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The clastogenic effects of chronic exposure to particulate and soluble Cr(VI) in human lung cells

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Abstract

Hexavalent chromium (Cr(VI)) is a well-designated human lung carcinogen, with solubility playing an important role in its carcinogenic potential. Although it is known that particulate or water-insoluble Cr(VI) compounds are more potent than the soluble species of this metal, the mechanisms of action are not fully elucidated. In this study, we investigated the hypothesis that the difference in potency between particulate and soluble Cr(VI) is due to more chronic exposures with particulate chromate because it can deposit and persist in the lungs while soluble chromate is rapidly cleared. Chronic exposure to both insoluble lead chromate and soluble sodium chromate induced a concentration and time-dependent increase in intracellular Cr ion concentrations in cultured human lung fibroblasts. Intracellular Pb levels after chronic exposure to lead chromate increased in a concentration-dependent manner but did not increase with longer exposure times up to 72 h. We also investigated the effects of chronic exposure to Cr(VI) on clastogenicity and found that chronic exposure to lead chromate induces persistent or increasing chromosome damage. Specifically, exposure to 0.5 μg/cm^2 lead chromate for 24, 48 and 72 h induced 23, 23 and 27% damaged metaphases, respectively. Contrary to lead chromate, the amount of chromosome damage after chronic exposure to sodium chromate decreased with time. For example, cells exposed to 1 μM sodium chromate for 24, 48 and 72 h induced 23, 13 and 17% damaged metaphases, respectively. Our data suggest a possible mechanism for the observed potency difference between soluble and insoluble Cr(VI) compounds is that chronic exposure to particulate Cr(VI) induces persistent chromosome damage and chromosome instability while chromosome damage is repaired with chronic exposure to soluble Cr(VI).

Keywords: Chromium; Chromate; Lead chromate; Sodium chromate; Chromosome aberrations; Cell culture

1. Introduction

Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen. Solubility plays a key role in the carcinogenic potential of Cr(VI). Epidemiological, animal and *in vitro* studies show that water-insoluble

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or 'particulate' Cr(VI) compounds are more potent carcinogens than the soluble species of this metal [1–4]; however, the mechanism for this difference is unknown.

Lead chromate is commonly used as the prototypical particulate Cr(VI) compound, because it is the most insoluble. Previous reports have shown that lead chromate particles dissolve in the extracellular fluid, releasing the lead (Pb) cation and the chromate anion which then enter the cell [7–9]. The internalized Cr ions induce chromosome aberrations, DNA adducts and DNA double strand breaks [10–15], but there appears to be little effect of the Pb cation. The internalized Pb ions are non-genotoxic and do not interfere with Cr-induced cytotoxicity or inhibition of growth [8,16–18]. Thus, it appears that the differences in genotoxic potency between particulate and soluble Cr(VI) compounds may not be related to the role of their cations.

A reasonable explanation for the difference in potency among Cr(VI) compounds may be due to differences in exposure. The clearance of particulate chromate is very slow compared to soluble species, therefore cells are exposed to lead chromate longer. This hypothesis is further supported by previous observations that particulate chromate accumulates at the bronchial bifurcation sites where they are deposited, persist for long periods of time and induces tumors [5,6]. Almost all of the *in vitro* genotoxicity studies conducted on chromium so far considered treatments of 24 h or shorter time points [8,10–16] and only 1 or 2 involving a 48 h exposure. Furthermore, there are no *in vitro* genotoxicity study investigating Cr(VI) treatments longer than 48 h. Thus, the purpose of this study was to investigate the effects of more chronic exposures to particulate and soluble Cr(VI) compounds when human lung cells were treated for more than 24 h.

2. Materials and methods

2.1. Chemicals and reagents

Lead chromate, sodium chromate, colcemid and potassium chloride were purchased from Sigma/Aldrich (St. Louis, MO) and giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA). Sodium dodecyl sulfate (SDS) was purchased from American Bioanalytical (Natick, MA). Trypsin/EDTA, sodium pyruvate, penicillin/streptomycin, L-glutamine and Gurr's buffer were purchased from Invitrogen Corporation (Grand Island, NY). Methanol, acetone and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ). Dulbecco's minimum essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture was purchased from Mediatech Inc. (Herndon, VA). Cosmic calf serum (CCS) was purchased from Hyclone (Logan, UT). Tissue culture dishes, flasks and plasticware were purchased from Corning Inc. (Acton, MA).

2.2. Cells and cell culture

WTHBF-6 cells were used in this study. They are a clonal cell line generated from normal human bronchial fibroblasts transfected with hTERT. These cells have similar clastogenic and cytotoxic responses to metals compared to their parent cells [20]. The cells were maintained as subconfluent monolayers in DMEM/F-12 supplemented with 15% CCS, 2 mM L-glutamine, 100 U/ml penicillin/100 μg/ml streptomycin and 0.1 mM sodium pyruvate and incubated in a 5% CO_2 humidified environment at 37 °C. They were fed three times a week and subcultured at least once a week using 0.25% trypsin/1 mM EDTA solution. All experiments were performed on logarithmically growing cells.

2.3. Preparation of chemicals

Lead chromate, a model particulate Cr(VI) compound, was administered as a suspension in acetone and sodium chromate, a model soluble Cr(VI) compound, was administered as a solution in water, as previously described [11]. Sodium chromate treatment was refreshed every 24 h to ensure continuous exposure to Cr(VI) and not Cr(III).

2.4. Clastogenicity

Clastogenicity was determined by measuring the amount of chromosome damage in treatment groups and controls as previously described [11]. One hundred metaphases per data point were analyzed in each experiment. Each experiment was repeated at least three times.

2.5. Determination of intracellular lead and chromium ion levels

The intracellular concentrations of the metals were determined as previously described [16,17]. Briefly, a monolayer of cells was treated with 0.1, 0.5 and 1 μg/cm^2 lead chromate for 0, 24, 48 and 72 h or 0.5, 1 and 2.5 μM sodium chromate for 24, 48 or 72 h. Cells were then harvested and placed in a hypotonic solution followed by 2% SDS to degrade the cell membrane. This solution was then sheered through a needle seven times and filtered in order to remove remaining undissolved lead chromate particles. Cr and/or Pb ion concentrations of the samples were then measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) as described previously [17]. Zero hour harvests were performed with lead chromate to account for the possibility that some undissolved lead chromate particles passed through the filter. The final concentrations were corrected for this possible confounding factor by subtracting 0 h values from the 24, 48 and 72 h values as previously described [16].