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Particulate and soluble hexavalent chromium are cytotoxic and genotoxic to human lung epithelial cells

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Abstract

Particulate hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen. It is currently a major public health concern, there is widespread exposure to it in occupational settings and to the general public. However, despite the potential widespread exposure and the fact that the lung is its target organ, few studies have considered the toxic effects of particulate Cr(VI) in human lung cells. Accordingly, we used lead chromate as a model particulate Cr(VI) compound and determined its cytotoxicity and genotoxicity in cultured human bronchial epithelial cells, using BEP2D cells as a model cell line. We found that lead chromate induced concentration-dependent cytotoxicity in BEP2D cells after a 24 h exposure. Specifically, the relative survival was 78, 59, 53, 46 and 0% after exposure to 0.5, 1, 5, 10 and 50 μg/cm^2 lead chromate, respectively. Similarly, the amount of chromosome damage increased with concentration after 24 h exposure to lead chromate. Specifically, 0.5, 1, 5 and 10 μg/cm^2 damaged 10, 13, 20 and 28% of metaphase cells with the total amount of damage reaching 11, 15, 24 and 36 aberrations per 100 metaphases, respectively. Lead chromate (50 μg/cm^2 lead chromate) induced profound cell cycle delay and no metaphases were found. In addition we investigated the effects of soluble hexavalent chromium, sodium chromate, in this cell line. We found that 1, 2.5, 5 and 10 μM sodium chromate induced 66, 35, 0 and 0% relative survival, respectively. The amount of chromosome damage increased with concentration after 24 h exposure to sodium chromate. Specifically, 1, 2.5 and 5 μM damaged 25, 34 and 41% of metaphase cells with the total amount of damage reaching 33, 59 and 70 aberrations per 100 metaphases, respectively. Ten micromolar sodium chromate induced profound cell cycle delay and no metaphases were found. Overall the data clearly indicate that hexavalent Cr(VI) is cytotoxic and genotoxic to human lung epithelial cells.

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

1. Introduction

The first association of hexavalent chromium (Cr(VI)) with respiratory cancer was in 1890 when a case report described a chrome pigment worker with an adenocarcinoma in his nasal turbinate bone [1]. Subsequently, numerous epidemiological studies showed that exposure to Cr(VI) causes an 18–80-fold increased risk of lung cancer [2–4]. These data are supported by toxicological studies showing that Cr(VI) induces tumors and neoplastic transformation [2–5]. Consequently, Cr(VI) is an established human lung carcinogen.

However, there are important distinctions between Cr(VI) compounds. For example it is now clear that the particulate salts are the carcinogenic form of Cr(VI) [2–5]. Only the particulate compounds induce tumors

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in experimental animals [2–4] and neoplastic transformation of C3H10T1/2 mouse embryo cells [5]. Studies in human lung fibroblasts indicate particulate Cr(VI) is genotoxic inducing chromosome aberrations, DNA strand breaks, and Cr-DNA adducts [6,7]. Further data indicate that these genotoxic effects are mediated by the extracellular dissolution of particulate Cr(VI) releasing soluble chromate anions that enter the cell causing DNA damage, growth arrest and cytotoxicity [8–15]. But these data are all reflective of effects and mechanisms in human lung fibroblasts. Much less is known about the effects of particulate Cr(VI) on human lung epithelial cells. This data gap is significant, while effects on fibroblasts provide important mechanistic insight and damage to fibroblasts contribute to carcinogenesis, the epithelial cells are the ones that transform and become tumors. Thus it is essential that data also be obtained in human lung epithelial cells.

In fact, only two studies have considered the genotoxicity of Cr(VI) in human lung epithelial cells [16,17] and of these, only one considered particulate Cr(VI) [16]. Specifically, the one particulate study found that two concentrations (0.4 and 0.8 $\mu g/cm^2$) of particulate Cr(VI) induced DNA adducts in primary human small airway epithelial cells. The one soluble Cr(VI) study reported DNA–protein crosslinks in primary human bronchial epithelial cells [17]. There are no studies of the effects of the clastogenic effects of either particulate or soluble Cr(VI) in human lung epithelial cells. Accordingly, the purpose of this study was to improve our current understanding of Cr(VI) by studying the cytotoxicity and genotoxicity of its particulate and soluble forms in cultured human bronchial epithelial cells.

2. Materials and methods

2.1. Chemicals and reagents

Lead chromate, sodium chromate, colcemid and potassium chloride (KCl) were purchased from Sigma Chemical (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). LHC8, HBS, EPET, SBTI and DNase were purchased from Biosource (Camarillo, CA). Gurr's buffer was 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

A bronchial epithelial cell line, BEP2D, was used in all experiments. These cells were immortalized using the human papilloma viruses E6 and E7 [18]. Cells were routinely cultured in LHC8 medium. Cells were maintained as adherent subconfluent monolayers by feeding at least twice a week and subculturing at least once a week using EPET and SBTI. Cells were tested routinely for mycoplasma contamination. All experiments were conducted on logarithmically growing cells.

2.3. Preparation of Cr(VI) compounds

Sodium chromate (CAS #7775-11-3, ACS reagent minimum 98% purity) was used as a model soluble Cr(VI) salt. Solutions of sodium chromate were prepared by weighing out the desired amount of sodium chromate, and dissolving it in double distilled water. Dilutions were made for appropriate treatment concentrations, and then filter sterilized through a 10 ml syringe with a 0.2 μM filter.

Lead chromate (CAS #7758-97-6, ACS reagent minimum 98% purity) was used as a model particulate Cr(VI) salt. Suspensions of lead chromate particles were prepared by rinsing twice in double-distilled water to remove any water-soluble contaminants and then twice in acetone to remove any organic contaminants. Air-dried particles were weighed, placed in acetone (for sterilization) in a borosilicate scintillation vial and stirred overnight with a magnetic stir bar as described previously [19]. Previous studies have shown that this preparation method reduces the particles to a size range of 0.2–2.3 μM with a mean size of 0.7 μM [19,20]. Further, it has been shown that Cr(VI) particles cannot be separated into more homogeneous sizes as their physical chemistry causes them to aggregate [19,20]. The particles were kept in suspension using a vortex mixer and diluted into appropriate suspension for specific treatments. Dilutions were also maintained as a suspension using a vortex mixer and treatments were dispensed into cultures directly from these suspensions.

2.4. Cytotoxicity assays

Cytotoxicity was determined by a clonogenic assay, which measures a reduction in plating efficiency in treatment groups relative to the controls. One million cells were seeded in 5 ml of medium on a 60-mm tissue-culture dish, and allowed to grow for 24 h. The cultures were then treated for 24 h with suspensions of either lead chromate or sodium chromate. After 24 h the treatment medium was collected (to include any loosely adherent mitotic cells); the cells were rinsed twice with Hepes buffered saline (HBS); and then removed from the dish with EPET. The trypsinized cells were added to the collected medium and centrifuged at 1000 rpm for 5 min. Three hundred microlitres of soybean trypsin inhibitor was added to stop the trypsin. The resulting pellet was resuspended in 10 ml of medium; counted with a Coulter Multisizer III; and reseeded at colony forming density (500 cells per dish). The colonies were allowed to grow for 14 days; fixed with 100% methanol; stained with crystal violet; and the colonies counted. There were four dishes per treatment group and each experiment was repeated at least three times.