Effects of Beetroot-Derived E162 on Cr(VI)-Induced Cytotoxicity

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Background

- Hexavalent Chromium (Cr(VI)) is a known human lung carcinogen.
- As a major occupational hazard, over 1.3 million workers in the US and Europe are exposed to Cr(VI).
- Cr(VI) has many uses: metal plating, anti-corrosives, and paint pigments. Associated industries release chromium into the environment, making chromium a major environmental pollutant.
- Cr(VI) is most dangerous to humans in its particulate form.
- The exact mode of action for chromium-induced carcinogenesis is not known. However, Cr(VI) is reduced in cells leading to reactive oxygen species (ROS) production, chromosome damage, and inflammation.

https://www.niehs.nih.gov/health/topics/agents/hex-chromium/index.cfm

- E162 is a food dye derived from beetroot with no known toxicity in humans.
- E162 has anti-inflammatory and anti-oxidative effects.
- A study showed mice given low doses of E162 have lower rates of cancer.

https://www.livingplate.org/wp-content/uploads/2018/03/beets.jpg

https://i.ebayimg.com/images/g/~-wAAOSw6JVddv4p/s-l300.jpg

Further Reading

1. Wise SS, Holmes AL, Liou L, Adam RM, Wise JP Sr. Hexavalent chromium induces chromosome instability in human urothelial cells. Toxicol Appl Pharmacol. 2016 Apr 1;296:54-60. doi: 10.1016/j.taap.2016.02.015. Epub 2016 Feb 18. PMID: 26908176; PMCID: PMC4886549.
2. Lechner, John F, and Gary D Stoner. "Red Beetroot and Betalains as Cancer Chemopreventative Agents." *Molecules*, vol. 24, no. 8, ser. 1602, Apr. 2019. *1602.*
3. Proctor, Deborah M, et al. "Assessment of the Mode of Action for Hexavalent Chromium-Induced Lung Cancer Following Inhalation Exposures." *Toxicology*, ser. 325, 2014, pp. 161–176. *325.*

Cell Viability

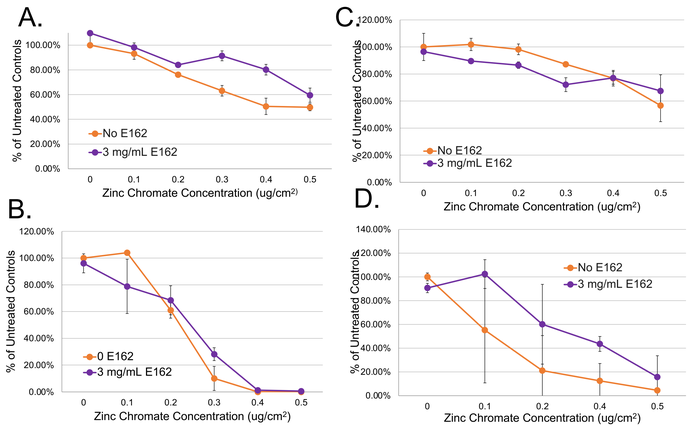

Figure 1. BEP-2D and WTHBF-6 Cell Viability as Determined by CCK8 Assay. A and B. BEP-2D cells were administered Cr(VI) and with or without E162 for 24 and 120 hours, respectively. C and D. WTHBF-6 cells were treated with Cr(VI) and with or without E162 for 24 and 120 hours, respectively. Cr(VI) decreased cell viability which was rescued to some degree by E162. Data are mean±SD.

Cytokine Production

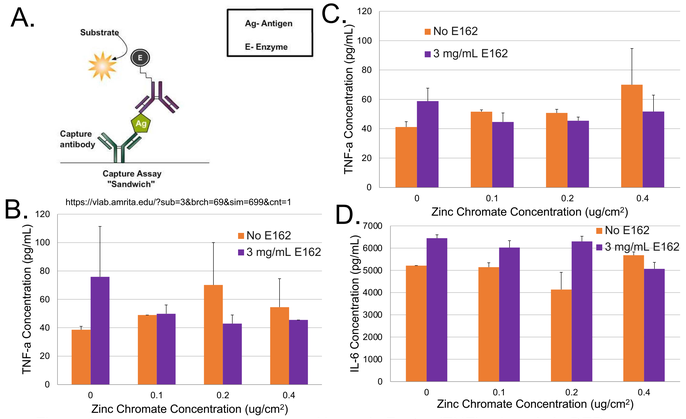

Figure 3. Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-a Levels in BEP-2D and WTHBF-6 Cell Culture Supernatants as Determined by ELISA. A. Brief principle of sandwich ELISA. B. TNF-a levels in BEP-2D cells administered Cr(VI) with or without E162 for 24 hours. C and D. TNF-a and IL-6 levels, respectively, in WTHBF-6 cells treated as in B. E162 reduced Cr(VI)-induced release of TNF-a. An opposite trend was found with IL-6. Data are mean±SD.

Clone Formation Ability

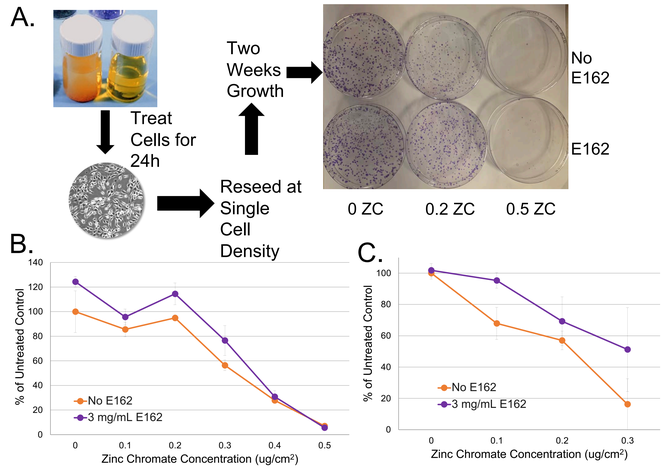

Figure 2. Clone Formation Abilities of BEP-2D and WTHBF-6 cells as Determined by Clonogenic Assay. A. Overview of experimental setup and representative image showing BEP-2D results. B and C. Quantitation of clones formed by BEP-2D and WTHBF-6 after treatment for 24 hours.

Inflammation-Related Proteins

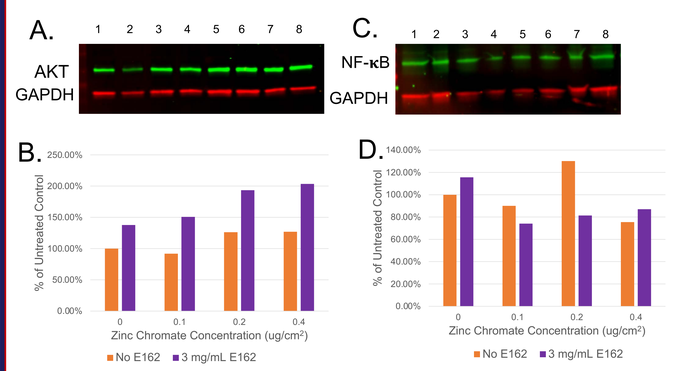

Figure 4. AKT and NF-κB Protein Levels in Cell Lysates as Determined by Western Blot. A. Image Showing AKT (60 kDa, green) and GAPDH (36 kDa, red) Protein Bands. B. Quantitation of A. C. Image Showing NF-κB (64 kDa, green) and GAPDH (36 kDa, red) Protein Bands. D. Quantitation of C. The relative expression of the target proteins was obtained based on GAPDH as a loading control.

Overview

Research Question

Does E162 reduce Cr(VI)-induced cytotoxicity? Is this achieved by E162's anti-inflammatory properties?

How did we do it?

- 2 Cell Lines Used:
  - Human Lung Epithelial Cells (BEP-2D cells)
  - Human Lung Fibroblasts (WTHBF-6 cells)
- Source of Cr(VI) Used: Zinc Chromate
- Experiments Performed:
  - Clonogenic Assay
  - Cell Viability Assay using Cell Counting Kit8
  - Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of the pro-inflammatory cytokines IL-6 and TNF-a
  - Western Blot for detection of NF-κB (a transcription factor involved in inflammation, cell proliferation, and cancer) and AKT (a protein kinase involved in inflammation and cancer among other cellular processes)

Take Home Message

Our preliminary data show that E162 can alleviate the toxic effects of Cr(VI) in human lung epithelial cells and fibroblasts. Specifically, E162 reduces the amount of TNF-a and increases cell viability and cell survival. This suggests a potential chemoprotective effect for E162 in Cr(VI)-induced cancer.

What's Next?

Future studies will consider the effects of E162 on the inflammatory response and the reduction of reactive oxygen species (ROS). We will more thoroughly quantify the pro-inflammatory cytokines and their pathways involved. We will also consider these effects in macrophages and delineate their role in the Cr(VI)-induced inflammation and cancer.

Acknowledgements

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