Research Brief 148: Dual Role for Vitamin C in Cr(VI) Toxicity

Background:

Hexavalent chromium [Cr(VI)] is listed by the EPA as a known human carcinogen, yet under normal physiological conditions it is unreactive toward DNA and other important biological macromolecules. Cr(VI) must be reduced to trivalent chromium [Cr(III)] to induce biological damage. The molecular mechanisms underlying the cytotoxic, mutagenic and carcinogenic effects of Cr(VI) involve complicated intracellular chemistry, multiple intracellular targets and multiple pathways.

Reduction of Cr(VI) to Cr(III) can occur via non-enzymatic reactions with cysteine and glutathione, but in the target tissues of chromate toxicity, ascorbate (reduced vitamin C; Asc), a two-electron donor, is the primary reducer of Cr(VI). Interestingly, while human lung tissue has ~1.3 mM Asc, human cells in culture contain no or barely detectable amounts Asc – simply because the majority of synthetic media formulations do not include vitamin C. As a result, mechanisms of Cr(VI) genotoxicity in cultured cells have not been studied under physiologically relevant conditions.

Advances:

Dr. Anatoly Zhitkovich of the Brown University SBRP is conducting basic research to identify biomarkers of genetic damage following exposure to Cr(VI). As part of this work, his team developed a method to load human and other cultured cells with Asc. They pre-treat cells with dehydroascorbic acid, the oxidized form of vitamin C, which readily enters cells and is then reduced to Asc, rapidly restoring physiological concentrations of Asc.

Dr. Zhitkovich's research group used this test system to examine intracellular responses of human lung cells following exposure to Cr(VI). They found that restoration of physiological Asc levels in these cells strongly increased chromosomal damage following even low-dose Cr(VI) exposure in an Asc-concentration-dependent manner.

They investigated the effect of cellular Asc on the genotoxic potential of Cr(VI) by comparing the numbers of biomarkers of DNA double strand breaks (DSB) in cells preloaded with a range of Asc concentrations. They found that preloading of primary human bronchial epithelial cells with Asc greatly enhanced the ability of low doses of Cr(VI) to cause DSB, increasing the yield of biomarker-containing cells by 8-20-fold. Assessment of the impact of cellular Asc on repair of chromosome breaks revealed a more than 10-fold increase in the levels of unrepaird DSBs in Asc loaded cultures.

The researchers conducted studies to see if increased genetic damage due to cellular Asc results from the changes in the types of DNA lesions and/or altered cellular responses to the same Cr-DNA damage. They added Asc to Cr-exposed test cultures after sufficient time for the completion of Cr(VI) reduction in Asc-free cells. No effect on the formation of DSB or the amount of unrepaird chromosomal damage was detected when Asc was introduced following exposure and reduction of Cr(VI). This indicates that Asc must be present in cells during Cr(VI) reduction – Asc does not change biological responses to Cr-DNA damage that is already formed. Therefore, the targets of Asc are Cr(VI) metabolism and resulting DNA damage rather than cellular damage response mechanisms.

Dr. Zhitkovich's group also investigated whether the increase in chromosome damage by Asc was a direct result of the formation of highly toxic DNA lesions or if it was caused by cellular responses to Asc-mediated Cr-DNA damage. They focused on mismatch repair (MMR) proteins, which ensure genomic stability by correcting mutagenic mismatches arising during DNA replication. The researchers determined that suppression of MMR essentially eliminated the formation of DSB. Thus, unrepaired chromosomal breaks are apparently caused by MMR proteins mis-processing Asc promoted Cr-DNA damage.

They also examined apoptotic responses following Cr exposure using three biochemical markers of apoptosis. They found that the presence of Asc during the reduction of Cr(VI) enhances Cr-induced apoptosis. Induction of apoptotic responses to many types of DNA damage is usually mediated by increased expression of p53 dependent genes. Dr. Zhitkovich's group examined the role of p53 in Cr(VI)-associated cell death and found that depletion of p53 had no significant effect on the levels of Cr(VI)-induced cytotoxicity, both in the presence and absence of intracellular Asc. These findings indicate that cellular vitamin C should enhance the ability of Cr(VI) to select for mismatch repair-
deficient cells in a p53-independent manner and are in agreement with a low incidence of p53 mutations in chromate-associated human lung cancers. The majority of Cr-induced human lung carcinomas have inactive mismatch repair, which was proposed by Dr. Zhitkovich to result from selective outgrowth of MMR-deficient cells due to their resistance to killing by Cr(VI).

**Significance:**

Vitamin C has been used as an antidote in industrial accidents and other instances when large amounts of chromium are ingested. It works by rapidly reducing Cr(VI) to Cr(III), which enters cells much slower than Cr(VI), thus reducing chromium's intracellular toxic effects. In contrast, Dr. Zhitkovich's research demonstrates that cellular Asc acts as a potent amplifier of Cr(VI)'s genotoxic activities. Thus, Asc plays a dual role in Cr(VI) toxicity: protective outside the cell and potentiating inside the cell.

This work is important as it identifies the mechanism of Asc-induced increases in DNA breakage, but also because it discovered that increasing Asc concentrations generates progressively more mutations and DSB — revealing the genotoxic potential of otherwise nontoxic doses of Cr(VI). When combined with Asc, Cr(VI) caused genetic damage in cells at doses four times lower than current federal standards and these findings might have potential policy implications.

For More Information Contact:

Anatoly Zhitkovich, Ph.D.
Brown University
Department of Pathology and Laboratory Medicine
70 Ship Street, Box G-E507
Phone: 401-863-2912
Email: Anatoly_Zhitkovich@brown.edu

To learn more about this research, please refer to:
