Original Paper

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Effect of thiamine and vitamin C on tissue lead accumulation following experimental lead poisoning in *Cyprinus carpio*

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Keywords

lead, tissue, common carp, thiamine, vitamin C

Abstract

The present study was conducted to evaluate the possible ameliorative effects of vitamin C and thiamine on lead accumulation in kidney, liver, muscle, brain and gill of experimentally lead-poisoned common carp. At the beginning of the experiment, fish (n=120) were divided into 4 groups randomly with group 1 being considered as the control group. Groups 2, 3 and 4 were exposed to lead acetate (5 mg/L, 15 days); groups 3 and 4 received vitamin C (500 mg/kg feed) and thiamine (50 mg/kg feed) during lead acetate exposure, respectively. Following this, it was observed that lead exposure caused a significant ($p < 0.05$) increase in lead content in all examined tissues of fish in group 2 in comparison to control group. It was also found that thiamine supplementation slightly decreased the augmented levels of lead in the muscle, brain and gill tissues, which was not significantly different from that of the control group. Similarly, vitamin C supplementation reduced the augmented concentrations of lead in the muscle to the levels that were not significantly different from that of the control group. Based on the present results, neither thiamine nor vitamin C was effective in providing a significant reduction of tissue lead burden in groups 3 and 4 as compared to group 2. Thus, monotherapy with such vitamins cannot be proposed as a suitable therapeutic approach for the effective reduction of the tissue lead burden in common carp. However, further investigations using other dosing regimens of each vitamin or combined treatment with chelators are required to reach such a conclusion.

Abbreviations

Pb: Lead
DMSA: DiMercaptoSuccinic Acid
Na_2Ca-EDTA: Sodium Calcium Edetate

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Introduction

Lead (Pb) is an environmental pollutant and its pollution has increased drastically in the last century resulting in lead poisoning becoming one of the greatest concerns of the world [1, 2]. Due to its physical and chemical properties, lead has been utilized in many industries; as a result, through industrial discharges, sewages, batteries etc., lead has found its way into fresh waters [3-6]. Having an accumulative behavior, exposure to prolonged low levels of heavy metals can induce their high accumulation in tissues without causing mortality in fish [6-8].

Several adverse effects of lead toxicity including neurological, hematological, gastrointestinal, reproductive, circulatory and immunological dysfunctions, as well as its carcinogenic effects have been reported [9, 10]. Since heavy metal pollutants build up in food chain, consumption of seafood may pose a high risk, especially to human consumers [6, 11]. Although the currently approved approach against lead toxicity is using chelating agents which will bind with and withdraw lead from lead-burdened tissues, some toxic effects of such agents necessitate research on alternative therapeutic approaches, particularly using natural compounds [12-14]. Vitamin C is a free radical scavenger and is used as a prophylactic agent against lead induced oxidative stress by quenching reactive oxygen species. It has been also proposed that vitamin C could have a chelation capacity for lead [9, 15, 16]. Thiamine can also make readily excretable complexes and expedite lead elimination [13, 15].

The present work seeks to evaluate the effects of thiamine and ascorbic acid on reducing lead accumulation in some tissues of experimentally lead-poisoned common carp. To our knowledge, this is the first study concerning the effect of thiamine and ascorbic acid on the tissues lead content in carp. These studies may be helpful in providing practical approaches against lead toxicity in polluted freshwaters in order to diminish some seafood hazards threatening animals and human health.

Results

Mortality was not observed among experimental groups during the experiment. Lead accumulation in various tissues following lead acetate and vitamin administration is shown in figures 1 and 2 as mean ± SEM. Lead exposure caused a

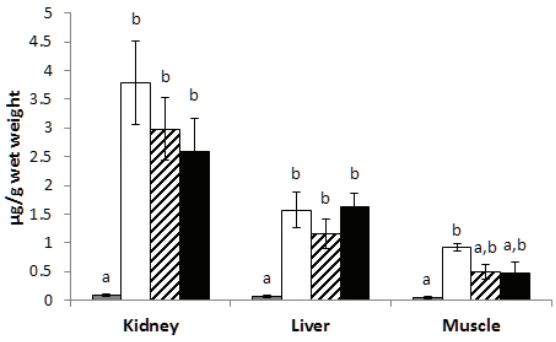

Figure 1
Effect of thiamine and ascorbic acid on tissue lead concentrations (µg/g wet weight) of kidney, liver and muscle in lead exposed common carp. Each value represents mean ± SEM (n=10). Gray, white, black, and cross-hatched bars indicate control, lead, lead and vitamin C, and lead and thiamine groups, respectively. Values with no common superscript differ significantly ($p < 0.05$).

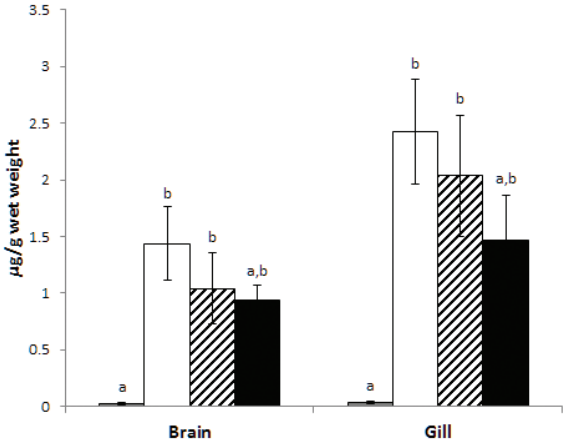

Figure 2
Effect of thiamine and ascorbic acid on tissue lead concentrations (µg/g wet weight) of brain and gill in lead exposed common carp. Each value represents mean ± SEM (n=10). Gray, white, black, and cross-hatched bars indicate control, lead, lead and vitamin C, and lead and thiamine groups, respectively. Values with no common superscript differ significantly ($p < 0.05$).

significant ($p < 0.05$) increase in the lead content of the kidney, liver, brain, muscle and gill of fish in group 2 in comparison to the control group. The highest tissue lead accumulation following lead acetate treatment was observed in the kidney followed by the liver, muscle, gill and brain (Figures 1 and 2).

As the results show, neither thiamine nor ascorbic acid caused a significant declining effect on tissue lead burden as compared to group 2. Thiamine supplementation in group 4 decreased the augmented levels of lead in muscle, brain and gill tissues to the levels that were not significantly dif-

ferent from that of the control group. In a similar manner, ascorbic acid supplementation decreased the augmented concentrations of lead in the muscle, but not in other examined tissues, to the levels that were not significantly different from that of the control group (Figures 1 and 2).

Discussion

The existence of high levels of heavy metals in food animals is of great concern. Lead exposure has been shown to decrease vitamin content in various tissues and administration of some vitamins has also been proposed to reduce the toxic symptoms of lead [17, 18]. The use of dietary constituents with possible chelating properties may be considered as a potential approach to alleviating health problems related to toxic metals exposure. In this experiment, the possible declining effects of ascorbic acid and thiamine on tissue levels of lead in Pb-exposed carp have been investigated.

Accumulation of heavy metals in fish organs may be affected by several parameters such as exposure dose and time, route of administration, age of fish, interaction with other metals, water chemistry and metabolic activity of the fish [19]. In fish, gill and intestine can be considered as the main sites of Pb uptake [20, 21]. Based on the present results, the order of Pb accumulation in different organs of fish following lead acetate exposure was kidney > liver > muscle > gill > brain. The organ distribution of lead in our study is to some extent reminiscent of previous work documented in carp [22]. Heavy metals affinity for liver and kidney may be attributed to the higher metabolic activity of these organs and their detoxifying role. However, it has been shown that the highest concentration of lead in lead-exposed Tilapia zilli was observed in the gill followed by liver, brain and then muscle [23].

Many of the chelating agents used to reduce lead content of tissues have not been approved in food animals [24]. Both thiamine and vitamin C have been suggested to have chelating capacities for lead; they can also reduce intestinal lead absorption. Vitamin C could even act as a free radical scavenger which magnifies its importance against lead-induced tissue damages [9, 12, 15, 25]. It has been previously reported that ascorbic acid and thiamine partly exhibited protection against Pb-induced tissue biochemical alterations in carp as manifested by alleviation of toxicant-mediated rise in lipid and protein oxidation markers and metabolic enzyme activities in some organs [26].

Our findings indicate that thiamine supplementation decreased the augmented levels of lead in muscle, brain and gill tissues to the levels that were not significantly different from that of the control group. However, thiamine administration could not significantly reduce the tissue lead burden as compared to group 2. This agrees with the observations of Coppock et al. (1991) showing that thiamine alone was not effective in reducing Pb concentration while administration of Na2,Ca-EDTA alongside thiamine was proven as an effective treatment for experimentally induced environmental lead poisoning in cattle [27]. Moreover, the low efficacy of thiamine alone on the retention of lead in tissues has also been reported in mice [25] and goat [28]. In contrast to our findings in common carp, Ghazaly (1991) showed the effectiveness of thiamine in preventing lead deposition in blood, kidney, liver, brain and muscle of lead-exposed Tilapia Zilii [29]. Furthermore, it has been reported that thiamine administration could decline the blood and ovaries' lead content in subacute lead poisoning in sheep which was not the case with the spleen and brain [13].

Indeed, ascorbic acid supplementation decreased the augmented concentrations of muscle lead to the levels that were not significantly different from that of the control group; however, it did not have the same effect on other examined tissues of carp. Although the efficacy of vitamin C in providing effective reduction of oxidative stress has been reported in lead-exposed rats, its effect on reducing the lead burden in liver, kidney and brain was not significant. Besides, co-administration of vitamin C during chelation with meso-2,3-dimercaptosuccinic acid (DMSA) or monoisoamyl DMSA had little or no additive effect on the depletion of lead compared to the effect of chelators alone [17]. However, Vij et al. (1998) indicated that vitamin C supplementation in lead-exposed male rats significantly reduced the level of lead in liver, kidney, and blood [18]. Ascorbic acid increased urinary elimination of lead and reduced the hepatic and renal lead burden in rats [30]. It has been proposed that the effect of vitamin C on lead absorption and excretion may be more obvious in low-exposed subjects with higher vitamin C supplementation. On the other hand, in human and animals exposed to high concentrations of lead,

the decline of lead burden following vitamin C administration is less significant [31]. As noticed above, some variations exist in the literature concerning the effects of thiamine and vitamin C on the tissue lead accumulation that might be associated with the differences in animal species, sample size, utilized doses, route and timing of exposure, experimental situations and procedures or other unknown factors.

In view of our results, it can be suggested that neither thiamine nor ascorbic acid were effective in providing significant reduction of tissue lead burden in lead-exposed common carp. Thus, monotherapy with such vitamins cannot be proposed as a suitable therapeutic approach for an effective reduction of body lead burden in common carp. However, further investigations using other dosing regimens of each vitamin or combined treatment with chelators are required to reach such a conclusion.

Materials and methods

Chemicals

Lead acetate was supplied by Merck (Darmstadt, Germany). Thiamine and ascorbic acid were purchased from Hakim Co. (Tehran, Iran) and Chemifarma Co. (Tehran, Iran). Sulfuric acid and nitric acid were supplied by Sigma (St. Lewis, MO, USA) and Merck (Darmstadt, Germany).

Experimental design and sampling

Healthy common carp (*Cyprinus carpio*; total, n=120), weighing 100 ± 10 g (mean ± SD), were obtained from a local commercial farm. Fish were divided randomly into four groups of 30 each and were held in four glass aquaria, each containing 250 L of fresh water. Fish were acclimatized for 15 days prior to the commencement of the experiment and were fed daily with commercial fish food at 2% total body weight at a fixed time. Physicochemical conditions of the water during the experimental period were dissolved oxygen, 5.5–6 ppm; temperature, 25 ± 1 °C; and pH, 7 ± 0.5. The photoperiod was a 12:12 light/dark cycle. The water in the aquaria was renewed every 48 h. The fish in group 1 were reared in normal freshwater and served as the control group. Group 2 received lead acetate (5.0 mg/L) while group 3 were exposed to lead acetate (5.0 mg/L) and received vitamin C (500 mg/kg feed). Also, group 4 received lead acetate (5.0 mg/L) and thiamine (50 mg/kg feed).

At the end of each exposure (15 days), ten fish of every aquarium were randomly selected and euthanized using tricaine methanesulfonate. Then, the liver, kidney, muscle, brain and gill were quickly removed, cleaned free of extraneous material and washed with physiological saline. Tissue samples were frozen in liquid nitrogen and stored at −70°C until analysis.

Analysis and measurements

Tissue preparation was performed using a slight modification of the wet-ashing technique [32] as described by Najarnezhad et al. (2010). Weighed pieces of tissues were digested in a 1:1 mixture of 98% sulfuric acid and 70% nitric acid. Ten milliliter of acid mixture per gram of tissue wet weight was used. Samples were heated at 120°C for 4 h, with acid mixture added as needed in a drop-wise manner to prevent charring until the organic matter was completely destroyed and finally the volume of solution reached 50 ml.

Lead concentrations in prepared samples were determined (in Toxicology Laboratory of Imam Reza hospital, Mashhad, Iran) by atomic absorption spectrophotometer (Perkin-Elmer3030) at 283.3-nm wavelength using a graphite furnace. The limit of detection for this analysis was 5 ng/ g and recovery for spiked samples was >%90. The results were expressed as µg/g wet weight of tissue samples.

Statistical analysis

All experimental values are represented as mean ± standard error of the mean (SEM). All results were analyzed using one way analysis of variance (ANOVA), followed by Bonferoni multiple comparisons test. The level of significance was set at $p < 0.05$. All calculations were performed using SPSS/PC software.

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Author Contributions

Conceived and designed the experiments: D.S., H.B. Performed the experiments: K.N., D.S., H.B. Analyzed the data: H.B. Contributed reagents/materials/analysis tools: H.B. Wrote the paper: K.N., H.B.

Conflict of Interest

None.

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