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Acute Effect of Cadmium Chloride on Chromosomal Abnormalities in the Nile Tilapia Fish *In Vivo*

Bundit Tengjaroenkul [1, 2] and Lamyai Neeratanaphan [1, 3*]

[1] *Research Group on Toxic Substances in Livestock and Aquatic Animals,* [2] *Faculty of Veterinary Medicine,* [3] *Division of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*

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Abstract

The cytotoxic potential of cadmium chloride ($CdCl_2$) in *Oreochromis niloticus* was conducted by assessment of chromosomal abnormalities (CA). Treatment fish were intraperitoneally injected with 5, 10, 20 and 30 mg/L $CdCl_2$ solution, and the CA effects were compared with those of control fish. After 48 h, muscles of the fish were analyzed for cadmium (Cd) concentration, and the kidneys were evaluated for CA. Cd concentrations in the fish from the control and treatments revealed as lower than detection limits, 0.04±0.02, 0.11±0.01, 0.20±0.06 and 0.17±0.04 mg/kg, respectively, whereas Cd was not detected in water samples. Eleven types of CA were demonstrated as single chromatid gap (SCG), sister chromatid gap, sister chromatid fragment, fragmentation, single chromatid breaks, deletion, dicentric, centromere separate, C-mitotic, centric fusion and pericentric invertion. The most CA in the treated fish was SCG. The percentages of CA cells in the control and treatments were 0, ٢٠.٦٦±6.80, 21.66±4.04, 25.00±5.29 and 37.66±2.08, respectively. The number of CA and the percentage of CA cells in the control and the treatments were statistically significant ($p<0.05$). The treatment fish injected with 30 mg/L was significant from the other treatments ($p<0.05$). This study demonstrates that acute toxicity with relatively low concentrations (5 mg/L) of $CdCl_2$ can induce CA in the Nile tilapia fish.

Keywords: Chromosomal abnormality, Cadmium chloride, Cytotoxicity, Fish, Tilapia.

1. Introduction

Aquatic ecosystem contamination by heavy metals from industrial, domestic and agriculture wastewater has been gaining increased attention. Heavy metals contamination can harm life of aquatic organisms after being absorbed through contaminated water, sediment and the food chain. Accumulation of heavy metals could produce adverse effects on structures and functions of cells and tissues of the exposed aquatic creatures. Cadmium (Cd) is one of nonessential metals in all living creatures (Besirovic *et al*., 2010); however, it is widely used in numerous industrial processes, including electroplating, smelting, making batteries and production of color as well as plastic (Waisberg *et al*., 2003). In case of leaching into aquatic ecosystem, Cd can be bioaccumulated, and cause detrimental consequences to various creatures along the aquatic food chain, including snail, shrimp and fish as well as to human as the top consumer in the food chain. Cd is generally accepted as one of hazardous agents to human health, and has been documented as teratogenic, apoptotic and genotoxic, hepatotoxic, pancreatotoxic, nephrotoxic and carcinogenic agent (Ahmed and Abdel-Wahhab, 2000; Horiguchi *et al*., 2000; Hovland *et al*., 2000; Shimada *et al*., 2000; Banerjee and Flores-Rozas, 2005; Kim *et al*., 2005; Mondal *et al*., 2005; Goodale *et al*., 2008). Cd exerts toxicological effects, mostly in the kidneys and liver (Stoeppler, 1991; Cai *et al*., 2001). Previous reports have shown that exposure to Cd at relatively high levels results in diseases, disorders and life threatening conditions (Othumpangat *et al*., 2005).

Presently, numerous research reports have revealed accumulation and toxicity of Cd in fish, including oxidative effects, morphology and physiology changes, osmoregulation and immune response and endocrine disruptions (Romeo *et al*., 2000; Dang and Wang, 2009; Garcia-Santos *et al*., 2011; Guardiola *et al*., 2013; Li *et al*., 2014). Fish, including the Nile tilapia, *Oreochromis niloticus*, are major aquatic creatures in the food chain of aquatic ecosystems, and are often used as biomarkers in toxicological studies. When they ingest organisms contaminated with heavy metals, deleterious health effects occur (Clearwater *et al*., 2002; Giusto *et al*., 2012; Mustafa *et al*., 2012; Al-Bairuty *et al*., 2013; Intamat *et al*., 2016). Among pollutants, Cd is one of hazardous toxicants to fish when absorbed via diet or the water medium (Kalman *et al*., 2010; Sriuttha *et al*., 2017). Currently, we have little knowledge of CA resulting from acute Cd exposure (Kamunde and MacPhail, 2011). The aim of this study was to investigate CA in *O. niloticus* resulting from acute Cd exposure (48 h) at four different concentrations.

* Corresponding author. e-mail: hlamya@kku.ac.th.

2. Materials and Methods

2.1. Experimental fish specimens

Oreochromis niloticus with weight 15-20 g received from a private fish farm in Khon Kaen city, Thailand, were bathed with $KMnO_4$ solution (0.05% v/v) to avoid skin infection (Pandey *et al*., 2011). They were acclimatized under experimental conditions for 10 days before the Cd toxicity study *in vivo*.

2.2. Exposure concentration of Cd

The acclimatized fish in the control were intraperitoneally injected with 0.8% sterilized normal saline, whereas the fish in four treatments were injected with 5, 10, 20 and 30 mg/L at a volume of 100 µL of cadmium chloride ($CdCl_2$). The experimental fish were kept for 48 h before the CA assessment.

2.3. Cd concentration in water

After 48 h, a 25-mL water sample was put in a glass container, and nitric acid (1.25 mL) was added. The container was set in water bath at 90±5°C, for 30 min. Deionized water was later added to the acid digested water sample to make a 25 mL final volume before being filtered using standard 11 µm filter paper. Cd in the water was evaluated by inductively coupled plasma optical emission spectrometry (ICP-OES) (Promsid *et al*., 2015).

2.4. Cd concentration in O. niloticus

The fish muscle weighed 1 g was homogenized, and mixed with 7 mL of nitric acid. After adding 1 mL of hydrogen peroxide, the samples were set in water bath at 90±5°C for 2 h. After cooling, deionized water samples were adjusted to make a suspended mixture of 25 mL. Then, the mixture was passed through an 11 µm cellulose filter paper. Cd concentration in fish muscle was measured using ICP-OES (Promsid *et al*., 2015) with a wavelength of 226.502 nm and a detection limit of 0.001 mg/L.

2.5. Quality assurance

Following standards of quality assurance, detection and measurement for Cd contamination were conducted at every tenth sample. The blank Cd concentrations were < 5% of the average of analyzed concentrations. Accuracy as well as precision of the analyses were confirmed by the replication of analyzed samples against Cd standard reference (APHA, 2005).

2.6. Chromosome preparation

The fish chromosomes were collected following conventional method (Srikacha *et al*., 2018; Tengjaroenkul *et al*., 2018). Each experimental fish was injected intramuscularly using 1 ml colchicine solution (0.05%) per 100 g fish weight, kept approximately 1 h, and anesthetized in ground ice. The kidneys were excised, made to small pieces and mixed with 0.075 M KCl. Sediment cells of 8 mL was incubated at 25°C for 30 min, before being fixed in cool methanol-acetic acid fixative at ratio 3:1. Later, the cells were centrifuged for 4 times at 1500 rpm for 10 min with 8 mL of the cool fixative. For chromosomal study, the cleaned sediment was added with 1 mL of the cool fixative, dropped to a glass slide, air dried and then stained with Giemsa solution (20%) for 30 min (Intamat *et al*., 2016).

One hundred with clear and well spread chromosomes in each treatment were photographed. Number of CA cells was recorded. All parameters as well as fundamental number (number of chromosome arms or NF) were used for karyotyping. Cytotoxicity was evaluated by determining the percentage of CA cells of the fish (Intamat *et al*., 2016).

2.7. Statistical analysis

Levels of Cd in water and *O. niloticus* muscles, as well as number and the percentage of CA cells were statistically analyzed using Analysis of Variance as well as Turkey's post hoc test (at 95% confidence).

3. Results

3.1. Cd concentration in water and O. niloticus

Cd in all experimental water samples was not detected. The Cd concentration in *O. niloticus* is demonstrated in Table 1. The highest Cd level in the fish muscle was shown in the treatment injected with 20 mg/L. Statistical results demonstrated that Cd concentrations of the fish in the control and the treatments revealed significant difference ($p<0.05$), and only the treatment received 5 mg/L was significantly different ($p<0.05$) from the other treatments.

Table 1. Cd concentration in *O. niloticus* samples.

$CdCl_2$ concentration (mg/L)	Cd concentration in fish muscle (mg/kg) experimental unit			Average Cd concentration
	1	2	3	(mg/kg)
Control	ND	ND	ND	ND
٥	0.02	0.04	0.06	0.04±0.02[a]
١٠	0.12	0.10	0.11	0.11±0.01[b]
٢٠	0.27	0.16	0.17	0.20±0.06[c]
٣٠	0.21	0.15	0.14	0.17±0.04[c]

ND: Not detected

[a, b, c] Values in the same column with different letters are significantly different ($p<0.05$).

3.2. Assessment of chromosomal abnormalities

The current study used a CA test to evaluate cytotoxic consequences on the Nile tilapia (*O. niloticus*) after acutely (48 h) injected with Cd. The diploid chromosome number (2*n)* of the Nile tilapia is 44. The karyotype of the tilapia fish consisted of two submetacentric, twelve acrocentric and thirty telocentric chromosomes (Figure 1). The different categories of CA found in the current study were single chromatid gap (SCG), single chromatid breaks (SCB), sister chromatid gap (SSCG), sister chromatid fragment (SSCF), fragmentation (F), deletion (D), dicentric (DC), centromere separate (CS), C-mitotic, centric fusion (CF) and pericentric invertion (PI) (Figure 2 and Table 2). The type of CA found in *O. niloticus* was different among levels of Cd concentrations. The control fish had no CA, whereas the treatment fish injected with Cd at 5 and 10 mg/L revealed nine types of CA (SCG, SSCG, F, D, DC, CS, C-mitotic, CF and PI). The treatment fish injected with Cd at 20 and 30 mg/L revealed eleven types of CA (SCG, SSCG, SCB, F, SSCF, D, DC, CS, C-mitotic, CF and PI).

The highest total number of CA was demonstrated in the treatment injected with 20 mg/L, while the highest number of cells with CA was demonstrated in the

treatment injected with 30 mg/L (Table 2). Statistical analyses indicated that there were significant differences in the number of CA and the number of CA cells between the control and the treatments ($p<0.05$), except for the fish treated with 5 mg/L. Among the numbers of CA cells in the treatments, there were significant differences between the fish treated with 30 mg/L and the other treatments ($p<0.05$) (Table 2).

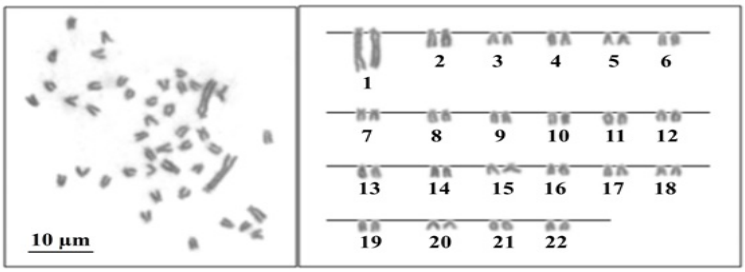

Figure 1. Karyotypes of diploid chromosome ($2n=44$) of the *O. niloticus* in the control.

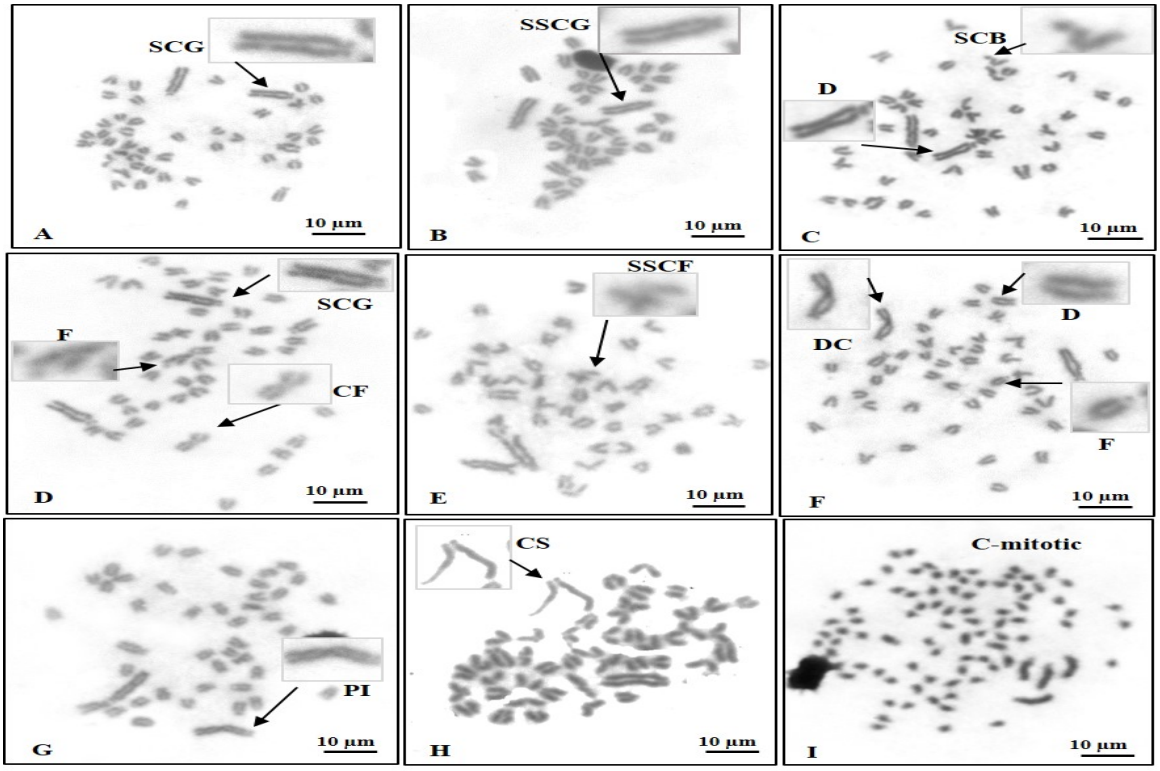

Figure 2. Examples of different chromosomal abnormalities in *O. niloticus* (*2n*=44): single chromatid gap (SCG), sister chromatid gap (SSCG), single chromatid breaks (SCB), centric fusion (CF), fragmentation (F), sister chromatid fragment (SSCF), deletion (D), dicentric (DC), pericentric invertion (PI), centromere separate (CS) and C-mitotic.

Table 2. Number and percentage of CA cells of *O. niloticus* in the control and the $CdCl_2$ treatments.

$CdCl_2$ concentrations	Number of CA											Total number of CA	Number of cells with CA	Percentage of cells with CA
	SCG	SSCG	SCB	SSCF	CF	F	D	PI	DC	CS	C-mitotic			
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0[a]
5	5	2	0	0	6	1	5	2	7	3	6	36	21	٢٠.٦٦±6.80[b]
10	9	4	0	0	2	2	2	2	7	1	3	33	21	21.66±4.04[b]
20	9	5	1	1	5	2	4	3	7	4	7	48	25	25.00±5.29[b]
30	7	3	1	1	7	2	4	4	7	4	7	47	32	37.66±2.08[c]

[a, b, c] Values in the same column with different letters are significantly different ($p<0.05$).

4. Discussion

The Cd concentration in water samples was not detected. This result could be due to the distribution and elimination of toxicants in fish. As Cd elimination in fish is low, resulting in Cd accumulation in liver, gill and muscle of various fish species (Jayakumar and Paul, 2006; Cyrille *et al*., 2012). Fish muscle, a valuable edible protein source, was selected as a target organ to detect low levels of Cd deposition. Previous studies have reported that the main target organs for Cd accumulation in fish samples are

the kidneys, liver, gills and skin. A potential reason that the muscle accumulated Cd as the lowest level is that it is not directly contact to the water medium. Another probable reason is that muscle has no function related to detoxification of xenobiotics, and consequently, accumulation of Cd in the muscle as compared with the liver and kidney is less likely to occur (Jayakumar and Paul, 2006). In addition, an accumulation of Cd in fish could be long in their life time; this is supported by Agency for Toxic Substances Disease Registry (1999) who reported half-life of Cd in the liver approximately 4-19 years.

The diploid chromosome number of *O. niloticus* is similar to previous studies (Vervoort, 1980; Sofy *et al*., 2008; Intamat, 2016; Sriuttha *et al*., 2017). This result implies that numerical of chromosome is not changed after acutely injected with Cd in the Nile tilapia fish.

The results using CA assessment revealed that *O. niloticus* injected with Cd had greater in both of the number of CA cells and the percentage of CA cells as the Cd concentration increased. Eleven types of CA indicate that Cd, particularly at higher levels is more effective on changing chromosome structures. The results of this study were different from other findings. For example, Intamat (2016) studied cytotoxic effects of sodium arsenite on *O. niloticus* on an experimental scale, and found five types of CA, including SCG, SCB, CG, F and D. Sriuttha *et al*. (2017) demonstrated that heavy metals of *O. niloticus* in domestic wastewater canals could induce six types of CA, including SCG, SCB, CG, F, D and DC. This variable CA information could imply that the CA may correlate to types, toxicities as well as the contact time of the exposed heavy metals. Several heavy metals have adverse effects on genetic materials (Achanzar *et al*., 2002; Asmuss *et al*., 2000; Hartwig and schwerstle, 2002; Fatur *et al*., 2003; Jin *et al*., 2003; Potts *et al*., 2003; Waisberg *et al*., 2003). Our results revealed that the different levels of Cd concentrations were associated with different types of CA. The most abnormality of chromosome from the tilapia kidney cells was SCG (Table 2). This result is similar to previous studies. Intamat *et al*. (2016) and Sriuttha *et al*. (2017) reported that SCG was the most found CA in kidney cells of the tilapia fish collected from both laboratory experiments and heavy metal contaminated areas. SCG has been demonstrated probably due to a lack of folding of the metaphase chromosome fiber into a chromatid. Palitti (2004) mentioned that SCG could occur as results of protein or DNA damages.

Cd compounds can cause breaking of DNA strand and CA that demonstrate less mutagenic in mammalian cells (Waalkes, 2003). Ashmawy *et al*. (2015) demonstrated that micronuclei formation was lower at low concentrations and shorter exposure times of Cd than at higher concentrations and longer exposure times. Toxic effects of heavy metal are generally produced at high exposure concentrations. In contrast, Cd exhibits both acute and chronic toxicity at very low exposure levels (NCM-WHO, 2003). In this study, Cd could demonstrate acute toxicity in term of CA at relatively low concentrations (5 mg/L) in the Nile tilapia fish. Similarly, Parveen and Shadab (2012) found that 5 mg/L of $CdCl_2$ caused genotoxicity as CA in *Channa punctatus* specimen. This provided evidence that duration of exposure of treatment can affect the genomic system of *O. niloticus* exposed to Cd at several concentrations (16, 18, 20 and 22 mg/L) and at different time periods (1, 7, 14, 21 and 28 days). Similarly, Jindal and Verma (2015) reported that a comet assay showed a greater value of mean percentage of DNA collected from the tail of the freshwater fish, *Labeo rohita*, after contacted to 0.37 and 0.62 mg/L $CdCl_2$ for 100 days. The genotoxicity of Cd has been described by indirect mechanisms involving in cell proliferation, free radical reactivity, tumor-suppression functions and DNA-repairing processes (Stohs *et al*., 2001; Pagliuca *et al*., 2003; Lutzen *et al*., 2004; Youn *et al*., 2005; Valko *et al*., 2006). Furthermore, Cd inhibited DNA repair mechanisms, including excision of nucleotide, default of nuclotide repair and deletion of the DNA precursor (Asmuss *et al*., 2000; Achanzar *et al*., 2002; Hartwig and schwerstle, 2002; Fatur *et al*., 2003; Jin *et al*., 2003; Potts *et al*., 2003). Cd reacted to signal transduction pathways, mainly with mitogenic signaling. Submicromolar levels of Cd have induced DNA synthesis and caused cell divisions of myoblast and macrophage (Misra *et al*., 2002). In addition, on an experimental scale, Cd stimulates a release of the secondary messenger of calcium, mitogenic kinases, factors related to transcription and translation, and oncogene expression (Waisberg *et al*., 2003). These mechanisms could be the major roles in the CA formation in tilapia fish cells.

5. Conclusion

Acute exposure at different concentrations of $CdCl_2$ causes significant difference in the numbers of cells with CA and the percentages of cell with CA between *O. niloticus* in the control and the treatments. The CA could be as potential indicator of acute Cd cytotoxicity in the tilapia fish. Further investigations coping with cytotoxic effects of $CdCl_2$ at various exposure times and at more variable concentrations are required.

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