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Methylation capacity of arsenic and skin lesions in smelter plant workers

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ABSTRACT

Potential occupational arsenic exposure is a significant problem in smelting plants. The metabolites containing arsenic with an oxidation of +3 have been considered more cytotoxic and genotoxic than their parent inorganic species. The current study examined the capacity of arsenic methylation and its risk on skin lesions. The primary aim of this study is to determine if methylation capacity, as measured by urinary arsenic metabolites, differed in workers with skin lesions compared to workers without skin lesions. Hydride generation-atomic absorption spectrometry was used to determine three arsenic species in urine of workers who had been working in arsenic plants, and primary and secondary methylation indexes were calculated. Skin lesions were examined at the same time. Many workers had obvious skin lesions (36/91). The mean concentrations of inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in urine of workers are obviously higher than those of the control group. There are more iAs, MMA, and DMA in urine, higher MMA%, lower iAs% for workers with skin lesions compared with those without skin lesions. Workers with skin lesions have the lowest SMI (3.50 ± 1.21), and they may be in danger. Our results support the viewpoint that individuals who metabolize inorganic arsenic to MMA easily, but metabolize MMA to DMA difficulty have more risk of skin lesions.

1. Introduction

Potential occupational arsenic exposure is a significant problem in smelting plants, especially in arsenic smelting (Wen et al., 2011). Ingested inorganic arsenic is known to have adverse health effects and contributes to complex diseases such as skin lesions, diabetes, cardiovascular, and cancers of several organs (Kumagai and Sumi, 2007; Shi et al., 2004). Human and rodent studies have indicated that chronic exposure to iAs are linked to several adverse health effects including premalignant skin lesions, such as hyperkeratosis on the palms and soles or hyperpigmentation combined with small areas of hypopigmentation on the neck and back. Some evidence suggests that arsenic-induced skin lesions are early biomarkers of other outcomes such as nonmelanoma skin cancer and cancer of the internal organs (Liao et al., 2010; Rahman et al., 2006; Bhattacharjee, 2007).

Trivalent arsenic compounds most likely exist in arsenic plant. In the pH range of 4–10, trivalent arsenic compounds are neutral in charge and pentavalent arsenicals are negatively

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charged (Cohen et al., 2006; Tseng, 2009). Therefore trivalent arsenics are more ready to cross cell membrane and enter the cells at physiologic pH. After exposure, arsenic is biotransformed into the body in a serial of reactions where methylation plays a crucial role. Before being excreted in the urine, iAs undergoes a series of reductions of the pentavalent species to the trivalent species followed by oxidative methylation to yield pentavalent methylated species. In humans, methylated and dimethylated arsenic are mainly found as metabolites in urine (Thomas et al., 2007; Meno et al., 2009; Fujihara et al., 2010; Chen et al., 2005). The methylated metabolites containing arsenic with an oxidation of +3 have been considered to be more genotoxic than their parent inorganic species, but the role of this metabolic process is under discussion (Hernández et al., 2008; Fujihara et al., 2009). Urinary levels of MAs^{III}, the most toxic species among identified metabolites of iAs, may serve as an indicator to identify individuals with increased susceptibility to toxic and cancer-promoting effects of arseniasis (Valenzuela et al., 2005). In humans, inorganic arsenic metabolism is incomplete, and limited evidence suggests that the ability to fully metabolize arsenic into DMA influences susceptibility to disease (Kile et al., 2011).

The current study examined the methylation capacity of arsenic and its risk on skin lesions.

2. Materials and methods

Study population. We recruited 91 workers from two arsenic plants which produce arsenic trioxide by smelting arsenic ore, in an outlying mountain area of Yunnan province, China. 55 individuals in the control group resided in villages distant from the two plants more than 50 km from the arsenic plants and that had a similar social economic status to the 91 workers. All participants receive the same physical examination looking for skin lesions, and so on.

We had complied with the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (World Medical Association 1989). All 146 recruited subjects gave informed consent before participating. A questionnaire was used to obtain information of each individual including age, gender, type of work, service length, smoking and alcohol use (current smoker who smoke more than 10 cigarettes each day, any consumption of alcohol), and other potential chemical exposures (lead, carbon monoxide, silicon dioxide, and so on), and data on health status, dietary habits, history of chronic disease, family members, place of birth, race, education, economic conditions, and reproductive conditions. Trained physical medical doctors conducted examinations according to the Diagnosis Standards on Arsenic of China (WS/T211-2001, Ministry of Health 2001) to obtain information.

Environmental monitoring. In order to determine the concentration of arsenic in work place air, environmental monitoring was performed according to Determination of Toxic Substances in Work place air-arsenic and its chemical compounds (GBZ/T 160.31-2004, China). ZGF-2OH dust sampling instrument (Science precision apparatus and meter limited company, China) was used to collect air arsenic samples. There were 7 samplers used in each plant, arsenic was detected in the dust at all posts that were measured. The air samples were collected over 8 h daily when the plant was in full operation for 3 days. The samplers were located in the breathing zone. There were 7 posts sampled and 21 samples collected in each plant. The size fraction of dust collected was inhalable particles (also known as particulate matter $\leq 10\,\mu$ns in aerodynamic diameter, PM10).

Dust samples were treated by microwave digestion in the presence of a 25% nitric acid solution. Arsenic was analyzed by the hydride-evolution technique.

Skin examination and definition of cases. A manual of diagnosis and mitigation was developed after a series of consultation meetings with dermatologists. All study personnel were given 2 weeks training in the field. Skin lesions examined by trained health personnel reconfirmed by trained expert with digital photographs and revisits.

Definitive cases. Hyperkeratosis on both palms and soles with or without hypo and/or hyperpigmentation on skin unexposed to sunlight.

Sample collection. Written instructions regarding the hygienic conditions for collection of samples, 500 ml polyethylene containers treated with hydrochloric acid and rinsed with deionized water were provided to all participants. Subjects were asked to provide the first morning void urine. Urine samples were immediately sent to the laboratory and stored at 4 °C within 6 h after collection. Then, samples were taken to Yunnan Provincial Center for Disease Control and Prevention in Kunming City, and stored frozen at −80 °C. Typically, all urine samples, kept on dry ice, were sent to the Laboratory of Arsenic Analysis in China Medical University (Shenyang, Liaoning province, China).

Reagents and standards. Arsenate ($Na_3AsO_4{\cdot}12H_2O$), arsenite ($NaAsO_2$), HCI, NaOH, and $NaBH_4$ were purchased from Shanghai Chemical Co. (Shanghai, China). All reagents used in this study are analytical grade and As free (<0.01 mg/L). We used a mixed As standard of 1000 mg/L monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Tri Chemical Laboratories Inc., Yamanashi, Japan). We acquired an iAs standard of 1000 mg/L from the National Center for Standard Reference Materials (Beijing, China). Standard reference material of freeze-dried urine (SRM 2670) for toxic metals was obtained from the U.S. National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Determination of As metabolisms. We determined As species (iAs, MMA, and DMA) in urine using atomic absorption spectrophotometer (AA-6800) with an As speciation pretreatment system (ASA-2SP, Shimadzu Co., Kyoto, Japan). Speciation analysis was based on the well-established hydride generation of volatile arsines, followed by cryogenic separation in liquid nitrogen. The limit of detection of 1 ng ± <5% for each of the four As species was determined using hydride generation-atomic absorption spectrometry (HGAAS). Briefly, 1 ml urine that had been stored at −80 °C was thawed at room temperature and digested with 1 N NaOH solutions at 100 °C for 3 h in a 15-ml polymethylpentene test tube (Sarstedt), followed by dilution with Milli-Q water (Millipore, Yonezawa, Japan). This digestion procedure did not alter the distribution of iAs or methylated arsenicals (Yamauchi and Yamamura, 1984). The absorbance of As in the digested urine samples was determined at 193.7 nm.