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Cadmium-induced changes in the corpuscles of Stannius of a freshwater teleost, *Heteropneustes fossilis*

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Abstract

Freshwater fish *Heteropneustes fossilis* were subjected to 288 mg/L (0.8 of 96 h LC_{50}) and 72 mg/L (0.2 of 96 h LC_{50}) of cadmium chloride for short-term and long-term experiments, respectively. After sacrificing the fish, the blood was collected at 24, 48, 72 and 96 h (short-term) or after 7, 14, 21, and 28 days (long-term) and analysed for plasma calcium levels. At each time interval, the corpuscles of Stannius were fixed.

In the short-term experiment, a decrease in the plasma calcium levels was recorded at 48 h, persisting until the end of the experiment (96 h). In the long-term experiment, cadmium exposure provoked a decrease in plasma calcium after 7 days, progressively decreasing until the end of the experiment (28 days).

There was no change in the histological structure of corpuscles of Stannius of the fish exposed to cadmium after 48 h, but after 72 and 96 h, the AF-positive cells of the corpuscles of Stannius exhibited increased granulation, but no change in nuclear volume. The AF-negative cells of the corpuscles of Stannius also showed no change in nuclear volume.

The corpuscles of Stannius were unaffected up to 7 days following exposure. After 14 days, the nuclear volume of AF-positive cells decreased. Following 21 days exposure the AF-positive cells possessed increased granulation, and the nuclear volume decreased further. After 28 days these changes were exaggerated and a few degenerating cells were encountered. Up to 14 days following cadmium exposure the AF-negative cells of corpuscles of Stannius exhibited no change, but later these cells increased in nuclear volume.

Keywords: cadmium; plasma calcium; corpuscles of Stannius; heavy metal; teleost

Introduction

Corpuscles of Stannius (CS) produce stanniocalcin (STC), a major antihypercalcemic hormone in fish (Wendelaar Bonga & Pang 1986, Ishibashi & Imai 2002). These glands were considered to be unique to holostean and teleostean fishes. Although no homologous organs for CS have been identified in higher vertebrates, some investigators have reported hypocalcemia after injecting CS-extract to non-piscine vertebrates (Srivastav & Swarup 1982, Pandey et al. 1982, Hasan *et al.* 1987). These reports suggest a functional STC receptor in non-piscine vertebrates. It is possible that STC is conserved through the evolution of fish to mammals, and that STC homologs may also be present in tetrapods (Ishibashi & Imai 2002). However, only mammalian STC homologs (STC1 and STC2) have been identified, expressed in many tissues – kidney, ovary, uterus, pancreas, prostate and bladder of human and other mammals (Ishibashi & Imai 2002, Song *et al.* 2006, Sazonova *et al.* 2008).

Loretz (2008) has reported extracellular calcium–sensing receptors in the CS of fish similar to the tetrapods. Recently STC1 is gaining more and more importance, and has been shown to activate antioxidant pathways in endothelial cells and macrophages, thus displaying cytoprotective and anti-inflammatory actions (Sheikh-Hamad 2010). Moreover, high expression of STC1 has been associated with several cancers including ovarian cancer (Liu *et al.* 2010). These data indicate that STC proteins have significant roles in metabolism, reproduction and development, and may be more widespread among the vertebrates than is presently believed.

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It is apparent that heavy metal toxicity is becoming a global problem (Benaduce *et al.* 2008; Oner *et al.* 2008). Cadmium is an environmental contaminant caused by mining and processing, as well as by the agricultural use of pesticides (Chowdhury *et al*. 2005). It is very toxic to aquatic organisms particularly to fish. After exposure to cadmium, fishes exhibit – (i) perturbed ion balance (Garcia-Santos *et al.* 2006), (ii) decreased Ca^{2+} uptake (Baldisserotto *et al.* 2004a,b), (iii) inhibited larval growth (Lizardo-Daudt & Kennedy 2008), (iv) accumulation of Cd in various tissues (Rangsayatorn *et al.* 2004, Wangsongsak *et al*. 2007, Oner *et al*. 2008), and (v) serious damage in vital organs - gill, liver and kidney (Rangsayatorn *et al.* 2004, Wangsongsak *et al*. 2007). As there are reports regarding the inhibition of Ca^{2+} uptake as well as decreased blood calcium concentrations of fish after cadmium exposure, we performed the present study to see the changes in the corpuscles of Stannius after exposure of cadmium in the freshwater catfish *Heteropneustes fossilis.* It is well known that in fish, branchial Ca^{2+} uptake is mainly controlled by plasma Ca^{2+} levels and by the hormone stanniocalcin.

Materials & Methods

Freshwater fish *H. fossilis* (body wt. 37-46 gm) were collected and acclimatized for 15 days in 500 L plastic pools. The experiments were performed over the short-term (96 h) and long-term (28 days). In the short-term experiment, fish were subjected to 288 mg/L of cadmium chloride (0.8 of the 96-h LC_{50} value), whereas in the long-term experiment, fish were subjected to 72 mg/L of cadmium chloride (0.2 of the 96-h LC_{50} value). Simultaneously, a control group was also run for comparison. Fish were kept in groups of 10 in 30 L media. Six fish were killed at each time interval from both control and experimental groups, in the short-term experiment at intervals of 24, 48, 72, and 96 h, and in the long-term experiment at intervals of 7, 14, 21, and 28 days following the treatment. Blood samples were collected by sectioning of the caudal peduncle, and plasma calcium levels analysed by Sigma kits.

After collection of blood samples, the corpuscles of Stannius along with the adjoining portion of the kidney were removed from the fish and fixed in aqueous Bouin's fluid. Tissues were routinely processed in a graded series of alcohols, cleared in xylene and embedded in paraffin wax. Serial sections were cut at 6 μm and stained with hematoxylin-eosin (HE) and aldehyde fuchsin (AF). Nuclear indices (maximal length and maximal width) were taken with the aid of an ocular micrometer and then the nuclear volume was calculated as – volume = 4/3 π ab^2 where 'a' is the major semi-axis and 'b' is the minor semi-axis. In the gland, when there are degenerating nuclei, only the indices of intact nuclei were measured.

All data are presented as the mean ± SE of six specimens. A two-way Analysis of Variance (Anova) of treatment (control vs treated) x time period tested the overall significance; the three degrees of freedom for the groups was split into three 1-df specific contrasts of the experimental compared to the control at the same time period: Student's t tests were used to determine the statistical significance of these contrasts.

Results

Short-term cadmium chloride exposure: analysis of variance indicated that the levels of plasma calcium were significantly different among groups (among time intervals $F_{3,40} = 3.97$, $p<0.014$; between treatments $F_{1,40} = 34.53$, $p<0.0001$). A decrease in the plasma calcium levels was recorded in cadmium-exposed fish first at 48 h ($p<0.003$), persisting until the end of the experiment (96 h; Fig. 1) ($p<0.006$ at 72 h and $p<0.003$ at 96 h).

The corpuscles of Stannius of control fish are enveloped by a thick connective tissue capsule which isolates them from the rest of the renal tissue. From the capsule, connective tissue layers extend into the gland, thus dividing the corpuscles into several complete or incomplete cords or lobules. These cell cords contain epithelial cells which possess oval or

rounded nuclei located near the middle of the cells. These nuclei possess many sharp staining chromatin granules and often a small central nucleolus. When subjected to aldehyde fuchsin staining technique, the corpuscles exhibit two cell types – AF-positive and AF-negative (Fig. 2).

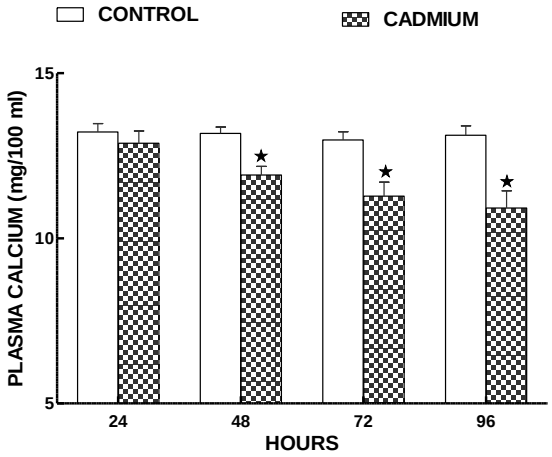

Figure 1: Plasma calcium levels of short-term cadmium chloride treated *Heteropneustes fossilis*. Values are mean ± SE (n=6). Asterisks indicate significant differences ($p<0.05$) of the contrast with the control at the same time.

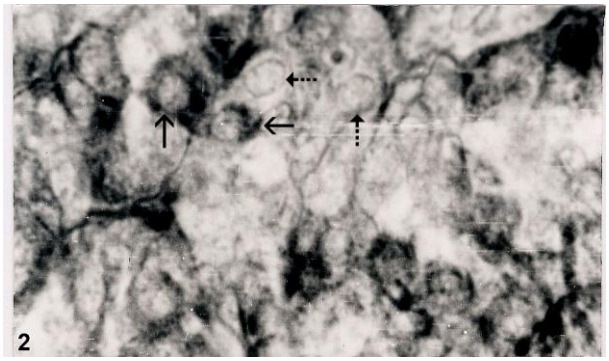

Figure 2: Corpuscles of Stannius of control *Heteropneustes fossilis* showing AF-positive and AF-negative cells. AF x 800.

There was no change in the histological structure of corpuscles of Stannius in fish exposed to cadmium for 48 h. After 72 and 96 h, the AF-positive cells exhibited increased granulation (Fig. 4). No change in the nuclear volume of these cells was noticed. Analysis of variance indicated that the nuclear volume of AF-positive cells were not significant among groups (among time intervals $F_{3,40} = 0.04$, ns; among treatments $F_{1,40} = 0.04$, ns).

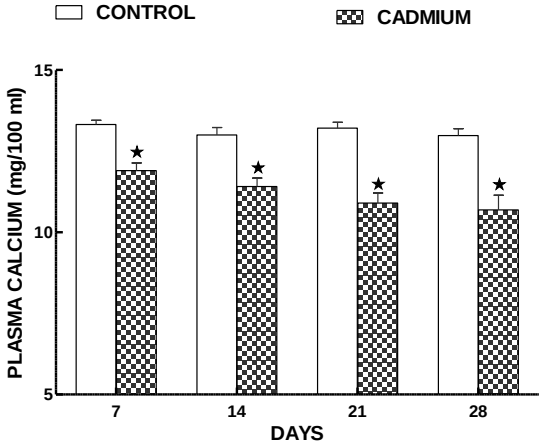

Figure 3: Plasma calcium levels of long-term cadmium chloride treated *Heteropneustes fossilis*. Values are mean ± SE (n=6). Asterisks indicate significant differences ($p<0.05$) of the contrast with the control at the same time.

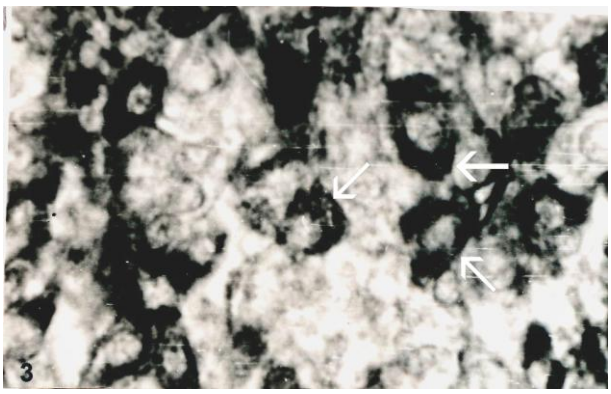

Figure 4: Corpuscles of Stannius of 96-h cadmium-chloride-treated *Heteropneustes fossilis* exhibiting increased granulation in AF-positive cells. AF x 800.

The AF-negative cells of corpuscles of Stannius of cadmium-exposed fish showed no changes in nuclear volume. Analysis of variance indicated that the nuclear volume of AF-negative cells were not significant among groups (among time intervals $F_{3,40} = 0.01$, ns; among treatments $F_{1,40} = 0.02$, ns).

Long-term cadmium chloride exposure: Analysis of variance indicated that the levels of plasma calcium were significantly different among groups (among time intervals $F_{3,40}$ = 2.98, p<0.043; between treatments $F_{1,40}$ = 101.0, p< 0.0001). Cadmium exposure provoked a decrease in the plasma calcium level after 7 days (p<0.0004), and thereafter levels progressively decreased until the end of the experiment (28 days; Fig. 3) (p<0.001 at 14 days; p< 0.001 at 21 days; p< 0.001 at 28 days).

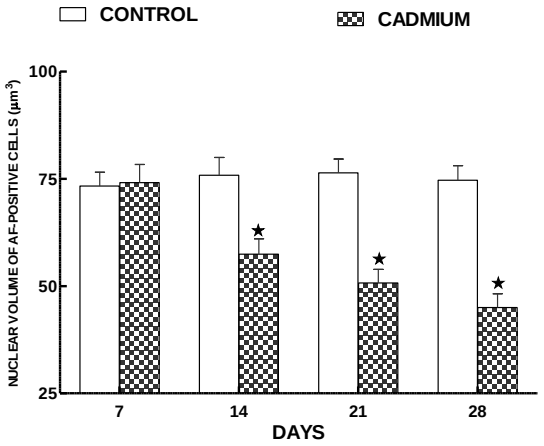

Figure 5: Nuclear volume of AF-positive cells of long-term cadmium chloride treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) as compared with control.

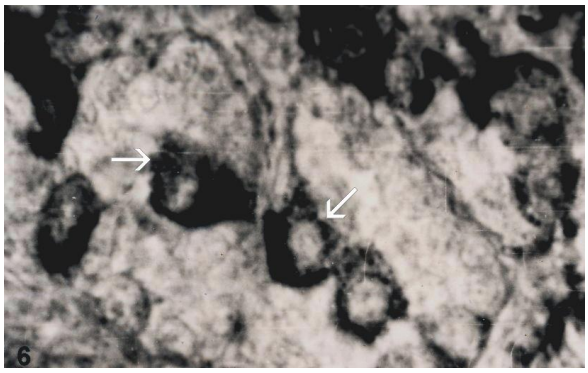

Figure 6: Corpuscles of Stannius of 21 days cadmium chloride treated *Heteropneustes fossilis* exhibiting increased granulation in AF-positive cells. AF x 800.

Analysis of variance indicated that the nuclear volume of AF-positive cells were significantly different among groups (among intervals $F_{3,40}$ = 5.54, p<0.003; between treatments, $F_{1,40}$ = 53.07, p<0.0001). The corpuscles of Stannius of cadmium-treated fish remained unaffected up to 7 days following the exposure. After 14 days the nuclear volume of AF-positive cells decreased (p<0.007) (Fig. 5). Following 21 days exposure the AF-positive cells possess increased granulation (Fig. 6) and the nuclear volume decreased further (p<0.002). (Fig.5). After 28 days these changes were exaggerated (p<0.0001), and some degenerating cells were encountered (Fig. 8).

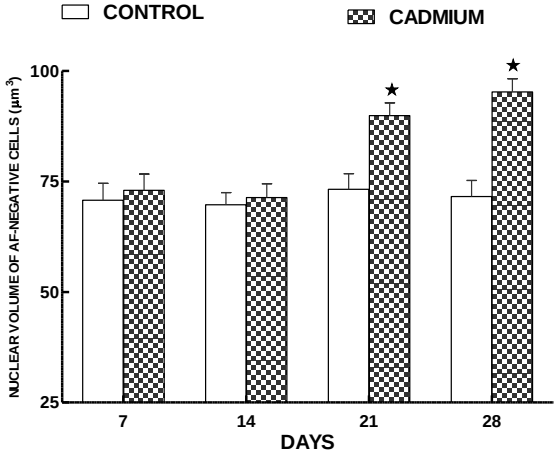

Figure 7: Nuclear volume of AF-negative cells of long-term cadmium chloride treated *Heteropneustes fossilis*. Values are mean ± SE (n=6). Asterisks indicate significant differences (p<0.05) in the contrast with the control.

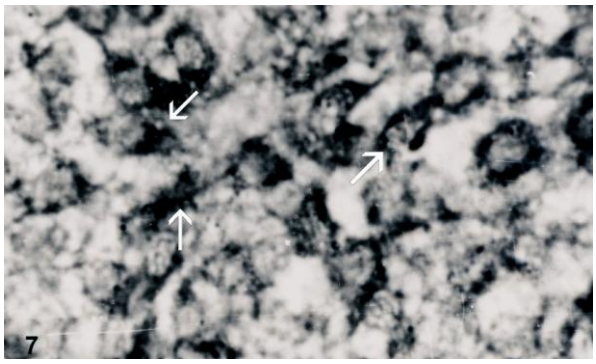

Figure 8: Corpuscles of Stannius of 28 days cadmium chloride treated *Heteropneustes fossilis* exhibiting degeneration of AF-positive cells. AF x 800.

Analysis of variance indicated that the nuclear volumes of AF-negative cells were significantly different among groups (among time intervals, $F_{3,40} = 7.82$, $p<0.0001$; between treatments $F_{1,40} = 22.10$, $p<0.0001$). Up to 14 days following cadmium exposure, the AF-negative cells of corpuscles of Stannius exhibited no change. They increased their nuclear volume after 21 ($p<0.004$) and 28 days ($p<0.0005$) of cadmium exposure (Fig. 7).

Discussion

Cadmium treatment provoked hypocalcemia in *H. fossilis*, as other investigators have observed in fish treated either with cadmium (Garcia-Santos *et al.* 2006, Pratap & Wendelaar Bonga 2007), aldrin (Singh *et al.* 1996), malachite green (Srivastava *et al.* 1995), propoxur (Singh *et al.* 1997), formothion (Singh *et al.* 1997) or cypermethrin (Mishra *et al.* 2001). However, no effect on blood/plasma calcium levels was reported by Haux (1979) in DDT-exposed flounders *Platichthys flesus,* and by Oner *et al.* (2008) in cadmium-exposed *Oncorhynchus niloticus.* In contrast, elevation of plasma calcium levels was noticed in fish treated with pesticides (Sharma *et al.* 1982) and tributyl tin (Suzuki *et al.* 2006).

The corpuscles of Stannius of cadmium-treated fish exhibited signs of inactivity, expressed by increased granulation and decreased nuclear volume among AF-positive cells. AF-positive cells have been associated with the release of stanniocalcin, which functions as a hypocalcemic hormone in teleosts (Wendelaar Bonga & Pang 1991, Srivastav & Srivastav 1988, Srivastav *et al.* 1997). In the present study, the observed increased granulation in AF-positive cells may be attributed to the perpetual hypocalcemia induced by the cadmium challenge to the fish. Pratap & Wendelaar Bonga (2007) reported an increase in the size of the corpuscles of Stannius in *Oreochromis mossambicus* acclimated to high calcium and then exposed to cadmium, and suggested that cadmium does not mediate stimulation of the corpuscles of Stannius. The present study derives support from the studies of Singh (1990) and Tiwari (1993), who reported accumulation of secretory granules in the AF-positive cells of fish which have been made hypocalcemic by maintaining them in calcium-deficient freshwater. Accumulation of secretory granules among mammalian calcitonin cells (which secrete a hypocalcemic hormone) has been reported in response to induced hypocalcemia (Biddulph & Maibenco 1972, Swarup *et al.* 1980).

AF-negative cells of the corpuscles of Stannius exhibited increased nuclear volume after cadmium treatment. Contrary to this, no cellular damage was noticed in type-2 (AF-negative) cells of cadmium-exposed *Oreochromis mossambicus* (Pratap & Wendelaar Bonga 2007). We noticed degeneration of AF-positive cells, but no cellular damage was observed in type-1 (AF-positive) cells of cadmium-treated *Oreochromis mossambicus* (Pratap & Wendelaar Bonga 2007). The degeneration of AF-positive cells in cadmium-exposed *H. fossilis* may be due to the perpetual hypocalcemia caused by cadmium treatment.

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الملخص العربي

التغيرات التى يحدثها الكادميوم فى الخلايا الدموية لأحد أنواع أسماك المياه العذبة (*Heteropneustes fossilis*)

روبى راع ، ساروشنى تريباثى ، ديواكار ميشرا ، سينيل كومار سريفاستاف ، أشاى كومار سريفاستاف

قسم علم الحيوان – جامعة جوراخبار – جوراخبار - الهند

تم تعريض سمكة *Heteropneustes fossilis* إلى 288 ملجم / لتر (0.8 من نصف التركيز المميت خلال 96 ساعة) و 72 ملجم / لتر (0.2 من نصف التركيز المميت خلال 96 ساعة) من كلوريد الكادميوم خلال التجربتين قصيرة وطويلة الأجل على التوالي. وبعد أن تم ذبح السمك، جمعت عينات من دمائه بعد 24 و 48 و 72 و 96 ساعة (التجربة قصيرة الأجل) أو بعد 7 و14 و21 و 28 يوماً (التجربة طويلة الأجل) لتحليل البلازما لمعرفة مستويات الكالسيوم بها. وعند كل فترة زمنية كان يتم تثبيت كريات ستانيوس. في التجربة قصيرة الأجل سجل انخفاضاً في مستويات الكالسيوم في البلازما بعد 48 ساعة استمر حتى نهاية التجربة (بعد مرور 96 ساعة). أما في التجربة طويلة الأجل فقد أدى التعرض للكادميوم إلى انخفاض الكالسيوم في البلازما بعد 7 أيام وظل في انخفاض تدريجي حتى نهاية التجربة (بعد مرور 28 يوماً).

لم يطرأ أي تغيير في تركيب الهستولوجى لنسيج كريات ستانيوس في الأسماك المعرضة للكادميوم بعد 48 ساعة ، ولكن بعد 72 و96 ساعة أظهرت خلايا أف الإيجابية لكريات ستانيوس تحبيب متزايد دون أي تغير في حجم النواة ، كما لم تظهر خلايا أف السلبية لكريات ستانيوس أي تغير في حجم النواة.

لم تتأثر كريات ستانيوس لمدة تصل إلى 7 أيام بعد التعرض للكادميوم. وبعد مرور 14 يوماً انخفض حجم النواة لخلايا أف الإيجابية . وبمرور 21 يوم أظهرت خلايا أف الإيجابية حبيبات متزايدة، كما انخفض حجم النواة أكثر.ولقد تضاعفت هذه التغيرات بعد مرور 28 يوماً كما تدهورت بعض الخلايا تماماً. بعد مرور ما يصل إلى 14 يوماً عقب التعرض للكادميوم لم تبدي خلايا أف السلبية لكريات ستانيوس أي تغير، ولكن بعد ذلك زاد حجم النواة لهذه الخلايا.