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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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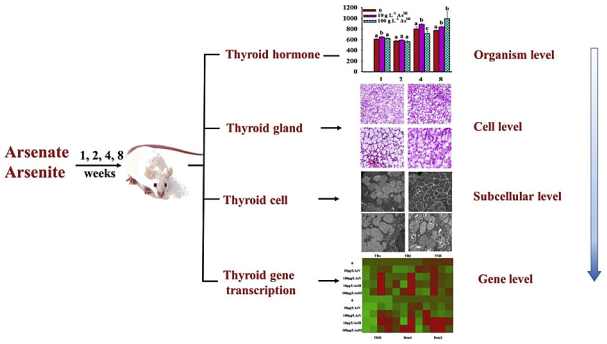
HIGHLIGHTS

- 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.

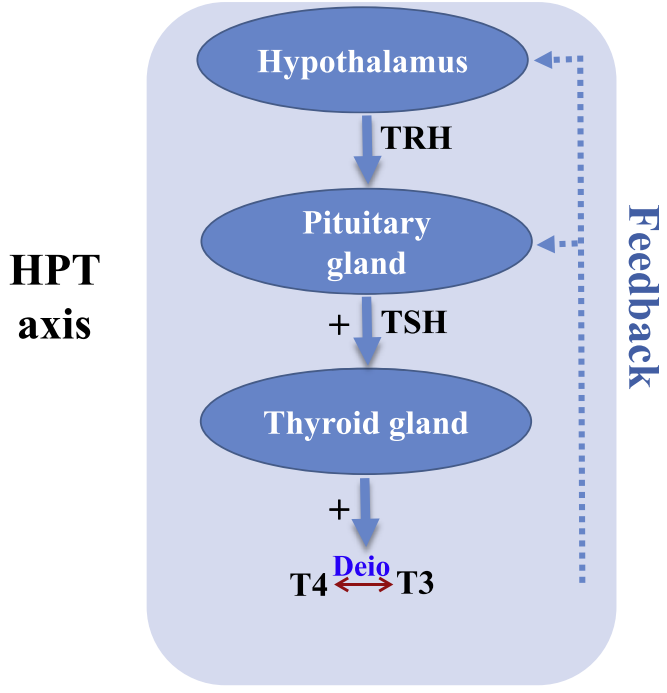

Fig. 1. The axis of hypothalamus-pituitary-thyroid (HPT). TRH = thyrotropin releasing hormone, TSH = thyroid stimulating hormone, Deio = iodothyronine deiodinases, T4 = thyroxine, and T3 = triiodothyronine.

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}.