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Heavy Metal Toxicity Perturbs Neuroendocrine Function, Cardiac Morphology, and Cardiac Functions in the Zebrafish Model

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Abstract

Heavy metal pollution is an increasing form of environmental contamination, in recent times, caused by toxic metals such as copper, zinc, lead, cadmium, chromium, *etc*. It has become a major cause of concern due to the adverse effects it is causing around the world. Heavy metals enter water bodies from industrial and consumer wastes and cause damage to different life forms that are dependent on the water body. The impact of these heavy metals can be assessed using various animal models, such as the Zebrafish (*Danio rerio*). It is a widely used model in toxicological studies for evaluating the alterations that occur in the endocrine system upon exposure to endocrine-disrupting chemicals, which include heavy metals. Apart from damaging the endocrine system, these metals can also damage the central nervous system, resulting in progressive neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and cancer on long-term exposure. There have also been reports of alterations in the neurobehavior of zebrafish and their larvae on exposure to different doses of various heavy metals, as found in several behavioural assays. In stress conditions, the alteration in the neuroendocrine system and cardiac function takes place, which leads to anxiety, depression, and bradycardia. Exposure to heavy metals also leads to a delayed hatch rate of zebrafish embryos. Since zebrafish shares 86% of similar genes associated with human diseases, it can be used to study the effects of heavy metal exposure on the neuroendocrine and cardiovascular systems. In this review, we elucidate the mechanisms by which heavy metals alter the neuroendocrine system and cardiac function of zebrafish and their embryos, which include induction of oxidative stress, disruption in the neurotransmitter pathways, and interference with calcium signalling, along with the dose-dependent impact on cardiac gene expression profile and neurobehavioral markers.

Keywords: Cardiac Function, Embryos, Heavy Metals, Neuroendocrine Disruption, Zebrafish

1. Introduction

Recent industrialisation and urbanisation have drastically increased the usage of heavy metals in different manufacturing fields, including the paper industry, pesticide production, welding, battery production, paint production, *etc*[1]. The wastewater discharge from these industries pollutes the adjacent water bodies, thereby affecting the aquatic organisms. As these heavy metals have the property of bioaccumulation, their concentration keeps increasing in the living system. This bioaccumulation and the presence of heavy metals in

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river ecosystems cause adverse effects on different organs of fish[2]. They cause deformities in fish, which affect the survival rate and appearance of fish in both laboratory and natural conditions. However, they serve as an excellent biomarker in quantifying heavy metal pollution in the environment[3]. Zebrafish serve as an excellent model for neurodegenerative disease research because of their genetic homology with humans, sharing up to 86% of human genes associated with neurodegenerative diseases[4]. Further, the zebrafish embryos also show developmental toxicity when exposed to various concentrations of several heavy metals, such as differences in hatching, heart rate, and survival rate of larvae[5]. In this review, we elucidate the mechanism by which heavy metals alter the neuroendocrine system and cardiac function of zebrafish and their embryos.

2. Endocrine Disruption due to Heavy Metals

Several studies were conducted to analyse the endocrine-disrupting effects of heavy metals, using zebra fish models. It is reported that copper suppressed the growth of the fish and affected the structure of the gonads of both sexes. Further, the hormonal levels and expression of genes associated with the HPG axis of zebra fish were altered[6]. Chronic cadmium exposure to the parental generation reduced the hatching rate and survival rate of offspring. Cadmium is a very potent antiestrogen and acts by inhibiting the action of estrogen in the brains of developing zebrafish[7], besides causing malformations in the offspring. It is reported that the regulation of the expression of thyroid axis-related genes is disturbed by cadmium exposure and that it is intergenerational[8]. It is reported that chronic uranium exposure causes thyroid dysfunction. In the zebrafish larvae, a significant reduction in the triiodothyronine (T3) levels and an increase in the thyroxine (T4) levels were observed. The expression of HPT axis-related genes, NIS, Deio1, Deio2, TRα, TSHβ, and UGT1ab was also significantly down-regulated after uranium exposure[9]. It was also found that uranium exposure disturbed the thyroid feedback system. Furthermore, reports on the effects of chromium contamination on zebrafish show that the intake of chromium-contaminated feed by female fish led to transmission of chromium to offspring and corresponded to orange yolk colour along with poor larval health and survival[10]. On exposure to mercurial fungicide, the zebrafish produced fewer eggs, and the hatching rate was decreased as well[11]. All these studies shed light on the endocrinological effects of different heavy metals on zebrafish.

3. Bioavailability and Uptake Kinetics of Different Heavy Metals in Zebrafish

The bioavailability and uptake kinetics of heavy metals in zebrafish are determined by a multifaceted interplay of chemical forms, environmental context, and biological factors, all of which critically influence toxicity outcomes. Metals such as cadmium, lead, copper, and nickel are commonly present in aquatic environments as free ions, nanoparticles, or bound to organic and inorganic ligands, and their chemical form dictates how readily they are absorbed by zebrafish. Ionic metals like Cd^{2+} and Pb^{2+} compete with essential ions such as Ca^{2+} and Na^{+} in the gill and intestinal epithelia, thereby disrupting ion regulation and increasing acute toxicity. Silver and cadmium sulfide nanoparticles exhibit even higher bioavailability due to their small size and surface reactivity, and can easily penetrate biological barriers, resulting in accumulation in tissues, as demonstrated by pronounced histopathological changes in gills and liver[12,13]. Embryos were partially protected by the chorion, which adsorbs a significant portion of metals, but post-hatching larvae exhibit much higher permeability and less developed detoxification systems, resulting in greater susceptibility to metal uptake and accumulation. The chorion membrane acts as a physical and chemical barrier that reduces the direct exposure of the toxicants on embryos, but post-hatching, the toxicants are directly exposed to the larval skin and gills, which are more permeable than the chorion. Furthermore, the liver and certain enzymes that help in neutralisation of the toxins are not fully developed to detoxify[14]. Chronic and combined exposures, such as those involving metals and antibiotics, can further increase bioaccumulation and oxidative stress, thereby compounding developmental and behavioural defects[15,16]. Tissue-specific accumulation is well documented, with metals concentrating in metabolically active organs like the gills, liver, gonads, and brain, leading to a spectrum of adverse outcomes from neuroendocrine disruption to cardiac dysfunction[15,17]. Mechanistically, metals disrupt sodium and potassium homeostasis, induce

diffusive ion loss, and can damage epithelial integrity, while co-exposure to other pollutants can exacerbate these effects through synergistic interactions[16]. These dynamics underscore the importance of considering metal speciation, environmental chemistry, and organismal biology in both experimental design and ecological risk assessment, as they are essential for interpreting toxicity data and establishing relevant water quality standards.

4. Effect of Heavy Metals on Developmental Cardiac Morphology and Cardiac Function of Zebrafish

Heavy metals, apart from causing dysfunction of the endocrine system, also cause damage to the cardiac system, either directly or indirectly. The cardiac function of zebrafish is analysed by measuring the heart rate of zebrafish larvae, and one of the common effects of exposure to almost all heavy metals is the dose-dependent alteration in the heart rate of zebrafish. The larvae's heart rates from the group exposed to arsenic were increased by approximately 50% compared with those of untreated fish[18]. Furthermore, in the zebrafish embryos treated with arsenite, immunostaining with MF20 and S46, to visualise the ventricle and atrium, respectively, revealed that the MF20-stained ventricle appeared small in size when compared to the control. It also influences the change in the heart rate of the embryos, leading to bradycardia[19]. The heart rate for cadmium-treated larvae was also found to be increased with concentration, and was significant even at a low concentration of 0.01µM[20]. On the contrary, the embryos exposed to silver nanoparticles (Ag-NP) showed reduced heart rate and alterations in the morphology compared to control and embryos exposed to platinum nanoparticles (Pt-NP). The heart of untreated embryos appeared normal with intact atria and ventricles and normal pumping, whereas thin and distorted chambers were seen in the Ag-NP exposed embryos, which slows down the pumping of blood, thereby reducing the heart rate[12]. It was reported that the down-regulated expression of amhc, vmhc, cmlc, and tnnt2 in Ag-NP exposed embryos might be the reason for reduced heart rate in embryos, as mutation in these genes leads to reduced cardiac contraction and defective cardiac function[21]. Contrastingly, upregulated expression of *amhc* and *nppa* was observed in the embryos exposed to Carbon Black Waste (CBW), which constitutes Nickel and Vanadium. Upregulation of the amhc gene is linked to cardiomyocyte hypertrophy as an alteration in Myosin Heavy Chain (MHC) composition takes place, resulting in lower ventricular systolic resistance in the heart. The upregulation of the *nppa* gene alters the heart and the cardiac performance of zebrafish, as it is responsible for cardiac muscle proliferation. The cardiac performance of zebrafish exposed to CBW is explained by the action of these two genes[22]. Further, in Au-NP-treated embryos, a large pericardial sac region and a shrunken cardiac ventricle were observed, which contributes to a reduction in heart rate[23]. Figure 1 summarises the genes involved in decreasing the heart rate of the larvae during development.

Exposure to CBW and AgNP leads to reduced heart rate due to the deregulation of several genes. The genes *amhc* and *nppa* are upregulated by CBW exposure, leading to cardiomyocyte hypertrophy and cardiac muscle proliferation, respectively. AgNP exposure downregulates the expression of *vmhc* and *cmlc*, leading to distorted heart chambers and reduced cardiac contraction, respectively.

Abbreviations: CBW, Carbon black waste; AgNP, Silver nanoparticle; amhc, Atrial myosin heavy chain; nppa, natriuretic peptide A; vmhc, Ventricular myosin heavy chain; cmlc, cardiac myosin light chain

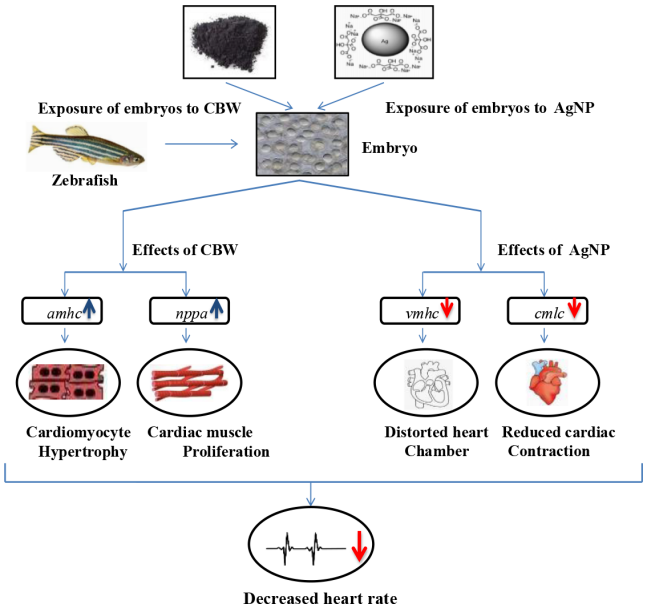


Figure 1. Schematic representation of genes involved in decreasing the heart rate of the larvae during development.

5. Effect of Heavy Metals on the Neurobehavior of Zebrafish

As already discussed, heavy metals act as endocrine disruptors that affect the proper functioning of the hormones. Additionally, as the working of the endocrine system is intertwined with that of the nervous system, with a bi-directional communication happening between the systems, the nervous system is also affected by heavy metal exposure. Several studies reported the effects of heavy metals on the nervous system. Prolonged exposure to mercury led to disruptions in neurocognitive functions and stress-related responses. Similarly, chronic exposure to mercury led to locomotor defects and anxiety-related behaviour, which was evident through a reduction of swimming speed and increased resting time. This defect induced by $HgCl_2$ largely contributes to impairment in the behaviour of the fish[24]. Exposure to iron reduced the ability of larvae to elicit a fear response, along with other drastic effects on the neurophysiological performance of zebrafish. It also led to a rise in the oxidative stress response[25]. Chronic exposure to lead in the form of $PbCl_2$ decreases aggressiveness and leads to high anxiety and freezing behaviour. Exposure to lead was also reported to cause Posttraumatic Stress Disorder (PTSD) and was related to increased secretion levels of catecholamines and cortisol, a hormone known to increase stress response[26]. Lead (Pb) and Cadmium (Cd) tend to concentrate in many of the CNS regions, with a greater extent in the hippocampus, and these have been shown to increase the levels of serotonin in Zebrafish. The plausible mechanism by which the concentration of Pb in the body affects the secretion and thereby the levels of serotonin is well reported. Pb tends to impede the metabolism of tryptophan by altering the activity of tryptophan pyrolase. The decreased activity of tryptophan pyrolase is due to the replacement of Iron (Fe) by Pb in the heme protein. Tryptophan, being the precursor molecule for serotonin and other important regulatory molecules, plays a major role in neurobehavior, and any changes in its levels can cause detrimental effects on the nervous system. Pb and Cd have also been shown to affect the calcium regulation capacity of calmodulin in the brain. Ca^{++}-calmodulin complex indirectly regulates the activation of Tyrosine Hydroxylase (TH) via phosphorylation. Owing to the effects of Pb, TH is activated in excess, and this facilitates 5-HT synthesis. This further results in depression or anxiety-like behaviour due to the altered 5-HT synthesis[27]. Figure 2 represents the behaviour and biochemical changes in adult zebrafish exposed to lead chloride.

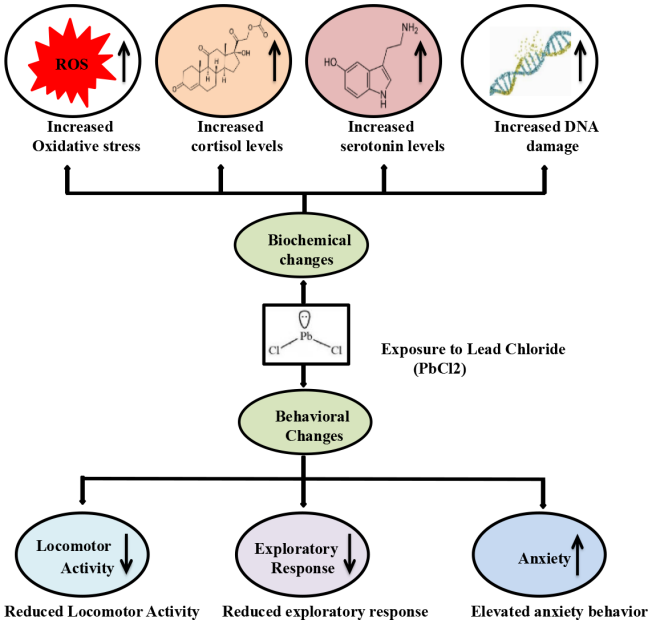


Figure 2. Schematic representation of behaviour and biochemical changes on chronic exposure of $PbCl_2$ to adult zebrafish.

Exposure to lead chloride leads to several biochemical and behavioural changes in the zebrafish. While biochemical assessment has revealed the presence of increased oxidative stress, cortisol levels, and serotonin levels, along with DNA damage, following lead chloride exposure, behavioural assessment has shown reduced locomotor activity, exploratory response, and increased anxiety behaviour with lead chloride exposure.

6. Conclusion

The consequence of heavy metal exposure has been widely analysed through various developmental and neurobehavioral endpoints in zebrafish, and the potential effects of this exposure have been inferred from a wide range of studies, imposing the need for safe discharge of heavy metals from industries. These studies have helped in identifying several endocrine-disrupting chemicals and elucidating the possible adverse health effects. However, further studies are required to elucidate the mechanisms of action responsible for these adverse effects. Additionally, studies are required to analyse the effects of a mixture of heavy metals, rather than one single metal, as environmental and occupational exposure are often complex. Zebrafish can act as an effective animal model for such studies, as they help to evaluate the impact

of heavy metals at the developmental, behavioural, and molecular levels.

7. References

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