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Molecular mechanism of Cr(VI)-induced carcinogenesis

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Abstract: We hypothesize that reduction of Cr(VI) to its low oxidation states, Cr(V) and Cr(IV), is an important step in the mechanism of Cr(VI)-induced carcinogenesis. These chromium intermediates are able to generate reactive oxygen species (ROS), which initiate Cr(VI)-induced carcinogenesis. Through free radical reactions, Cr(VI) can activate multiple carcinogenic processes. (1) Cr(VI) causes activation of nuclear transcription factor kappa B (NF-κB). Among ROS, hydroxyl radicals (·OH) are responsible for Cr(VI)-induced NF-κB activation. (2) Cr(VI) is able to activate activator protein-1 (AP-1). (3) Cr(VI) causes p53 activation and ·OH radicals function as messengers for the activation of this tumor suppressor protein. (4) Cr(VI) is capable of inducing apoptosis via both p53 and ROS-mediated reactions. (5) Cr(VI) causes over-expression of oncogenes (jun-B and raf), up-regulation of antioxidants (glutathione peroxidase), activation of certain enzymes involved in mitogen-activated protein kinase (MAP kinase) signal pathways (MAPKAP kinase), stimulation of enzymes involved in cell cycle control and checkpoint mechanisms (checkpoint suppressor 1), and activation of enzymes responsible for Cr(VI) reduction, such as NAD(P)H dependent dihydrolipoamide dehydrogenase.

Cr(VI)-containing compounds are considered to be well established carcinogens (1). They are potent inducers of tumors in experimental animals and active agents in causing DNA damage such as DNA strand breakage. We have hypothesized that reduction of Cr(VI) to its low oxidation states, Cr(V) and Cr(IV), is an important step (2). These chromium intermediates are able to generate ROS, which initiate Cr(VI)-induced carcinogenesis. This article summarizes our studies on Cr(VI) reduction and related free radical generation and the role of free radical reactions in various potential mechanisms for the initiation of carcinogenesis induced by this metal.

Cr(VI) reduction: Various low-molecular-weight cellular constituents have been shown to be able to reduce Cr(VI) *in vitro* at physiological pH. A variety of enzymatic and nonenzymatic factors function as Cr(VI) reductants (2, 3). These factors include microsomes, mitochondria, cytochromes, and several flavoenzymes, such as glutathione reductase.

Using electron spin resonance (ESR) with a low-frequency microwave bridge and a cyclinder-shaped loop gap resonator, we were able to show that a Cr(V) intermediate can be generated by one electron reduction of Cr(VI) in living animals (4). The Cr(V) was found predominantly in the liver with a small amount in the blood. The Cr(V) intermediate was identified to be a Cr(V)-NADPH complex with an oxygen bond to Cr(V). Pretreatment of the animals with ascorbate or GSH decreased the Cr(V) formation, while pretreatment with NADPH enhanced it. These results suggest that NADPH/flavoenzymes and not GSH or ascorbate are the major one-electron Cr(VI) reductants *in vivo*.

Free radical generation: Chromium is able to generate many different kinds of free radicals through its reactive intermediates, Cr(V), Cr(IV), Cr(III), and Cr(II), upon reactions with different agents. For example, reaction of Cr(VI) with glutathione (GSH) generates GSH-derived free radicals, while reaction with ascorbate generates ascorbate-derived free radicals (3, 5). Although all of these radicals can cause cell injury, hydroxyl radical (·OH) is especially important due to its high reactivity. In our

earlier studies on the enzymatic reduction of Cr(VI), we have found that ·OH can be generated upon reaction of Cr(V) with hydrogen peroxide (H_2O_2) through a Fenton-like reaction. It has been suggested that this radical may be the species responsible for Cr(VI)-induced carcinogenesis (2). Further studies have demonstrated that various chromium oxidation states can be reduced by superoxide radical ($O_2^{\cdot-}$) to generate chromium intermediates at lower oxidation states, which react with H_2O_2 to generate ·OH through Haber-weiss reactions (2). The scheme that summarizes chromium-mediated ·OH generation is illustrated in Figure 1.

DNA damage by free radical reactions: Using λ Hind III DNA digest, our laboratory assessed DNA damage induced by a mixture of Cr(VI) and ascorbate. A significant amount of DNA strand breaks occurred when the DNA was incubated with Cr(VI) and ascorbate. Ascorbate-derived free radicals play a major role in this type of DNA damage. Addition of H_2O_2 to the reaction mixture generated ·OH radical and drastically enhanced the DNA damage. In addition to strand breaks, ·OH radicals can also react with guanine residues at several positions to generate a range of products, of which the most studied one is 8-hydroxyl-deoxyguanosine (8-OHdG). The formation of this adduct is considered a biomarker to implicate ROS in the mechanism of carcinogenesis induced by a variety of agents. Using HPLC with electrochemical detection, we have found that ·OH radicals generated by Cr(V) and Cr(IV)-mediated reaction caused 2'-deoxyguanosine (dG) hydroxylation to form 8-OHdG.

NF-κB activation: Nuclear transcription factor (NF)-κB is considered a primary oxidative response factor that functions to enhance the transcription of a variety of genes. In several cell types, ROS have been shown to activate this transcription factor. It has been shown that Cr(VI) is able to induce NF-κB activation (6). The reduction of Cr(VI) to low oxidation states is required for the NF-κB activation. Hydroxyl radicals generated by Cr(V)- and Cr(IV)-mediated Fenton-like reactions play a prominent role in the mechanism of Cr(VI)-induced NF-κB activation. It is possible that NF-κB activation and a subsequent expression of proto-oncogenes, such as c-*myc*, may play a role in the induction of neoplastic transformation by Cr(VI).

AP-1 activation: Nuclear transcription factor AP-1 is also an oxidative stress response transcription factor. It is a complex protein composed of homodimers and heterodimers of oncogene proteins of the Jun and Fos families. The results obtained from our recent studies have shown that Cr(VI) is able to induce activation of this transcription factor. This induction requires mitogen activated protein (MAP) kinase p38 and c-jun-N-terminal kinase (JNK), but not extracellular-signal-regulated kinase (ERK). Aspirin, a newly established antioxidant, inhibited the AP-1 activation. Inhibition of p38 and IκB kinase (IKK) attenuated the Cr(VI)-induced AP-1 activation. The results suggest that ROS may serve as a common up-stream signal initiating the AP-1 activation in response to Cr(VI) stimulation, whereas p38 and IKK act as down-stream executive kinases for the activation of this transcription factor.

Activation of p53: The p53 is also an important oxidative response transcription factor. It is involved in various biological processes, such as regulation of genes in the cell cycle, cell growth arrest after DNA damage, and apoptosis. Our recent studies have shown that Cr(VI) is able to induce the activation of p53 protein in the epithelial cell line, A549. Hydroxyl radical plays a key role in this Cr(VI)-induced p53 activation. Hydroxyl radical is generated by a Cr(VI)-mediated Fenton-like reaction. H_2O_2 was generated via $O_2^{\cdot-}$ dismutation. The $O_2^{\cdot-}$ radical was produced from molecular oxygen during the Cr(VI) reduction.

Apoptosis: Apoptosis is a programmed cell death mechanism to control cell number in tissues and to

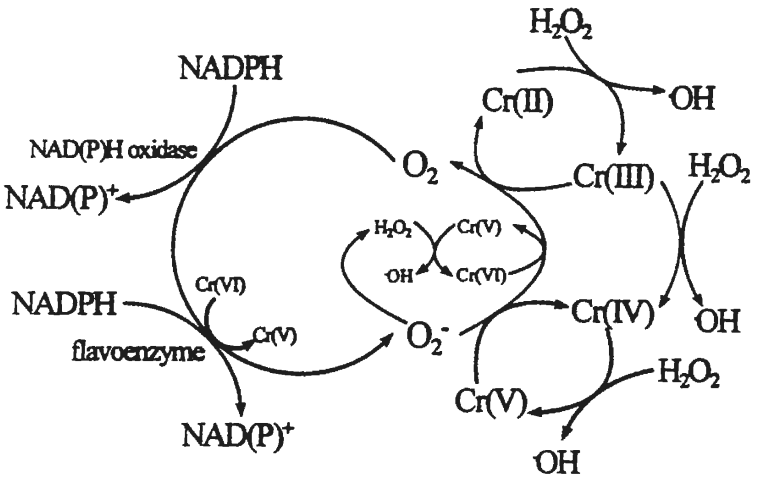

Figure 1. A scheme of chroimium-mediated 'OH generation.

eliminate individual cells that may lead to disease states. Because of the fundamental importance of apoptosis in the regulation of tissue growth, alteration of this pathway is important in carcinogenesis. Our study has shown that Cr(VI) is able to induce apoptosis (8). In the apoptotic signaling pathway, ROS generated from both Cr(VI) reduction and p53 activation play an important role. The Cr(VI)-derived ROS initiate apoptosis before activation of p53 protein. Cr(VI) induces apoptosis through both p53-dependent and p53 independent pathways. ROS generated by Cr(VI) may play a dual role in the mechanism of Cr(VI)-induced carcinogenesis: genetic damage and apoptosis. The cancer development may depend on the delicate balance of these two opposite processes.

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