ZINC AND CALCIUM SUPPLEMENTATION TO COMBAT CADMIUM INDUCED BIOACCUMULATION IN FRESH WATER TELEOST OREOCHROMIS MOSSAMBICUS (TILAPIA)

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Received: 01 Aug 2016 Revised and Accepted: 09 Sep 2016

INTRODUCTION

Heavy metals are considered the most important constituents of pollution from the aquatic environment. These metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Accumulation levels vary considerably among metals and species [1-3]. Studies carried out with different fish species have revealed that non-essential metals can produce toxic effects in fish by disturbing physiological activities [4], biochemical processes [5], reproduction and growth [6]. Among the heavy metals, Cd is one of the most toxic, non-essential heavy metal and accumulates in tissues of aquatic organisms like fish [7, 8]. Problems with Cd bioaccumulation were studied by many authors in aquatic animals [9-14].

Cd was readily bioaccumulated and bio-concentrates in aquatic organisms. Tissue Cd concentrations build up at the site of exposure, gills in a waterborne exposure or gastrointestinal tract in a diet borne exposure and are transferred via the circulation to other tissues. Cd accumulates in nearly all tissues and organs of liver, kidney and gill reaching relatively high levels. Available reports indicate that the kidney, liver and gills were the critical targets for Cd in fishes [15-17] in which they have been reported to cause significant metabolic, biochemical and physiological effects. The bioaccumulation of heavy metals in the different fish tissues has been studied by several investigators [18-24].

Despite many years of research, we are still far from an effective treatment of heavy metal poisoning. The main therapeutic option for metal poisoning relies in chelation therapy. Chelating agents are capable of linking together metal ions to form complex structures which can be easily excreted from the body. Cd can compete and interact metabolically with essential nutrients such as selenium (Se), Ca, Zn, copper (Cu) and iron (Fe) in the body [25-27]. Zn is the most abundant trace intracellular element required for a number of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals [28]. Indeed, increasing evidence suggests that zinc plays an important role as an antioxidant and protects cellular components from oxidation. Zn is one of the most important nutritional factors influencing the metabolism and toxicity of heavy metals, including Cd.

Ca is an important element needed for the maintenance of membrane integrity and ion regulation. It plays a diverse role in the living organisms. In most of the vertebrates, it is a major component of the skeleton, but it also has vital functions in the body fluids and soft tissues [29]. Ca supplementation protected against accumulation of Cd in kidney, liver and other selected tissues of fish [30]. Cd accumulation was reduced with the increase of waterborne and dietary Ca [31-33]. Higher water Ca levels reduce the amount of Cd binding to gills [27] and reduce bronchial Cd uptake rates resulting in lower accumulation in the kidney, liver and other tissues of fish. Hence the present study was carried out to know whether the supplementation of Zn and Ca either individually or in combination would reduce the Cd bioaccumulation in the selected tissues of Cd-exposed Oreochromis mossambicus.

MATERIALS AND METHODS

Chemicals

Cadmium as cadmium chloride (CdCl₂), zinc as zinc chloride (ZnCl₂) and calcium as calcium chloride (CaCl₂) were purchased from Merck (Dornstadt, Germany). The other chemicals which were used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St Louis, Mo, USA), SD Fine Chemicals. The chemicals used for this study were of the highest purity.

Maintenance of animals (Fish)

Fish O. mossambicus (Tilapia) weighing 10±2 gm were collected from the local fresh water ponds and acclimatized to laboratory conditions. After acclimatization, fishes were exposed to 1/10th of LC₅₀ of Cd i.e. 5 ppm for 7, 15 and 30 days (d) long sojourn. After 15d Cd exposure, fish were divided into three groups. The 1st group was supplemented with Zn at a dose of 1 ppm; Group–II was supplemented with Ca at the dose of 1 ppm and Group–III received a combination of both Zn and Ca at the above said doses for 7, 15 and 30d. After specific time intervals, fish were sacrificed and liver, kidney, brain, gill and muscle tissues were isolated in ice cold conditions. Then the tissues were used for bioaccumulation studies.

RESULTS

A significant (P<0.05) elevation was observed in bioaccumulation levels during Cd exposure. The high amount of Cd accumulation was found in 30d Cd-exposed kidney (2.2353±0.410 µg/g) followed by other tissues. After supplementation with Zn and Ca, Cd accumulation was progressively decreased in all the test tissues. The maximum percentage of Cd depletion was found in 30d Ca-supplemented muscle tissue.

CONCLUSION

Our findings clearly envisage that the Zn and/or Ca supplementation is very effective in reducing the Cd toxicity in the teleostean fish, Oreochromis mossambicus.
conditions for a week in separate troughs. The experiment was carried out in the laboratory of Department of Zoology, Sri Venkateswara University, Tirupati. The laboratory temperature was maintained at 28°C ± 2°C. The fish were fed ad libitum with groundnut cake and water was renewed for every 24 h with routine changing of troughs leaving no fecal matter. The protocol and animal use have been approved by the Institutional Animal Ethics Committee (Resol. No.10 (II)/a/CPCS/IAEC/SVU/AUR-JO dt 22-12-2008), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Experimental design

Fish were divided into four groups, the first group as control and other groups as experimental. The experimental groups were exposed to sublethal concentration of CdCl₂, i.e., 5 ppm (1/10th of LC₅₀) by following the method of Kanno et al.,[34]. After the specified time intervals the test tissues like liver, kidney, muscle, brain and gill were isolated and then immediately they were washed with saline (0.9%) and 50 mg of each tissue was digested in an acid mixture of Nitric acid: Perchloric acid (3:2 V/V) for overnight. The acid mixture was then subjected to evaporation and the residue obtained was dissolved in 5 ml of double distilled water. From this 1 ml was withdrawn and analyzed for Cd concentrations by using Atomic Absorption Spectrophotometer (Schimadzu AA 6300).

Data analysis

The data was subjected to statistical analysis such as mean, standard deviation and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 11.5) package. All values are expressed as mean±SD of 6 individual samples. Significant differences were indicated at P<0.05 level.

RESULTS

The accumulation of Cd significantly increased in the selected tissues with the Cd exposure when compared to control (table 1). The maximum level of Cd accumulation was observed in 30d fish kidney (22.53±0.41 µg/g). Further liver showed high Cd concentrations when compared to other tissues. Low level of Cd accumulation was found in the brain and muscle tissues of fish over a period of 30d. Among all the selected tissues, the lowest concentration of Cd was observed in the 30d muscle tissue (4.96±0.23 µg/g).

Bioaccumulation studies

The Cd concentration levels in the selected tissues were measured by following the method of Kanno et al. [34]. After the specified time intervals the test tissues like liver, kidney, muscle, brain and gill were isolated and then immediately they were washed with saline (0.9%) and 50 mg of each tissue was digested in an acid mixture of Nitric acid: Perchloric acid (3:2 V/V) for overnight. The acid mixture was then subjected to evaporation and the residue obtained was dissolved in 5 ml of double distilled water. From this 1 ml was withdrawn and analyzed for Cd concentrations by using Atomic Absorption Spectrophotometer (Schimadzu AA 6300).

Table 1: Cd accumulation (µ g/g wet weight of the tissue) in different tissues of O. mossambicus after Cd exposure

| S. No. | Tissue | Control | Cd-exposed |
|-------|--------|---------|------------|
|       |        | 7d      | 15d        | 30d        |
| 1.    | Kidney | 1.28±0.080 | 6.38±0.520 | 14.0±0.123 | 22.3±0.410 |
| 2.    | Liver  | 1.41±0.028 | 4.02±0.330 | 11.0±0.263 | 15.7±0.370 |
| 3.    | Muscle | 0.63±0.015 | 1.71±0.266 | 3.0±0.124  | 4.9±0.230  |
| 4.    | Brain  | 0.83±0.013 | 2.82±0.353 | 4.0±0.339  | 9.1±0.357  |
| 5.    | Gill   | 1.72±0.018 | 3.04±0.326 | 6.1±0.258  | 11.5±0.314 |

Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan’s Multiple Range Test (DMRT), All experimental mean values are significant at P<0.05 level over control.

After supplementation with Zn and/or Ca, Cd bioaccumulation levels were progressively decreased in all the test tissues. 30d Ca-supplemented fish Muscle tissue showed the maximum percentage of depletion in Cd accumulation (0.45±0.196 µg/g) than the other tissues (table 2).

However, with Zn alone supplementation, all the test tissues showed moderate levels of Cd accumulation for all the time intervals (table 3). Moreover, low level of depletion in Cd accumulation was found in the test tissues under combined supplementation of Zn and Ca compared to other modes of supplementation (table 4). From the above results, it is clearly understood that the individual supplementation of Ca could tremendously reduce the Cd body burden in the test tissues under 30d long sojourn.

Table 2: Cd accumulation (µ g/g wet weight of the tissue) in different tissues of O. mossambicus after Ca supplementation

| S. No. | Tissue | 15d Cd | Ca supplementation |
|-------|--------|--------|-------------------|
|       |        | 7d     | 15d    | 30d    |
| 1.    | Kidney | 14.06±0.123 | 11.5±0.363 | 9.8±0.414 | 5.9±0.424 |
| 2.    | Liver  | 11.0±0.263 | 8.4±0.451 | 6.6±0.368 | 3.8±0.468 |
| 3.    | Muscle | 3.0±0.124  | 2.1±0.466 | 1.2±0.390 | 0.4±0.196 |
| 4.    | Brain  | 4.0±0.339  | 2.7±0.500 | 1.8±0.458 | 0.9±0.294 |
| 5.    | Gill   | 6.1±0.258  | 4.5±0.418 | 2.9±0.612 | 1.4±0.254 |

Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan’s Multiple Range Test (DMRT). All mean values are significant at P<0.05 level over 15d Cd exposure.

Table 3: Cd accumulation (µ g/g wet weight of the tissue) in different tissues of O. mossambicus after Zn supplementation

| S. No. | Tissue | 15d Cd | Zn supplementation |
|-------|--------|--------|--------------------|
|       |        | 7d     | 15d    | 30d    |
| 1.    | Kidney | 14.06±0.123 | 12.18±0.334 | 10.6±0.311 | 6.1±0.421 |
| 2.    | Liver  | 11.0±0.263 | 8.8±0.513  | 7.0±0.46  | 4.0±0.400 |
| 3.    | Muscle | 3.0±0.124  | 2.3±0.438  | 1.4±0.412 | 0.5±0.246 |
| 4.    | Brain  | 4.0±0.339  | 2.9±0.316  | 2.3±0.333 | 1.5±0.339 |
| 5.    | Gill   | 6.1±0.258  | 4.9±0.408  | 3.2±0.502 | 2.4±0.398 |

Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan’s Multiple Range Test (DMRT). All mean values are significant at P<0.05 level over 15d Cd exposure.
DISCUSSION

Bioaccumulation of heavy metals in aquatic and terrestrial ecosystems is of special concern in the interest of human welfare. Toxic elements such as heavy metal are known to bioaccumulate in the tissues of freshwater animals like fish. In particular, the bioaccumulation of Cd, arsenic (As), manganese (Mn), mercury (Hg) and lead (Pb) in the trophic food chain is the cause of concern since they can have deleterious effects on human health [35-37]. Furthermore, fish were one of the main links between the heavy metal present in the environment and the human exposure [38, 39]. The results revealed that Cd concentrations were significantly increased in all the test tissues at all exposure periods. Maximum accumulation of Cd was observed in kidney and liver of O. mossambicus (22.35±0.41µg/g and 15.79±0.44µg/g). The increased accumulation of Cd in the liver and kidney over time could be due to the involvement of these organs in the detoxification and removal of toxic substances circulating in the stream. Moreover, since these organs are the major organs of metabolic activities including detoxification of xenobiotics [40], Cd might also be transported into these organs from other tissues like the gills and muscle, for the purpose of subsequent elimination. The kidney is thus the final destination of all the Cd from various tissues as it has also been shown that Cd-MT is filtered through the glomerulus and is reabsorbed by the proximal tubular cells, possibly by endocytosis. Within these cells, the complex is taken up by lysosomes and degraded by proteases to release Cd, which may result in renal accumulation of the metal. Thus, these factors might have accounted for the raised level of the heavy metal in the kidney during the exposure periods. These findings corroborate those of Asagba et al., [41] studies on freshwater catfish (Clarias gariepinus) and accumulation in fish can be proportionally higher through dietary exposure than through waterborne exposure [42, 43].

Gill also accumulates a higher proportion of Cd (11.580±0.314 µg/g). Several reasons have been proposed to justify the gills as the primary site for Cd uptake, such as proximity to toxics due to its external position, it's highly branched structural and vascular nature with the resultant highly increased surface area through which large volumes of water pass through the gill surface amongst other tissues [44].

In the brain, Cd inhibits enzymes such as Mg²⁺-ATPase and Na⁺-K⁺-ATPase causing metabolic effects and disrupting neurotransmitter uptake [45]. In several situations, acetylcholine is not broken and accumulates within synapses causing physiologic impairment and alterations in fish swimming behavior [46]. The reason for the consistent low-level accumulation of Cd in the brain (3.27±0.40 µg/g) is offered with certainty. However, a possible reason is that the blood-brain barrier restricts the entry of Cd into the brain [47].

The muscle of fish accumulated lowest concentration of Cd (2.65±0.30 µg/g), even after 30 d of exposure. This may not be disconnected with the fact that the muscle is not concerned with detoxification and metals like Cd and Pb spread uniformly over the muscle tissue and this may be the reason for the low level of Cd accumulation in the muscle [48].

The current study revealed interesting interactions between Ca supplementation and the response to Cd exposure. Among all exposure periods for the 30 d Ca supplementation, there was a maximum reduction in tissue Cd accumulation. It is indicated that the extra Ca present in aquatic media inhibited water born Cd accumulation in the selected tissues of the experimental animal.

It is clear from the present study that the toxicity of metal is affected by Ca which in turn reduces the toxic effect of metal through competitive inhibition at the gill surface. The non-toxic Ca competes with the toxic metals for the same binding sites [49]. If Ca occupies the sites, the lamellae are protected from deterioration. Increased Ca levels in the medium resulted in a slower transfer of Cd from the gills to the blood and the rate of Cd accumulation was lowered in liver, kidney and other tissues. Similar findings were also reported in rainbow trout by Hollis et al., [50] and in Carinbba irrerula by Ghosh and Adhikari [51].

From the data obtained in the present investigation, Zn also plays a major role in reducing the Cd body burden as Ca supplementation. Supplementation of Zn either alone or in combination with Ca reduced the Cd body burden in the tissues [52-54]. Zn functions as a complex antioxidant. It has the ability to form coordinating bonds with electronegative atoms [55]. It regulates MT synthesis. Zn inhibited oxidative stress induced by Cd [56]. Zn prevented damage to the tissues from Cd exposure. This suggests Cd interference with Zn-related metabolic functions. The competitive mechanism of interaction is a plausible mechanism of Zn in relation to Cd toxicity. Interactions between Cd and Zn occurs as early as in an intestine during absorption, but more intensive interactions take place during accumulation in the tissues. It has been shown that Cd may inhibit Zn activities at many stages interfering with its absorption, distribution to different tissues, transport into cells and/or transport into several intracellular structures [57-59]. The most compelling reason for the protective effects of Zn against Cd toxicity is that Zn induces the synthesis of the metal binding protein, MT in the tissues [60, 61]. Interaction of Zn with Cd results in a decrease in the excretion of Cd. This has been proposed as a mechanism by which Zn protects against Cd toxicity [62] because Zn and Cd compete for a common transport mechanism in the organisms. Thus, Zn supplementation has showed beneficial effects on Cd toxicity [63-65]. This may be the reason for the reduced Cd accumulation in the test tissues supplemented with Zn in the present study.

CONCLUSION

It could be therefore concluded that Zn and Ca supplementation might play a vital role in reducing the Cd tissue burden of fresh water fish thereby mitigating the risk of potential hazards to human health.

ACKNOWLEDGEMENT

The authors are highly thankful to the CSIR, New Delhi for the financial support rendered with the award of Major Research Project (No. 37(1450)/10/EMR-II, dated 09-12-2010) to Prof. A. Usha Rani, Dept. of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

CONFLICT OF INTERESTS

Declared none

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Table 4: Cd accumulation (µg/g wet weight of the tissue) in different tissues of O. mossambicus after Zn+Ca supplementation

| S. No. | Tissue | 15d Cd | Zn+Ca supplementation |
|-------|--------|--------|------------------------|
|       |        | µg/g   | µg/g                  |
| 1.    | Kidney | 14.06±0.123 | 12.75±0.306 |
| 2.    | Liver  | 11.01±0.263 | 9.25±0.451  |
| 3.    | Muscle | 3.016±0.124 | 2.654±0.308  |
| 4.    | Brain  | 4.076±0.339 | 3.27±0.404   |
| 5.    | Gill   | 6.175±0.258 | 5.43±0.399   |

Values are expressed as mean±SD (n = 6 rats in each group). Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan’s Multiple Range Test (DMRT). All mean values are significant at P<0.05 level over 15d Cd exposure.
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How to cite this article

Obaiah Jamakala, A Usha Rani. Zinc and calcium supplementation to combat cadmium-induced bioaccumulation in freshwater teleost Oreochromis mossambicus (Tilapia). Int J Pharm Pharm Sci 2016;7(11):186-190.