Short-term exposure of arsenite disrupted thyroid endocrine system and altered gene transcription in the HPT axis in zebrafish

Hong-Jie Sun a, Hong-Bo Li a, Ping Xiang a, Xiaowei Zhang a, Lena Q. Ma a, b, *

a State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Jiangsu 210046, China
b Soil and Water Science Department, University of Florida, Gainesville, FL 32611, USA

A R T I C L E I N F O

Article history:
Received 6 April 2015
Received in revised form 22 May 2015
Accepted 27 May 2015
Available online xxx

Keywords:
Arsenite
Oxidative stress
Zebrafish
Thyroid endocrine
Gene transcription
Hypothalamic–pituitary–thyroid axis

A B S T R A C T

Arsenic (As) pollution in aquatic environment may adversely impact fish health by disrupting their thyroid hormone homeostasis. In this study, we explored the effect of short-term exposure of arsenite (AsIII) on thyroid endocrine system in zebrafish. We measured As concentrations, As speciation, and thyroid hormone thyroxine levels in whole zebrafish, oxidative stress (H2O2) and damage (MDA) in the liver, and gene transcription in hypothalamic–pituitary–thyroid (HPT) axis in the brain and liver tissues of zebrafish after exposing to different AsIII concentrations for 48 h. Result indicated that exposure to AsIII increased inorganic As in zebrafish to 0.46–0.72 mg kg−1, induced oxidative stress with H2O2 being increased by 1.4–2.5 times and caused oxidative damage with MDA being augmented by 1.6 times. AsIII exposure increased thyroxine levels by 1.3–1.4 times and modulated gene transcription in HPT axis. Our study showed AsIII caused oxidative damage, affected thyroid endocrine system and altered gene transcription in HPT axis in zebrafish.

Published by Elsevier Ltd.

1. Introduction

As a ubiquitous metalloid, arsenic (As) is widely distributed in the environment resulting from both natural processes and anthropogenic activities (Kubota et al., 2001). Aquatic environment, which has received discharge from domestic and industrial wastewaters for years, may contain elevated As concentrations. Hence, As contamination in aquatic environment is becoming a critical issue in several regions around the globe. For example, Cáceres et al. (1992) reported high As concentrations up to 21 mg L−1 in Lao River, Chile, Culioli et al. (2009) found As concentrations of 18–2330 μg L−1 in Presa River, France, and Wang et al. (2010) showed As levels of 147–177 μg L−1 in Yangzonghai Lake, China. The elevated As concentrations in aquatic environment are far above the USEPA provisional guideline value of 150 μg L−1, potentially adversely impact aquatic environment (USEPA, 1999). Fish, as the top predators in aquatic environment, are exposed to more external contamination than other species. As an important food resource for humans from aquatic environment, it is necessary to examine As toxicity in fish.

Arsenic exists in four oxidation states: arsenate (+5), arsenite (+3), elemental As (0) and arsine (−3). In the aquatic environment, inorganic As is present as arsenite (AsII) and arsenate (AsV), which can be accumulated and metabolized in fish. Studies demonstrated that fish can contain high As concentrations, mainly as the nontoxic arsenobetaine (AsB) (Slejkovec et al., 2004). Due to its influence on fish including growth, endocrine homeostasis and reproduction, As toxicity has been studied in recent years. Considering its greater toxicity than AsV, AsIII toxicity has received more concern than AsV (Kubota et al., 2001; Sun et al., 2014a).

Many studies have investigated AsIII toxicity on fish and found that AsIII exposure caused oxidative stress in fish (Bagnyukova et al., 2007; Ventura-Lima et al., 2009a). Furthermore, the potential endocrine-disrupting effects of As have recently received some attention (Davey et al., 2008; Watson and Yager, 2007). The effect of As on steroid receptors and estrogen hormones has been reported (Davey et al., 2007, 2008). However, thyroid endocrine system, an important endocrine system in fish, has received limited attention. The effect of As on thyroid hormone in pig and mice has been reported (Allen and Rana, 2007; Mohanta et al., 2014), but limited information is available on fish.

Thyroid endocrine system, i.e., hypothalamic–pituitary–thyroid (HPT) axis, is responsible for releasing and stimulating hormones for the production and release of thyroid hormones (THs)
including tri-iodothyronine (T3) and thyroxine (T4), which plays a crucial role in regulating THs dynamics by coordinating their synthesis, secretion, transport and metabolism (Fig. 1) (Carr and Patino, 2011; Porazzi et al., 2009).

Zebrafish (Danio rerio) has been used as a robust platform for toxicology research. This is attributed to its fast reproductive capacity, well-defined development processes, and known sequenced genome. In this study, zebrafish was employed as a model and they were exposed to various concentrations of AsIII for 48 h. The objectives of our research were to: (1) determine As accumulation and metabolism in zebrafish; (2) understand the impact of AsIII on antioxidative system of zebrafish; (3) examine the effect of AsIII on the content of thyroid hormone thyroxine; and (4) explore the impact of AsIII on gene transcription in HPT axis of zebrafish.

2. Materials and methods

2.1. Experimental fish and chemicals

Adult male zebrafish (D. rerio) (345 ± 21 mg) were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The fish were maintained at 28 ± 0.5 °C with a 14/10 light/dark cycle in an aerated tap-water. The water used had the following characteristics: pH = 6.7 ± 0.5, dissolved oxygen = 6.4 ± 0.4 mg L⁻¹, conductivity = 0.252 ± 0.006 mS/cm, and total hardness = 194 ± 12.0 mg L⁻¹ CaCO₃ (Feng et al., 2013). Before As exposure, zebrafish were fed with commercial food (Inchgold, China; crud protein: >45%, crud fat: >5%, crud fiber: <3%, crud ash, <12%, calcium: >1.1%, total phosphorus: >1.5%, lysine: >1.5%, moisture: <10%) and acclimated in 15 L glass tanks (20 cm × 25 cm × 30 cm; containing 5 L tap water) for 7 d. The fish were fed twice a day. After feeding for 30 min, excess food was removed from the tanks. Fish were fasted for 24 h before the experiment.

2.2. Lethal AsIII concentrations in zebrafish

Sodium arsenite (NaAsO₂, Sigma–Aldrich, ≥90%) was dissolved in Milli-Q water to make AsIII solutions. To determine the proper exposure concentrations of AsIII, we conducted a preliminary experiment based on 48-h exposure. In the test, AsIII concentrations were 0, 30, 40, 45, 50, and 60 mg L⁻¹ according to Liao et al. (2003). Ten fish were used per tank and each concentration was run in triplicate. For the experiment, the test solutions in each tank was refreshed every 12 h. Dead fish were removed and survival was recorded after 8 h. In the preliminary experiment, all zebrafish survived in the control group. However, mortality occurred in zebrafish exposed to AsIII at ≥30 mg L⁻¹ for 48 h. Their survival decreased with increasing AsIII concentrations, showing a dose–response relationship (Fig. 2). The 48 h median lethal concentration (48 h-LC₅₀) was calculated according to Chen et al. (2013). The three-parameter logistic model, \[ Y = a / (1 + (X/X₀)^{b}), \] was chosen to fit the data of survival rate, where Y is the index, X is the AsIII concentration, a is the value of the index at zero AsIII concentration,
X₀ is the concentration that reduces the index by 50%, and b is the form parameter determining curve shape. The calculated 48 h-LC₅₀ value of AsIII for zebrafish was 42 mg L⁻¹ (Fig. 2). The safe AsIII concentration was 10% of the 48-LC₅₀ according to Sprague (1971), which was the highest AsIII concentration used for fish exposure experiment.

Based on the preliminary experiment, AsIII concentrations at 0, 0.1, 0.5, 1.0, 2.1, and 4.2 mg L⁻¹ were used for short-term AsIII exposure. Ten fish were exposed in each glass tank, and each concentration was run in duplicate. The test solutions in each tank was refreshed every 12 h. After 48 h exposure to AsIII, the fish were used for the following biochemical analyses.

2.3. As concentration and speciation in zebrafish

Zebrafish after AsIII exposure were prepared for As analysis (Zhang et al., 2012). After storing at −80 °C overnight, the whole fish were freeze-dried (Freeze Dry System, Labconco, USA), and ground to powder in a mortar under liquid nitrogen. For total As analysis, −0.5 g of fish powder was weighed into vials, mixed with 10 ml of 1:1 HNO₃:H₂O on a Hot Block Digestion System (Environmental Express, USA) and stayed overnight. They were then heated at 105 °C for 2 h, removed from the block and cooled for 3 min. The samples were added 1 ml 30% H₂O₂ slowly, and then heated for another 15 min. The samples were cooled completely, and then diluted up to 20 ml with Milli-Q water. The final solution was stored at 4 °C before analysis with inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer NEXION 300X, USA). The blank and certified reference material (GBS-21, Chinese geological reference materials) were used for quality control. The mean ± standard error was 4.68 ± 0.36 μg/kg, which was comparable with the certified value of 4.5 μg/kg. The internal standards were carried to ensure accuracy and precision. Standard solution at 1 μg/L As was measured after every 20 samples to monitor the stability of ICP-MS, which has a detection limit of 0.5 μg/L.

For As speciation, −0.5 g of fish powder was weighed into a 15 ml centrifuge tube and 10 ml of 1:1 methanol:water mixture was added to the sample. All samples were extracted ultrasonically for 2 h at 25 °C and centrifuged at 4000 rpm for 15 min, subsequently the supernatant was collected in a 50 ml centrifuge tube. The process was repeated twice and three supernatants were mixed. After filtration, extracts were stored at −80 °C and analyzed for As speciation within 24 h.

As speciation was measured by high performance liquid chromatography (Waters 2695, USA) coupled with ICP-MS. A guard column connected to a PRP-X100 10 μm anion-exchange column (Hamilton, UK) was used to separate As species using 7.5 mM (NH₄)₂HPO₄ and NH₄NO₃ mobile phase at pH 6.2. Sample of 50 μL was injected at 1 ml/min flow rate (Ren et al., 2014). A 10 μg/L standard mix of arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), was run to obtain retention time. All working standards and samples were diluted from stock solution with mobile phase on the day of analysis. Before injecting into chromatographic columns, all solutions were sonicated and filtered (0.22 μm). The spike recovery efficiency for As was 102 ± 3.2% (n = 3).

2.4. Extraction of H₂O₂ and malondialdehyde in zebrafish liver

To determine the oxidative stress and damage in zebrafish after AsIII exposure, H₂O₂, malondialdehyde (MDA) and protein were extracted from fish livers (Sun et al., 2011). Three zebrafish from each treatment were over-anaesthetized with MS222. Their livers were removed and rinsed with ice-cold physiological saline (0.68%). Livers were homogenized at tissue:solution of 1:9 using physiological saline solution, and the homogenate was centrifuged at 4000 g for 10 min at 4 °C to remove cellular debris and cartilage fragments. Samples were kept on ice during the entire procedure. The supernatant was then divided into aliquots and stored at −80 °C for MDA and H₂O₂ assays. The content of H₂O₂, MDA, and total protein in supernatant were measured using a commercial chemical assay kit (Nanjing Jiancheng Bioengineering Institute, China).

2.5. Extraction of thyroid hormone thyroxine (T₄) in whole fish

The extraction of T₄ was performed according to Yu et al. (2010) with modification. We chose T₄ because its content is generally higher than T₃ (Power et al., 2000). Briefly, three whole zebrafish in each treatment were homogenized after added to the enzyme-link immuno sorbent assay (ELISA) (tissues/total was 10%) with a basic homogenizer. The samples were disrupted for 5 min by spasmic sonication on ice, and followed by 10 min oscillation. After vortexing for 10 min, samples were centrifuged at 5000 g for 10 min at 4 °C. Subsequently, the supernatants were collected and prepared for following measurement. Specifically, took 50 μL solution and diluted into 1 x, 2 x, 5 x and 10 x and compared with standard curve to find out that 5 x dilution was suitable. The T₄ levels were measured using ELISA with a commercial kit for fish (Usclfne, Wuhan, China). It was based on competitive binding enzyme immunoassay technique, with a detection limit of 0.3 μg L⁻¹.

2.6. RNA isolation and cDNA synthesis for genes in HPT axis using fish brains and livers

After 48 h exposure to AsIII, five zebrafish from each treatment were over-anaesthetized with MS222. Zebrafish were kept on ice during the entire procedure. Their brains and livers were rapidly removed and rinsed with ice-cold 0.68% physiological saline solution, and then homogenized using TRizol reagent (TaKaRa, Japan). RNA isolation and cDNA synthesis were performed according to Sun et al. (2014b). They were kept at 4 °C for 10 min and centrifuged at 12,000 g for 10 min at 4 °C. The supernatant was transferred into sterile centrifuge tubes containing 0.2 ml of chloroform. After vortexing for 15 s, the mixtures were kept at room temperature for 5 min and centrifuged at 12,000 g for 15 min at 4 °C. Aqueous supernatant was carefully removed into a new tube with minimum disturbance and mixed with 0.5 ml of isopropanol. The mixtures were kept at room temperature for 10 min and centrifuged at 12,000 g for 10 min at 4 °C. The pellets were washed with 1 ml of
2.7. Quantification of gene expression in HPT axis by RT-PCR

The selection of primer sequences were based on Yu et al. (2010) (Table 1). Expression of the 8 target genes and internal control (18sRNA) were measured by quantitative RT-PCR. All PCR reactions (20 μl) comprised of 10 μl of SYBR Premix Ex TaqII (TaKaRa, Japan), 1 μl of cDNA, 10 μM of each forward and reverse primer and 7 μl ultrapure water. The PCR reaction comprised of an initial denaturation step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 30 s, 60 °C for 20 s, and 72 °C for 1 min. Thermal cycling and fluorescence detection were performed in Corbett Real-time PCR Machine with Rotor-Gene 6000 Series Software 1.7 (QIAGEN, Netherlands). The relative expression levels of different genes were calculated according to the 2^ΔΔCT method (Livak and Schmittgen, 2001).

2.8. Statistical analysis

All data were presented as mean ± standard error (SE) and were evaluated by one-way analysis of variance followed by Duncan’s multiple range test (α = 0.05). All statistical analyses were carried out with SigmaPlot 11.0.

3. Results and discussion

In our study, the median lethal concentration of AsIII (48 h-LC50) for zebrafish was 42 mg l⁻¹ (Fig. 2), which was within the range of reported values. For example, Rankin and Dixon (1994) reported the 96 h-LC50 of As for 4.5 g rainbow trout (Oncorhynchus mykiss) at 21.0 mg l⁻¹, and Liao et al. (2003) showed the 48 h-LC50 of As for ~149 g tilapia (Oreochromis mossambicus) at 52 mg l⁻¹. These different results between the present study and previous studies are probably due to different experimental conditions and fish species.

3.1. Effect of AsIII on As concentration and speciation in zebrafish

After exposing to AsIII at 0, 0.1, 0.5, 1.0, 2.1, or 4.2 mg l⁻¹ for 48 h, the As content in zebrafish increased with increasing AsIII concentrations (Fig. 3a). The As content in the control was negligible, while As content in the fish increased from 0.74 mg kg⁻¹ at AsIII0.1 to 2.08 mg kg⁻¹ at AsIII4.2. The data indicated that higher As concentrations induced more As uptake by zebrafish. Similar conclusion was obtained by Liu et al. (2006) who reported that after exposure of 1–10 mg l⁻¹ AsV for 90 d, As content in fish increased from 0.24 to 5.15 mg kg⁻¹. Zhang et al. (2011) also found that As content increased in grunt (Terapon jarbua) as the medium As concentrations increased from 0.5 to 50 μg l⁻¹, suggesting that higher As influx rate into fish is responsible for the high As content in fish.

Besides total As concentrations, four As species were also detected including two inorganic (AsIII and AsV) and two organic As (MMA and DMA) (Fig. 3b). With increasing AsIII concentration, inorganic As content in zebrafish did not vary significantly (P > 0.23) but organic As showed significant increase (P < 0.004) at AsIII concentrations ≥2.1 mg l⁻¹. The data suggested that zebrafish can tolerate low AsIII concentrations (≤1 mg l⁻¹), however, higher AsIII concentrations (≥2.1 mg l⁻¹) probably exceeded their capacity, as a result, excess inorganic As was transformed to MMA and DMA. In addition, significant difference was observed between total As content (0.74–2.07 mg kg⁻¹) and the sum of organic and inorganic As species (0.56–0.75 mg kg⁻¹, Fig. 3). The difference between total As content and the sum can be attributed to the fact that parts of As was transformed to arsenobetaine. Le et al. (2004) pointed that transformation of inorganic As into arsenobetaine is a typical detoxification pathway of fish. Zhang et al. (2012) reported that 89–97% AsIII can rapidly transform to the less toxic arsenobetaine in grunt. Slejkovec et al. (2004) manifested that the main As compound in several freshwater fish was arsenobetaine.

Although zebrafish tolerated As, it might have induced detrimental effect on zebrafish. To further understand the effect of AsIII, we investigated the oxidative stress, oxidative damage and the changes in T4 content of zebrafish after 48 h exposure to AsIII.

3.2. Effect of AsIII on oxidative stress and thyroid hormone thyroxine (T4)

It is known that As can cause zebrafish to produce more reactive oxygen species (ROS) in fish, such as H2O2 and suffer from lipid peroxidation as indicated by MDA level (Ventura-Lima et al., 2009b; Sun et al., 2012). In our study, the H2O2 concentrations showed significant increase after exposure to AsIII (P < 0.001, Fig. 4a). It indicated that AsIII exposure caused imbalance between oxidative stress and the antioxidant defense system, resulting in...
increasing AsIII was probably due to the decrease in total polyunsaturated fatty acids, which was prone to oxidative damage (Sun et al., 2012), demonstrating that AsIII-N2 treatment probably induced more severe damage on zebrafish. Besides, we also found that these data were consistent with As speciation in fish where organic As appeared in treatments with AsIII ≥2.1 mg L⁻¹ (Fig. 3b). This was probably because zebrafish were compelled to improve the metabolic capacity to decrease the toxic effect of higher AsIII concentrations, indicating that inorganic As was responsible for the oxidative stress in zebrafish.

AsIII exposure not only caused oxidative damage, but also exerted pressure on thyroid hormone in fish. In our study, thyroxine (T4) level in the control was 43.8 ng g⁻¹, which was significantly elevated to 55.5−62.5 ng g⁻¹ in AsIII treatments (P = 0.004, Fig. 4c). This finding was consistent with Allen and Rana (2007) who reported that the thyroxine content in rats increased from 20 to 52 nmol L⁻¹ after exposing to 40 mg kg⁻¹ AsIII body weight. This was probably because zebrafish needed to enhance thyroxine content to counter As toxicity. Lam et al. (2005) reported that there is a close relation between thyroxine and immune system, and zebrafish may enhance its immunity to overcome damage via improving thyroxine level. However, the low correlation between thyroxine levels and ROS in zebrafish (r² = 0.34) indicated that, although AsIII-induced ROS elevated thyroxine level in zebrafish, it was probably not the main reason. Hence we hypothesized that there was other pathway for AsIII-induced toxicity on thyroid in zebrafish. To better elucidate AsIII toxicity on thyroid, we performed real-time PCR assays to analyze the changes in gene transcription in thyroid synthesis, transport, binding, and regulation of HPT axis.

3.3. Effect of AsIII on genes involved in HPT axis of zebrafish

Thyroid endocrine system is responsible for regulating thyroid hormones level (Liu et al., 2010). Thyroid hormones control the development and physiology of fish primarily by interacting with specific nuclear protein, i.e., thyroid hormone receptors (TRs). TRs have two receptor isofoms, including TRα and TRβ (Fig. 5). In our study, AsIII significantly down-regulated transcriptions of TRα and TRβ (P < 0.048, Fig. 5). This could be due to the increase of thyroid hormone thyroxine. Chen et al. (2012) showed the similar phenomenon who manifested that after exposing to decabromodiphenyl ether, the transcription of TRs in zebrafish increased corresponding to reduction in thyroxine. However, with increasing AsIII concentration, the mRNA transcription of TRα and TRβ showed different changes (Fig. 5). TRα mRNA transcription decreased with increasing AsIII concentration, i.e., it decreased by 41% in AsIII-1 than the control. However, TRβ mRNA transcription showed little dose-dependence with increasing AsIII. This may be because they were responsible for different functions in zebrafish. TRα is responsible for controlling thyroid hormone in early life stage (embryos and ovary) while TRβ doesn’t play a role in this period (Liu et al., 2000).

In fish, corticotropin-releasing hormone (CRH) and thyroid-stimulating hormone (TSH) secretions act as common regulators of the thyroidal axis as feedback mechanism, which is triggered by changes in the concentrations of circulating thyroid hormones (Yu et al., 2011). Since TSH is encoded by the gene TSHβ, TSHβ was used to assess TSH function. In our study, TSHβ and CRH mRNA transcription significantly decreased with increasing AsIII concentration (P < 0.001, Fig. 5). The transcription levels of TSHβ and CRH mRNA in the AsIII-2 treatment decreased by 35% and 43% compared to the control. The down-regulation of mRNA transcriptional levels was regarded as a compensatory response to increasing thyroid hormone contents in zebrafish (Fig. 4c). This was consistent with Yu production of more ROS. Flora (2011) pointed out that arsenic can induce ROS production via several pathways in fish. Bhattacharya and Bhattacharya (2007) demonstrated that the H₂O₂ levels in Clarias batrachus increased after exposed to AsIII₁₂ and AsIII₁₄ for 10 d. However, compared with control, there was no significant difference in AsIII₁₀ in our study. This may be due to the accumulation of low level of inorganic As (AsIII and AsV) in AsIII₁₀, which results in less ROS production (Figs. 3 and 4a). In addition to ROS, AsIII exposure also significantly increased MDA content in fish (P = 0.001, Fig. 4b). Unlike H₂O₂, MDA levels didn’t show significant change after exposing to low AsIII concentrations (≤1 mg L⁻¹), however, at AsIII₁₂, MDA levels increased by 56% (Fig. 4b). It indicated that AsIII₁₂ induced production of excess ROS in zebrafish, resulting in oxidative damage on zebrafish. Matés (2000) demonstrated that oxidative damage occurs when the physiological antioxidant protection fails to counteract the elevated ROS levels. Altikat et al. (2014) reported that after exposing to 1 mg L⁻¹ AsIII for 15 d, the MDA levels in Carp livers (Cyprinus carpio) increased from 4.1 to 5.7 nmol mg⁻¹ protein. In our study, compared with control group, MDA levels showed no significant difference in AsIII₁₂ treatment (Fig. 4b). The decrease in MDA content with
et al. (2011) who reported that augmented thyroid hormone levels suppress gene transcription of TSHβ in zebrafish after exposing to polybrominated diphenyl ethers. In addition, Zoeller et al. (2007) also reported that thyroid hormone exerts a negative feedback effect on the activity of hypothalamic, CRH neurons and the gene transcription of CRH. In this study, the variation of the transcription levels of CRH was similar to TSHβ gene, this is because biosynthesis and secretion of TSH was regulated by CRH. TSH is not only impacted by thyroid hormone, but also accepts regulation from thyroglobulin (Gauthier et al., 1999). As a precursor in thyroid hormone synthesis, thyroglobulin has been suggested as a marker of thyroid abnormalities (Vejbjerg et al., 2009). In this study, gene transcription of thyroglobulin was up-regulated upon AsIII exposure ($P = 0.001$, Fig. 5). Thyroglobulin mRNA transcription was the highest in the AsIII$_{4.2}$ treatment, increased by 41% compared to the control. The elevation of thyroglobulin mRNA transcription was probably because thyroglobulin was needed to degrade more thyroid hormone to counter the effect of AsIII (Suzuki et al., 1998). Meanwhile, the significant increase of transthyretin transcription was observed ($P = 0.044$, Fig. 5). This was because zebrafish needed to transport excessive thyroid hormone to various target tissues, which is regulated by transthyretin (Power et al., 2000).

Iodothyronine deiodinases (deio1 and deio2) are responsible for both activation and inactivation of thyroid hormones and play a critical role in maintaining the ratio of T4 and T3 (Orozco and

Fig. 5. Change in transcription levels of genes involved in HTP axis of zebrafish under different AsIII concentrations. Vertical lines represent ± SE, and different letters denote significant difference at $p < 0.05$ (Note: $n = 3$). TR – thyroid hormone receptor, TSH – thyroid stimulating hormone, TTR – transthyretin, CRH – corticotropin-releasing hormone, TG – thyroglobulin, and Deio – iodothyronine deiodinases.
Valverde-R 2005). In our study, deio1 and deio2 mRNA transcription were significantly down-regulated with increasing AsIII (P = 0.001, Fig. 5). They decreased by 55–68% compared to the control. This result was consistent with previous report that hyperthyroidism decreased expression levels of deio1 and deio2 mRNA in killifish (Fundulus heteroclitus) exposed to 3,5-diiodothyronine for 24 h (García-G et al., 2004). The increasing transcription of deio2 may be associated with increasing levels of circulating T4, whereas decreased deio1 may be attributed to the decreased thyroid hormone T3 levels. However, different deiodinases are responsible for different function during maintenance of thyroid hormone homeostasis in zebrafish.

4. Conclusion

Our study showed short-term exposure of AsIII to zebrafish induced significant oxidative stress (elevated H2O2 content being 37.9–67 nmol mg−1 protein) and caused oxidative damage (increasing MDA content to 1.12 nmol mg−1 protein). Furthermore, we also found that short-term exposure of AsIII to AsIII significantly increased thyroid hormone thyroxine levels. Besides, the mRNA transcription levels of genes in the HPT axis were significantly changed by increasing AsIII. The data indicated that AsIII exposure modulated gene transcription levels in the HPT axis as well as affected thyroid hormone levels in zebrafish. This observation was of importance for further study of As toxicity on thyroid endocrine system. Some lakes are at risk of As contamination, causing impact on fish habitats to have elevated As. These results provided evidences to better understand the impact of As on thyroid endocrine system in fish.

Acknowledgment

This project was supported in part by Jiangsu Provincial Innovation Fund and Jiangsu Provincial Double-Innovation Fund.

References

