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Thyrotoxicity of arsenate and arsenite on juvenile mice at organism, subcellular, and gene levels under low exposure

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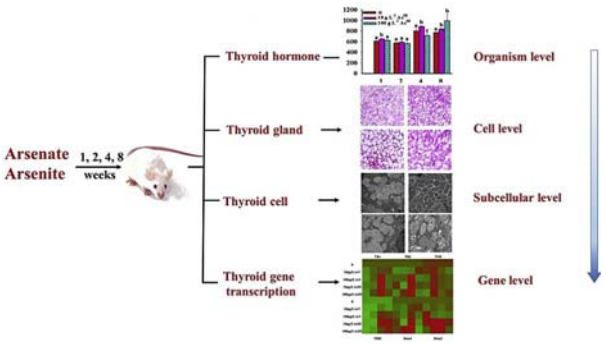
HIGHLIGHTS

- Thyrotoxicity of 10 or 100 μg L^{-1} AsV or AsIII was tested using a mouse model.
- 10 μg L^{-1} AsV or AsIII increased thyroxine level after 4–8 week of exposure.
- 100 μg L^{-1} AsIII damaged the thyroid tissues after 8 week of exposure.
- AsV and AsIII affected gene transcription involved in thyroid hormone homeostasis.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic contamination in drinking water is a worldwide issue, posing threat to human health. Arsenic is an endocrine system disruptor, however, limited information is available regarding its long-term effects on thyroid endocrine system at low exposure. In this study, we assessed the thyroid toxicity of arsenate (AsV) and arsenite (AsIII) at 10–100 μg L^{-1} in juvenile mice after 8-week of exposure via drinking water. After 1–2 week, AsV and AsIII had little influence on thyroxine (T4) level (56.3–64.7 μg L^{-1}) in mouse blood compared to control mice at 57.3–60.7 μg L^{-1}. However, after 4–8 weeks, 10 μg L^{-1} AsIII or AsV increased T4 levels to 83.8–88.8 μg L^{-1} compared to control treatment at 77.2–80.0 μg L^{-1}, while 100 μg L^{-1} AsV or AsIII decreased T4 levels except for 100 μg L^{-1} AsIII for 8 weeks. Based on transmission electron microscopy, exposure to 100 μg L^{-1} AsIII or AsV for 8 weeks caused thyroid gland damage. In addition, exposure to AsV or AsIII at 10 or 100 μg L^{-1} impacted gene transcription of hypothalamic-pituitary-thyroid axis including thyroid stimulating hormone and iodothyronine deiodinases. Our data demonstrated that exposing to low levels of AsIII or AsV disrupted T4 homeostasis, influenced the related gene transcription and damaged the thyroid glands in juvenile mice.

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1. Introduction

Arsenic (As) is a highly toxic metalloid and is classified as a Group I carcinogen (IARC, 1991). It is released into aquatic system from both anthropogenic and natural activities, causing As contamination in water and posing threats to public health (Halder et al., 2012; Abdul et al., 2015). Its limit in drinking water is 10 μg L^{-1} As based on WHO (2008). At present, over 200 million people around the world suffer from As contamination in drinking water (Naujokas et al., 2013), resulting in health problems including cancers (Rahman et al., 2009). Recently, investigators proposed that As is a potential endocrine disruptor, and the effect of As on endocrine system has attracted more attentions (Xun et al., 2012; Jain, 2014).

Thyroid endocrine system, one of endocrine systems in humans, plays a crucial role in maintaining normal life processes including growth, development, metabolism, and reproduction via regulating thyroid hormones including triiodothyronine (T3) and thyroxine (T4) (Fig. 1). Thyroid hormones exert their functions on human basal metabolism primarily by interacting with thyroid hormone receptors (TRs including TRα and TRβ). TRs not only mediate the activity of thyroid hormones, but also feed information back to hypothalamus to control thyrotropin releasing hormone (TRH), which transmits the information to pitutary to govern the thyroid stimulating hormone (TSH) and thyroid hormones level, i.e., hypothalamic-pituitary-thyroid (HPT) axis. Besides, iodothyronine deiodinases (Deio) are responsible for conversion of T3 and T4, being crucial in maintaining thyroid hormone levels. Since T4 level secreted by thyroid is more than 20 times than that of T3, T4 level has been used as the indicator of thyroid hormone, while TRs, TRH, TSH, and Deio are typical indicators of the thyroid endocrine system (Sun et al., 2015, 2016b).

Recently, several studies have revealed the toxic effects of As on thyroid hormones. For example, Meltzer et al. (2002) reported that an As-rich fish diet impacted thyroid hormones in humans, showing decreased plasma levels of T3 and T4 and increased T4/T3 ratio following daily As consumption of 260 μg for 15 weeks. Ciarrocca et al. (2012) investigated thyrotoxicity of arsenite (AsIII) in Italians via a health survey, showing decreased T3 and T4 levels in higher AsIII exposure group. Moreover, Mohanta et al. (2014) assessed AsIII thyrotoxicity in swine as a surrogate model for humans, showing decreased T3 and T4 levels in swine fed with food containing 50 mg kg^{-1} AsIII for 11 weeks. However, most of these studies investigated thyrotoxicity of As at organism levels, focusing on thyroid hormones levels, with limited information being available on the molecular mechanisms of As-induced thyrotoxicity.

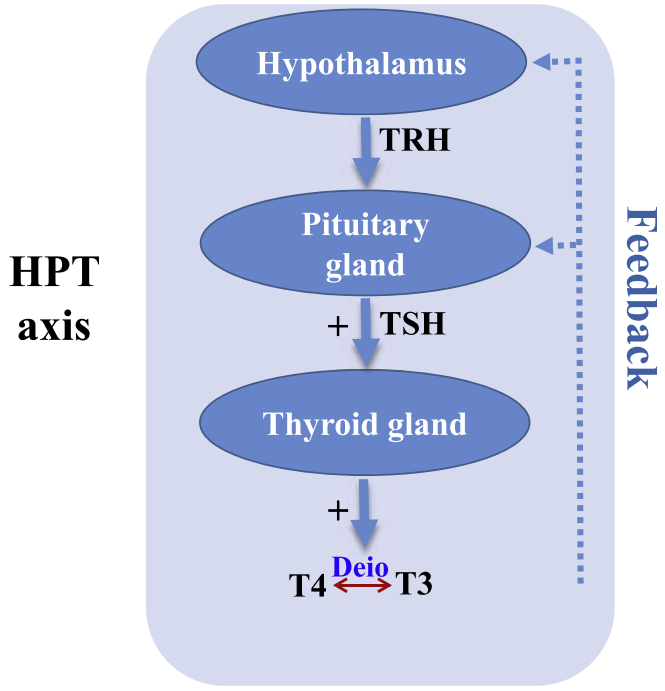

Fig. 1. The axis of hypothalamus-pituitary-thyroid (HPT). TRH = thyrotropin releasing hormone, TSH = thyroid stimulating hormone, Deio = iodothyronine deiodinases, T4 = thyroxine, and T3 = triiodothyronine.

Based on adult zebrafish, our study showed that exposure to 0.1–4.2 mg L^{-1} As for 48 h disrupted their thyroid endocrine system via disrupting secretion, transportation, and conversion of thyroid hormones (Sun et al., 2015). However, it is a short-term study with relatively high As concentrations. Since humans are exposed to long-term low concentration of As, to better understand the long-term effects of low As exposure on thyroid endocrine system of human, we used mouse as a test organism to determine the thyrotoxicity of inorganic As at organism, cell, subcellular, and gene levels.

Specific objectives of this study were to determine the long-term effects of low AsV or AsIII exposure on (1) T4 levels in mouse thyroid tissues at organism level; (2) histopathological changes of mouse thyroid tissues at cell/subcellular levels; and (3) the mRNA transcription of genes related with synthesis, secretion, and metabolism of thyroid hormones. Revealing the thyrotoxicity of As from different levels and species facilitates a better understanding of As toxicity to humans.

2. Materials and methods

2.1. Mouse acclimation and As exposure

Female Balb/c juvenile mice (11.3 ± 0.2 g body weight, 3 weeks old) were used following Li et al. (2014). Briefly, mice were acclimated in cages (25 cm × 10 cm × 15 cm) on dry woodchips for 3 days prior to experiment, with room conditions being kept at 25 °C, 50% humidity, and 12/12 h light/dark cycle. Rodent diet (Qinglongshan Experimental Animal Breeding Farm, Nanjing, China) and Milli-Q water were provided *al libitum*. All animal care and experimental procedures were in accordance with the principles and guidelines of Nanjing University.

Inoragnic arsenite (AsIII; $NaAsO_2$, Sigma-Aldrich, ≥90%) and arsenate (AsV; $Na_2HAsO_4{\cdot}7H_2O$, Sigma-Aldrich, ≥98%) were dissolved in Milli-Q water to prepare AsIII and AsV stock solutions. Based on the WHO guideline in drinking water, drinking water containing 10 or 100 μg L^{-1} AsIII or AsV was prepared from the stock solutions and supplied to mice over a 8-week period. To maintain constant As speciation, drinking water was refreshed every 48 h. After exposing to 1, 2, 4, or 8 weeks, mice were sacrificed to collect the blood, brain, and thyroid samples. Each treatment had 4 replicates.

2.2. Thyroxine in blood and histopathologyical changes in thyroid tissues

Following collection, blood samples were centrifuged at 1550 g for 10 min and plasma was separated to determine thyroid hormone thyroxine levels (T4). Briefly, 50 μL of plasma was diluted 10 times with phosphate-buffered saline and then measured for T4 levels using an enzyme link immune sorbent assay with a commercial kit for mouse (Uscnlife, Wuhan, China). It was based on competitive binding enzyme immunoassay technique, with a detection limit of 0.3 μg L^{-1}.

To analyze the histopathological changes, the thyroid tissues were dissected with ~0.2 g fresh weight being fixed in 10% formaldehyde. After 48 h, the samples were dehydrated in a grade alcohol series (70%→80%→90%→95%→100% alcohol) and embedded in paraffin wax. Samples of 4–5 μm thickness were stained with hematoxylin-eosin for pathological studies. The samples were examined with an Olympus BX60 microscope and visualized through the color-View camera (Olymupus, Tokyo, Japan).

In addition to hematoxylin-eosin staining, thyroid tissues were also examined with transmission electron microscopy (TEM, Hitachi H-600, Japan). Briefly, ~0.2 g fresh weight of thyroid tissues was fixed with 2% glutaraldehyde and 2% formaldehyde in phosphate-buffered saline solution at 4 °C overnight and then gradually dehydrated with ethanol and propylene oxide. The tissues were infiltrated in an embedding medium containing 50% (v/v) propylene oxide for 1 h, and then 33% propylene oxide overnight followed by 100% embedding resin Embed 812 for 2 h at room temperature. Tissue blocks were then polymerized in an oven at 60 °C for 24 h. Ultrathin sample sections were cut with an ultramicrotone (Ultra, Diatome, Switzerland) prior to TEM analyses.

2.3. RNA isolation and cDNA synthesis for genes in HPT axis in brain and thyroid samples

In our study, the effect of As on genes related in HPT axis was measured. At the end of exposure, mouse brains were collected to measure the gene expression of TRH and TSH, while thyroids were collected to measure the gene expression of TRα, TRβ, Deio1, and Deio2. The brains and thyroids were firstly rinsed with ice-cold 0.86% physiological saline solution, and then homogenized using TRIzol reagent (TaKaRa, Japan). RNA isolation and cDNA synthesis were performed at 4 °C for 10 min according to Sun et al. (2013). Following centrifuged at 12,000 *g* for 10 min, 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. Aqueous supernatant was carefully removed into a new tube with minimum disturbance and mixed with 0.5 mL of isopropanol. The mixtures were kept for 10 min and centrifuged at 12,000 *g* for 10 min. The pellets were washed with 1 mL of 75% ethanol and centrifuged at 7500 *g* for 5 min before being air-dried for 5 min and finally resuspended in RNase-free water. The RNA contents in mouse tissues were measured via absorbance at 260/280 nm using a UV–vis spectrophotometer (NanoDorp, 2000; ThermoFisher, USA). RNA of 1 μg was subjected to cDNA synthesis with *SuperScript II* reverse transcriptase according to the manufacturer's protocol (TaKaRa, Japan).

2.4. Quantification of gene expression in HPT axis by RT-PCR

Considering their importance, the expression of genes relevant in HPT axis (i.e., TRα, TRβ, TSH, TRH, Deio1, and Deio2) and internal control (β-actin) were measured by quantitative RT–PCR following Sun et al. (2014). Briefly, all PCR reactions (20 μL) comprised of 10 μL of SYBR Premix *Ex Taq*II (TaKaRa, Japan), 1 μL of cDNA, 10 pM of each forward and reverse primers, and 7 μL of ultrapure water (Table 1). 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 a Real-time PCR Machine (Bio-RAD CFX™ Connect, USA). The relative expression levels of different genes were calculated according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Table 1
Real-time PCR primers used in the present study.

Name		Sequence
β-actin	Forward	5′- TCCGCCTTAATACTTCATT -3′
	Reverse	5′- ACCAAAGCCTTCATACAT -3′
TRα	Forward	5′- GAGTTTACCAAGATCATCA -3′
	Reverse	5′- GTGTCATCCAGGTTAAAG -3′
TRβ	Forward	5′- GGACATAGACAATACTCAA -3′
	Reverse	5′- AGCAATATCACTTAACACT -3′
TSH	Forward	5′- CATACAGAGACTTCATCTAC-3′
	Reverse	5′- TCGTTCTATTCCAGGTAA -3′
TRH	Forward	5′- GAGGAGTAAGGTTAGAGT -3′
	Reverse	5′- AAGTTATACCAGGTCAGT -3′
Deio1	Forward	5′- GTGACAACCAGAAATAAAG -3′
	Reverse	5′- ACCAGTTCAGCATTATAC -3′
Deio2	Forward	5′- TGAGAACTGAACTGAATC -3′
	Reverse	5′- GCTGAGACAATACACTTA -3′

2.5. Statistical analyses

Data are presented as mean ± standard deviation and were evaluated by one-way analysis of variance followed by Duncan's multiple range test ($\alpha = 0.05$). All statistical analyses were carried out using SigmaPlot 11.0.

3. Results and discussion

3.1. Impact of As on T4 levels in mouse blood

Thyroid hormones regulate the metabolism and development of animals, thus abnormal levels can cause growth problems, resulting in cardiovascular, skeletal, and neurological illnesses (Epstein et al., 2001; Pop et al., 2003). In this study, in control mice without As exposure, the T4 levels in mouse blood after 4 and 8 weeks of exposure were significantly higher (77.2–80.0 μg L^{-1}) than that after 1–2 weeks (57.3–60.7 μg L^{-1}), while there was no difference between 4 and 8 weeks (Fig. 2), indicating that thyroid hormone levels increased with mouse growth. After 1–2 weeks of exposure, neither AsV nor AsIII at 10 or 100 μg L^{-1} had effects on T4 levels, which were 56.3–64.7 μg L^{-1} (Fig. 2), suggesting mice can stand As stress for short period. Similar results have been reported, showing ≤100 μg L^{-1} AsIII or AsV had little effect on T4 level in bighead carp in early life stage (Sun et al., 2016b).

However, as exposure increased to 4–8 weeks, 10 μg L^{-1} AsV or AsIII increased T4 levels in mouse blood to 83.8–88.8 μg L^{-1} compared to control at 77.2–80.0 μg L^{-1} (Fig. 2). It was reported that thyroid hormone homeostasis is closely related to mouse immune system (Klein, 2006), so we speculated that mouse probably enhanced their immune systems by elevating thyroid hormone levels to alleviate adverse effects of low As exposure (Lam et al., 2005). However, compared to low AsV at 10 μg L^{-1}, higher AsV at 100 μg L^{-1} after 4–8 weeks of exposure decreased T4 levels (79.1–79.3 μg L^{-1}), similar to control. Similar to AsV, after 4 weeks of exposure, T4 level was significantly lower for 100 μg L^{-1} than 10 μg L^{-1} AsIII (71.8 vs. 88.6 μg L^{-1}). These suggested higher As exposure decreased T4 levels compared to low As exposure. It was possible that high As exposure caused damage to mouse thyroid, influencing its capacity to deal with As stress. In vitro experiment by Palazzolo and Jansen (2008) showed that after exposure to 0.1–1 mg L^{-1} AsIII for 10 min, the activity of thyroid peroxidase was inhibited. As the major enzyme involved in multiple processes of hormonogenesis of thyroid hormones, the abnormal level of thyroid peroxidase disturbs the thyroid hormones homeostasis in mouse (Hughes et al., 2003).

Interestingly, after 8 week of exposure, higher AsIII exposure at

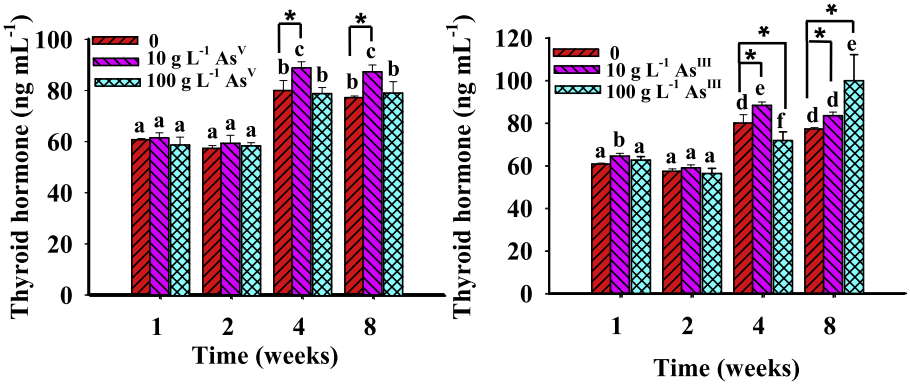

Fig. 2. Impacts of 10 or 100 mg L^{-1} of AsV (A) and AsIII (B) in drinking water on thyroxine hormone levels in mouse blood after 1, 2, 4, or 8 weeks of exposure. Bars represent mean and standard deviation of 4 replicates. Different letters indicate significant differences between treatments and control; * mean significant differences between treatments for same exposure duration.

100 μg L^{-1} increased the T4 level to 99.8 μg L^{-1}, significantly higher than that at 10 μg L^{-1} AsIII or control mice (83.8 and 77.2 μg L^{-1}) (Fig. 2). The data suggested that, compared to AsV, AsIII exerted higher toxicity to mouse, so mouse dealt with the As toxicity via elevating the T4 levels. This is because T4 can inhibit As accumulation in mouse and decrease the As toxicity (Allen and Rana, 2003; Rana and Allen, 2006). Besides, the different toxicity mechanisms of AsV and AsIII as well as associated discussion can be found at Sun et al. (2016a).

3.2. *Effect of As on histopathological changes in mouse thyroid*

To provide novel insight into As thyrotoxicity, the morphological and histological changes in thyroid gland via histopathological analyses were assessed using TEM. The follicle shape, follicular epithelium, and mesenchyme of thyroid gland in the control group were well developed (Fig. 3A–D), with no apparent damage on thyroid tissues being observed at 10 μg L^{-1} AsIII or AsV (data not shown). However, after 8 weeks, exposing to 100 μg L^{-1} AsIII increased the cellularity of the thyroid gland, depletion of colloids, and irregularities in follicle shape including invaginations and protrusions of the epithelial layer (Fig. 3E and F). Besides, decreased colloid with some follicles containing pale, pink, and lacking proteinaceous material were observed (Fig. 3GH). Similar results were observed for 100 μg L^{-1} AsV exposure (data not shown). The data indicated that, at 100 μg L^{-1}, inorganic As exposure damaged thyroid gland in mice, thereby influencing its thyroid hormone homeostasis.

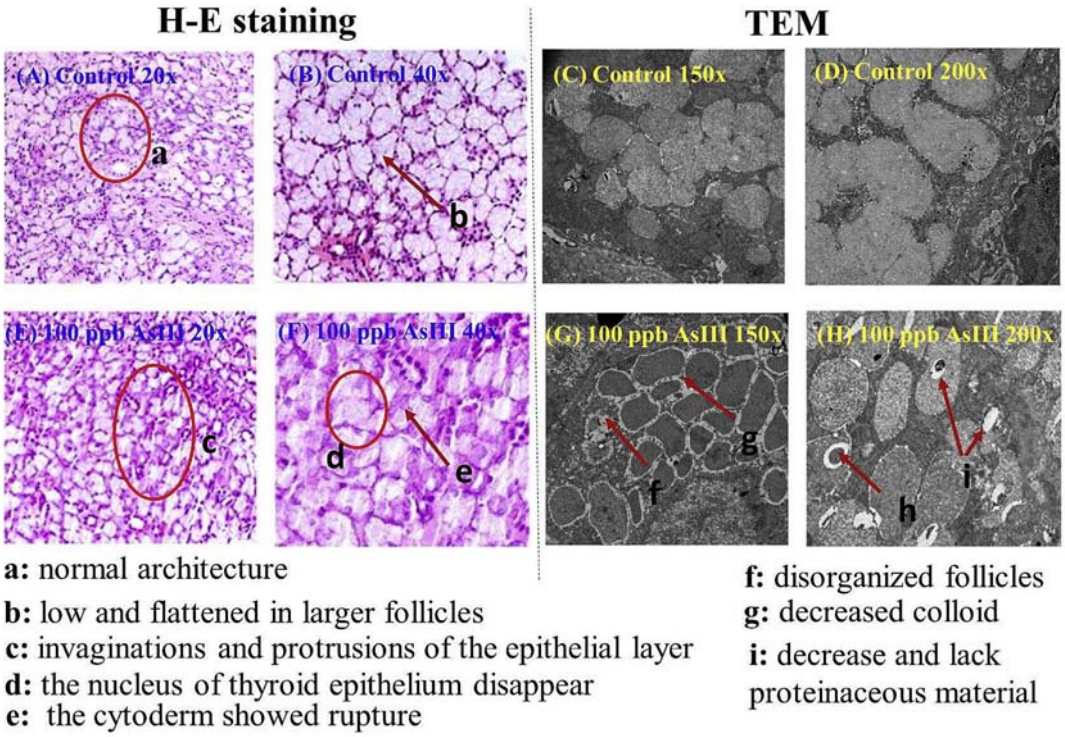

Fig. 3. Histopathological changes in mouse thyroid tissues following 8 weeks of exposure to 100 μg L^{-1} AsIII via drinking water. A–D: mouse thyroid without AsIII exposure; and E–H: mouse thyroid with AsIII expsosure.

3.3. Impact of As on thyroid hormone receptor transcription in mouse thyroid

Thyroid hormone receptor (TRs), the mediator of thyroid hormones, are encoded by two genes, TRα and TRβ (Harvey and Williams, 2002). In this study, after 1-week of exposure, both AsV and AsIII significantly inhibited TRα mRNA transcriptional levels (Fig. 4AB). This was similar with our previous study where little change in T4 levels was observed after bighead carp embryo was exposed to 100 μg L^{-1} AsV for 78 h, but TRα mRNA transcription was inhibited (Sun et al., 2016a). However, changes at 2–8 weeks were variable with no consistent trend with either increasing As concentration or exposure time (Fig. 4AB). It was possible that mouse increased the T4 level via augmenting the mRNA transcription to resist the As threat, however, the change was concentration- and time-dependent. For example, at 100 μg L^{-1}, AsV elevated TRα mRNA transcription after 4 weeks but reduced its mRNA transcription after 8 weeks of exposure. This was probably due to the detrimental effect of As with increasing exposure, with higher toxicity inhibiting TRα mRNA transcription. Similar observations was also reported by Xie et al. (2015) who found that 1.0 mg L^{-1} microcystin-RR significantly repressed TRα mRNA transcription in zebrafish larvae though with no effect on T4 level. The changes in TRs transcription are the principal mechanisms for animals to handle the change in thyroid hormone (Baos et al., 2006).

Though different from TRα, the impact of AsV and AsIII on TRβ mRNA transcription was also concentration- and time-dependent (Fig. 4CD). At 10 μg L^{-1} AsV, the transcription level of TRβ mRNA significantly decreased after 1–4 week of exposure compared to control, but the level increased after 8-week. For 100 μg L^{-1} AsV, TRβ mRNA transcription increased after 4-week of exposure but decreased after 8 weeks (Fig. 4C). Meanwhile, 100 μg L^{-1} AsIII inhibited the transcriptional levels of TRβ mRNA after 1 week, and then elevated the TRβ mRNA transcriptional level afterwards. When exposure increased to 8 weeks, TRβ mRNA transcriptional level was repressed (Fig. 4D). This may be due to different toxic effect at different As concentrations, both AsV and AsIII toxic effect increased with increasing exposure time to 8 weeks, displaying significant change. Besides, compared with AsV, AsIII exhibited higher toxic effect and caused more influence on TR transcriptional levels.

3.4. Impact of As on gene transcription of TSH and TRH in mouse brain

To further understand the effect of As on thyroid hormones, we also measured transcriptional levels of thyroid stimulating hormone (TSH). After 1 week of exposure, 10 μg L^{-1} AsV increased the transcription level of TSH mRNA while 100 μg L^{-1} AsV decreased TSH mRNA transcription (Fig. 5A). This may be due to different toxicity at different As concentrations, causing different responses of TSH. After 2–4 weeks, 10 μg L^{-1} AsV had little effect on TSH mRNA transcription while 100 μg L^{-1} AsV elevated its mRNA transcription levels. This maybe because that after exposure to 100 μg L^{-1} for 4 weeks, mouse elevated T4 level via increasing the transcriptional level of TSH mRNA to improve its capacity to counter As toxicity. However, after 8 weeks, TSH mRNA

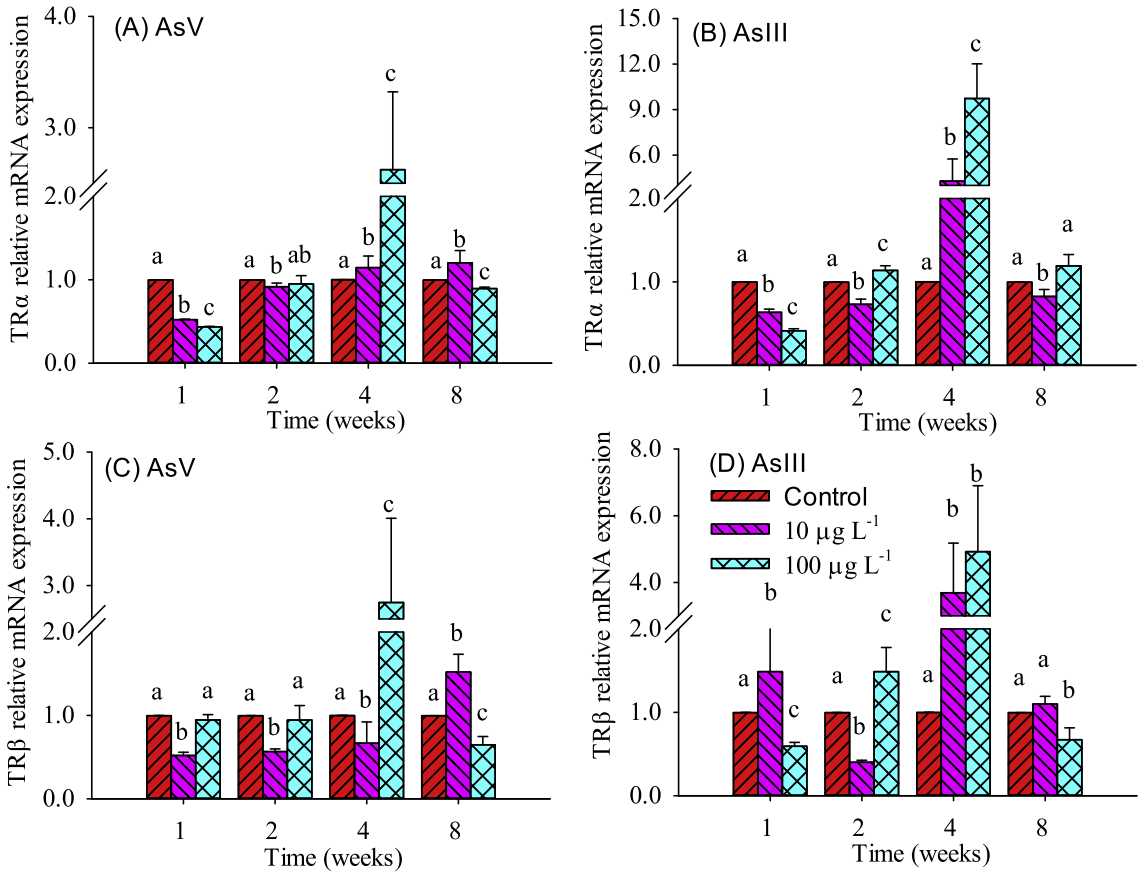

Fig. 4. Changes in TRα (A and B) and TRβ (C and D) after exposing to 10 or 100 μg L^{-1} AsV and AsIII via drinking water for 1, 2, 4 or 8 weeks. Bars represent mean and standard deviation of 4 replicates. Different letters indicate significant differences between treatments and control.

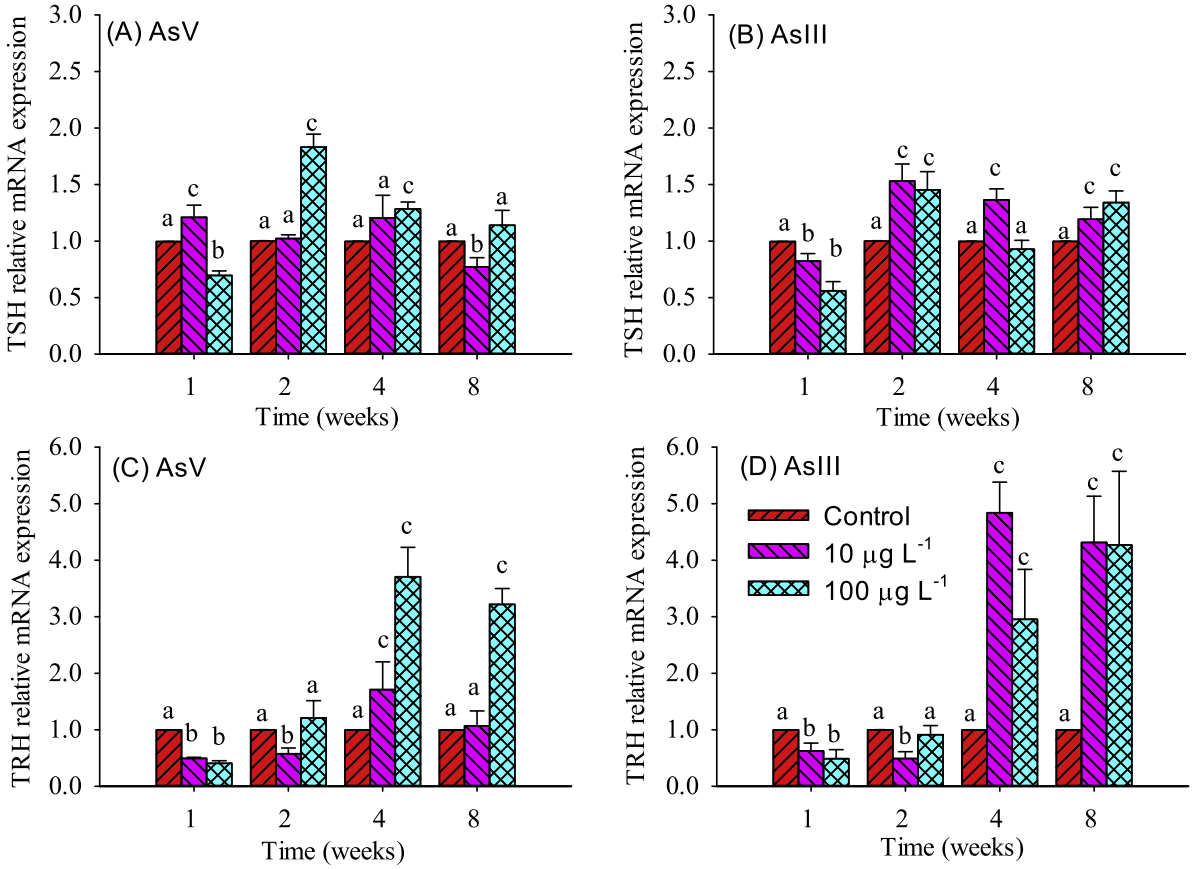

Fig. 5. Changes in TSH (A and B) and TRH (C and D) after exposing to 10 or 100 μg L^{-1} AsV and AsIII via drinking water for 1, 2, 4 or 8 weeks. Bars represent mean and standard deviation of 4 replicates. Different letters above bars indicate significant differences among treatments in the same exposure duration.

transcription exhibited different trend. While 10 μg L^{-1} AsV significantly repressed TSH mRNA transcription, 100 μg L^{-1} AsV had no effect on the transcription level. This was because that after 8 weeks of exposure, more As was accumulated in mouse (Hughes et al., 2003), elevating the T4 contents and resulting in feedback effect on TSH to decrease its transcription levels.

Regarding AsIII, both 10 and 100 μg L^{-1} lowered TSH mRNA transcription after 1-week of exposure (Fig. 5B), while during 2–8 weeks, AsIII exposure increased TSH mRNA transcription, which was responding to increased T4 levels. This may be because toxic effect of As increased with increasing exposure duration, resulting in elevated transcriptional level of TSH mRNA. In addition, the feedback loop of thyroid endocrine system also played a crucial role in this process. (Chiamolera and Wondisford, 2009). Besides TSH, we also measured the transcriptional levels of TRH. From Fig. 5, we can see that 10 μg L^{-1} AsV inhibited TRH mRNA transcriptional level after 1–2 weeks of exposure, and it then elevated the transcriptional level of TRH mRNA at 4 weeks, with the transcriptional level recovering to control levels after 8 weeks. The data indicated that 10 μg L^{-1} AsV had limited effect on the transcriptional level of TRH mRNA, so mouse can overcome the threat from 10 μg L^{-1} AsV. However, unlike 10 μg L^{-1} AsV, 10 or 100 μg L^{-1} AsIII or 100 μg L^{-1} AsV showed more toxic effect on the transcriptional level of TRH mRNA, with continous increasing TRH mRNA transcription with exposure time (Fig. 5CD). The data indicated that As induced toxic effect on mouse and caused detrimental effect on thyroid endocrine system.

3.5. Impact of As on gene transcription of Deio in mouse thyroid

The iodothyronine deiodinases are responsible for both activation and inactivation of thyroid hormones. In this study, 10 μg L^{-1} AsV inhibited the transcriptional levels of Deio1 after 2-week of exposure, while it increased the transcriptional levels of Deio1 after 4–8 weeks. However, at 100 μg L^{-1}, AsV also repressed the mRNA transcription of Deio1 after 2 weeks and increased after 4 weeks of exposure, but it returned to normal levels after 8 weeks (Fig. 6A). These results indicated that Deio1 displayed different transcriptional performance to confront the stress caused by As after different exposure period, showing higher toxic effect after 4–8 weeks of exposure. At 10 μg L^{-1} AsIII, Deio1 mRNA transcription levels increased after 1 and 4 week of exposure, however, it decreased after 2 and 8 weeks. At 100 μg L^{-1}, the transcriptional level of Deio1 mRNA increased after 2 and 4 weeks, but decreased at 8 weeks of exposure (Fig. 6B). These results showed that AsIII showed higher toxic effect than AsV. The transcriptional level decreased after 8 weeks of exposure because of the greater damage on thyroid tissues, which is consistent with TEM data (Fig. 4GH).

Regarding Deio2, it didn't show apparent change except it decreased after exposing to 10 μg L^{-1} AsV for 1 week. At 100 μg L^{-1}, the transcriptional level displayed inconsistent change. The Deio2 mRNA transcription increased and then decreased with increasing exposure (Fig. 6C). These results indicated that 10 μg L^{-1} AsV showed less toxic effect, it only repressed the transcriptional levels of Deio2 mRNA after 1 week of exposure, mice probably can overcome the stress at 10 μg L^{-1} AsV. At 100 μg L^{-1}, higher AsV dose

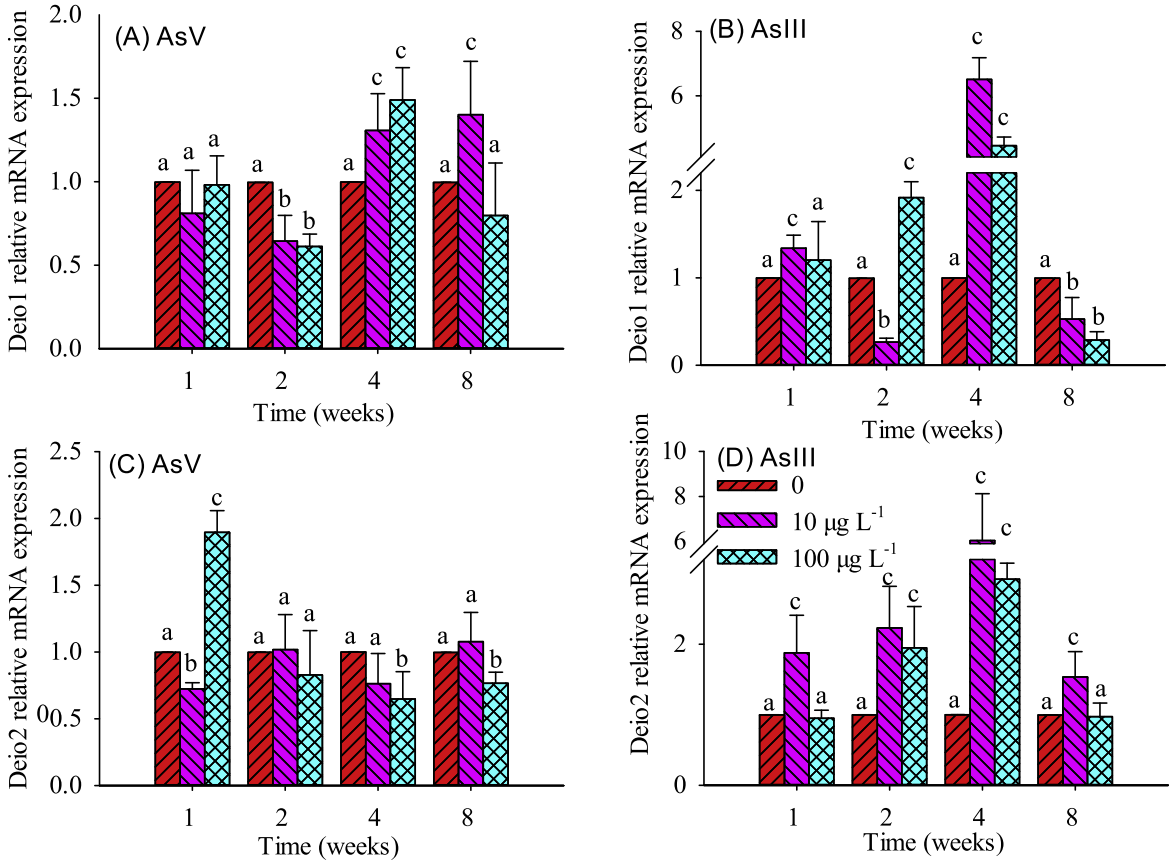

Fig. 6. Changes in Dieo1 (A and B) and Dieo2 (C and D) after exposing to 10 or 100 μg L^{-1} AsV and AsIII via drinking water for 1, 2, 4 or 8 weeks. Bars represent mean and standard deviation of 4 replicates. Different letters above bars indicate significant differences among treatments in the same exposure duration.

caused mice to augment the Deio2 transcription to deal with AsV toxicity, with higher toxic effect with increasing exposure by decreasing the transcriptional levels. Different from AsV, AsIII elevated the transcriptional level of Deio2 mRNA, except 100 μg L^{-1} AsIII after 8 weeks of exposure (Fig. 6D). This was attributed to AsIII-induced damage of thyroid tissue.

In short, both Deio1 and Deio2 mRNA transcription exhibited different trends with time after exposure to AsIII or AsV (Fig. 6). This was because of their different function. Deio1 plays an important role in activating or inactivating T4, while Deio2 is responsible for generating the active form of T3 via deiodination of T4.

4. Conclusion

In this study, we assessed the impacts of different concentrations of AsV and AsIII on thyroid hormone T4 levels and HPT-aixs related gene transcription after different exposure time (1, 2, 4, and 8 weeks). We observed that AsIII showed more effect on disrupting T4 levels and HPT related genes expression, attributing to its higher toxic effect. Furthermore, this study also manifested that 10 μg L^{-1} AsV or AsIII slightly elevated the T4 content by 11–13% after 4 and 8 weeks of exposure. At 100 μg L^{-1}, however, AsIII inhibited T4 content by 10% after 4 weeks, and elevated T4 content by 29% at 8 weeks. Furthermore, As had significant effects on HPT axis related gene transcription. Besides, we detected thyroid gland damage after 100 μg L^{-1} AsIII exposure. This study demonstrated that both AsV and AsIII disrupted T4 homeostasis, influenced the related gene transcription and damaged the thyroid glands after low levels of exposure.

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