Particulate Hexavalent Chromium Causes DNA Double Strand Breaks and RAD51 Inhibition, Leading to Increased Chromosome Instability in Human Bronchial Epithelial Cells

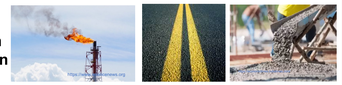
Idoia Meaza, Jennifer H. Toyoda, Haiyan Lu, Aggie R. Williams, J. Calvin Kouokam and John Pierce Wise Sr.

Wise Laboratory of Environmental and Genetic Toxicology, School of Medicine, University of Louisville.

Background

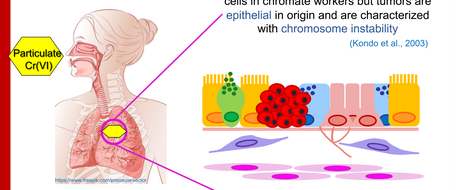
A

Cr(VI) is a major human health concern



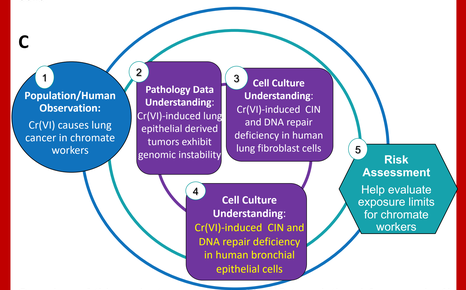
Hexavalent chromium [Cr(VI)] is a major human health concern. It is a widespread environmental and occupational contaminant and a known human carcinogen. Anthropogenic sources of Cr(VI) consist of burning fossil fuels, chrome plating, electroplating, cement work, leather tanneries, dyes and pigments.

B



Cr(VI is classified by IARC as Group 1 "known cause of cancer in humans". Cr(VI) particles settle at bifurcation sites resulting in tumor formation. Biopsies of tumors from chromate workers have shown that Cr accumulates on lung fibroblasts whereas tumors originate from epithelial cells.

C



Overview of this project according to NIEHS translational framework: **1)** Population and human observations showed that Cr(VI) causes lung cancer in chromate workers. **2)** Pathology observations have shown Cr-induced lung epithelial derived tumors exhibit genomic instability **3)** Cell culture studies further showed that Cr(VI)-induced CIN and DNA repair deficiency in human lung fibroblasts **4) Does Cr(VI) induce CIN and DNA repair deficiency in human lung epithelial cells? 5)** The outcomes of this project could help contribute to risk assessment and ultimately policy changes and help evaluate exposure limits for chromate workers.

Further Reading

- Ishikawa, Y.; Nakagawa, K.; Satoh, Y.; Kitagawa, T.; Sugano, H.; Hirano, T.; Tsuchiya, E. "Hot Spots" of Chromium Accumulation at Bifurcations of Chromate Workers' Bronchi. *Cancer Res.* 1994, *54* (9), 2342–2346
- Qin, Qin, Hong Xie, Sandra S. Wise, Cynthia L. Browning, Kelsey N. Thompson, Amie L. Holmes, and John Pierce Wise. "Homologous Recombination Repair Signaling in Chemical Carcinogenesis: Prolonged Particulate Hexavalent Chromium Exposure Suppresses the Rad51 Response in Human Lung Cells." *Toxicological Sciences* 142, no. 1 (November 1, 2014): 117–25.
- Kondo, K., Takahashi, Y., Ishikawa, S., Uchihara, H., Hirose, Y., Yoshizawa, K., Tsuyuguchi, M., Takizawa, H., Miyoshi, T., Sakiyama, S., & Monden, Y. (2003). Microscopic analysis of chromium accumulation in the bronchi and lung of chromate workers. *Cancer*, *98*(11), 2420–2429.

Project Overview

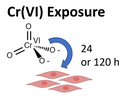
Research Question

Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen with environmental and occupational exposure risks. Tumors from chromate workers show Cr(VI) targets chromosomes by inducing chromosomal instability (CIN), which has been supported by animal and cell culture studies. CIN is the proposed driver of carcinogenesis and is a hallmark of lung cancer. Homologous recombination repair is the major DNA repair pathway that prevents development of CIN by repairing DNA double strand breaks with high fidelity. Previous work from our lab showed RAD51, a key protein in homologous recombination repair pathway, is inhibited after prolonged exposure to Cr(VI) in human lung fibroblasts and accumulates in the cytoplasm, which prevents it from being functional. However, Cr(VI)-induced lung tumors are epithelial in origin, and it is unknown if Cr(VI) targets RAD51 in lung epithelial cells.

What are the effects of particulate Cr(VI), the most potent form of Cr(VI), on RAD51 and chromosome instability in human lung epithelial cells?

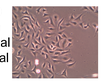
Overall Study Design

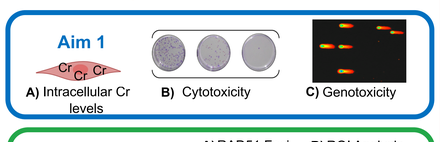
Cr(VI) Exposure



Representative chromate compound: Zinc chromate

Cell Model: BEP2D Human bronchial epithelial cells





This study investigated our research question through 3 aims:

1) Characterize the cytotoxic and genotoxic effects of particulate Cr(VI) in human lung epithelial cells
2) Investigate the RAD51 response after particulate Cr(VI) exposure to human lung epithelial cells
3) Study chromosome instability after particulate Cr(VI) exposure in human lung epithelial cells.

Take Home Message

Prolonged Cr(VI) exposure inhibits RAD51 foci formation and increases inappropriate cytoplasmic accumulation indicating break repair is compromised. Consequently, prolonged Cr(VI) exposure increases CIN.

These data match epidemiological findings showing in tumors from chromate workers Cr accumulates in human lung fibroblast and epithelial cells are characterized with CIN.

Next Steps

Investigate the mechanism by which Cr(VI) inhibits RAD51 after prolonged exposures in lung epithelial cells.

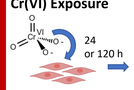
Aim 1: Cytotoxic and Genotoxic Effects of Particulate Cr(VI) in Human Lung Epithelial Cells

Why we did it

Cr(VI) causes DNA double strand breaks, which if left unrepaired can progress to CIN. Cr(VI) induces repair deficiency and CIN in human fibroblast cells, but whether Cr(VI) causes these effects in epithelial cells is unknown. In this aim we characterized the toxicological effects of Cr(VI) exposure in human bronchial epithelial cells by studying cell survival and DNA double strand breaks. Intracellular levels of Cr were also measured to account for differential uptake of Cr.

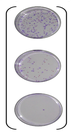
How we did it

Cr(VI) Exposure

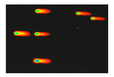


A) Intracellular Cr levels

B) Cytotoxicity

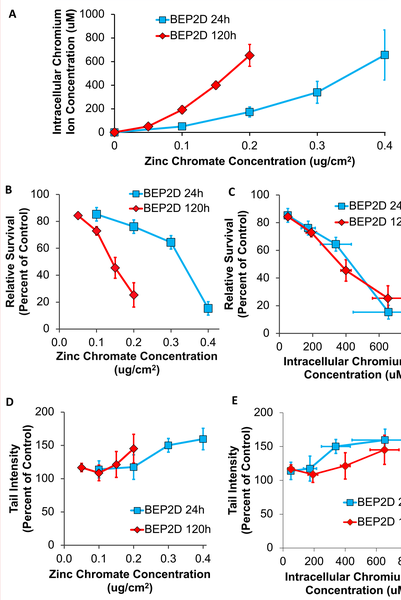


C) Genotoxicity



BEP2D cells were treated with zinc chromate for varying time periods (24 or 120 h) and concentrations (0.1 to 0.4 ug/cm^2). We measured cytotoxicity with a clonogenic assay, genotoxicity with a neutral comet assay and intracellular Cr levels with atomic absorption spectroscopy.

What we found



Particulate Cr(VI) exposure is cytotoxic and genotoxic to human bronchial epithelial cells. Data represent the mean of at least three independent experiments ± standard error of the mean. **A)** Intracellular Cr ion levels based on administered dose **B)** Relative survival based on administered dose. **C)** Relative survival based on intracellular chromium ion levels. **D)** Tail intensity based on administered dose. **E)** Tail intensity based on intracellular chromium ion levels.

What does it mean

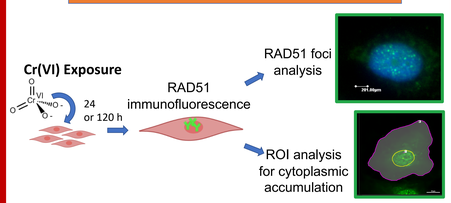
Assessing cytotoxicity and Cr uptake allowed us to choose concentrations of Cr(VI) that are environmentally and occupationally relevant, without being highly lethal to the cells. Additionally, we confirmed Cr(VI) is genotoxic to lung epithelial cells.

Aim 2: RAD51 Response After Particulate Cr(VI) Exposure to Human Lung Epithelial Cells

Why we did it

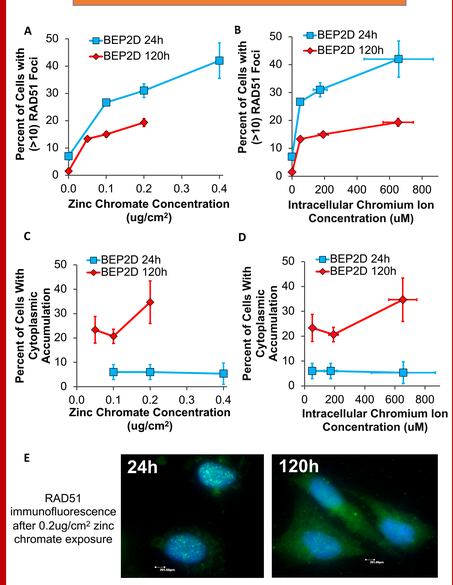
Once appropriate concentrations of zinc chromate, our representative particulate Cr(VI), were selected, we investigated the status of RAD51 after Cr(VI) exposure in epithelial cells. RAD51 inhibition occurs after prolonged exposure to Cr(VI) in fibroblast cells and therefore we tested whether this outcome occurs in epithelial cells.

How we did it



Immunofluorescence of RAD51 was performed in BEP2D after treatment with zinc chromate for either 24 or 120 h. Foci were measured by fluorescence microscopy and cytoplasmic accumulation was measured by confocal imaging and region of interest (ROI) analyses.

What we found



E

RAD51 immunofluorescence after 0.2ug/cm^2 zinc chromate exposure

Prolonged exposure to particulate Cr(VI) inhibits RAD51 foci and increases inappropriate accumulation in the cytoplasm. Data represent the mean of three independent experiments ± standard error of the mean. **A)** RAD51 foci based on administered dose. **B)** RAD51 foci based on intracellular chromium ion levels. **C)** Cytoplasmic accumulation based on administered dose. **D)** Cytoplasmic accumulation based on intracellular chromium ion levels. **E)** Representative images of RAD51 immunofluorescence after 24h or 120h exposure.

What does it mean

Prolonged Cr(VI) exposure inhibits RAD51 foci formation and increases inappropriate cytoplasmic accumulation indicating break repair is compromised. Outcomes translate from fibroblast cells to epithelial cells.

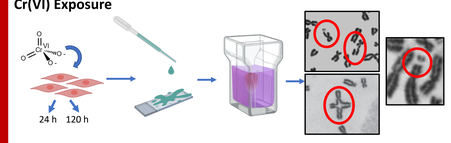
Aim 3: Chromosome Instability After Cr(VI) Exposure in Human Lung Epithelial Cells

Why we did it

Unrepaired DNA double strand breaks can result in the development of CIN. We confirmed in Aim 1 that Cr(VI) causes DNA double strand breaks in human bronchial epithelial cells and DNA repair is deficient at prolonged exposures (Aim 2). Therefore, in this aim we sought to investigate the effects of Cr(VI) on CIN.

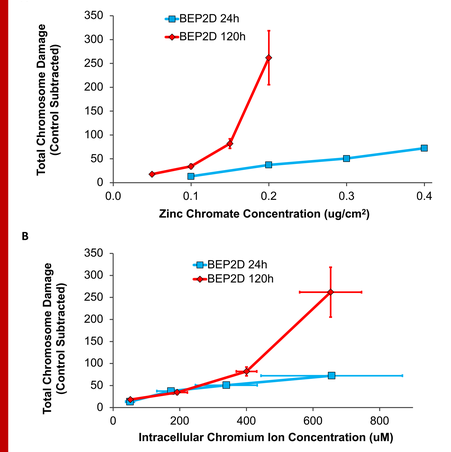
How we did it

Cr(VI) Exposure



A chromosome aberration assay was performed in BEP2D cells after treatment with zinc chromate for either 24 or 120 h. Total chromosome damage and the percent of metaphases with chromosome damage were quantified.

What we found



Particulate Cr(VI) induced chromosome instability in epithelial cells. Data represent the mean of three independent experiments ± standard error of the mean. **A)** Total chromosome damage based on administered dose. **B)** Total Chromosome damage based on intracellular chromium ion levels.

What does it mean

CIN translates from fibroblast cells to epithelial cells. Prolonged particulate Cr(VI) exposure increases CIN in epithelial cells. In comparison, epithelial cells contain more chromosome damage than fibroblast cells.

Acknowledgements

Research reported in this publication was supported by the National Institute of Environmental Health Sciences [R01ES016893, P30ES030283 and R35 ES032876 to JPW], [T32ES011564 to JPW, JHT], the Jewish Heritage Foundation for Excellence [JPW]. The content is solely the responsibility of the presenters and does not necessarily represent the official views of the National Institute of Environmental Health Sciences or National Institute of Health.