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Oxidative DNA damage of peripheral blood polymorphonuclear leukocytes, selectively induced by chronic arsenic exposure, is associated with extent of arsenic-related skin lesions ☆

Qiuling Pei [a,*], Ning Ma [d], Jing Zhang [a], Wenchao Xu [a], Yong Li [a], Zhifeng Ma [a], Yunyun Li [a], Fengjie Tian [a], Wenping Zhang [a], Jinjun Mu [b], Yuanfei Li [c], Dongxing Wang [a], Haifang Liu [a], Mimi Yang [a], Caifeng Ma [a], Fen Yun [a]

[a] Department of Toxicology, Public Health College, Shanxi Medical University, No 56 Xin Jian Nan Lu, Taiyuan (030001), China
[b] The Second Hospital, Shanxi Medical University, Taiyuan (030001), China
[c] The First Hospital, Shanxi Medical University, Taiyuan (030001), China
[d] Faculty of Health Science, Suzuka University of Medical Science, Suzuka, 510-0293, Japan

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ABSTRACT

There is increasing evidence that oxidative stress is an important risk factor for arsenic-related diseases. Peripheral blood leukocytes constitute an important defense against microorganisms or pathogens, while the research on the impact of chronic arsenic exposure on peripheral blood leukocytes is much more limited, especially at low level arsenic exposure. The purpose of the present study was to explore whether chronic arsenic exposure affects oxidative stress of peripheral blood leukocytes and possible linkages between oxidative stress and arsenic-induced skin lesions. 75 male inhabitants recruited from an As-endemic region of China were investigated in the present study. The classification of arsenicosis was based on the degree of skin lesions. Arsenic levels were measured in drinking water and urine by Atomic Fluorescence Spectroscopy. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) was tested by Enzyme-Linked Immunosorbent Assay. 8-OHdG of peripheral blood leukocytes was evaluated using immunocytochemical staining. 8-OHdG-positive reactions were only present in polymorphonuclear leukocytes (PMNs), but not in monocytes (MNs). The 8-OHdG staining of PMN cytoplasm was observed in all investigated populations, while the 8-OHdG staining of PMN nuclei was frequently found along with the elevated amounts of cell debris in individuals with skin lesion. Urinary arsenic levels were increased in the severe skin lesion group compared with the normal group. No relationship was observed between drinking water arsenic or urine 8-OHdG and the degree of skin lesions. These findings indicated that the target and persistent oxidative stress in peripheral blood PMNs may be employed as a sensitive biomarker directly to assess adverse health effects caused by chronic exposure to lower levels of arsenic.
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Introduction

Arsenic (As), widely distributed in nature, is a common environmental contaminant (Cullen and Reimer, 1989). Elevated arsenic concentrations can occur in environmental media (air, soil, water) from natural sources and anthropogenic activities. More than 100 million people in Bangladesh, India, and China, as well as large populations in North and South America currently are exposed to drinking water containing >10 μg As/L (Schuhmacher–Wolz et al., 2009). Although a number of epidemiological studies have demonstrated that chronic exposure of humans to high concentrations of arsenic in drinking water is associated with skin lesions, peripheral vascular disease, cardiovascular and cerebrovascular diseases, diabetes mellitus, neurological diseases, and a high risk of cancers (EPA, 2007; IARC, 2004; Schuhmacher–Wolz et al., 2009), the biomarker of early effect, which may be of significance in identifying and preventing the arsenic-related diseases, remains unclear.

There is increasing evidence that the induction of reactive oxygen species (ROS) like superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxy radical ($^{\bullet}OH$) plays a crucial role in arsenic toxicity (Flora, 2011; IARC, 2004). Experimental results have shown the generation of $O_2^{\bullet -}$ and H_2O_2 after arsenic exposure in some cell lines such as human vascular smooth muscle cells (Lynn et al., 2000), vascular endothelial cells (Barchowsky et al., 1999), and keratinocytes (Corsini et al., 1999). Furthermore, DNA damage mediated by arsenic-induced ROS generation too has been reported (Nesnow et al., 2002). Apart from the direct

Abbreviations: PMNs, polymorphonuclear leukocytes; MNs, monocytes; ROS, reactive oxygen species; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; WBC, peripheral blood leukocytes; As, arsenic; AsW, drinking water As; AsU, urine As; LOE, length of exposure.

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* Corresponding author at: Department of Toxicology, Shanxi Medical University, No 56 Xin Jian Nan Lu, Taiyuan (030001), China.
E-mail address: 924969007@qq.com (Q. Pei).

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evidence of arsenic-induced ROS, indirect evidence too has been revealed. The increased lipid peroxidation and decreased glutathione levels have been observed in arsenic-treated animals (Shi et al., 2004). A few epidemiological data exhibit that oxidative stress is associated with chronic arsenic toxicity. The incremental 8-OHdG concentrations were detected in the urine of individuals with an acute arsenic intoxication (Yamauchi et al., 2004). Individuals exposed to arsenic via drinking water showed high levels of ROS and lipid peroxide, but low levels of glutathione in plasma (Schoen et al., 2004; Wu et al., 2001).

8-OHdG, a DNA base-modified product generated by ROS, is widely accepted as a marker of oxidative DNA damage and oxidative stress (Flora, 2011). Blood is one of the targets of arsenic toxic action (Cohen et al., 2006; Pott et al., 2001; Schuhmacher-Wolz et al., 2009). Normal DNA repair removes 8-OHdG adducts which are measurable in urine, blood, and tissues. Although a few previous studies have reported that 8-OHdG concentrations increase in plasma of arsenic-exposed humans (Schoen et al., 2004; Wu et al., 2001), the expression and subcellular distribution of 8-OHdG in peripheral blood leukocytes, possibly in situ to estimate an early response to arsenic toxicity, have not been investigated in arsenic-exposed population.

The purpose of this study was to investigate whether chronic arsenic exposure can cause oxidative DNA damage of peripheral blood leukocytes and evaluate the relationship between the expression and subcelluar distribution of 8-OHdG in peripheral blood leukocytes and the extent of skin lesions in humans exposed to lower arsenic concentrations. Furthermore, we explored the relevance of arsenic exposure levels and individual characteristics to the extent of skin lesions in the investigated population.

Materials and methods

Site and participant selection. In an arsenic endemic rural region in Datong of Shanxi province, China, well water with high arsenic levels is clustered in the Sanggan River coastal area. The residents in the region have used the household-owned well water as their primary drinking source for more than 20 years. In September 2010, the health examinations of residents and well water arsenic concentrations in three villages of the region were investigated by Shanxi Medical University. Overall, 256 individuals (mean age: 51.67 ± 13.05; range: 20–79-year-old; men: 89; women: 167) were recruited from this region. All subjects were free of any acute clinical symptoms such as inflammatory diseases and drug administration in one week. The behavioral and demographic questionnaires were obtained by an in-person interview.

Our results (unpublished data) and other reports showed that men are susceptible to chronic arsenic exposure and that age, smoking, alcohol consumption, and sun exposure are confounding factors (Ahsan et al., 2006; Hakozaki et al., 2008; Schuhmacher-Wolz et al., 2009). To control these confounding factors as far as possible, the cohort in the present study was restricted to 75 men who live in the region for more than 30 years and have similar living habits in daily life.

On account of Datong rural area in a wide range of arsenic poisoning, 12 healthy male residents, 55 to 70-year-old, have been recruited from an arsenic-unexposed area in Linfen village, Shanxi. Age and life habits of these residents were matched with the arsenic-exposed.

Ethics Committee at Shanxi Medical University approved the protocol for this study. Informed consent was obtained prior to participation.

Blood, urine, and water collections. Fasting peripheral venous blood was collected from the participants. Blood smear was immediately prepared, fixed in methanol for 10 min, and stored at 4 °C until use. Water samples were taken directly from the tube well each family, collected in acid-washed polypropylene containers, and preserved at −20 °C. Spot morning urine samples were collected in polypropylene containers. Urine samples were stored at −80 °C until analysis.

Dermal examination. The dermal examination of participants was carried out by two trained dermatologists. The number of dyspigmentation/keratosis on trunk/palms and soles was recorded. Because an untypical keratosis is not easily distinguished from work-related thickening of corneum layer in the present study, the extent of skin lesions was evaluated based on dyspigmentation. The number of whitish patches on the trunk of the exposed person was calculated and evaluated semi-quantitatively as the levels of skin lesions as the following: normal = undetectable; mild = sporadic, ≤10; moderate = scattered, 10–20; severe = pervasive, ≥20.

Determination of arsenic concentration in urine and drinking water. The modified method was used for As detection as previously described (He et al., 2002; Wei and Liu, 2007). Briefly, a mixed solution of urine, nitric acid, and perchloric acid (1:0.25:0.75) was heated on hot plate until the liquid became cleared and crystallized, which was eluted with hydrochloric acid. The eluted solution and 50 g/l of a mixed solution of thiourea and ascorbic acid, and deionized water (0.25:0.25:0.50) were mixed as a sample solution. The preparation process of drinking water, arsenic standard solution, and control water were the same as the eluted solution of urine sample.

Arsenic concentrations were determined by an AF-610A atomic fluorescence spectrophotometer (Beijing Ruili Analytical Instrument Co., Beijing, China). The hollow cathode lamp was operated at 60 mA and the PMT voltage was set at 270 V. Argon was used as carrier gas with the flow rate of 800 mL/min. A personal computer was fitted with the software of AFS 610 for the control of the AFS procedure and the integration of the peak areas.

The coefficient of arsenic recovery was 96.8%–101%, and the interassay variation was <0.5% (replications = 11). Each sample was subjected to two replicate analyses. Arsenic concentrations in urine were corrected according to urine creatinine value. All reagents were at least of analytical reagent grade, and Milli-Q water was used throughout the determination procedure. Arsenite stock solution (1 mg/L) was purchased from National Standard Substance Center in China.

Immunocytochemical determination of 8-OHdG in peripheral blood leukocytes. Blood smears on poly-L-lysine-coated slides were hydrated with PBS, incubated in 1% skim milk for 30 min in PBS, and then incubated with a mouse anti 8-OHdG monoclonal antibody (1:200, Japan Institute for Control of Aging, Fukoroi, Japan) overnight at room temperature. After being thoroughly washed three times with PBS, the slides were incubated with Alexa 488 (594)-labeled goat antibody against mouse IgG (1:400 Molecular Probes, Eugene, Oregon, USA) for 3 h. The slides were mounted with Dapi-Fluoromount-G following washing with PBS. The stained sections were examined using fluorescence microscopy (BX53, Olympus, Tokyo, Japan). All of the interpretations of the immunocytochemical data were scored by two pathologists blindly and independently and based on the method described as follows. 100 leukocytes for every slide were examined on the basis of nuclear staining. The numbers of 8-OHdG positive cells and total leukocytes (cell nuclei) were calculated respectively. The percentage (0–100%) of 8-OHdG cells were evaluated as the rate of positive cells to total nuclei. In addition, we observed the subcellular distribution of 8-OHdG in blood leukocytes. The results of nuclear or cytoplasmic staining were recorded separately.

The previous study was undertaken to examine the cellular distribution of 8-OHdG and to confirm the specificity of the antibody. Addition of 100 μM 8-OHdG in the primary antibody reaction completely eliminated the immunofluorescent signal (Toyokuni et al., 1999).

Determination of urinary 8-OHdG levels. Before analysis, urine samples were centrifuged at 2000 *g* for 10 min to remove any suspended cell debris. The supernatants were used for the determination of 8-OHdG levels using a competitive enzyme-linked immunosorbent assay kit (ELISA;

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