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Imbalanced immune responses involving inflammatory molecules and immune-related pathways in the lung of acute and subchronic arsenic-exposed mice

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ABSTRACT

Inorganic arsenic has been claimed to increase the risk of pulmonary diseases through ingestion, as opposed to inhalation, which makes it a unique and intriguing environmental toxicant. However, the immunotoxic effects of lung, one of the targets of arsenic exposure, have not been extensively investigated in vivo. In the present study, we first confirmed that 2.5, 5 and 10 mg/kg $NaAsO_2$ orally for 24 h dose-dependently triggered the infiltration of neutrophils, lymphocytes and macrophages in BALF. Not only the transcription activity, but also the secretion of proinflammatory cytokines IL-1β, IL-6 and TNF-α were consistently raised in the lung and BALF of acute arsenic-exposed mice. Acute oral administration of $NaAsO_2$ also raised pulmonary MPO activity and mRNA levels of chemokine *Mip-2* and *Mcp-1*. Meanwhile, obvious histopathological damages with inflammatory cells infiltration and erythrocyte aggregation around the capillaries were verified in the lung of mice drank arsenic-rich water freely for 3 months. Furthermore, we affirmed notable disturbance of $CD4^+$ T-cell differentiation in the lung of acute arsenic-exposed mice, as demonstrated by up-regulated mRNA levels of regulator *Gata3* and cytokine *Il-4* of Th2, enhanced *Foxp3* and *Il-10* of Treg, down-regulated *T-bet* and *Ifn*-γ of Th1, as well as lessened *Ror*-γ*t* and *Il-23* of Th17. However, impressive elevation of cytokine *Ifn*-γ *and Il-23*, as well as moderate enhancement of *Il-4* and *Il-10* were found in the lung by subchronic arsenic administration. Finally, our present study demonstrated that both a single and sustained arsenic exposure prominently increased the expression of immune-related p38, JNK, ERK1/2 and NF-κB proteins in the lung tissue. While disrupting the pulmonary redox homeostasis by increasing MDA levels, exhausting GSH and impaired enzyme activities of CAT and GSH-Px, antioxidant regulator NRF2 and its downstream targets HO-1 and GSTO1/2 were also up-regulated by both acute and subchronic arsenic treatment. Conclusively, our present study demonstrated both acute and subchronic oral administration of arsenic triggers multiple pulmonary immune responses involving inflammatory molecules and T-cell differentiation, which might be closely associated with the imbalanced redox status and activation of immune-related MAPKs, NF-κB and anti-inflammatory NRF2 pathways.

Abbreviation: AHR, Alleviated airway hyper-responsiveness; BCA, Bicinchoninic acid assay; BSA, Bovine serum albumin; BALF, Broncho-alveolar lavage fluid; CAT, Catalase; COPD, Chronic obstructive pulmonary disease; JNK, c-Jun N-terminal kinases; DMA, Dimethylarsinic acid; ELISA, Enzyme-linked immunosorbent assay; ERK1/2, Extracellular-signal-regulated kinases ½; GSH, Glutathione; GSH-Px, Glutathione peroxidase; GR, Glutathione reductase; GSTO1/2, GST-glutathione-S-transferase1/2; H & E, Hematoxylin-eosin; HO-1, Heme oxygenase-1; HCl, Hydrochloric acid; IL-1β, Interleukin-1β; LDH, Lactate dehydrogenase; LSD, Least-Significant Difference; LD, Limit of detection; MDA, Malondialdehyde; MMP-9, Matrix metalloproteinase; MMA, Monomethylarsonic acid; MPO, Myeloperoxidase; NRF2, NF-E2-related factor 2; *Nrf2*$^{-/-}$, *Nrf2*-deficiency; NF-κB, Nuclear factor kappa B; ANOVA, One-way analysis of variation; OVA, Ovalbumin; PBS, Phosphate buffered saline; PVDF, Polyvinylidene fluoride; ROS, Reactive oxygen species; Treg, Regulatory T; Th1, T helper type 1; TBAR, Thiobarbituric acid reaction; TIMP-1, Tissue inhibitor of metalloproteinase; T-As, Total arsenic; TNF-α, Tumor necrosis factor-α; γ-GCSc, γ-glutamyl cysteine synthetase

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1. Introduction

Inorganic arsenic is a naturally occurring element ubiquitously presenting in the environment, particularly in groundwater (Humans, 2004, 2012). A variety of adverse health effects to humans, such as dermal changes, hepatic, renal, respiratory, cardiovascular, neurological, carcinogenic and many other harmful effects are closely related to arsenic exposure. Among them, the lung is already confirmed as one of the major target organs (Mandal and Suzuki, 2002). Particularly, the ability of inorganic arsenic to increase the risk of lung diseases through ingestion, as opposed to inhalation, makes it a unique and intriguing lung toxicant (Smith et al., 2009). Increasing lines of epidemiological studies have indicated that chronic arsenic exposure through drinking water with high concentrations of arsenic could result in increased risks of a variety of pulmonary diseases, including lung cancers, tuberculosis, bronchiectasis and other respiratory illnesses in human populations (Mazumder et al., 2000; Parvez et al., 2008; Raqib et al., 2009; Smith et al., 2006).

Recent studies have expanded the concept that inflammation is a critical component of pulmonary diseases including asthma, chronic obstructive pulmonary disease (COPD), tuberculosis and lung cancers (Coussens and Werb, 2002; Gorska et al., 2016; Leepiyasakulchai et al., 2012). It has been indicated that inorganic arsenic is capable of stimulating the release of inflammatory molecules in liver, kidney, spleen and thymus, as well as in many cell types in vitro (Bourdonnay et al., 2011; Das et al., 2005; Singh et al., 2015; Zhang et al., 2014). Some epidemiologic literatures have also reported the enhancement of pulmonary inflammation and respiratory function impairments in certain arsenic-exposure populations, especially in infants born by arsenic-exposed mothers (Olivas-Calderon et al., 2015). Matrix metalloproteinase (MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) in sputum, two sensitive markers of lung inflammation, are found to be abnormal in adults exposed to 20 μg/L arsenic for at least 3 years (Josyula et al., 2006). Another analysis of the broncho-alveolar lavage fluid (BALF) was performed in 10 patients of chronic arsenic poisoning for a mean duration of 17.31 ± 7.83 years, and the macrophage counts, lactate dehydrogenase (LDH) levels and nitric oxide activity are shown to be elevated apparently in those patients with pulmonary diseases than in those without (De et al., 2004). As to the animal models, Kozul et al. reports that 100 μg/L arsenic treatment for 5 weeks could significantly decrease the amounts of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, as well as reduce the number of dendritic cells in the mediastinal lymph nodes, which impair the immune responses of C57BL/6 mice to respiratory influenza A infection (Kozul et al., 2009a). In addition, alleviated airway hyper-responsiveness (AHR), abrogated airway eosinophil recruitment and increased macrophages were also confirmed in the BALF of ovalbumin (OVA)-immunized mice by 2.5 and 5 mg/kg As_2O_3 intraperitoneally for 7 days (Chu et al., 2010). Although the links between inorganic arsenic exposure and pulmonary immune dysfunction have been identified to some extent, more intensive and systemic studies on the immunotoxic effects as well as the involved molecular mechanisms of this environmental toxicant are still needed to be clarified.

Our previous study has confirmed the inflammatory responses and T-cell differentiation of acute arsenic exposure in the mice immune system (Duan et al., 2017), and we further suggested that arsenic-induced activation of extracellular-signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinases (JNK), p38, and their downstream transcription factor nuclear factor kappa B (NF-κB) might be involved in the immune reactions of spleen and thymus. However, the immunomodulatory and inflammatory effects of lung, one of the major targets of arsenic exposure, have not been extensively investigated in vivo. On the other hand, many studies recently have linked the responsiveness of NF-E2-related factor 2 (NRF2)-directed antioxidant pathways to various pulmonary inflammatory disorders (Wang et al., 2017; Zhao et al., 2016). It is demonstrated that *Nrf2*-deficiency ($Nrf2^{-/-}$) could induce the augmentation of OVA-driven airway inflammation with more pronounced lung mucus cell hyperplasia, the increase of eosinophilic infiltration, as well as the suppression of multiple antioxidant effects relative to $Nrf2^{+/+}$ mice (Rangasamy et al., 2005).

Comprehensively, the present study focused on the changes of the potential inflammatory molecules and immune-related pathways in the pulmonary system. We set up acute arsenic exposure models by treating mice with an oral administration of 2.5, 5 and 10 mg/kg $NaAsO_2$, and determined the inflammatory cellular profiles in BALF, inflammatory molecules levels, $CD4^+$ T cells differentiation and oxidative status, as well as the expressions of immune-related MAPKs/NF-κB and anti-inflammatory NRF2 signaling pathways in mice lung. In addition, our present study further observed the pathologic changes and immune-related responses in lung tissues after repeated oral arsenic exposure for 3 months.

2. Materials and methods

2.1. Reagents and chemicals

Sodium arsenite ($NaAsO_2$, ≥ 99.0%) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). $NaAsO_2$ was dissolved in distilled water and diluted to the desired concentrations. Real-time polymerase chain reaction (real-time PCR) kits were from Takara Co (Japan). IL-1β ELISA kit (eBscience, Lot#4297869), IL-6 ELISA kit (eBscience, Lot#E09358-1646) and TNF-α ELISA kit (eBscience, Lot#4314355) were all purchased from eBiosciences (eBiosciences, San Diego, CA). Phosphate buffered saline (PBS) was from Gibco-Invitrogen (Carlsbad, CA). Myeloperoxidase (MPO), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione (GSH) assay kits were purchased from Jiancheng Biological Institute (Nanjing, China). Primary antibodies of p-P38 (#9211), P38 (#9212), p-JNK (#9251), JNK (#9252), p-ERK1/2 (#9101), ERK1/2 (#9102) were purchased from Cell Signaling Technology (Cell Signaling, Danvers, USA). NF-kB (C-20: sc-372), NRF2 (H-300: sc-13032), heme oxygenase-1 (HO-1) (H-105: sc-10789), GST-glutathione-S-transferase1/2 (GSTO1/2) (FL-241: sc-98560), γ-glutamyl cysteine synthetase (γ-GCSc) (GCLC) (H-300: sc-28965), Glutathione reductase (GR) (H-120: sc-32886), β-actin (I-19: sc-1616) and corresponding secondary antibodies were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Potassium hydroxide (KOH), hydrochloric acid (HCl) and potassium borohydride (KBH_4) were purchased from Shanghai Chemical Co. (Shanghai, China) with arsenic free (< 0.01 mg/L). All other chemicals used were of the highest grade commercially available. Water used in all the preparations was distilled and deionized.

2.2. Animals and experimental procedures

Female C57BL/6 and Kunming mice (weighing 14–18 g, 6–8 weeks old) were obtained from the Center for Experimental Animals at China Medical University (Shenyang, China) with a National Animal Use License number of SCXK-LN2013-0007. Animal use has been approved by Animal Use and Care Committee at China Medical University with a protocol number of CMU62043006. All experiments and surgical procedures were approved by the Animal Care and Use Committee at China Medical University, which complies with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the numbers of animal used and their suffering.

Mice were group-housed in stainless steel cages (10 mice per cage), and were fed a standard animal diet and water ad libitum under controlled temperature conditions with 12-h light-dark cycles. The doses of $NaAsO_2$ were selected on the basis of previously published studies as well as our preliminary experiments. Acute arsenic-exposed mice were exposed to 2.5, 5 and 10 mg/kg $NaAsO_2$ intragastrically for 12 and 24 h, respectively. Subchronic arseic-exposed mice drank water with