

ASSESSING THE IMPACT OF LEAD AND CADMIUM ACCUMULATION ON THE DEVELOPMENT OF DANIO RERIO

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ABSTRACT:

The accumulation of heavy metals like lead (Pb) and cadmium (Cd) by biological pathways causes certain effects on the organism. This study explores the effects of lead treatment on Danio Rerio embryos (Zebrafish). Embryos were exposed to lead and cadmium in various concentrations at 0.1 µg/l, 1 µg/l, 10 µg/l, 20 µg/l, 100 µg/l. These results revealed that lead exposure can cause abnormalities in the structure of Zebrafish embryos.

Keywords: Danio Rerio, cadmium, lead, embryos, cardio activity.

1. Introduction

The Zebrafish has emerged to become a powerful vertebrate animal model for cardiovascular research in recent years. Its advantages include easy genetic manipulation, transparency, small size, low cost, and the ability to survive without active circulation at early stages of development. Mouse is the established mammalian model for cardiovascular research. Knockout mouse models made it possible to investigate numerous congenital heart defect (CHD) types (Li J 2015, Ta-Shma A 2016).

Embryonic chick is a commonly used vertebrate model with the advantage of resembling human heart structure with four-chamber/four-valve configuration and enabling clinically relevant surgical manipulations. Embryonic Zebrafish has emerged more recently for cardiovascular research to be used in high-throughput studies. Even though Zebrafish heart is different from human heart with only systemic circulation, heart structure and

physiology are similar between the two species. Unique characteristics for Zebrafish embryos make them particularly attractive for cardiovascular research (Huseyin C. Yalcin 2017).

The most important feature of zebrafish as a model is the fact that its genome has been fully sequenced. A body of evidence reveals that 71.4% of human genes are related to zebrafish genes (Howe 2013). Analysis of mapping data showed conservation between zebrafish and human genes and revealed significant degrees of synteny between conserved genes (Barbazuk 2000, Woods 2000). It has also been shown that major zebrafish tissues and organs share many features with human counterparts at the anatomical, physiological and molecular levels, including brain, heart, muscles, kidney, and liver (Bassett 2003, Drummond 2005, Bakkers 2011, Santoriello 2012, Kalueff 2015).

Heavy metals are among the most widespread pollutants in environment and have a high potency to accumulate in sediments (Burton Jr 1991,

Chapman 1998). It has been well documented that excessive aqueous cadmium (Cd) or copper (Cu) exposure could lead to dramatic effects on fish embryos such as high mortality, low hatchability, delay in time to hatch, reduced length, weight and head height, cardiac activity disturbances and numerous developmental impairments including craniofacial alterations, yolk sac abnormalities, spinal deformities, oedemata, cardiovascular disturbances and lack of pigmentation (reviewed by (Jezińska 2009)). In addition, both heavy metals have been reported as potent genotoxicants in several fish cell lines, generating reactive oxygen species (ROS) that can induce DNA strand breaks and trigger apoptosis and/or cell death

2. Material and methods

2.1. Embryo collection

Embryo collection and lead treatment Zebrafish were bred in a 14 h:10 h Light: Dark cycle. The fish were mated with a male/female ratio of 1:2 in a baffle tank which separated males and females. In the next day, the septum is removed for fish mating. The embryos were collected after 30 mins of mating. Embryos were bred in 250 ml Erlenmeyer flask and treated with lead in different concentration, including 0.1 µg/l, 1 µg/l, 10 µg/l, 20 µg/l, 100 µg/l.

2.2. Chemicals

CdNO₃. 2H₂O, phosphate-buffered saline (PBS), nitric acid 65% and normal melting point agarose (NMP) and Pb standard 1000 mg Titrissols (PbNO₃ in H₂O) was purchased from RANGNGEN Company.

2.3. Fish breeding and embryos collection

Mature fish were reared and maintained in 40 L tanks with a male: female ratio of 1:2, at a temperature of 26°C with a photoperiod of 12 h light:12 h dark. Tanks were cleaned using a siphon and 30% was renewed with stalling water (1/3 24 h-dechlorinated tap water and 2/3 purified water by reverse osmosis) twice a week. Fishes were fed once a day with dry flakes (Tetramins Tropical, Tetra, Melle, Germany) and once a day with brine shrimp *Artemia* sp. nauplii (Ocean Nutrition, Assen, Belgium). It was visually checked daily. During the spawning stimulation period, medaka genitors were reared at 28 °C under a 16 h:8 h light: dark photoperiod and fed four times a day. Females

were examined for eggs 1 h after onset of light and egg clusters were gently removed by hand. Harvested eggs were pooled and then individualised by rolling them softly to sever attaching filaments. Finally, they were staged according to (Iwamatsu 2004, Gonzalez-Doncel 2005) under a stereomicroscope and cold light source to discard unfertilised or dead individuals and only pre-morula stage embryos (2– 4 hours post fertilisation (hpf)) were selected for the study.

2.4. Evaluation of live embryos

The live embryo showed a transparency, round shape, normal chorion, and uniformly yolk. The dead embryos were removed from experiments. The ratio of live embryo was estimated at 48 h and 72 h. The heart rate of embryos was recorded and counted under microscope.

2.5. Statistical analysis

The data were analysed for statistical significance by one-way ANOVA where $P \leq 0.05$ was considered statistically significant (Tuan Van Ngo 2021).

2.6. Results

Figure 2 showed that there was no difference in the ratio of live embryo from control group and other groups for 48 h lead and cadmium treatment. After 48 h, an increase of lead and cadmium treatment began to reduce the number of live embryos.

Cadmium and lead tested concentrations did not induce lethal effects on both embryos and larvae, viability for lead and Cadmium exposed fish ranged from 85% to 96% at the embryonic stage. Larval survival ranged from 75% to 89% for the various treatments (Table1).

Almost all viable embryos hatched before the end of the experiment for both heavy metal exposures. As a result, hatching rates were very close to embryonic viability and no significant difference was observed in the treatments. Indeed, mean hatching success ranged from 80% to 91% for Cd-exposed embryos and from 93% to 99% for Pb-exposed embryos (Fig. 2). Absorption of lead is higher than cadmium according to exposure conditions in the oropharyngeal stage.

In this study, we also assess the effects of lead treatment on heart rate of zebra fish embryo. As seen in the Figure 2, an increase of lead induced

the heart rate for 48 h and 72 h treatment. After 48 h of treatment, the heart rate of control group was 113.3 bpm. The group of 0.1 µg/l lead treatment showed a higher heart rate (121.3 bpm) than control group. Groups of 1 µg/l, 10 µg/l and 20 µg/l lead treatment showed an increase of heart rate (127.3 bpm, 128.0 bpm, and 130.7 bpm, respectively)

2.7. Genotoxic effects

The mean percentage of tail DNA measured in control groups was inferior to 10% which confirmed that cells, even after dissociation treatment, were in satisfactory conditions for comet assay analysis (Collins 2004).

Cadmium and lead exposure led to a significant DNA damage induction from the lowest concentration as shown by the significant increase in the percentage of tail DNA from 7% for the control group up to 48-60% in average for contaminated groups (data not show). Only Pb-100 M and Cd-100 M concentrations induced significant DNA damage increase in comparison to control group (5% of tail DNA in control Fig. 3)

Zebrafish have been used successfully to understand the biological activity of genes orthologous to human disease-related genes in greater detail 3-5. To investigate the number of potential disease-related genes, we compared the list of human genes possessing at least one

Figure 1: Zebrafish (*Danio Rerio*)



zebrafish orthologue with the 3,176 genes bearing morbidity descriptions that are listed in the Online Mendelian Inheritance in Man (OMIM) database. Of these morbid genes, 2,601 (82%) can be related to at least one zebrafish orthologue. A similar comparison identified at least one zebrafish orthologue for 3,075 (76%) of the 4,023 human genes implicated in genome wide association studies (Kerstin Howe, Philip Howden et al. 2013)

2.8. Discussion

These results are in compliance with observations reported in the literature which describe hatching as usually occurring between 9 and 12 dpf at 25-26°C for the Japanese medaka (Gonzalez-Doncel 2003, Farwell 2006, Gonzalez-Doncel 2008).

Table 1: The heart rate of embryos

		Control	0,1 µg/l	1 µg/l	10 µg/l	20 µg/l	100 µg/l
Live embryos (48h)	Pb ²⁺	114	120	128	130	132	136
		112	120	126	126	130	134
		114	124	128	128	130	132
	Cd ²⁺	114	120	128	130	132	136
		111	120	126	128	130	134
		113	122	126	124	128	130
Oropharyngeal (72h)	Pb ²⁺	120	128	136	142	144	148
		124	128	132	138	142	150
		122	124	132	138	144	146
	Cd ²⁺	121	126	135	138	141	147
		124	125	130	139	140	146
		122	126	132	140	141	148

Figure 2: The effect of lead and cadmium treatment on the ratio of live embryo (48h)

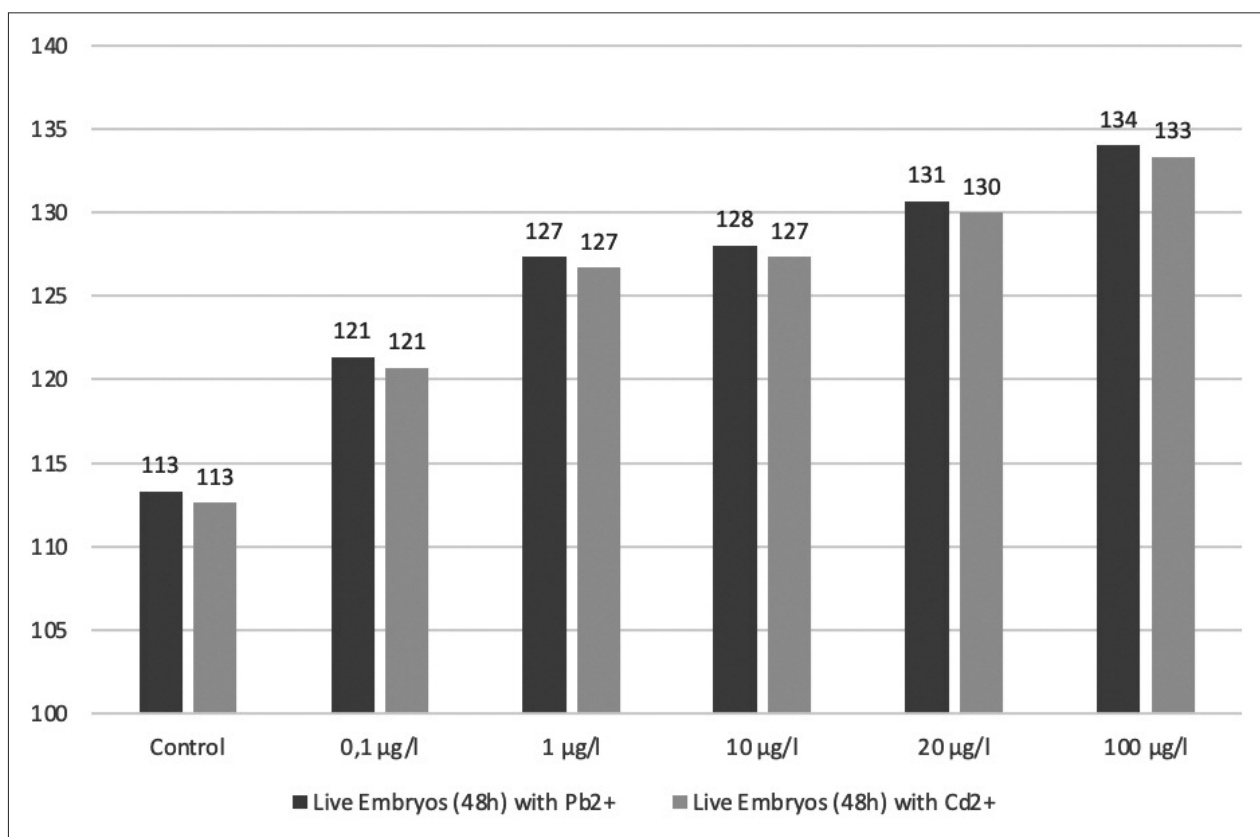
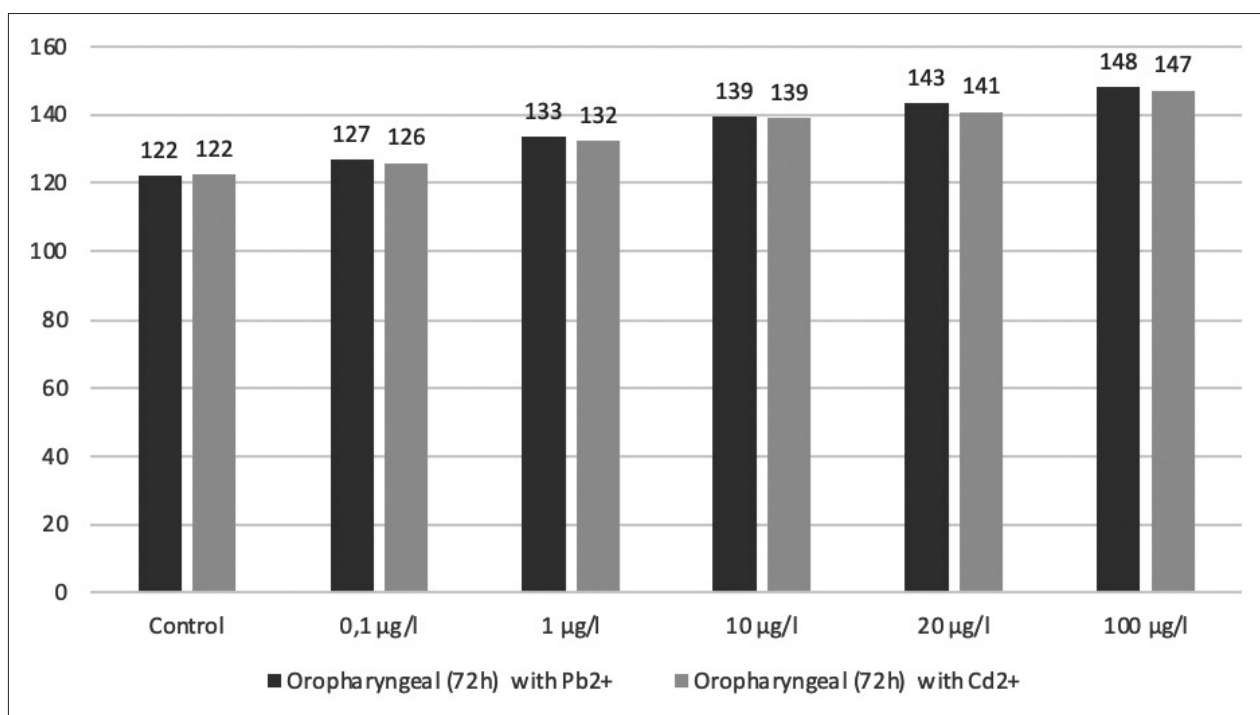


Figure 3: The effect of lead and cadmium treatment on the ratio of Oropharyngeal (72h)



The previous studies reported the toxicity of heavy metals on fish such as oxidative stress (Liu J 2009), respiratory problems (Wang H 2013) and morphological deformations of the fish (Jezierska 2009, Boglione C 2013). In the present work, an increase of lead treatment showed the reduction of zebrafish embryo development. Lead treatment not only affects embryo development, but also increases heart rate of zebrafish.

Embryos that were allowed to develop in direct contact to control sediment in our test conditions showed survival rates around 90% and 80% at embryonic and larval stages, respectively, and hatching success superior to 85%. These results are in compliance with the guidelines for ELS assays which set minimum hatching success and survival rate after hatching at 80% each (OECD 1992)

Average times to hatch observed in control treatment were very similar in both experiments (10.2 dpf for both Cd and Pb exposures) with more than 75% of embryos hatching at 10 dpf.

3. Conclusion

Observations on control embryos demonstrate that the sediment-contact exposure protocol performed in this study provides acceptable conditions for embryo development. This study demonstrates the evaluation of sediment hazardous potency at environmental concentrations of metals. Moreover, it confirms the importance of performing both chemical analyses and pertinent toxicity tests to evaluate hazards of pollutants accumulated in sediments since low concentrations of heavy metals could result in non-negligible deleterious effects on early developmental stages of various fish species. (Iris Barjhoux 2012) ■

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ĐÁNH GIÁ TÁC ĐỘNG CỦA VIỆC TÍCH LŨY CHÌ VÀ CADIMI ĐỐI VỚI SỰ PHÁT TRIỂN CỦA CÁ NGỰA VẦN

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TÓM TẮT:

Sự tích lũy kim loại nặng chì (Pb) và cadimi (Cd) bằng con đường sinh học gây ra những ảnh hưởng nhất định đến cơ thể sinh vật. Nghiên cứu tập trung vào tác động của việc xử lý chì đối với phôi *Danio Rerio* (Cá ngựa vằn). Phôi được tiếp xúc với chì và cadmium ở các nồng độ khác nhau ở 0,1 µg/l, 1 µg/l, 10 µg/l, 20 µg/l, 100 µg/l. Những kết quả này cho thấy việc tiếp xúc với chì có thể dẫn đến sự bất thường về cấu trúc của phôi cá ngựa.

Từ khóa: cá ngựa vằn, cadimium, chì, phôi cá, hoạt động của tim.