±1.2**
	15	28.6±2.2	26.4±3.7	25.7±2.1	23.5±0.9**

Value are means±SE (n = 7). Differences from control: *$P<0.05$, **$P<0.01$. RBC: red blood cell, Hb: hemoglobin, Ht: hematocrit. [1]PBS injection.

Table 2. Changes of serum Cl, P, Mg, Ca and osmolaity levels in common carp, *Cyprinus carpio* L. exposed to inorganic mercury

Parameter	Day	Mercury administration dose (mg Hg Kg b.w.$^{-1}$)			
		Control[1]	1	5	10
Cl (mM)	3	140.2±6.6	143.6±3.6	135.3±7.4	121.5±3.8**
	7	138.4±3.5	142.3±2.9	121.3±3.7**	119.3±2.1**
	15	143.6±1.9	139.3±3.1	128.6±3.1**	124.5±3.9**
P (mM)	3	2.49±0.04	2.53±0.08	2.38±0.04	2.41±0.11
	7	2.47±0.05	2.49±0.03	2.51±0.08	2.49±0.05
	15	2.53±0.09	2.44±0.07	2.47±0.07	2.48±0.09
Mg (mM)	3	1.45±0.04	1.55±0.06	1.48±0.06	1.48±0.08
	7	1.49±0.03	1.51±0.03	1.42±0.04	1.43±0.05
	15	1.46±0.05	1.48±0.05	1.51±0.11	1.51±0.07
Ca (mM)	3	2.76±0.03	2.84±0.04	2.78±0.02	2.21±0.04**
	7	2.95±0.02	2.42±0.02	2.22±0.03**	2.13±0.02**
	15	2.69±0.05	2.72±0.07	2.43±0.07*	1.89±0.03**
Osmol (mOsm)	3	245.7±5.3	254.2±7.9	224.6±7.3*	229.5±5.9*
	7	254.9±7.3	236.1±7.1	236.3±6.9*	194.2±7.1**
	15	239.4±6.1	225.2±9.3	214.2±9.2**	200.3±7.1**

Value are means±SE (n=7). Differences from control: *$P<0.05$, ** $P<0.01$. Cl: chloride, P: phosphate, Mg: magnesium, Ca: calcium. Osmol: osmolality. [1]PBS injection.

concentration of mercury causes a decrease in Ht in a time dependent manner. Especially significant reduction ($P<0.01$) in Ht have been noticed in all treatment group of 10 mg Hg Kg b.w.$^{-1}$ after 3 days exposure in comparison to control group. Our studies provide evidence that inorganic mercury effect on erythrocyte hemolysis. Decline in RBC count, hemoglobin concentrations and hematocrit presumably reflect erythrocyte hemolysis and/or irreparable damage of gill morphology and function (Gupta and Dua 2002). The decrease in hemoglobin concentration may be due to either an increase in the rate at which hemoglobin is destroyed or a decrease in the rate of hemoglobin synthesis.

2. Serum inorganic components

Result of blood serum inorganic components profile of both the control and treatment carp in the present study are given in Table 2. As shown in Table 2, There was a clear trend of decreasing serum chloride and calcium concentration. Significant decrease ($P<0.01$) of chloride ion concentration was observed at 5 mg and 10 mg Hg dose-exposed group of fish after day 3 and day 7 the exposure, respectively, in the laboratory investigation. However, magnesium and phosphate indices revealed a marginal or no deviation from control values (Table 2). Many laboratory studies have documented inhibitory effects of various metals on gill function of fish (Evans, 1987; Watson and Benson, 1987) and crab (Bjerregarrd and Vislie, 1985). Indeed, The gills of fresh water teleosts function as the primary site for the active absorption of ions from the external media and for the respiratory exchange of gases.

Mercury can cause altered osmoregulation in both marine and freshwater fish. In this study, carp of control group normally maintain a blood osmolality between 239 and 254 mOsm kg^{-1} (Table 2). Following the 7 days exposure to mercury, all treated groups had significantly lower mean blood osmolalities ($p<0.05$) when compared to a control group. This result can be explained by direct mercury-induced osmoregulation failure. Lock *et al.* (1981) suggested that mercury cause osmoregulatory effects primarily by producing an increase in the permeability of the gills to water. Report from Stinson and Mallatt (1989) have described increasing permeability of the gills in lamprey response to mercury poisoning.

3. Hepatic enzyme assay

Aquatic organisms posses different defense mechanisms to counteract the impact of aquatic pollutants.

Table 3. Changes of hepatic catalase and glutathione peroxidase activities in common carp, *Cyprinus carpio* L. exposed to inorganic mercury

Parameter	Day	Mercury administration dose (mg Hg Kg b.w.$^{-1}$)			
		Control[1]	1	5	10
CAT (μM mg protein^{-1} min^{-1})	3	78.9±5.9	72.6±2.2	73.6±4.9	71.6±3.7
	7	71.9±5.3	84.9±5.9*	58.3±4.6*	46.3±6.1**
	15	81.4±3.7	77.9±4.3	42.6±4.2**	47.5±2.8**
GPx (nM mg protein^{-1} min^{-1})	3	6.9±0.5	8.9±0.7*	5.6±0.5*	5.8±0.5*
	7	7.1±0.3	8.1±0.5	5.3±0.5**	6.1±0.6*
	15	6.7±0.6	7.1±0.5	4.9±0.5**	3.6±0.6**

Value are means±SE (n=7). Differences from control : *$P<0.05$, ** $P<0.01$. CAT : catalase, GPx : glutathione peroxidase. [1]PBS injection.

In case of heavy metal, it accumulated in internal organ may trigger redox reactions that generate reactive oxygen species. These compounds may induce physiological and biochemical alteration in fish. Moreover, hepatic antioxidant enzyme has been reported as a biomarker for evaluation of the physiological status. The current carp hepatic GPx activity results demonstrated that only low Hg levels promote increasing the hepatic GPx activity, where high Hg concentration, such as 5 and 10 mg Hg Kg b.w.$^{-1}$, induced inhibition of enzyme activity. Several studies have established that the activity of GPx in response to mercury was suppressed in rat kidney (Chung *et al.* 1982) and increased in *Dicentrarchus labrax* erythrocytes (Gwozdzinski *et al.* 1992). However, Heisinger and Scott (1985) reported that mercury did not modify either selenium-dependent or selenium-independent hepatic GPx activities of *Ictalurus melas*. In this study, hepatic catalase activity of carp exposed to higher concentration of mercury (5, 10 mg Hg Kg b.w.$^{-1}$) showed significant decreasing in activities after 7 days exposure. Hepatic GPx activity of higher concentration of mercury (5 mg and 10 mg Hg) was significantly decreased compared to control group ($P<0.05$). While an elevation in GPx activity was observed in lower treatment group (1 mg Hg) than that of control fish. According to Aksnes and Njaa (1981), fish, being more susceptible to oxidative damage, have high GPx activity in general, whereas in rainbow trout GSH -depleted liver, no peroxide-stimulated GSH disulfide release has been observed (Bell *et al.* 1986). The present study concerning 3 days exposure to lower Hg concentration shows that *C. carpio* hepatic GPx activity increase is possibly due to increased H_2O_2 production as well as enzyme-inducing effects, whereas 7 days exposure shows a suppression of GPx activity, which may be attributed to the influence of higher Hg concentration. Similarly, in fish erythrocytes exposed to 1 mM Zn, decreased activities of CAT and GPx were also observed, along with a decrease in the thiolic group content by Akahori *et al.* (1999). In conclusion, sublethal exposure of inorganic mercury may be responsible for the disruption of homeostasis of blood chemistry, osmoregulatory functions and inhibition of hepatic antioxidant enzyme in carp.

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