Toxicology and Applied Pharmacology 274 (2014) 117–123

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Urinary arsenic metabolism in a Western Chinese population exposed to high-dose inorganic arsenic in drinking water: Influence of ethnicity and genetic polymorphisms

Songbo Fu [a,1], Jie Wu [b,1], Yuanyuan Li [a], Yan Liu [c], Yanhui Gao [a], Feifei Yao [a], Chuanying Qiu [d], Li Song [a], Yu Wu [a], Yongjian Liao [e], Dianjun Sun [a,*]

[a] Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, Key Lab of Etiology and Epidemiology, Education Bureau of Hei Long Jiang Province & Ministry of Health (23618104), Harbin 150081, China
[b] Laboratory of Medical Genetics, Harbin Medical University, Harbin 150081, China
[c] Department of Health Statistics, Harbin Medical University, Harbin 150081, China
[d] Dongcheng District Center for Disease Control and Prevention, Beijing 100009, China
[e] Gansu Center for Disease Control and Prevention, 730020, China

ARTICLE INFO

Article history:
Received 23 June 2013
Revised 28 October 2013
Accepted 5 November 2013
Available online 15 November 2013

Keywords:
Arsenic
Methylation metabolism
Genetic polymorphism
AS3MT
GSTO1
GSTO2

ABSTRACT

To investigate the differences in urinary arsenic metabolism patterns of individuals exposed to a high concentration of inorganic arsenic (iAs) in drinking water, an epidemiological investigation was conducted with 155 individuals living in a village where the arsenic concentration in the drinking water was 969 μg/L. Blood and urine samples were collected from 66 individuals including 51 cases with skin lesions and 15 controls without skin lesions. The results showed that monomethylated arsenic (MMA), the percentage of MMA (%MMA) and the ratio of MMA to iAs (MMA/iAs) were significantly increased in patients with skin lesions as compared to controls, while dimethylated arsenic (DMA), the percentage of DMA (%DMA) and the ratio of DMA to MMA (DMA/MMA) were significantly reduced. The percent DMA of individuals with the Ala/Asp genotype of glutathione S-transferase omega 1 (GSTO1) was significantly lower than those with Ala/Ala. The percent MMA of individuals with the A2B/A2B genotype of arsenic (+3 oxidation state) methyltransferase (AS3MT) was significantly lower than those with AB/A2B. The iAs and total arsenic (tAs) content in the urine of a Tibetan population were significantly higher than that of Han and Hui ethnicities, whereas MMA/iAs was significantly lower than that of Han and Hui ethnicities. Our results showed that when exposed to the same arsenic environment, different individuals exhibited different urinary arsenic metabolism patterns. Gender and ethnicity affect these differences and above polymorphisms may be effectors too.

Introduction

From a public health perspective, drinking water is the primary source of exposure to inorganic arsenic (iAs), and arsenic concentrations in the drinking water of millions of residents throughout the world are greater than 100 μg/L (Smith et al., 2000). Chronic exposure to high-dose iAs in drinking water leads to cancer and other forms of physical damage (IARC, 2004; WHO, 2001). To ameliorate these circumstances, many studies have focused on the methylation metabolism of iAs in the body (Meza et al., 2007). However, different arsenides with different toxicities are produced during this complex process, of which monomethylarsonous acid (MMA^{III}) has a higher toxicity than its precursor arsenite (As^{III}) (Chung et al., 2009; Del et al., 2001; Kitchin, 2001), potentially reflecting the effects of certain factors induced by chronic arsenic exposure (Del et al., 2001; Lindberg et al., 2008). In areas of high-dose iAs in drinking water, the differences in arsenide content in the urine of individuals are highly variable (Thomas et al., 2004; Vahter, 2002; Valenzuela et al., 2005). A number of studies have shown higher proportions of MMA in urine, which might reflect the retention of highly toxic MMA^{III} in body tissues, and these individuals have an increased risk of physical damage related to arsenic toxicity (Chen et al., 2005; Chung et al., 2009; Lindberg et al., 2008; Tseng, 2007). These differences in urinary arsenic metabolism among individuals show that different individuals have different iAs methylation metabolism abilities and that these differences result in variable susceptibilities to body damage due to chronic arsenic exposure (Drobna et al., 2004; Vahter, 2002).

* Corresponding author at: Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, 157 Baojian Road, Harbin 150081, China. Fax: +86 451 8665 7674.
E-mail address: hrbmusdj@163.com (D. Sun).
[1] These authors contributed equally to this work.

0041-008X/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.taap.2013.11.004

As arsenic methylation metabolism in the body requires a variety of enzyme catalysts, genetic polymorphisms should be considered to further address the effects of related genetic polymorphisms on the arsenic methylation metabolism level. Metabolism in the human body involves a series of reduction and methylation processes, and glutathione S-transferase omega 1 (GSTO1), glutathione S-transferase omega 2 (GSTO2) and arsenic (+3 oxidation state) methyltransferase (AS3MT) are the metabolic enzymes that play an important role in these processes (Drobna et al., 2009; Marnell et al., 2003; Schlawicke Engstrom et al., 2007; Schmuck et al., 2005; Zakharyan et al., 2001). The effects of genetic polymorphisms in these enzymes on arsenic metabolism in the human body have also been confirmed in many studies (Agusa et al., 2009; Chung et al., 2002; Valenzuela et al., 2009; Whitbread et al., 2003). Many in vivo and in vitro studies have shown that AS3MT oxidizes all trivalent arsenides through methylation (iAs^{III}, MMA^{III} and dimethylarsinous acid—DMA^{III}) (Schlawicke Engstrom et al., 2007), which is crucial to the transformation of a variety of intermediate products from iAs into the corresponding methylates (Drobna et al., 2005). Both GSTO1 and GSTO2 can restore monomethylarsonic acid (MMA^{V}) to MMA^{III} (Schmuck et al., 2005; Zakharyan et al., 2001), and this reduction reaction is the rate-limiting step in the biological transformation of human arsenic (Zakharyan et al., 2001). In addition, some factors, such as the arsenic concentrations in drinking water, race, gender and age, might also affect individual arsenic methylation metabolism levels (Chowdhury et al., 2003; Loffredo et al., 2003; Meza et al., 2004).

Because the methylation of iAs in the body exhibits increased activity and toxicity, more extensive studies on the patterns of methylation metabolism in individuals exposed to different levels of arsenic and various influential factors are required. In the present study, the arsenic concentration in drinking water was 969 μg/L (Qiu et al., 2010), this concentration is nearly one hundred times higher than 10 μg/L, which is the highest standard for arsenic in drinking water according to the recommendations of the World Health Organization (WHO, 2003). Because the exposure time lasted for 5 years, more than half of the villagers in the current study have suffered typical skin lesions induced by exposure to arsenic in drinking water. The characteristics of urinary arsenic methylation metabolism in individuals with high prevalence and high-dose iAs in drinking water have not been determined. Therefore, this study examined the differences in urinary arsenic metabolism patterns in populations exposed to high-dose iAs in drinking water and the roles of gender, age, ethnicity and genetic polymorphisms, such as GSTO1, GSTO2 and AS3MT, in these differences.

Materials and methods

Research area and populations. The Sasuoma Village of Wanggeertang in Xiahe County in the Gannan Tibetan Autonomous Region, Gansu Province is a multinational village with a total of 252 individuals of Hui, Tibetan and Han ethnicities. No unified water source existed in this region. The villagers drank spring, well and snow water. In 2002, the villagers began drinking water from a concentrated water-engineering source for individuals and livestock that originated from spring water in a nearby mountain. The arsenic concentration in the drinking water was 969 μg/L, which was the arithmetic mean by measuring water samples of 41 households in 2007 (Qiu et al., 2010). From August 18 to 24, 2007, the Endemic Disease Control Center of the Chinese Center for Disease Control and Prevention organized professionals to collect water and urine samples at the Gansu Center for Disease Control and Prevention. On August 24, 2007, the water source was discontinued. From October 4 to 19 of the same year, the Endemic Disease Control Center of the Chinese Center for Disease Control and Prevention, together with professionals and clinical doctors of the Gansu Center for Disease Control and Prevention, conducted an epidemiological investigation, a clinical investigation and blood sample collection.

The results of the epidemiological investigation and clinical examination showed that the total population of the village was 155, including 56 males and 99 females, ranging from 3 to 82 years of age. The ethnicity distribution included 89 Hui, 50 Tibetan and 16 Han individuals. Approximately 90 villagers suffered typical skin lesions induced by arsenic exposure in the drinking water, with a morbidity rate of 58%.

Sample collection. The Medical Ethics Committee of Harbin Medical University approved this research. All participants provided written informed consent. The questionnaire included gender, age, living conditions, dietary habit, smoking, alcohol consumption, illness, etc. According to the national standard (Standard of Diagnosis for Endemic Arsenism, WS/T-211-2001), trained clinical doctors analyzed skin lesions and graded the lesions as mild, moderate or severe. Of the 155 individuals, 90 presented skin lesions. Migrant workers who had returned from the city, children under 6 years old, elderly individuals over the age of 70 and villagers who had suffered fever, infection or autoimmune diseases or who had recent occupational exposure to arsenic (1 month) or X-ray (within 6 months) were excluded from the blood sample collection. Approximately 3 mL of fasting venous blood was extracted from the villagers who were willing to provide blood samples in the morning; this blood was blended and collected in EDTA anticoagulation tubes for mixing. After collection, the specimens were immediately transported to a laboratory, placed in a −18 °C car refrigerator and stored in a −80 °C low-temperature refrigerator. A total of 106 blood samples were collected, including 65 patients with skin lesions. Of these 65, 12 patients suffered from mild skin lesions, 31 patients exhibited moderate skin lesions and 22 individuals had severe skin lesions. The patients included 25 males and 40 females, with a mean age of 38.95 years. The remaining 41 individuals without skin lesions were exposed to the same arsenic environment and included 12 males and 29 females, with a mean age of 30.95 years.

The urine samples were collected before the drinking water containing high-dose iAs was discontinued, at an interval of 2 months from the collection of the blood samples. The samples were obtained at the Gansu Center for Disease Control and Prevention. Approximately, 15 mL of morning urine was collected using a clean polypropylene plastic bottle, and the collected specimen was immediately placed in a 4 °C car refrigerator and subsequently stored at −80 °C in a cryogenic refrigerator at a laboratory at the Gansu Center for Disease Control and Prevention. The collected urine and blood samples were frozen and shipped to the Endemic Disease Control Center of the Chinese Center for Disease Control and Prevention for testing. A total of 66 urine samples, corresponding to all of the blood samples above, were collected on a separate occasion from the collection of the blood samples. The 51 skin lesion patients comprised the cases and included 13 mild skin lesion patients, 20 moderate skin lesion patients and 18 severe skin lesion patients. These patients included 11 males and 40 females, with a mean age of 35.55 years. The controls consisted of 15 individuals without skin lesions who were exposed to the same arsenic environment and included 4 males and 11 females with a mean age of 26.87 years. These samples were used to examine urinary arsenic metabolism and various influential factors.

The examination of arsenic metabolism products. IAs^{III}, arsenate (iAs^{V}), MMA^{V} and dimethylarsinic acid (DMA^{V}) in the 66 urine samples were measured using high-performance liquid chromatography (HPLC) for separation and hydride generation atomic fluorescence methods for detection (Le and Ma, 1998; Le et al., 2000b). Each form of standard arsenic (with a purity greater than 99.9%) was obtained from Sigma. Before quantification, the urine samples were thawed naturally and centrifuged for 10 min at 12,000 rpm. Subsequently, the supernatant was filtrated through a 0.45 μm membrane.