Published in final edited form as:

Curr Opin Toxicol. 2019 April ; 14: 1–7. doi:10.1016/j.cotox.2019.05.003.

Mechanisms of Chromium-Induced Toxicity

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Abstract

Chromium is a pervasive environmental contaminant that is of great importance because of its toxicity. Hexavalent chromium is a classified group 1 carcinogen with multiple complex mechanisms by which it triggers cancer development. Increased levels of oxidative stress, chromosome breaks, and DNA-adduct formation are some of the major mechanisms by which Cr(VI) causes cellular damage. Trivalent chromium is another species of chromium that is described as a non-essential metal, and is used in nutritional supplementation. Evidence on nutritional benefit is conflicting which could suggest that humans absorb enough Cr(III) from diet alone, and that extra supplementation is not necessary. This review highlights the differences between Cr(VI) and Cr(III) from a chemical and toxicological perspective, describes shortcomings in nutritional research of Cr(III), and explains the multiple mechanisms by which Cr(VI) is involved in the process of carcinogenesis.

Keywords

Hexavalent chromium; trivalent chromium; oxidative stress; genotoxicity; carcinogenesis; epigenetics; supplementation

1. Introduction

The dispersion of metals in the environment are of major concern to global human health. Chromium (Cr) is a classified group 1 carcinogen by the International Agency for Research on Cancer (IARC) and is pervasive throughout the environment [1, 2]. This transition metal has 7 oxidation states (0–VI), with the metallic (Cr(0)), trivalent (Cr(III)), and hexavalent (Cr(VI)) states being the most common and thus most prevalent states found in the environment and in industrial settings [3]. Trivalent chromium has been found naturally in rocks and soil, and is readily taken up by plants. By these means, Cr(III) can enter the food chain and is part of the human diet [4]. Industrial processes such as metal refining, chrome-plating, stainless-steel production, leather tanning, and chemical dye production all use chromium [5]. These industrial processes are largely responsible for the release of Cr(VI)

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Declaration of Interest
None declared

into the air [6]. It is estimated that workers in these industries have a two-fold higher exposure levels than the entire general population [1].

The anthropogenic release of chromium into the environment caused population exposure to occur by inhalation of contaminated air, or ingestion of contaminated drinking water [7, 8]. Trivalent chromium is found in the soil, but environmental conditions like natural oxidation can convert Cr(III) to Cr(VI) in this medium if for example if there are high levels of manganese in the soil or the soil is very alkaline in pH [9, 10]. Due to the inherent toxicity and carcinogenicity of chromium containing substances, US EPA and US OSHA have determined exposure limits of 100 g/ L of total chromium for drinking water standards and 5 g/m³ of Cr(VI) timed weighted average for a normal work day [5]. While it is well documented that exposure to chromium in the environment is pervasive, and that hexavalent chromium is a potent carcinogen, there are multiple mechanisms by which chromium exposure induces cellular damage and adverse health effects. This review describes the chemistry of two predominant Cr oxidative states that humans are exposed to, their activity at the cellular level, and major theories by which Cr exposure induces cellular toxicity and DNA damage, through the activation of oxidative stress pathways, direct DNA damage, and epigenetic gene expression changes.

2. Essentiality of Trivalent Chromium.

Trivalent chromium (Cr(III))-containing compounds are components of many multivitamins, nutritional supplements, and even present in foods. Predominant forms of chromium (III) that are taken as supplementation include; chromium-picolinate, chromium-histidinate, chromium-dinicotinate, and niacin-bound chromium [11]. Chromium picolinate is the most prominent form of Cr(III) in nutritional supplementation because this form allows for optimal absorption. The Council for Responsible Nutrition (CRN) published data from 2017 revealing that 170 million adults in the United States take some form of nutritional supplementation, be that a multivitamin, specialty supplement, herbals or botanicals, sports nutrition supplements, or weight management supplements [12]. In 2016 chromium (III) supplements were ranked the fourth highest selling supplement in the USA, grossing 110 million dollars that year, only ranking behind calcium, magnesium, and iron [13]. Dietary intake alone of Cr(III) has been estimated to be within the range of 23-29 g/day and 39-54 g/day for women and men respectively [14]. These ranges fall within and also exceed the Adequate Intake (AI) values (25 g/day for women and 35 g/day for men) established by the Food and Nutrition Board of the National Academies of Sciences Engineering and Medicine [15]. With this level of intake from dietary chromium alone, it seems that individuals who also take additional chromium supplementation far exceed the AI for chromium. While dietary intake of chromium is described as nutritional supplementation, and some studies suggest that it is beneficial for diabetics and in the process of weight loss and muscle anabolism, the evidence is weak, conflicting, and some studies even suggest that excessive intake of Cr(III) formulations are carcinogenic [16, 17]

Unlike Cr(VI) uptake, ligand-bound Cr(III) is postulated to enter the cell via phagocytic mechanisms or through non-specific mechanisms of diffusion, making it difficult for independent researchers and government regulatory agencies to assess the direct impact of

Cr(III) on toxicity [18]. It is estimated that Cr(III) diffusion is about 1%, with most ingested chromium being excreted in the feces. Urinary excretion of Cr(III) is inversely related to Cr(III) intake, and the excretion rate increases under different physiological conditions such as stress, and during periods of exercise [19-21]. Urinary excretion of Cr(III) is also increased with increasing doses of Cr(III) [22].

Chromium (III) compounds are taken as supplementation because studies appear to demonstrate positive effects in regards to the potentiation of insulin action, and increased beneficial results from exercise including higher percentage of lean muscle mass and loss of fat [23, 24]. However, these findings are inconclusive and it is misleading to assert that supplementing Cr(III) into the diet can improve the lean-to fat body mass ratio in both animal models and adults [23, 25]. There also appears to be an optimal intake level for Cr(III) in which absorption peaks. (40 g/day). It is suggested that because of the dose-dependent increased excretion rates, if Cr(III) has any nutritional benefit, it is gained through normal diet, and supplementation has no nutritional benefit. Depending on the formulation, Cr(III) supplementation may even be toxic or carcinogenic, according to some studies on Cr-picolinate [26]. Because of the conflicting evidence regarding Cr(III) supplementation, it should be considered that Cr(III) is not an essential trace element for human health, and in addition, because increased supplementation seems excessive, perhaps normal dietary intake from healthy foods is all that is necessary for Cr(III). There are no concrete mechanisms that can define any essential role for Cr(III) in any form of life as we know exists today.

3. Uptake, Reduction, Genotoxic effects of Chromate

The Chemistry of chromium plays a major role in its cellular entry and toxic effects. Hexavalent chromium in the environment largely exists as the chromate oxyanion (CrO_4). Structurally, the chromate oxyanion is very similar to the sulfate oxyanion (SO_4) and thus utilizes general sulfate transporters on the cell surface to enter the cell [27]. Once inside the cell, Cr(VI) exerts its toxic effect, following reduction with ascorbate and biological thiols such as glutathione (GSH) or cysteine amino acid residues [3, 28, 29]. Step-wise two electron reductions with ascorbate generates Cr(IV) intermediates and this reaction usually occurs *in vivo*, but in tissue culture systems which have very low levels of ascorbate, Cr(VI) is reduced by GSH to reactive (Cr(V)) using one electron reduction and with either reductant Cr(VI) is ultimately converted to Cr(III) [30]. This process, especially with GSH reduction, can generate hydrogen peroxide and other free radical species which produce high levels of oxidative stress, causing damage to cellular lipids, proteins, and DNA [27, 31]. Furthermore, addition of ascorbate back into the cell culture medium to improve the intercellular concentration decreases overall oxidative stress, but induces DNA double strand breaks following formation of ternary DNA adducts containing Cr(III) crosslinked with histidine, cysteine, ascorbate or glutathione [18, 32, 33]. These different Cr- containing bulky DNA adducts are not easily repaired and thus are a main contributor to Cr- induced malignant cellular transformation [28]. Figure 1 depicts the uptake of Cr(VI) vs Cr(III) and its fate in the cellular environment, highlighting the reductive potential of chromium in different biological compartments, and its action on DNA targets.

Chromium has also been found to alter the epigenetic profile of cells at both the level of DNA methylation and histone modification [34],[35, 36]. Exposure to Cr(VI) is associated with changes in various histone marks including, decreased H3K27Me3, and increased levels of H3K4Me3 as well as H3K9Me2 and H3K9Me3 [37]. Interestingly, Cr(VI) appears to be linked to changes in the expression of the mismatch repair gene *MLH1*. Exposure to Cr(VI) induces the expression of the histone methyltransferase G9a, which is responsible for dimethylation of H3K9. Increased level of H3K9Me2 have been detected in A549 cells exposed to Cr(VI). This results in decreased expression of the *MLH1* gene, which limits the DNA repair capacity of the cell [37]. In addition, it has been found that Cr(VI)-toxicity is mediated by the presence of major MMR proteins at sites of adducts. MMR protein recruitment to these sites facilitated the induction of gamma H2AX foci, which resulted in DNA double strand breaks and induction of p53-mediated apoptosis rapidly following exposure (6-12 hours) [38]. These findings provide further evidence of the genotoxicity of Cr(VI). Inhibition of repair machinery or cells with inherently defective DNA repair capacities compound genotoxic events. Cr(VI) exposure can also alter the epigenetic landscape by direct interaction with chromatin and DNA-modifying enzymes. Cr(VI) has been shown to interfere with the activities of epigenetic machinery such as histone deacetylase (HDAC) enzymes, rendering them inactive [39, 40]. One study has demonstrated that Cr(VI) exposure can lead to the crosslinking of an HDAC-1 – DNMT1 epigenetic complex to the promoter region of the *Cyp1a1* gene. This was shown to inhibit the benzo[a]pyrene-induced activation of AHR [41].

3.1. Mutagenic Vs non Mutagenic mode of action of Cr(VI)

Most human and animal studies have shown that chromate induces lung cancer by inhalation, and in the 1980s there was a notion that this is the only type of cancer that was caused by chromate. However, an examination of the literature revealed that many other types of cancers were evident in epidemiological studies, [42, 43] but the main route was inhalation and from occupational exposures.

A number of early studies have demonstrated that hexavalent chromium is mutagenic in bacteria, [44] [45] in mammalian cell culture systems [46] and also induces mutations *in vivo* in experimental animals [47] [48, 49]. In fact, in the 1989 IARC assessment, Cr(VI) was considered to be one of the most active agents in mutation assays and in other assays of genotoxicity. Chromate is known to form adducts in DNA including DNA-protein crosslinks, and crosslinks with amino acids such as cysteine, GSH and ascorbate [50-59]. Most of these adducts involved the reduced Cr(III) which coordinates ligands to DNA in ternary complexes [52, 54, 59]. Chromate also induces chromosomal damage and formation of oxidized DNA adducts [60-63]. The cited studies are just a fraction of those in the literature that establish chromate as a mutagenic and genotoxic carcinogen. However, this does not mean that it does not have other effects in the cell such as changes in the epigenetic program and gene expression,[60]that may also be involved in its carcinogenicity [64, 65] [66]. For example chromate is known to inactivate DNA mismatch repair which may occur by selection [37] [33, 38, 67], epigenetic silencing, or mutations [67] [37]. It also causes changes in gene expression that reflect its toxic action such as activation of antioxidant pathways like Nrf2 and its associated genes.[68] [69, 70]

One of the major routes of human exposure to chromate is in drinking water, where the US EPA drinking water standard is 100 ppb total chromium. This is not a small amount of chromium since it equals about 2uM. If this chromium were all hexavalent it would be extremely toxic [71]. In 2007 the National Toxicology Program conducted a drinking water study cancer bioassay with chromate in mice and rats [72]. To summarize their findings: administration of sodium dichromate dihydrate in the drinking water to F344/N rats and B6C3F1 mice resulted in squamous cell carcinoma of the oral mucosa in rats and small intestinal tumors in the mice [72]. Despite all the strong evidence that chromate is a mutagen, which was also confirmed in the NTP study, [72] a number of studies funded by the chrome industry were initiated to show that that a non-mutagenic mode of action could be involved [73-75]. In fact, the conclusions from these industry-funded studies were that chromate *does not* have a mutagenic mode of action and therefore in risk assessment there should be a threshold below which it is safe to be exposed to chromate. If an agent has a mutagenic MOA there is no safe threshold and low dose linear extrapolation is applied. One of these studies indicates that the deposition of Cr and DNA damage is not uniform in the small intestine and most of the DNA damage and Cr is deposited at sites that are not involved with carcinogenesis [73]. They propose that chromate enhances cell proliferation to induce cancers and that this is a non-mutagenic mode of action [73]. Other studies showed that gene expression changes induced by chromate in the small intestine where NTP found tumors were consistent with a non-mutagenic MOA. They compared gene expression changes in the liver induce by other chemicals that had a non-mutagenic MOA to the changes induced by chromate and found that chromate induced similar gene expression changes as non- mutagenic carcinogens.[75]

However, given the high mutagenic and genotoxic activity of chromate (vide supra) how can these studies say that chromate is not mutagenic? There is no question that chromate has many mechanisms of action that do not involve mutations but, what reason is there to point to non-mutations as the true mechanism? The identification of these other mechanisms does not diminish the mutagenic activity of chromate.

These studies were supported by the Cr(VI) Panel of the American Chemistry Council, formerly known as the Chemical Manufacturers Association which includes member companies that use Cr(VI). If research can show that Cr(VI) produces cancer by a non-mutagenic mode of action then the permissible levels in drinking water and in air will be regulated differently and the allowable concentration levels will increase substantially. However, for most carcinogenic processes there are multiple mechanisms that participate in tumor formation and mutations, whether inherited or induced by mutagenic carcinogens, are certainly at the forefront. Some examples include: genomic instability due to DNA double strand break-induced G2 arrest [76], loss of mismatch repair due to epigenetic silencing, mutations, or selection [37], tissue injury, oxidative stress, oxidized DNA adducts, and inflammation [77, 78] may also play an important role. However, the most likely scenario is that there are a number of ways for chromate-induced carcinogenesis to occur and since chromate reacts with DNA directly and indirectly via oxidative stress, causes mutations, epigenetic changes, tissue injury, cell proliferation and inflammation, we must consider all of these events in playing a role. The mutagenic activity does not dissipate when chromate causes all these other effects.

Chromium-containing compounds are pervasive in environment. Trivalent chromium is present in many foods and supplementation products, popularly used by many people across the US and the world. Evidence that dietary intake of Cr(III) is inconclusive and questions the use of this metal in nutritional supplementation. Hexavalent chromium is a widely recognized carcinogenic metal which is dispersed throughout the environment from anthropogenic point sources. Exposure to Cr(VI) induces toxic and carcinogenic effects by a complex multi-front mechanism of action including oxidative stress, epigenetic changes, chromosome and DNA damage, and mutagenesis. Independent research has validated and expanded upon the IARC classification of mutagenic carcinogen for Cr(VI). Efforts by the chromium-industry have been made to suggest that contrary to this research, Cr(VI) is not mutagenic. It is clear that in addition to more research on Cr(VI) effects on humans and carcinogenesis needs to be undertaken, honest and transparent communication of these effects to the public is necessary as well.

Acknowledgments

Support

Research in Environmental Health Sciences (Center Grant) - ES000260-54

Arsenic Carcinogenesis and Interference with Histone mRNA - ES026138-03

Epigenetic Stress and Chromate Carcinogenesis - ES029359-01A1

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* Denotes of special interest

** Denotes of outstanding interest

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Figure 1.
General Mechanisms of Chromium Uptake and Cellular Fate