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Lung inflammation biomarkers and lung function in children chronically exposed to arsenic

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ABSTRACT

Evidence suggests that exposure to arsenic in drinking water during early childhood or *in utero* has been associated with an increase in respiratory symptoms or diseases in the adulthood, however only a few studies have been carried out during those sensitive windows of exposure. Recently our group demonstrated that the exposure to arsenic during early childhood or *in utero* in children was associated with impairment in the lung function and suggested that this adverse effect could be due to a chronic inflammation response to the metalloid. Therefore, we designed this cross-sectional study in a cohort of children associating lung inflammatory biomarkers and lung function with urinary As levels. A total of 275 healthy children were partitioned into four study groups according with their arsenic urinary levels. Inflammation biomarkers were measured in sputum by ELISA and the lung function was evaluated by spirometry. Fifty eight percent of the studied children were found to have a restrictive spirometric pattern. In the two highest exposed groups, the soluble receptor for advanced glycation end products' (sRAGE) sputum level was significantly lower and matrix metalloproteinase-9 (MMP-9) concentration was higher. When the biomarkers were correlated to the urinary arsenic species, negative associations were found between dimethylarsinic (DMA), monomethylarsonic percentage (%MMA) and dimethylarsinic percentage (%DMA) with sRAGE and positive associations between %DMA with MMP-9 and with the MMP-9/tissue inhibitor of metalloproteinase (TIMP-1) ratio. In conclusion, chronic arsenic exposure of children negatively correlates with sRAGE, and positively correlated with MMP-9 and MMP-9/TIMP-1 levels, and increases the frequency of an abnormal spirometric pattern. Arsenic-induced alterations in inflammatory biomarkers may contribute to the development of restrictive lung diseases.

Introduction

Chronic arsenic (As) exposure through drinking water has been correlated with increased incidence and mortality by cancers as well as nonmalignant diseases (Chiou et al., 1995; Hopenhayn-Rich et al., 1998). While arsenic can affect many tissues and organ systems, the lung seems particularly susceptible (NRC, 2001). Although the mechanism of As-induced respiratory illness is not fully understood, several studies have shown that a large amount of As is deposited and stored in the lung, especially in the epithelium (Gerhardsson et al., 1988; Saady et al., 1989). In rabbits, arsenic has been shown to accumulate in the lung more than in any other organ except the liver and kidney (Bertolero et al., 1981; Marafante et al., 1981).

Several studies have reported a high prevalence of respiratory symptoms in subjects highly exposed to As through drinking water such as chronic cough, abnormal chest sound, shortness of breath (Mazumder et al., 2000), lower forced expiratory volume measured in 1 s (FEV_1), and altered forced vital capacity (FVC) (von Ehrenstein et al., 2005). Additionally, De et al. (2004), reported that 57% of subjects chronically exposed to arsenic had respiratory symptoms with 53% having restrictive lung disease and 41% of the study participants having both obstructive and restrictive lung diseases. Mazumder et al. (2005), reported a 10-fold increased risk of chronic obstructive pulmonary disease (COPD), identified by high-resolution computed tomography in subjects chronically exposed to arsenic. In a study carried out by

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Smith et al. (2006), they found that exposure to arsenic in drinking water during early childhood or *in utero* has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults from both malignant and nonmalignant lung disease. Recently our research group demonstrated that exposure to arsenic through drinking water during *in utero* and early life was associated with a decrease in FVC and with a restrictive spirometric pattern in the children that suggested that these adverse effects could be due to a chronic inflammatory response to this metalloid (Recio-Vega et al., 2014). Therefore, it is very important to carry out studies with the aim to detect early lung diseases and so to decrease the frequency of pulmonary pathologies during the adulthood.

Several authors have suggested that the mechanism of action of As in the lungs is that it can enhance tissue inflammation (De et al., 2004; Nemery, 1990), induce respiratory function impairment by oxidative stress (Lantz and Hays, 2006) and/or produce or increase pulmonary fibrosis (von Ehrenstein et al., 2005; Nemery, 1990). Increased inflammatory responses have been reported in infants born to arsenic exposed mothers (Fry et al., 2007) and arsenic alters markers of inflammation (sRAGE, MMP-9 and TIMP-1) in adults exposed to 20 μg/L As. sRAGE has been recognized for its role in several chronic diseases, such as diabetes, atherosclerosis, coronary artery disease, and lung cancer (Bierhaus et al., 2005; Falcone et al., 2005; Hofmann et al., 2004). In a population-based study, Lantz et al. (2007), demonstrated a significant negative correlation between sRAGE sputum levels and total urinary inorganic As. MMPs and TIMP-1 are sensitive markers of lung inflammation in humans (Josyula et al., 2006) and both of them are continually secreted in the airways. *In vitro* models have shown that acute arsenic exposure increases activity and expression of MMP-9 in airway epithelial cells (Olsen et al., 2008).

Josyula et al. (2006) evaluated the changes in biomarkers of lung inflammation, as measured by the ratio of sputum metalloproteinase and antiprotease activity, in subjects exposed to arsenic and concluded that the increased sputum proteinase/antiprotease activity suggests a potential toxic mechanism for low-level arsenic exposure. Human and mouse models have shown that *in utero* and early life exposures to arsenic can result in alterations in adult lung function and lung disease. However, no reports exist concerning the relationship of long-term exposure to As with sRAGE, MMP-9 and TIMP-1 sputum levels or between these inflammation biomarkers and lung function in children. Studies at this age will provide relevant results and so establish different actions and preventive programs with the aim to abolish pulmonary diseases in children and later during the adulthood. In this study Mexican children from a rural area with arsenic present in their drinking water were examined for urinary arsenic levels, spirometric lung function, and inflammatory markers in their sputum.

Material and methods

Study population. The subjects included in this report are a subset of those reported in an earlier study (Recio-Vega et al., 2014). More than 500 children were evaluated; however since satisfactory sputum sample is not easy to obtain at this group-age, we only include in this study those children where acceptable sputum samples were obtained ($n = 275$) and this will allow us to better assess the risk facing children from arsenic exposure. The participants were females and males aged 6–12 years residing in four rural communities in which the highest arsenic tap water levels have been detected in the last 20 years (104–360 ppb) in the studied area. These communities received groundwater through the local water supply and the high As levels detected are due to an over water extraction from the ground for crops. At present, water is obtained from a depth of 200–300 m. These communities form part of the geographic area known as Comarca Lagunera, which is located in the north-central part of Mexico.

To aid in focusing on long-term and continuous arsenic exposure we only included subjects who were healthy, were conceived in the studied rural communities, whose mothers lived their entire pregnancies in these communities, and who have remained as permanent residents of the same communities. Subjects who agreed to participate in the study were divided in four study groups (quartiles) according with their total urinary arsenic level (Group 1: ≤63 μg/L; Group 2: 63–113 μg/L; Group 3: ≥114–181 μg/L; and Group 4: >181 μg/L).

Written informed consent was obtained from each participant and from their parents to obtain biological samples. The study protocol was approved by the Ethics Committee of the School of Medicine at Torreon, University of Coahuila, Mexico.

Questionnaire application. Information was collected through in-person interviews and included socio-demographic variables (education, socioeconomic status, type of kitchen, type of fuel used for cooking), lifetime residential history, lifestyle factors (secondhand smoke defined as someone smoking regularly in the same room at home, and exercise), parent's occupational history, water source types (municipal tap water, bottled, other), current medications, medical history, and diet. Water consumption habits were ascertained through a standardized questionnaire. Standardized questions were adapted to Spanish from the questionnaire used by the American Thoracic Society Division of Lung Disease (Ferris, 1978) to register the presence of respiratory symptoms. Asthmatic volunteers confirmed by a doctor were not included in the study. Written informed consent was obtained from each participant and from their parents to obtain biological samples, which were obtained at the time of interview.

As measurement in drinking water and urine. Three drinking water samples (school, home and well) were collected from each rural community included in the study and analyzed for inorganic arsenic levels. No other contaminants in the drinking water were assessed. Individual exposure was assessed based on urinary concentration of the sum of inorganic As. A first morning void urine sample was collected in sterile 120 mL screw-topped polypropylene container. The urine samples were obtained during the late autumn and winter seasons to avoid the hottest seasons when there is much higher water consumption and children have increased outdoor activities.

Urine samples were analyzed using the methodology described by U.S. Center for Disease Control (CDC, 2004) at the Arizona Laboratory for Emerging Contaminants, University of Arizona, Tucson, Arizona, U.S.A. Briefly, arsenic species in urine (As^V, As^{III}, monomethylarsonic acid (MMA^V), dimethylarsinic acid (DMA^V) and arsenobetaine) were separated by HPLC. Arsenic concentrations in water samples and urine were analyzed by inductively coupled plasma mass spectrometry utilizing the Standard Reference Water, SMR 1640 (NIST, Gaithersburg, MD, USA) and the freeze-dried Urine Reference Material for trace elements (Clinchek-control; RECIPE Chemicals instruments GmbH, Munich, Germany) for urine as quality control. Urinary arsenic concentration was adjusted for creatinine by expressing its level as a ratio with creatinine (μg/g creatinine). The creatinine levels were estimated in urine samples using the Jaffe reaction method. Additional exposure to DMA or to organic arsenic metabolized in the body to DMA, which usually is attributable to consumption of seafood such as bivalves and seaweeds, was considered minimal because such sea-foods are essentially never eaten in this area. Arsenic metabolism efficiency was calculated using the following formulas proposed by Del Razo et al. (1997), first methylation = $MMA^V / (As^V + As^{III})$; second methylation = DMA^V / MMA^V.

Lung function measurement. A detailed description of lung function measurements on children was reported in our previous study (Recio-Vega et al., 2014). Briefly: lung function was assessed according to American Thoracic Society guidelines (1995) using an EasyOne spirometer (NDD Medical Technologies, Zurich, Switzerland). The device was standardized to meet American Thoracic Society guidelines for lung function tests. Predicted values were those of the Mexican-Americans