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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 nonessential 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.

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Hexavalent chromium, Trivalent chromium, Oxidative stress, Genotoxicity, Carcinogenesis, Epigenetics.

Introduction

The dispersion of metals in the environment is of major concern to global human health. Chromium (Cr) is a group 1 carcinogen classified 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 the 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) into the air [6]. It is estimated that workers in these industries have a twofold higher exposure level 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 such as natural oxidation can convert Cr(III) to Cr(VI) in this medium if, for example, there are high levels of manganese in the soil or the soil is very alkaline in pH [9,10]. Owing to the inherent toxicity and carcinogenicity of chromium-containing substances, the US Environmental Protection Agency and US Occupational Safety and Health Administration have determined exposure limits of 100 μg/L of total chromium for drinking water standards and 5 $\mu g/m^3$ 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.

Essentiality of trivalent chromium

Trivalent chromium (Cr(III))—containing compounds are components of many multivitamins and nutritional supplements and even present in foods. Predominant forms of chromium (III) that are taken as supplementation include chromium picolinate, chromium histidinate, chromium dinicocysteinate, 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 published data from 2017 revealing that 170 million adults in the United States take some form of nutritional

supplementation, be it a multivitamin, a 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 μg/day and 39–54 μg/day for women and men, respectively [14]. These ranges fall within and also exceed the adequate intake values (25 μg/day for women and 35 μg/day for men) established by the Food and Nutrition Board of the National Academics of Sciences, Engineering, and Medicine [15]. With this level of intake from dietary chromium alone, it seems that additional chromium supplementation in individuals far exceeds the adequate intake of chromium. Although 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 and conflicting, and some studies even suggest that excessive intake of Cr(III) formulations is carcinogenic [16,17].

Unlike Cr(VI) uptake, ligand-bound Cr(III) is postulated to enter the cell via phagocytic mechanisms or through nonspecific 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].

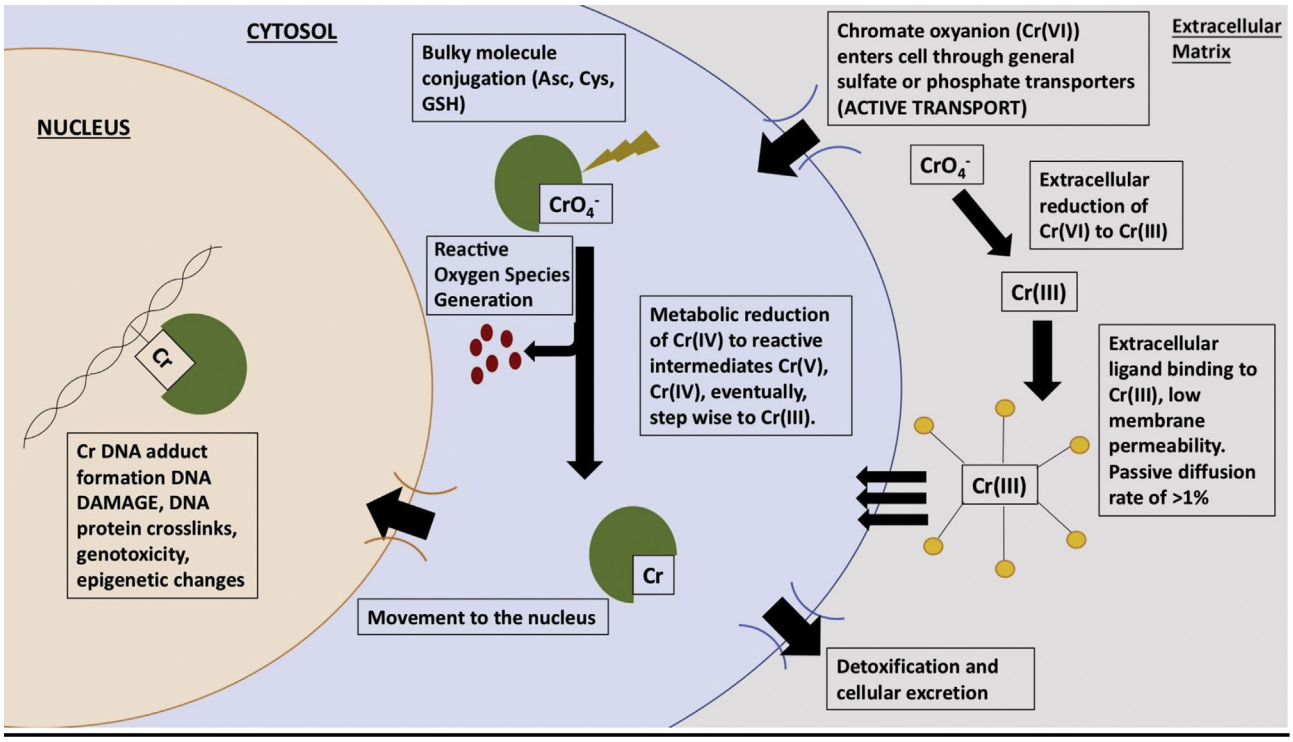
Chromium (III) compounds are taken as supplementation because studies appear to demonstrate positive effects in regard to the potentiation of insulin action and increased beneficial results from exercise including higher percentage of lean muscle mass and loss of fat [23,24].
However, these findings 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 of Cr(III) in which absorption peaks (40 μg/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 of Cr(III) in any form of life as we know exists today.

Uptake, reduction, and 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 uses 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]. Stepwise two-electron reductions with ascorbate generate Cr(IV) intermediates; this reaction usually occurs *in vivo*, but in tissue culture systems that 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 that 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) cross-linked with histidine, cysteine, ascorbate, or GSH [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 levels of DNA methylation and histone modification [34–36]. 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. An increased level of H3K9Me2 has 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

Figure 1



General mechanisms of chromium uptake and cellular fate.

addition, it has been found that Cr(VI) toxicity is mediated by the presence of major Mismatch DNA Repair proteins at the 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 h) [38]. These findings provide further evidence of the genotoxicity of Cr(VI). Inhibition of repair machinery or cells with inherently defective DNA repair capacities compounds 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 enzymes, rendering them inactive [39,40]. One study has demonstrated that Cr(VI) exposure can lead to cross-linking of an histone deacetylase 1–DNA Methyltransferase I epigenetic complex to the promoter region of the *Cyp1a1* gene. This was shown to inhibit the benzo [a]pyrene-induced activation of Aryl Hydrocarbon Receptor [41].

Mutagenic vs non mutagenic mode of action of Cr(VI)

Most human and animal studies have shown that inhalation of chromate induces lung cancer, 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 occupational exposures.

A number of early studies have demonstrated that hexavalent chromium is mutagenic in bacteria [44,45] and in mammalian cell culture systems [46] and also induces mutations *in vivo* in experimental animals [47–49]. 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 cross-links and cross-links 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], which may also be involved in its carcinogenicity [64–66]. For example, chromate is known to inactivate DNA mismatch repair that may occur by selection [37] [33,38,67], epigenetic silencing, or mutations [37,67]. It also causes changes in gene expression that reflects its

toxic action such as activation of antioxidant pathways such as Nrf2 and its associated genes [68–70].

One of the major routes of human exposure to chromate is through drinking water, where the US EPA drinking water standard is 100 ppb of total chromium. This is not a small amount of chromium because it equals about 2 uM. If this chromium was hexavalent, it would be extremely toxic [71]. In 2007, the National Toxicology Program conducted a drinking water study to a assess the carcinogenicity of Chromate in mice and rats [72]. Summarizing their findings, administration of sodium dichromate dihydrate in the drinking water for F344/N rats and B6C3F1 mice resulted in squamous cell carcinoma of the oral mucosa in rats and small intestinal tumors in mice [72]. Despite all the strong evidence that chromate is a mutagen, which was also confirmed in the National Toxicology Program study [72], a number of studies funded by the chrome industry were initiated to show that that a nonmutagenic 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 Mode of Action, 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 nonmutagenic 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 nonmutagenic MOA. They compared gene expression changes in the liver induced by other chemicals that had a nonmutagenic MOA with the changes induced by chromate and found that chromate induced similar gene expression changes as nonmutagenic 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 nonmutations 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 nonmutagenic mode of action, then the permissible levels in drinking water and 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, and are certainly at the forefront. Some examples including 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 because chromate reacts with DNA directly and indirectly via oxidative stress and causes mutations, epigenetic changes, tissue injury, cell proliferation, and inflammation, we must consider all of these events to be playing a role. The mutagenic activity does not dissipate when chromate causes all these other effects.

Chromium-containing compounds are pervasive in the environment. Trivalent chromium is present in many foods and supplementation products, popularly used by many people across the US and the world. There is evidence that dietary intake of Cr(III) is not essential for human life and therefor the practice of including Cr(III) as a nutritional supplement if vitamin pills, fitness supplements etc. is often questioned. Hexavalent chromium is a widely recognized carcinogenic metal that is dispersed throughout the environment from anthropogenic point sources. Exposure to Cr(VI) induces toxic and carcinogenic effects by a complex multifront mechanism of action including oxidative stress, epigenetic changes, chromosome and DNA damage, and mutagenesis. Independent research has validated and expanded on the IARC classification of mutagenic carcinogens 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 that needs to be undertaken, honest and transparent communication of these effects to the public is necessary as well.

Conflict of interest

The authors declare no conflict of interest.

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