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Effects of sub-chronic exposure to lead (Pb) and ascorbic acid in juvenile rockfish: Antioxidant responses, MT gene expression, and neurotransmitters

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HIGHLIGHTS

- Antioxidant responses were affected following exposure to dietary Pb.
- *MT* gene expression was induced following exposure to Pb.
- AChE activity was inhibited following exposure to Pb.
- AsA supplementation effectively attenuated these Pb-induced toxicity.

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ABSTRACT

Juvenile rockfish *Sebastes schlegelii* were exposed to varying levels of dietary lead (Pb^{2+}) at 0, 120 and 240 mg/L, and ascorbic acid (AsA) at 100, 200 and 400 mg/L for four weeks. Antioxidant responses such as superoxide dismutase (SOD), glutathione S-transferase (GST), and glutathione (GSH) were analyzed to assess oxidative stress. SOD and GST activity in the liver and gills were considerably elevated by dietary Pb. In contrast, GSH levels in the liver and gills were significantly reduced following Pb exposure. High levels of AsA supplementation attenuated the increase in SOD and GST activity and reduction in GSH levels. The metallothionein gene (*MT*) in the liver was notably stimulated by Pb exposure, and AsA supplementation attenuated this increase. With respect to neurotoxicity, acetylcholinesterase (AChE) activity was substantially inhibited in the brain and muscle following Pb exposure. AsA supplementation also attenuated AChE inhibition following Pb exposure. The results of this study presented Pb exposure affected rockfish as toxicity, and AsA was effective to alleviate toxic effects of Pb.

1. Introduction

Metal pollution in aquatic system has occur by many industrial and agricultural processes (Javed and Usmani, 2015a,b). High concentrations of metal exposure lead to metal accumulation in the tissues of fish, and the accumulations may catalyze reactions generating reactive oxygen species (ROS), which may induce environmental oxidative stress (Farombi et al., 2007). Among various toxic metals, lead (Pb) is one of the most toxic metals, and Vinodhini and Narayanan (2008) reported Pb was much higher accumulation in fish tissues than other metals such as cadmium (Cd), chromium (Cr), and nickel (Ni).

Oxygen is essential for aerobic organisms to carry out various metabolic processes. However, a reliance on oxygen forces aerobic organisms to withstand related toxicity, such as that observed with increased levels of ROS that lead to considerable cell damage (Ahmad et al., 2004). Oxidative stress associated with ROS attack occurs when antioxidant defenses are not effective in detoxifying ROS. Exposure of aquatic animals to toxic substances generally induces oxidative stress within the intracellular space, by generating ROS, such as the superoxide anion, hydrogen peroxide, and hydroxyl radical (Kim and Kang, 2015a). Alterations in antioxidant levels can be reliable and sensitive biomarkers of toxicants (Ferreira et al., 2005; Ahmad and Ahmad, 2016). Among the various toxicants, Pb is considered a critical inducer of oxidative stress (Mucha et al., 2003). The accumulation of heavy metals in aquatic animals could catalyze the production of ROS that can in turn cause the oxidative stress in aquatic animals (Farombi et al., 2007). Defensive

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mechanisms, including various antioxidant defense enzymes become activated to counteract ROS production (Tjalkens et al., 1998). Among the various antioxidant mechanisms, superoxide dismutase (SOD) is the first defense system to counteract oxidative stress caused by ROS production, and converts the superoxide anion into hydrogen peroxide (Vlahogianni et al., 2007). Glutathione S-transferase (GST) is an antioxidant and phase II detoxification enzyme that can be a reliable biomarker of oxidative damage in aquatic animals (Regoli and Principato, 1995). Glutathione (GSH) protects cells against ROS production due to exposure to toxic substances as it is the most abundant intracellular thiol-based antioxidant that also serves as a cofactor for antioxidant enzymes (Pandey et al., 2008).

As a metal-binding protein that is induced by metal toxicity, there are multiple metallothionein (*MT*), and *MT* plays a role in metal ion homeostasis, detoxification, and stress response of metals (Jenny et al., 2004). It is generally accepted that elevated intracellular metal accumulation following exposure of fish to metal ions, causes an increase of *MT* in target organs like the liver, kidney, and gills (Baudrimont et al., 2003). Given that *MT* is closely associated with metal exposure because of its ability to bind metals for detoxification (Ay et al., 1999), it can be a sensitive and reliable biomarker of metal contamination in aquatic environments (Chen et al., 2004).

Pb exposure can also cause neuronal injury in fish, a condition that has harmful effects on the central nervous system. Many authors have reported significant alterations in learning and memory as well as locomotor activity and sensorimotor responses in vertebrate species including fish, following exposure to Pb (Kuhlmann et al., 1997; Weber et al., 1997; Carvan et al., 2004). Among various neurotransmitters, acetylcholine (ACh) is associated with cognitive processes via the stimulation of muscarinic and cholinergic receptors. Acetylcholinesterase (AChE) maintains ACh levels by catalyzing ACh. Exposure to metal ions can induce neurodegeneration owing to oxidative stress (Senger et al., 2006). Pb is a neurotoxin that affects the developing central nervous system. Exposure to Pb even at low concentrations can induce behavioral abnormalities and impaired cognitive abilities in animals (Xu et al., 2005). Significant AChE inhibition is generally exhibited in fish exposed to various toxicants, and AChE inhibition can be a sensitive biomarker of neurotoxicity in fish (Modesto and Marinez, 2010).

Ascorbic acid (AsA, Vitamin C) is one of the most critical nutrients in fish that is essential for growth and immunity (Kim and Kang, 2015b). Fish tissues contain many polyunsaturated fatty acids (PUFAs) that are vital for proper membrane function. Fish must have effective antioxidant defenses, because PUFAs are highly susceptible to oxidative damage. Therefore, the dietary supply of essential antioxidants, such as vitamins, is in part necessary to protect them from oxidative damage (Martinez-Alvarez et al., 2005). Singh et al. (2014) suggest that AsA functions as an antioxidant that protects the cell from ROS both in vivo and in vitro, by scavenging aqueous ROS via rapid electron transfer that effectively reduces lipid peroxidation. AsA is also a cofactor for the biosynthesis of neurotransmitters, particularly the transformation of dopamine to norepinephrine, a reaction that is catalyzed by dopamine β-monooxygenase (Yousef, 2004).

In Korea, rock fish (*Sebastes schlegelii*), the experimental animal in the present study, is a major fish species cultured in marine net cages. The species is in high demand because of the quality of its flesh and its rapid growth (Kim and Kang, 2016). However, an insufficient number of studies have been conducted on the toxic effects of exposure to dietary Pb in this species. Based on AsA functions to improve immune and antioxidant responses (Yamaguchi et al., 1995; Chen et al., 2004), dietary AsA supplementation might protect *S. schlegelii* from harmful effects of dietary Pb exposure. To our knowledge, the protective role of AsA against Pb-induced alterations in antioxidant responses, *MT* gene expression, and the role of AChE in *S. schlegelii* has not been investigated. The purpose of this study was to evaluate toxic effects of dietary Pb and detoxification effects of AsA on the associated antioxidant responses, *MT* gene expression, and neurotransmitters in rock fish, *S. schlegelii*.

2. Materials and methods

2.1. Experimental fish and conditions

S. schlegelii were acclimatized for 2 weeks before the experiment. Experimental condition is demonstrated in Table 1. Similarly sized *S. schlegelii* (90 fish; body length 11.3 ± 1.2 cm, body weight 32.5 ± 4.1 g) were selected to conduct this study. The exposure experiment was conducted by feeding the diets containing lead (Pb) and ascorbic acid at a rate of 2% body weight daily (as two 1% meals per day) with 6 fish per treatment group. The glass tank water was thoroughly exchanged once per two days. At the end of 2 and 4 week exposure, fish dissection was conducted immediately after complete anesthesia using buffered 3-aminobenzoic acid ethyl ester methanesulfonate. All experimental animals used in this study were maintained under a protocol approved by the Institutional Animal Care and Use Committee of the Pukyong National University.

2.2. Feed ingredients and diets formulation

Compositions of diets are demonstrated in Table 2. Lead (II) nitrate was obtained from Sigma Chemical Co., Ltd. The respective lead (Pb) and ascorbic acid (AsA) concentrations were made with Pb premix and AsA premix. The respective concentrations were 0, 120, and 240 mg/kg (Pb) and 100, 200, and 400 mg/kg (AsA). In Korea, the Pb concentrations in the coastal sediment reached up to 92 mg kg-1 (Lim et al., 2007). Although the Pb concentrations are much higher than inhabited environment, this experiment can suggest the toxic effects of dietary Pb exposure. Pb premix was made up of 2 g lead with 98 g cellulose, and AsA premix was made up of 2 g ascorbic acids with 98 g cellulose. After blending all components, water was added to make stiff dough, and pellets were made by feed mill machine. All the diets were stocked into the deep freezer at - 20 °C until use after drying the diets for 24 h at room temperature (Kim and Kang, 2016). The actual Pb and AsA concentrations are demonstrated in Table 3. The actual Pb concentrations were determined by using ELAN 6600DRC ICP-MS (Perkin-Elmer), and the actual AsA concentrations were determined by using Aglient 1200 series HPLC (Aglient). The actual Pb and AsA concentrations in the diets were expressed as mg/kg dry wt.

Table 1
Chemical components of seawater used in this study.

Item	Value
Temperature (°C)	19.0 ± 1.0
pH	8.1 ± 0.5
Salinity (‰)	33.2 ± 0.5
Dissolved Oxygen (mg/L)	7.1 ± 0.3
Chemical Oxygen Demand (mg/L)	1.15 ± 0.2
Ammonia (μg/L)	10.3 ± 0.7
Nitrite (μg/L)	1.4 ± 0.2
Nitrate (μg/L)	9.27 ± 1.0