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Arsenic is cytotoxic and genotoxic to primary human lung cells

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ABSTRACT

Arsenic originates from both geochemical and numerous anthropogenic activities. Exposure of the general public to significant levels of arsenic is widespread. Arsenic is a well-documented human carcinogen. Long-term exposure to high levels of arsenic in drinking water has been linked to bladder, lung, kidney, liver, prostate, and skin cancers. Among them, lung cancer is of great public concern. However, little is known about how arsenic causes lung cancer and few studies have considered effects in normal human lung cells. The purpose of this study was to determine the cytotoxicity and genotoxicity of arsenic in human primary bronchial fibroblast and epithelial cells. Our data show that arsenic induces a concentration-dependent decrease in cell survival after short (24 h) or long (120 h) exposures. Arsenic induces concentration-dependent but not time-dependent increases in chromosome damage in fibroblasts. No chromosome damage is induced after either 24 h or 120 h arsenic exposure in epithelial cells. Using neutral comet assay and gamma-H2A.X foci forming assay, we found that 24 h or 120 h exposure to arsenic induces increases in DNA double strand breaks in both cell lines. These data indicate that arsenic is cytotoxic and genotoxic to human lung primary cells but lung fibroblasts are more sensitive to arsenic than epithelial cells. Further research is needed to understand the specific mechanisms involved in arsenic-induced genotoxicity in human lung cells.

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1. Introduction

Arsenic (As) is an abundant naturally occurring element found in earth crust [1]. It is also released into the environment from human activities such as mining, electronics manufacturing and farming. As a result, high arsenic levels can occur in ground water and food raising health concerns for millions of people worldwide. In 2001, the United States Environmental Protection Agency (EPA) revised its drinking water standard for arsenic from 50 μg/l to 10 μg/l to better protect people from the adverse effects of long-term arsenic exposure [2]. However, millions of people worldwide are still exposed to arsenic at concentrations greater than 50 μg/l in drinking water [3,4].

Arsenic has been classified as group 1 human carcinogen by the International Agency for Research on Cancer (IARC). Studies show that chronic inorganic arsenic exposure leads to the development of lung, skin, liver, kidney and urinary bladder cancers [5]. Among these cancers, lung cancer is a major public health concern due to its high incidence rate and mortality [6].

Arsenic was first found associated with lung cancer in smelter workers exposed to arsenic via inhalation [7,8]. A significant dose–response relationship between the ingestion of inorganic arsenic in drinking water and increased lung cancer risks was found in Bangladesh [4], Taiwan [9,10], and Chile [11]. A recent study reported that even after high arsenic exposure level (11–335 μg/l) had been reduced for decades, lung cancer risk were still high in the exposed population [12]. Evidence also shows that even moderate concentrations of arsenic (less than 7.5 ppm) significantly impact lung cancer incidence, suggesting non-occupational exposures or lower levers of environmental exposure to arsenic should also be of concern with respect to lung cancer [13].

Finding an animal model to study arsenic-induced lung cancer has been difficult. While some studies found higher lung cancer rates in arsenic-exposed animals, others show negative results [5]. These negative results may be due a variety of factors including low animal numbers, low doses or short exposure durations [7]. By contrast, most lung cell culture studies support the conclusion that arsenic is a lung carcinogen. The ability of inorganic arsenic to induce malignant cell transformation has been demonstrated in several human lung epithelial cell lines [14–16].

The mechanism of arsenic-induced lung cancer is uncertain. Several hypotheses have generally been proposed including genotoxicity, induction of oxidative stress and inhibition of DNA repair [17]. Among these, the genotoxic mode of action is of high interest but has been under studied in human lung cells [18–21]. Only two

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studies considered arsenic genotoxicity in human lung cells. They found arsenic induces DNA single strand breaks and DNA–protein crosslinks in human fetal lung fibroblasts [22,23]. Studies of the impact of arsenic on chromosomes in human lung cells have not yet been considered, despite the importance of chromosomes as a subcellular target in carcinogenesis. Thus, this study assesses the ability of arsenic to induce chromosomal aberrations and DNA double strand breaks in primary human lung cells.

2. Materials and methods

2.1. Chemicals and reagents

Sodium metaarsenite, demecolcine, and potassium chloride (KCl) were purchased from Sigma (St. Louis, MO). Giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA). Crystal violet, methanol and acetone were purchased from J.T. Baker (Phillipsburg, NJ). Dulbecco's minimal essential medium and Ham's F-12 medium (D-MEM/F-12) were purchased from Mediatech Inc. (Herndon, VA). BEGM bronchial epithelial cell growth medium and supplements were purchased from Lonza (Allendale, NJ). Cosmic calf serum (CCS) was purchased from Hyclone (Logan, UT). Gurr's buffer, trypsin/EDTA, sodium pyruvate, penicillin/streptomycin, and L-glutamine were purchased from Invitrogen Corporation (Grand Island, NY). Tissue culture dishes, flasks and plasticware were purchased from Corning Inc. (Acton, MA).

2.2. Cells and cell culture

We chose to use normal primary human bronchial fibroblasts, NHBF (Clonetics), and normal primary human bronchial epithelial cells, NHBE (Lonza) for these studies. The cell cycle time for both cell lines is 22–24 h. These cells are isolated from normal donor airway located above the bifurcation of the lungs. NHBF were cultured in a 50:50 mix of Dulbecco's minimal essential medium and Ham's F-12 medium plus 15% cosmic calf serum, 1% L-glutamine, 0.1 mM sodium pyruvate and 1% penicillin/streptomycin. NHBE were cultured in serum-free BEGM bronchial epithelial cell growth medium with supplements. All cells were maintained in a 37 °C, humidified incubator with 5% CO_2. Cells were routinely checked for mycoplasma contamination.

2.3. Chemical preparation and treatment

Solutions of sodium arsenite were prepared by weighing out the desired amount of $NaAsO_2$, and dissolving it in double distilled water and then sterile-filtering it through a 10 ml syringe with a 0.2 μm filter. Dilutions were made for appropriate treatment concentrations. Cells were seeded in culture dishes, and allowed to grow for 48 h to enter log growth phase. The cells were then treated for 24 h or 120 h with $NaAsO_2$.

2.4. Cytotoxicity assays

Cytotoxicity was determined by a clonogenic survival assay measuring the reduction in plating efficiency in treatment groups relative to controls as previously described [24]. There were four dishes per treatment group and each experiment was repeated at least three times.

2.5. Chromosome abnormality

Cells were prepared for chromosomal analysis as previously described [24]. Cells were analyzed for chromosome structure and numerical aberrations, centromere spreading, premature anaphase, and premature centromere division. At least 100 metaphases per data point were analyzed in each experiment. Each experiment was repeated at least three times.

2.6. Neutral comet assay

DNA double strand breaks were measured using a gel electrophoresis assay (comet assay) under neutral conditions based on our published method [25]. Briefly, the cell suspension was mixed with low-melting agarose (Trevigen) and spread on a 2 well CometSlide (Trevigen). The cells were then lysed in prechilled lysis solution (Trevigen) at 4 °C followed by digesting with proteinase-K (Amresco, Solon, OH) for 2 h at 37 °C. Next, cells were electrophoresed in freshly prepared electrophoresis buffer (300 mM sodium acetate, 100 mM Tris, pH 9.0) at 4 °C. All the steps described above were conducted under a reduced light level to prevent spurious DNA damage. Slides were then immersed in DNA precipitation solution (1 M ammonium acetate in ethanol) for 30 min at room temperature, air-dried and stained with SYBR Green. Comet images were captured using an Olympus fluorescence microscope equipped with a digital video camera and analyzed with the Comet Assay IV image analysis system (Perceptive Inc, UK). The tail intensity was the measurement used to quantify DNA double strand breaks. 100 nuclei were analyzed for each concentration.

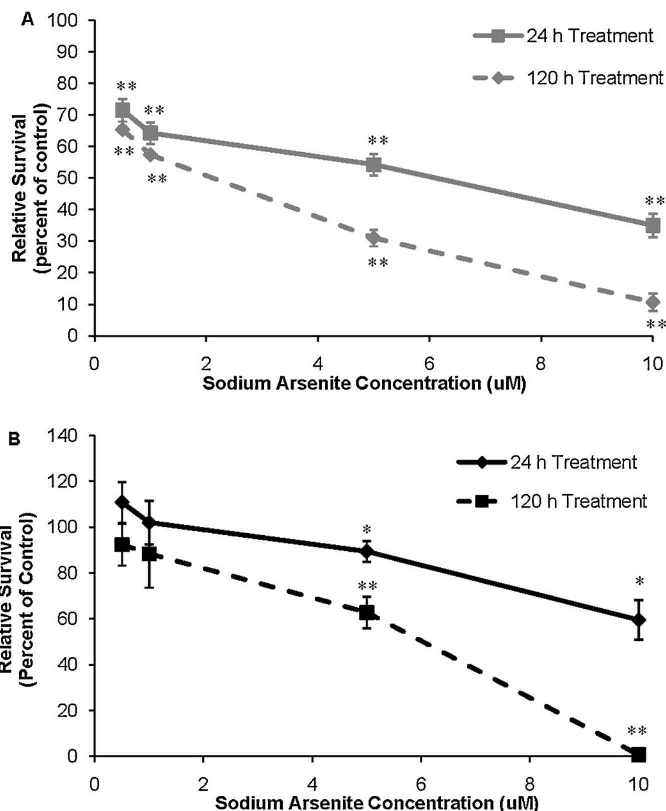

Fig. 1. Cytotoxicity of sodium arsenite in primary human lung cells. This figure shows that sodium arsenite induces a time- and concentration-dependent decrease in relative cell survival in human lung cells. Cells were treated with sodium arsenite for 24 h or 120 h. (A) Sodium arsenite decreases relative cell survival in human lung fibroblasts. (B) Sodium arsenite decreases relative cell survival in human lung epithelial cells. Data represent four independent experiments ± standard error of mean. *Statistically different from control ($p < 0.05$). **Statistically different from control ($p < 0.005$).

2.7. Immunofluorescence for gamma-H2A.X foci formation

We also used gamma-H2AX foci to detect the presence of arsenic-induced DNA double strand breaks based on our published methods [25]. Briefly, cells were grown on 8 well chamber slides. After treatment, the cells were fixed in 4% paraformaldehyde for 10 min, permeablilized with 0.2% Triton X-100 for 5 min and blocked with goat serum (Jackson Immunolaboratories) for 1 h. Cells were then incubated with anti-gamma-H2A.X antibody (Cell Signaling) at 4 °C overnight and followed by incubating with AlexaFluor 488-conjugated IgG secondary antibody for 1 h. Nuclei were counterstained with DAPI. The slides were mounted and viewed with an Olympus laser scanning confocal microscope (LSCM) using a 100× objective. Images of the same experiment were obtained using the same LCSM parameters (brightness, contrast, pinhall, etc.) and analyzed with Image J software. 100 nuclei per each treatment concentration were analyzed. Cells with more than 5 foci were considered double strand break positive.

2.8. Statistics

Values were expressed as the mean ± SEM (standard error of the mean) of triplicate experiments. Student's t-test was used to calculate p-values to determine the statistical significance of difference in means for each pair of concentrations. A 95% confidence interval for the difference in means of each pair of concentrations was constructed based on Student's t distribution.

3. Results

3.1. Cytotoxicity of arsenic

Arsenic induces a concentration-dependent decrease in relative survival in both human lung fibroblasts and epithelial cells. Specifically, 24 h exposure to concentration of 0.5, 1, 5 and 10 μM sodium arsenite reduced relative survival to 72, 64, 54 and 35% in lung

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