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Oxidative stress, neurotoxicity, and metallothionein (MT) gene expression in juvenile rock fish *Sebastes schlegelii* under the different levels of dietary chromium (Cr^{6+}) exposure

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ABSTRACT

Juvenile *Sebastes schlegelii* were exposed for 4 weeks with the different levels of dietary chromium (Cr^{6+}) concentration (0, 30, 60, 120 and 200 mg/kg). The superoxide dismutase (SOD) activity, glutathione S-transferase (GST) activity, and glutathione (GSH) level of liver and gill were evaluated after 4 weeks exposure. The SOD and GST activity of liver and gill was significantly increased **in the concentration of 240 mg/kg after 2 weeks and over 120 mg/kg after 4 weeks**, whereas a considerable decrease **in the concentration of 240 mg/kg after 2 weeks and over 120 mg/kg after 4 weeks** was observed in the GSH levels of liver and gill. In neurotoxicity, AChE activity was significatly inhibited in brain **in the concentration of 240 mg/kg after 2 weeks and over 60 mg/kg after 4 weeks** and muscle **in the concentration of 240 mg/kg after 2 weeks and over 120 mg/kg after 4 weeks**. Metallothionein (MT) gene in liver was considerably increased **over 120 mg/kg after 2 weeks and at 30, 120, and 240 mg/kg after 4 weeks** by dietary chromium exposure. The results indicate that dietary Cr exposure over 120 mg/kg can induce substantial alterations in antioxidant responses, AChE activity and MT gene expression.

1. Introduction

Heavy metals have been considered as one of the major toxicants in aquatic environment, which are also receiving increased worldwide attention due to their toxic effects on human health and other aquatic animals (Palaniappan and Karthikeyan, 2009; Kim and Kang, 2014). Most heavy metals are naturally in existence in the environment, but high levels of heavy metal exposure commonly occur by anthropogenic activity that is highly toxic to aquatic animals (Kim and Kang, 2015a).

Chromium is one of the most toxic heavy metals found in the effluents of various industries such as chrome-tanning industries, electroplating, textiles, dyeing, and photographic industries, whereas it is a crucial nutrient metal necessary for metabolism of carbohydrates, sugar, and lipid at a permissible amount (Kuykendall et al., 2006). Chromium (Cr) has two main states in aquatic environment such as trivalent chromium (Cr III) and hexavalent chromium (Cr VI). Hexavalent chromium (Cr VI) is the prominent toxic chromium, and its cellular uptake generally occurs by an anion (phosphate) transport mechanism in contrast to trivalent chromium (Cr III) that cannot enter cells by anion transport and seems to be biologically inactive (O'Brien et al., 2003). After the absorption and uptake of hexavalent chromium (Cr VI), the biological reduction of Cr (VI) to Cr (III) occurs by formation of DNA binding, which causes **tissue** damage (Sugiyama et al., 1991). The inhibition of ATPase-linked transport processes is one of the chromium toxic effects in fish, which results in the damage of respiratory and osmoregulatory functions in the absorptive organs such as the gills and intestines (Kuykendall et al., 2006). Heavy metals have been generally known as a critical inducer in generating reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and superoxide radical, which cause oxidative stress in addition to osmoregulatory dysfunctions and tissue damage (Dautremepuits et al., 2004; Kim and Kang, 2015b). Hexavalent chromium (Cr VI) produces the oxygen (O2) and hydroxide radical (OH^-) in the reaction of superoxide with hydrogen peroxide (Lushchak, 2008). Hexavalent chromium induces the generation of OH during the process of its reduction, and functions as a catalyst being able to enter reversible oxidation, which is related to ROS generation (Begum et al., 2006; Lushchak, 2011). Generally, oxidative stress occurs during the process to balance the ROS production with antioxidant defenses (Pena-Llopis et al., 2003). In response to ROS production against toxicants, superoxide dismutase (SOD) is commonly activated to scavenge the highly toxic superoxide as an antioxidant defense mechanism of organisms, and converts it to hydrogen peroxide. Glutathione S-transferase

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(GST) has an important function to catalyze the conjugation of pollutants to eliminate them from the cellular system. Glutathione (GSH) scavenges ROS directly by oxidizing reduced glutathione to oxidized glutathione (GSSG), which converts O_2^- to H_2O_2 (Dorval et al., 2003). The exposure to hexavalent chromium in aquatic animal clearly causes oxidative stress (Lushchak, 2011), and the evaluation of oxidative toxicity by xenobiotics can be a reliable indicator in environmental toxicology and ecotoxicology (Regoli et al., 2002). In biotransformation mechanism, the phase I functional transformation contains the oxidative, reductive, and hydrolytic reactions managed by cytochrome monooxygenase (CYP450) enzyme family, and the phase II conjugation such as GST is responsible for the alignment of the reactive substances generated in phase I or two compounds including covalent attachments of small polar endogenous molecules. This phase is responsible of the formation of water-soluble compounds (Dixon et al., 2010).

Acetylcholine (ACh) is one of the neurotransmitters, which is associated with cognitive processes, through the activation of muscarinic and cholinergic receptors. The reaction is catalyzed by acetylcholinesterase (AChE) to maintain the levels of ACh (Richetti et al., 2011). The heavy metal exposure induces neurochemical changes by its penetrating action across the blood brain barrier, which causes oxidative stress and alterations in the metabolism of some proteins involved in the neurodegeneration (Monnet-Tschudi 2006; Senger et al., 2006). The AChE inhibition results in the accumulation of acetylcholine at synaptic junctions, which causes acute cholinergic syndrome, and finally lead to death (Hsieh et al., 2001). Therefore, AChE is a critical biomarker to evaluate environmental contaminations.

Metallothioneins (MTs) are a heavy metal-binding protein, which is one of the mechanisms in aquatic organisms to resist metal toxicity through the detoxification process of toxic metals (Achard et al., 2004). It is widely known that the increased intracellular metal concentrations by heavy metal exposure to aquatic animals induce the increase in MT concentrations of target organs such as liver, kidney, and gill (Baudrimont et al., 2003). Considering it is one of the most important markers to monitor metal contamination in the environment (Linde et al., 2001), MT gene expression has been proposed as a sensitive and reliable biomarker for evaluating the toxic effects of metal exposure (George et al., 2004).

The hexavalent chromium exposure to fish in aquatic environment induces the behavioral symptoms such as erosion of scales, discoloration, mucus secretion, and surfacing and darting motion activity in addition to the elevated mortality (Vutukuru, 2005). In South Korea, the level of the chromium in coastal sediments ranges from 7.0 mg/L to 233.0 mg/L (Lim et al., 2007). In the aquatic environment, the metal absorption in fish under the metal exposure occur through the gill from waterborne status and through the ingestion from dietborne status, and the accumulation via the food chain is a major route of metal accumulation (Dural et al., 2007). The heavy metal contamination in the coastal sediments can affect the cultured fish in marine net. Rock fish, *Sebastes schlegelii*, is an economically major species largely cultured in marine net of Korea coast, **and they are carnivores**. But, the study about chromium toxicity has been insufficiently conducted. Therefore, the aim of the present study was to evaluate the toxic effects of dietary chromium exposure to the *S. schlegelii* on the oxidative stress, neurotoxicity, and metallothionein gene expression to assess the environmental stress and toxicity of chromium.

2. Materials and methods

2.1. Experimental fish and conditions

Juvenile rockfish (*S. schlegelii*) were obtained from a local fish farm in Tongyeong, Korea. The fish were acclimatized for 2 weeks under laboratory conditions. During the acclimation period, the fish were fed a Cr-free diet twice daily and maintained on a 12-h:12-h light/dark cycle and constant condition at all times (Table 1). After acclimatization, 60 fishes (body length, 13.7 ± 1.7 cm; body weight, 55.6 ± 4.8 g) were randomly selected for the study. **The 5 tanks for 2 weeks and 5 tanks for 4 weeks were prepared, and each tank had 6 fish for this study.** Dietary chromium exposure took place in 500 L circular tanks 6 fish per treatment group (5 tanks at 2 weeks and 5 tanks at 4 weeks). The dietary chromium concentrations were 0, 30, 60, 120, and 240 mg/kg (Table 2), and fish were fed each chromium concentration at a rate of 2% body weight daily (as two 1% meals per day). **During the feeding time, the intake state was monitored with the naked eye, and ensured that all fish were fed with the contaminated feed. The circular tank water was thoroughly exchanged once per two days using the draining system at the bottom of aquariums. All tanks were equipped with aeration system to maintain the proper dissolved oxygen concentration**. An extremely high dose of 240 mg/kg is a highly improbable occurrence in a real environment, but it provided an opportunity to evaluate lead toxicity in the experimental fish. Considering the probable effects level for hexavalent chromium in sediment (278 mg/kg) and the bio-magnification through food chain, the concentrations are suitable for the toxicity experiment to assess the dietary hexavalent chromium toxicity (Lee et al., 2008). At the end of each period (at 2 and 4 weeks), fish were anesthetized in buffered 3-aminobenzoic acid ethyl ester methanesulfonate (Sigma Chemical, St. Louis, MO).

2.2. Feed ingredients and diets formulation

Formulation of the diets is shown in Table 1. Potassium dichromate was obtained from Sigma Chemical Co., Ltd. All diets contained 33% casein, 23% fish meal, 5% corn starch, 2% vitamin premix, and 2% mineral premix. 10% fish oil was added to meet the essential fatty acids (EFA) requirements of rock fish. Chromium premix was made up of 1 g chromium with 99 g cellulose. Five isonitrogenous and isolipidic diets were formulated with supplementation of different dietary chromium concentrations of approximately 0, 30, 60, 120, and 240 mg/ kg diet. All ingredients were blended thoroughly. At last, water was added into the mixture to produce stiff dough. Then the dough was pelleted by experimental feed mill, and dried for 24 h at room temperature. After processing, all the diets were packed and kept-20 °C until use. For determination of total chromium concentrations in diets,

Table 1
The chemical components of seawater and experimental condition used in the experiments.

Item	Value
Temperature (°C)	20.0 ± 0.5
pH	8.1 ± 0.5
Salinity (‰)	33.2 ± 0.5
Dissolved Oxygen (mg/L)	7.1 ± 0.3
Chemical Oxygen Demand (mg/L)	1.21 ± 0.1
Ammonia (μg/L)	11.7 ± 0.8
Nitrite (μg/L)	1.6 ± 0.2
Nitrate (μg/L)	10.31 ± 1.0

Table 2
Formulation of the experimental diet (% dry matter).

Ingredient (%)	Chromium concentration (mg/kg)				
	0	30	60	120	240
Casein[a]	33.0	33.0	33.0	33.0	33.0
Fish meal[b]	23.0	23.0	23.0	23.0	23.0
Wheat flour[c]	20.0	20.0	20.0	20.0	20.0
Fish oil[d]	10.0	10.0	10.0	10.0	10.0
Cellulose[a]	5.0	4.7	4.4	3.8	2.6
Corn starch[c]	5.0	5.0	5.0	5.0	5.0
Vitamin Premix[e]	2.0	2.0	2.0	2.0	2.0
Mineral Premix[f]	2.0	2.0	2.0	2.0	2.0
Chromium Premix[g]	0.0	0.3	0.6	1.2	2.4
Actual Cr levels	2.3	29.1	61.8	116.7	237.3

[a] United States Biochemical (Cleveland, OH).
[b] Suhyup Feed Co., Ltd., Gyeong Nam Province, Korea.
[c] Young Nam Flour Mills Co., Pusan, Korea.
[d] Sigma Chemical Co., St. Louis, MO.
[e] Vitamin Premix (mg/kg diet): ascorbic acid, 240; dl-calcium pantothenate, 400; choine chloride 200; inositol, 20; menadione, 2; nicotinamide, 60; pyridoxine · HCl, 44; riboflavin, 36; thiamine mononitrate, 120, dl-a-tocopherol acetate, 60; retinyl acetate, 20000IU; biotin, 0.04; folic acid, 6; vitamin B_{12}, 0.04; cholecalcifero, 4000IU.
[f] Mineral Premix (mg/kg diet): Al, 1.2; Ca, 5000; Cl, 100; Cu, 5.1; Co, 9.9; Na, 1280; Mg, 520; P, 5000; K, 4300; Zn, 27; Fe, 40; I, 4.6; Se, 0.2; Mn, 9.1.
[g] Chromium Premix (mg/kg diet): 10,000 mg Cr/ kg diet.

ICP-MS measurements were performed using an ELAN 6600DRC ICP-MS instrument with argon gas (Perkin-Elmer). Total chromium concentrations were determined by external calibration. ICP multi-element standard solution VI (Merck) was used for standard curve. The chromium bioaccumulation in diet samples was expressed mg/kg dry wt.

2.3. Antioxidant enzyme analysis

Liver and gill tissues were excised and homogenized with 10 volumes of ice-cold homogenization buffer (0.1 M PBS buffer) using Teflon-glass homogenizer (099CK4424, Glass-Col, Germany). The homogenate was centrifuged at 4 °C 10,000*g* for 30 min under refrigeration and the obtained supernatants were stored at −80 °C for analysis.

Superoxide dismutase (SOD) activity was measured with 50% inhibitor rate about the reduction reaction of WST-1 using SOD Assay kit (Dojindo Molecular Technologies, Inc.). One unit of SOD is defined as the amount of the enzyme in 20 μl of sample solution that inhibits the reduction reaction of WST-1 with superoxide anion by 50%. SOD activity was expressed as unit mg protein^{-1}.

* WST-1 = 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H* -tetrazolium, monosodium salt

Glutathione-S-transferase(GST) activity was measured according to the method of modified Habig (1974). The reaction mixture consisted of 0.2 M phosphate buffer (pH 6.5), 10 mM GSH (Sigma) and 10 mM 1-chloro-2,-dinitrobenzene, CDNB (Sigma). The change in absorbance at 25 °C was recorded at 340 nm and the enzyme activity was calculated as 340 nm and the enzyme activity was calculated as nmol min^{-1} mg protein^{-1}.

Reduced glutathione was measured following the method of Beutler and Kelly (1963). Briefly, 0.2 ml fresh sample supernatant was added to 1.8 ml distilled water. Three ml of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 ml distilled water) was mixed with supernatants. The mixture was centrifuged at 4500*g* for 10 min. 1.0 mL of supernatant was added to 4.0 ml of 0.3 M NaHPO4 solution and 0.5 mL DTNB (5,5′-dithiobis-2-nitrobenzoic acid) was then added to this solution. Reduced glutathione was measured as the difference in the absorbance values of samples in the presence and the absence of DTNB at 412 nm. GSH value was calculated as μmol mg protein^{-1} in the tissues.

2.4. Inhibition of AChE activity

AChE activity was determined brain(1:25) and muscle(1:10) homogenate in 0.1 M phosphate buffer, pH 8.0. The homogenate were centrifuged 10,000*g* for 20 min at 4 °C. The supernatant was removed and used to test AChE activity. AChE activity was determined according to the method of Ellman et al. (1961). AChE activity was normalized to protein content and expressed as nmol min^{-1} mg protein^{-1}. Briefly, the activity on the homogenate was measured by determining the rate of hydrolysis of acetylthiocholine iodide (ACSCh, 0.88 mM) in final volume of 300 μl, with 33 μl of 0.1 M phosphate buffer, pH 7.5 and 2 mM DTNB. The reaction was started with the addition of the substrate acetylthiocholine, as soon as the substrate was added the hydrolysis and the formation of the dianion of DTNB were analyzed in 412 nm for 5 min (in intervals of 1 min) using a microplate reader. Protein concentration was determined using Bradford's method (1976), with a bovine plasma albumin (Sigma, USA) as standard.

2.5. MT gene expression

Total RNA was extracted from liver samples using RNA purification kit (Real Biotech Corporation, Taipei, Taiwan), and the quantity and quality of the total RNA were assessed using the Ultrospec 3100 pro (Amersham Bioscience, Amersham, UK). The 260/280 nm absorbance ratios of all samples ranged from 1.80 to 2.00, indicating a satisfactory purify of the RNA samples. Purified RNA was subjected to reverse transcription to cDNA by cDNA synthesis kit (Enzo Life Sciences Inc., NY, USA) according to the reagent's instructions. For real-time quantitative PCR analysis of MT gene expression, the real-time qPCR primer of MT gene and 18 s rRNA gene are shown in Table 3. Real-time PCR assay were carried out in a quantitative thermal cycler (LightCycler ® 480 II, Roche Diagnostics Ltd., Rotkreuz, Switzerland) in a final volume of 20 μl containing 10 μl 2 x Master Mix (LightCycler® 480 SYBR Green I Master, Roche Diagnostics Ltd., Rotkreuz, Switzerland), 1 μl of cDNA mix. MT gene-specific primers were applied to evaluate the mRNA levels of MT in liver. Reference 18s rRNA gene was used as internal control. The real-time qPCR amplification began with 5 min at 95 °C, followed by 45 cycles of denaturation of 10 s at 95 °C, annealing of 10 s at 60 °C, and extension of 10 s 72 °C. To analyze the mRNA expression level, the comparative CT methods ($2^{-\Delta\Delta CT}$ method) was used.

2.6. Statistical analysis

The experiment was conducted in exposure period for 4 weeks and performed triplicate. Statistical analyses were performed using the SPSS/PC+ statistical package (SPSS Inc, Chicago, IL, USA). Significant differences between groups were identified using one-way ANOVA and Duncan's test for multiple comparisons (Duncan, 1955). The significance level was set at $P < 0.05$.

Table 3
The primers used in this study for real-time qPCR.

Gene	Sequence	Product size
18s rRNA	Fw: TGAGAAACGGCTACCACATC Rv: CAATTACAGGGCCTCGAAAG	100 bp
MT	Fw: CAACTGCGGTGGATCCTG Rv: CCAGAGGCGCATTTAGGG	102 bp

3. Results

3.1. Antioxidant enzyme activity

Antioxidant enzyme activity (SOD, GST, and GSH) in liver and gill tissues of *S. schlegelii* is shown in Fig. 1. Liver and gill SOD activity of the *S. schlegelii* was considerably increased over 120 mg/kg Cr exposure. The liver SOD activity was significantly increased at 240 mg/kg of dietary Cr exposure after 2 weeks and over 120 mg/kg of dietary Cr exposure after 4 weeks. In case of gill SOD activity, a considerable increase was observed at 240 mg/kg of Cr exposure after 2 weeks and over 120 mg/kg of dietary Cr exposure after 4 weeks. Liver and gill GST activity of the *S. schlegelii* was also significantly increased over 120 mg/kg Cr exposure for liver and at the concentration of 240 mg/kg for gill. The liver GST activity was substantially increased at 240 mg/kg of dietary Cr exposure after 2 weeks and over 120 mg/kg of dietary Cr exposure after 4 weeks. The gill GST activity was increased at 240 mg/kg of Cr exposure after 2 weeks and 4 weeks. However, liver and gill GSH level were significantly decreased. The liver GSH was considerably decreased at 240 mg/kg of dietary Cr exposure after 2 weeks and over 120 mg/kg of dietary Cr exposure after 4 weeks. In case of gill GSH, a significant decrease was observed at 240 mg/kg of dietary Cr exposure after 2 weeks and over 120 mg/kg of dietary Cr exposure after 4 weeks.

3.2. Inhibition of AChE activity

AChE activities of brain and muscle tissues in rockfish, *S. schlegelii* exposed to dietary Cr are shown in Fig. 2. AChE activity in brain tissue was significantly inhibited in the concentration at 240 mg/kg after 2 weeks and over 60 mg/kg after 4 weeks. Brain

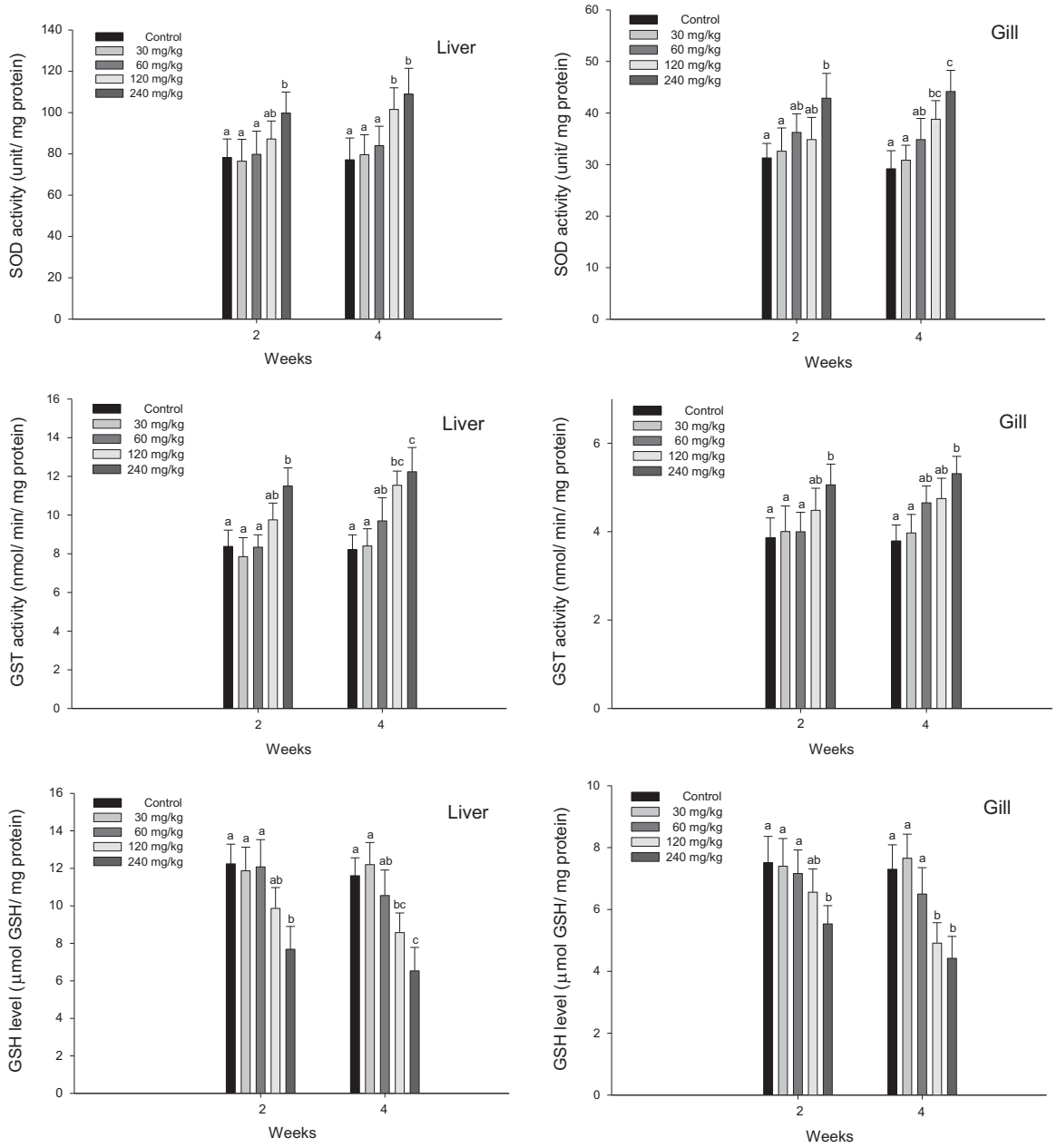

Fig. 1. Changes of antioxidant system (SOD activity, GST activity, and GSH level in liver and gill) of rockfish, *Sebastes schlegelii* exposed to the different concentration of chromium for 4 weeks. Vertical bar denotes a standard error. Values with different superscript are significantly different at 2 weeks and 4 weeks ($P < 0.05$) as determined by Duncan's multiple range test.

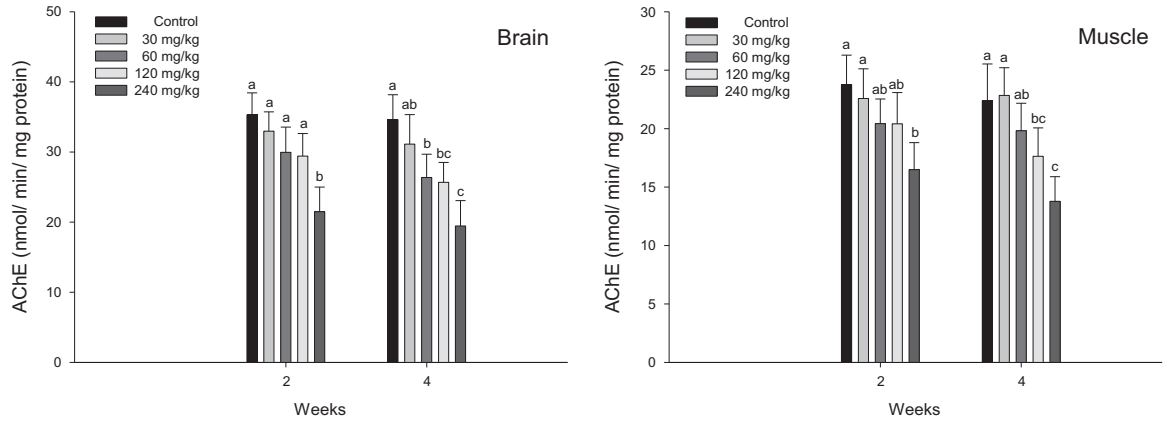

Fig. 2. AChE activity of rockfish, *Sebastes schlegelii* exposed to the different concentration of chromium for 4 weeks. Vertical bar denotes a standard error. Values with different superscript are significantly different at 2 weeks and 4 weeks ($P < 0.05$) as determined by Duncan's multiple range test.

AChE inhibition levels were 39% at 240 mg/kg after 2 weeks and 44% at 240 mg/kg after 4 weeks. In muscle tissue, AChE activity was significantly inhibited at 240 mg/kg after 2 weeks and over 120 mg/kg after 4 weeks. Muscle AChE inhibition levels were 31% at 240 mg/kg after 2 weeks and 38% at 240 mg/kg after 4 weeks, respectively.

3.3. *MT gene expression*

The MT gene expression of liver in rockfish, *S. schlegelii* is demonstrated in Fig. 3. The dietary Cr exposure considerably caused the increase of MT gene expression in liver, compared to the control. MT gene expression in liver was considerably increased over 120 mg/kg after 2 weeks and at 30, 120, 240 mg/kg after 4 weeks. The significant increase trend in MT gene expression was observed by the dietary Cr exposure.

4. Discussion

Aquatic toxicants leading to necrosis and cell degeneration in aquatic animals are one of the most critical factors to cause the lipid peroxidative damage which induces free radical's attack on biological structures, and the intracellular formation of reactive oxygen species (ROS) by the toxicants induces the oxidative damage to the organ system of aquatic animals (Taglian et al., 2004; Kim and Kang, 2015c). Among various toxicants, hexavalent chromium (Cr VI) is also one of the most strong inducer to causes ROS, because it transformed into pentavalent chromium (Cr V) in the liver, and the hydroxyl radical and lipid peroxidation occur through this process (Gunaratnam et al., 2002). Oxidative stress occurs when the rate of ROS generation exceeds that of their degradation by cellular defense mechanisms (Lushchak et al., 2008). Generally, cellular response to oxidative stress is connected with two processes; damage to cellular components and up-regulation of antioxidant defenses (Kubrak et al., 2010).

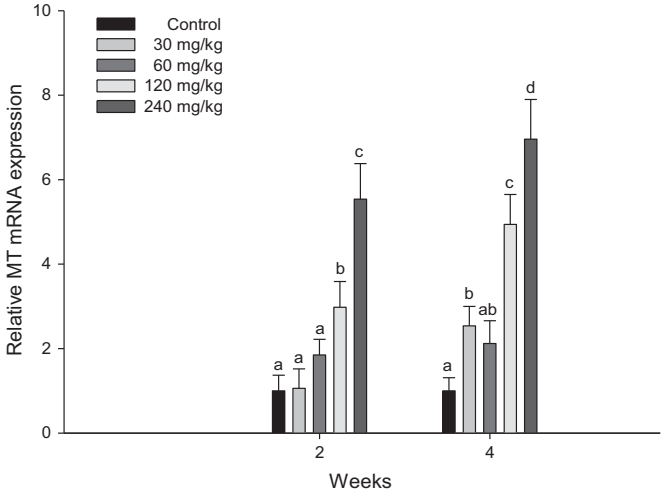

Fig. 3. Relative MT mRNA of rockfish, *Sebastes schlegelii* exposed to the different concentration of dietary chromium for 4 weeks. Values with different superscript are significantly different in 2 and 4 weeks ($P < 0.05$) as determined by Duncan's multiple range test.

Ahmad et al. (2006) reported that the Cr (VI) exposure clearly caused oxidative stress in gills and kidney of European eel, *Anguilla anguilla* L. Sridevi et al. (1998) also reported a significant SOD increase in freshwater field crab, *Barytelphusa guerini* exposed to hexavalent chromium. In the present study, the dietary hexavalent chromium exposure induced a significant increase of SOD activity in the liver and gill of *S. schlegelii* over 120 mg/kg Cr exposure, which may be a reaction to cope with free radical generation during chromium toxicity. Glutathione S-transferase is also one of the most important enzymes to protect organisms from oxidative stress, and it is used as an useful indicator of contaminants in aquatic organisms (Elia et al., 2003). Ciacci et al. (2012) reported a considerable increase GST of *Mytilus galloprovincialis* exposed to hexavalent chromium. Similarly, the GST activity in the liver and gill of *S. schlegelii* was considerably increased by the dietary chromium exposure over 120 mg/kg for liver and at 240 mg/kg for gill, which may depend upon the efficient role of organs in detoxification against metal exposure. Ahmad et al. (2006) reported a depleted GSH level in *Anguilla anguilla* L. exposed to chromium. Bagchi et al. (2002) also reported the chromium exposure reduced GSH concentration in gill of European eels. They suggested the depleted GSH occurs for protecting the cells from cytotoxicity during the detoxification of hexavalent chromium. The significant GSH decrease in liver and gill of *S. schlegelii* was observed by the dietary hexavalent chromium exposure over 120 mg/kg, which may result in oxidative damage by dietary Cr. These results indicated that the high level of dietary hexavalent chromium exposure significantly influences the antioxidant activity of *S. schlegelii* as oxidative stress in *S. schlegelii* by increased ROS production.

Contaminants by heavy metals in aquatic environment induce the neurotoxicity of aquatic animals at their main mode of action by most insecticides or the central nervous system along with other organ systems by most heavy metals (Lionetto et al., 2003). Contaminants commonly inhibit AChE as a neurotoxicity, which causes excessive ACh accumulation, disruption of nerve function,

and even death (Bhattacharya, 1993). Whereas AChE activity is commonly used as a critical biomarker for pesticide exposure, many authors have recently demonstrated AChE inhibition by metals (Tsangaris et al., 2007; Atting et al., 2010). Hexavalent chromium toxicity is related to severe damage to nervous tissues by the formation reactive oxygen species (ROS) (Kumari et al., 2011). Ciacci et al. (2012) reported a considerable decrease in AChE of *Mytilus galloprovincialis* exposed to hexavalent chromium. In the present study, AChE activity of *S. schlegelii* was significantly inhibited by the dietary Cr exposure over 60 mg/kg for brain and over 120 mg/kg for muscle, which indicates that the dietary Cr exposure affects the cholinergic signaling. Therefore, the high level of dietary Cr exposure may significantly influence *S. schlegelii* as neurotoxicity.

Metallothionein (MT) is low molecular mass cysteine with a high affinity for metal ions, and functions as an essential defense system for metal toxicity through the regulation of essential metals and the detoxification of non-essential metals (Creti et al., 2010), which is induced by free cytosolic metal ions. It is also known as an antioxidant (Hansen et al., 2006). Tom et al. (2004) suggested that the transcript of metallothionein gene in fish liver by metals is a sensitive biomarker for the metal toxicity. Therefore, MT can be a sensitive and reliable factor in metal resistance in aquatic animals against metal toxicity (Xie and Klerks, 2004). The over-expression of MT gene is generally observed in response to resistance to oxidative stress for metal exposure, but it can be adversely decreased by gene depletion enhanced sensitivity to oxidative injury (Atif et al., 2006). Kim et al. (2008) reported a significant metallothionein induction of pufferfish, *Takifugu obscurus* exposed cadmium. Similarly, the dietary Cr exposure to *S. schlegelii* induced a considerable induction of hepatic MT gene expression, which may indicate the induction of metal-binding proteins as a defense system for metal toxicity.

In conclusion, this work demonstrated that the high level of dietary chromium substantially affects the experimental fish, *S. schlegelii*. The antioxidant enzymes (SOD and GST) and GSH of *S. schlegelii* was significantly altered by the dietary Cr. The high level of dietary Cr exposure to *S. schlegelii* also induced the considerable inhibition of AChE activity. A significant Metallothionein gene expression of *S. schlegelii* was caused by the high level of dietary Cr exposure. **But, there was no significant difference according to the exposure time.** Considering the results of the alterations in antioxidant pathway, inhibition of AChE activity, and the increased MT gene expression, the high level of dietary Cr exposure should adversely affect the experimental fish, *S. schlegelii*.

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