THE EFFECTS OF SHORT-TERM EXPOSURE TO CADMIUM AND COPPER ON SIALIC ACID IN CARP (*Cyprinus carpio)* TISSUES

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ABSTRACT

The aim of the present study was to determine the effects of cadmium and copper on the sialic acid levels of liver, gill, muscle and kidney of *Cyprinus carpio* following a 7-days exposure period at static conditions. Sialic acids (N-acetylneuraminic acids, SAs) are negatively charged monosaccharides that are common constituents in the oligosaccharides of vertebrates and some invertebrate species. Quantitative and qualitative differences in sialic acid are seen in health and disease, and at different stages of cell growth, differentiation, aging and malignant transformation. In this study, adult carps were exposed to 0.5, 2.5 and 5.0 ppm copper and 0.1, 0.5 and 1.0 ppm cadmium, and also for interaction of 0.5+0.1 ppm copper+cadmium concentrations under static conditions for one week. At the end of 7 days, all carps were dissected into their liver, gill, muscle and kidney tissues for evaluation of heavy metal accumulation (Cu and Cd), and for analyzing the sialic acid level. Accumulation of Cu and Cd in the tissues investigated was increased with the dose of the metal. Under *in vitro* conditions, Cu, which is a useful ion for normal tissue function, has an antagonistic effect with sialic acid in the tissue. In contrast, Cd, which is not involved in any physiological function, has a synergistic effect with sialic acid. According to the results of this study, it could be suggested that tissue sialic acid interacts with metal ions under *in vitro* conditions. On the other hand, it is also possible that these results are due to direct effects of the metal ions on sialic acid metabolism.

KEYWORDS: Carp, *Cyprinus carpio*, copper, cadmium, accumulation, sialic acid, interaction.

INTRODUCTION

The expansion of industrial activity in recent years has led to a remarkable increase of the presence of heavy metals in the environment [1]. Pollutants such as heavy metals enter living organisms by way of the food chain, and they can accumulate in many tissues [2-4].

Copper (Cu) is an essential trace element for living organisms, and is used as a co-factor for structural and catalytic properties in a variety of enzymes, including catalase, cytochrome oxidase and superoxide dismutase [5]. Though required as an essential trace metal, high Cu concentrations can be toxic [6-9]. Copper is a widespread pollutant in aquatic systems [10, 11]. Aquatic contamination of Cu has both natural and anthropogenic causes. In particular, Cu is frequently used to control aquatic vegetation in fish culture systems [11].

Cadmium (Cd) is a widespread heavy metal continuously introduced into the atmosphere and soil from a variety of sources, including the smelting of ores, the burning of fossil fuels, waste incineration, urban traffic, and as a by-product of phosphate fertilizers. [13]. Cd does not have a physiological role in living organisms. However, it can enter the food chain as a result of bioaccumulation and induce health problems in organisms [13-15]. Cd may cause toxicity by disturbing the cellular homeostasis of essential metal ions, such as copper, zinc and calcium. Cd has a high affinity for zinc and calcium-binding sites and can displace these metals from preexisting complexes [16, 17].

The most common effects of acute and short-term exposure to Cd in animals are degenerative problems in the liver [18] and kidney [19], toxic effects on mice bone marrow [20] and tissue damage [7, 21]. It was reported that short-term and chronic exposure to Cu could alter many physiological parameters in rainbow trout, *Oncorhynchus mykiss* [21]; in frog, *Rana ridibunda* [6, 23, 22]; in *Haliotis rubra* [25], and in freshwater fish, such as *Oreochromis niloticus* [26] and *Cyprinus carpio morpha* [27].

Sialic acids (N-acetylneuraminic acids, SA) are negatively charged monosaccharides that are common constituents in the oligosaccharides of vertebrates and some invertebrate species. The majority of sialic acid in higher animals is bound up in glyco-conjugates. SA are possibly the most biologically important monosaccharide units of glyco-conjugates. SA often occurs as the terminal monosaccharide of oligosaccharide chains of glycoproteins, glycosphingolipids and GPI anchors. Both its negative charge and its terminal position make it critical in numerous biological processes. SA impart a net negative charge to the cell surface and are important in cell-to-cell and cell-to-matrix interactions [28, 29].

Quantitative and qualitative differences in SA are seen in health and disease, as well as at different stages of cell growth, differentiation, aging and malignant transformation [28-33]. In recent years, it has been reported that levels of sialic acid are increased in certain types of cancer [34-38], and it has been proposed that sialic acid may be a useful tumor marker for some cancer types [39].

It was reported that exposure to metal toxicity may cause an increase in plasma and tissue sialic acid concentrations [40-42]. Recent studies have shown that some metal cations form complexes with the membrane-bound SA under *in vivo* physiological conditions, and it was proposed that this interaction might be a cause of metal toxicity [43, 44].

Although the accumulation of Cu and Cd in many fish species has been studied [2, 3, 7, 11, 22], there is no information concerning the *in vivo* effects of these metal ions on the concentration of SA in tissues. Therefore, in the current study, accumulations of Cu and Cd were examined in the tissues of freshwater fish (*Cyprinus carpio)*. Additionally, the relationships between these metals and the tissue contents of SA were investigated.

MATERIALS AND METHODS

Experiment

The fish used in this study was transferred from forming DSI (The General Directorate of State Hydraulic Works) ponds (Ipsala, Edirne-Turkey) to a controlled laboratory environment, and acclimatized to laboratory conditions for 4 weeks in aquariums measuring 50x50x100 cm. The room temperature and photoperiod during the experiments were 20 ± 1 °C and 12 L:12 D, respectively.

Some of the other physical and chemical parameters of the aquarium environment are listed below:

pH : 8.17 ± 0.1

Total hardness : 268.7 ± 4.8 mg/L $CaCO_3$

Dissolved O_2 : 6.67 ± 0.6 mg/L

Seven aquariums, one of which was designed as a control, were used to conduct experiments. $CdCl_2.H_2O$ (Merck) and $CuSO_4.5H_2O$ (Merck) salts were used for the preparation of stock metal solutions. Six aquariums were filled with 100 L filtered (active carbon) tap water and metal stock solutions were added to each aquarium so that the final solutions were 0.5, 2.5 and 5.0 ppm Cu; 0.1, 0.5 and 1.0 ppm Cd, and 0.5+0.1 ppm Cu+Cd. The seventh aquarium was used as a control. Five fish were added to each aquarium. The aquariums were aerated and fish were fed with fish bait during the experiment. Every two days, the water in each aquarium was replenished to keep the metal concentrations constant.

At the end of seven days, the fish were anaesthetized with MS 222 (tricane methanesulphonate, 75 mg/L) for tissue (liver, kidney, gills, muscle) sampling.

Copper and cadmium determinations

The tissues were digested with concentrated nitric acid and perchloric acid (1:1, v/v) at 120 °C for 2 h in an autoclave. Following acidic digestion, all samples were analyzed for the two elements by atomic absorption spectrometry (UNICOM 929 AA). All digested samples were analyzed three times for each metal [45, 46].

Sialic acid determinations

Tissue samples were frozen at -70 °C until use. After melting, tissues were homogenized in phosphate buffer, pH 7. SA was liberated with perchloric acid hydrolysis [47, 48]. Spectrophotometric determination was carried out using the Shimadzu UV/vis spectrophotometer at 525 nm.

The chemicals used for spectrophotometric determinations were purchased from Merck.

Statistical analysis

Statistical analysis of data was performed using the SPSS statistical package program (version 11.0). As metal accumulation and tissue SA levels in replicate aquariums were not significantly different from each other, samples were pooled and two-way Anova was performed, followed by SNK test as post-hoc test. Groups were considered to be significantly different from each other if $p< 0.05$. Results were expressed as the mean ± standard deviation.

RESULTS

No mortality was observed at control group while the animals in aquariums containing 5 ppm Cu were killed after 5 days.

Metal accumulation in the tissues

The results of the metal accumulation in the fish tissues exposed to Cu, Cd and Cu/Cd are presented in Figs. 1-4. In comparison with the control group, Cu and Cd accumulated in the tissues, dramatically increasing in a dose-dependent manner (Tables 1-2).

TABLE 1 - Cu (mg/L) accumulation in carp (*Cyprinus carpio*) exposed to 0.0, 0.1 and 2.5 mg/L copper (mean ± standard deviation ,* p>0.05).

Dose	Tissues	Mean ±SD
Cu 0.1 ppm	Kidney	0.0032±0.0008 *
	Liver	0.0044±0.0011
	Muscle	0.0032±0.0008*
	Gill	0.0026±0.0005
Cu 2.5 ppm	Kidney	0.0078±0.0016*
	Liver	0.0058±0.0015
	Muscle	0.0068±0.0008*
	Gill	0.0044±0.0005
Control	Kidney	0.0000±0.0000
	Liver	0.0010±0.0007
	Muscle	0.0000±0.0000
	Gill	0.0000±0.0000

TABLE 2 - Cd (mg/L) accumulation in carp (*Cyprinus carpio*) exposed to 0.0. 0.1. 0.5 and 1.0 mg/L cadmium (mean ± standard deviation, * p>0.05).

Dose	Tissues	Mean ±SD
Cd 0.1 ppm	Kidney	0.01260±0.0052*
	Liver	0.01160±0.0021*
	Muscle	0.00260±0.0008*
	Gill	0.00520±0.0013*
Cd 0.5 ppm	Kidney	0.01620±0.0027*
	Liver	0.00820±0.0021*
	Muscle	0.00480±0.0004*
	Gill	0.00740±0.0011*
Cd 1.0 ppm	Kidney	0.02100±0.0015*
	Liver	0.01320±0.0052*
	Muscle	0.00720±0.0013*
	Gill	0.00700±0.0015*
Control	Kidney	0.00000±0.0000
	Liver	0.00000±0.0000
	Muscle	0.00000±0.0000
	Gill	0.00000±0.0000

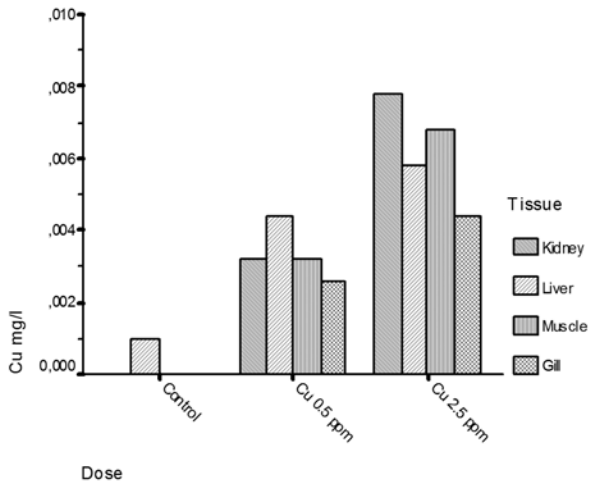

FIGURE 1 - Cu concentrations in the fish tissues.

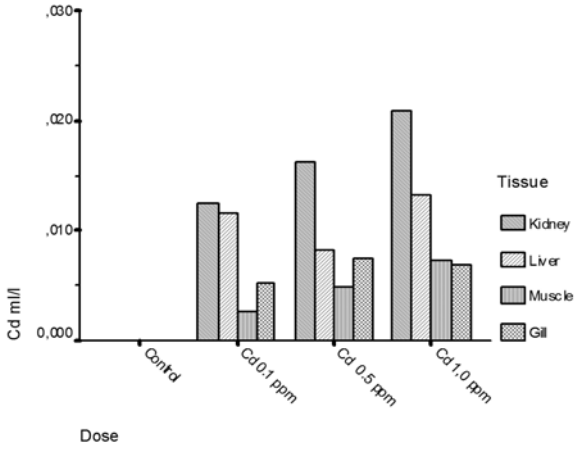

FIGURE 2 - Cd concentrations in the fish tissues.

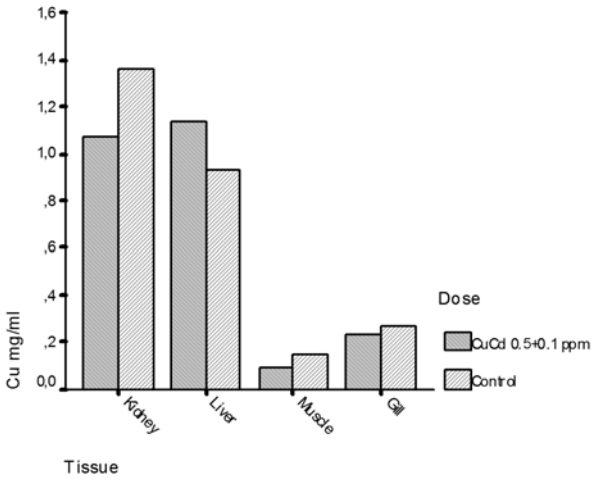

FIGURE 3 - Cu concentrations in the dose of Cu+Cd mixture.

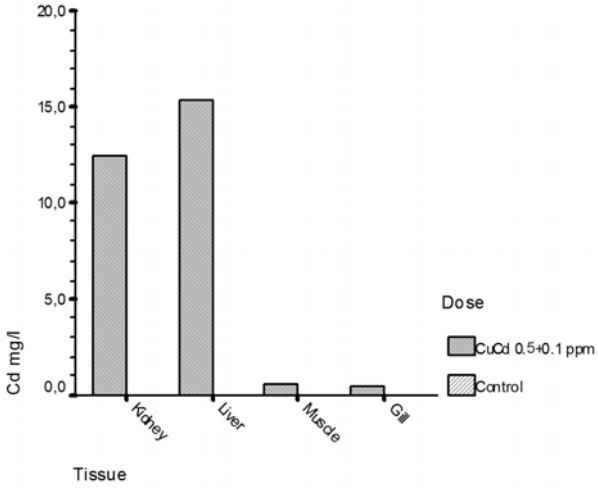

FIGURE 4 - Cd concentrations in the dose of Cu+Cd mixture.

Sialic acid levels in the tissues

The results of the tissue SA analysis are shown in Figs. 5-7.

1. Cu group. Except in the gills, tissue levels of SA at metal doses of 2.5 ppm were significantly decreased (Fig. 5). In the group treated with 0.5 ppm Cu, there were no statistical differences between the SA levels of the experimental and control groups.

2. Cd group. SA levels in the livers of the Cd groups were higher than the control group (Fig. 6). However, while the Cd levels were increasing dose-dependently, the SA levels were decreasing.

In the group treated with 0.1 ppm Cd, SA levels in the muscle were significantly increased. In the kidney, the level of SA was statistically decreased at a dose of 1.0 ppm. There was no significant difference in the gills.

3. Cu+Cd group. It was observed that the SA levels in tissues were not significantly different from the control

TABLE 3 - Sialic acid level in muscle, gill, liver and kidney tissues (mean ± standard deviation mg/ml).

	0.5 ppm Cu	2.5 ppm Cu	0.1ppm Cd	0.5 ppm Cd	1.0 ppm Cd	0.5+0.1 CuCd	Control
Muscle	0.070±0.037	0.077±0.031	0.315±0.227	0.130±0.005	0.101±0.030	0.089±0.035	0.151±0.118
Gills	0.210±0.122	0.179±0.017	0.273±0.163	0.211±0.008	0.224±0.103	0.227±0.170	0.271±0.161
Liver	0.966±0.353	0.614±0.402	2.142±0.699	1.488±0.983	1.357±0.464	1.138±0.428	0.932±0.354
Kidney	0.929±0.247	0.701±0.321	1.209±0.672	1.363±0.475	0.873±0.186	1.067±0.583	1.362±0.585

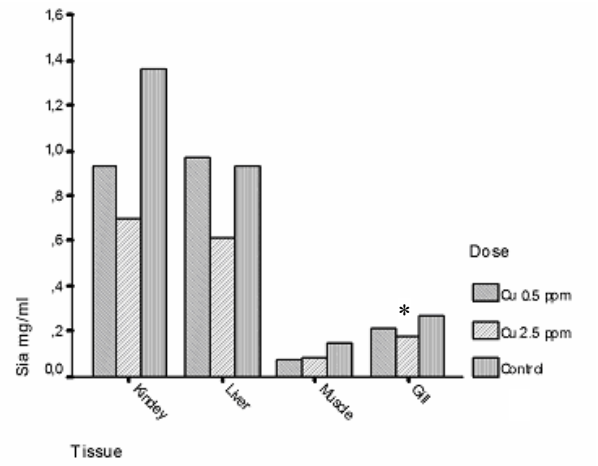

FIGURE 5 - Tissue SA concentrations in the Cu doses.

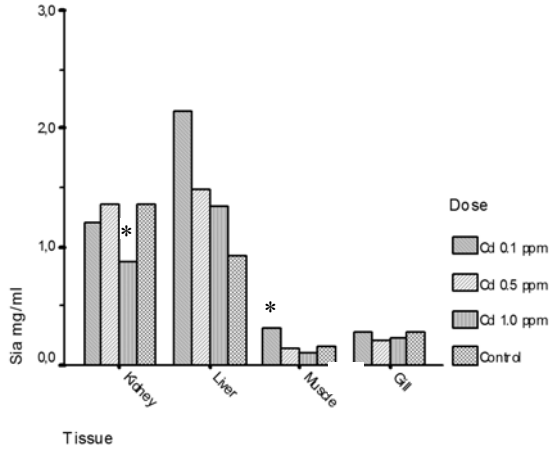

FIGURE 6 - Tissue SA concentrations in the Cd doses.

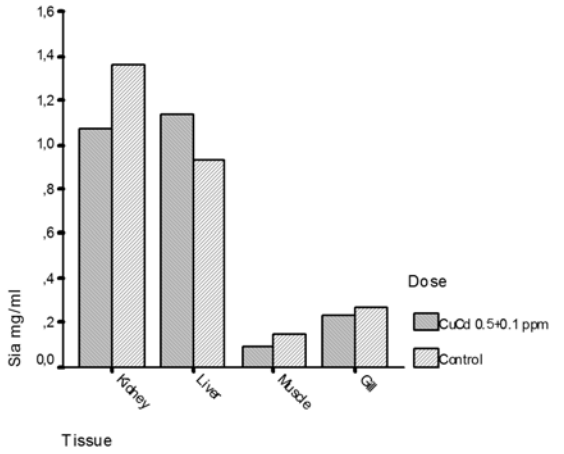

FIGURE 7 - Tissue SA concentrations in the doses of Cu+Cd mixture.

group (Fig. 7). However, the results are similar to those seen above in the Cd-treated groups (Fig. 6).

DISCUSSION

In this study, the distributions of Cd and Cu and the relationships between these metals and sialic acid were investigated in the tissues of C. carpio after 7 days of exposure to the metals. The results of the metal analysis demonstrated that the accumulation of Cd and Cu in the kidney, liver, muscle and gills increases with the dose of the metal in water (Fig. 1). Similarly, it was reported that Cd accumulation in the liver and kidney of *Carassius auratus* increases with the dose of the metal [7]. These researchers suggested that Cd accumulation in the liver (0.021±0.0015) and kidney (0.0132±0.0052) were much higher than that in the gill (0.007±0.0015; Fig. 2, Table 2). This result may be explained by the fact that the liver (an important organ in storage and detoxification) and the kidney (a waste metabolism/ regulation organ) can accumulate more Cd that is not involved in any physiological function [7].

In the present study, it was also observed that Cd and Cu accumulation in liver and kidney were higher than those in gills and muscles (Figs. 1-2, Table 1). In animals exposed to the mixture of Cd/Cu, there was no statistically significant change in the tissue Cu accumulation (Fig. 3). However, Cd levels were significantly increased in both the liver (15.414±1.217) and kidney (12.445±0.84) (Fig. 4). This difference may be explained by the fact that Cu absorption is prevented by Cd.

Sialic acid (N-acetylneuraminic acid) is one of the carbohydrates of an oligosaccharide unit in glycoproteins that compose cellular membranes. It contains α-hydroxycarboxylate moiety that is known to chelate cations [43]. With NMR in a potentiometric and spectroscopic study, Saladini et al. [43] reported that sialic acid has great affinity for the toxic bivalent metals Cd and Pb under near-physiological conditions. Additionally, they show that the high stability of the complex species formed with these metals may account for the mechanism of metallic toxicity. In another study, it was reported under in vivo conditions that Al(III) at a physiological pH is present in a complex with sialic acid [44]. According to these researchers, the toxic effect of Al(III) towards cellular membranes may be due to its coordination by protein-bound sialic acid.

The results of the present study are similar to the findings of the above ones. At a dose of 2.5 ppm Cu, metal accumulation in liver and kidney was increased (Fig. 1),

but levels of sialic acid were reduced (Fig. 5). This decrease in sialic acid may occur because Cu is in a complex with SA in tissues.

The accumulation of Cd in tissues was significantly increased depending on the dose (Fig. 2). Similarly, the SA in the liver was increased, but this change in SA was reduced when levels of Cd were raised (Fig. 6). In the kidney tissue, the level of SA was reduced at the highest doses of Cd, similarly to the group treated with Cu. According to these findings, it could be suggested that tissue sialic acid interacts with metal ions under in vivo conditions. The analysis of SA in the Cu/Cd group yielded findings that were parallel to the Cd group (Fig. 7). These results also suggest that Cu absorption is prevented by Cd.

Finally, according to the results of this study, in *Cyprinus carpio*:

1. Accumulation of Cu and Cd in the tissues investigated was increased with the dose of the metal.
2. It was observed that these metals have different effects on the sialic acid content of tissues. Under *in vivo* conditions, Cu, which is a useful ion for normal tissue function, has an antagonistic effect on Cd-induced change in sialic acid. In contrast, Cd, which is not involved in any physiological function, has a synergistic effect with sialic acid.
3. It was indicated that these metals are in a complex with sialic acid in the membranes of the tissues examined, and this interaction between the metal ions and the sialic acid, as explained by other researchers [44], may actually cause the cellular toxicity of the metal. On the other hand, it is also possible that these results are due to direct effects of the metal ions on sialic acid metabolism. To clarify the mechanism of toxicity for Cu and Cd, it is necessary to complete a more detailed *in vitro* analysis.

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