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In vivo reduction of chromium (VI) and its related free radical generation

Ke Jian Liu[1] and Xianglin Shi[2]

[1]*College of Pharmacy, University of New Mexico, Albuquerque, NM;* [2]*Pathology and Physiology Research Branch, National Institute For Occupational Safety and Health, Morgantown, WV, USA*

Abstract

Chromium (VI) compounds are widely recognized as human carcinogens. Extensive studies *in vitro* and in model systems indicate that the reactive intermediate, Cr (V), generated by cellular reduction of Cr (VI), is likely the candidate for the ultimate carcinogenic form of chromium compounds. Here we review our current understanding of the in vivo reduction of Cr (VI) and its related free radical generation. Our results demonstrate that Cr (V) is indeed generated from the reduction of Cr (VI) *in vivo*, and that Cr (V) thus formed can mediate the generation of free radicals. Cr (V) and its related free radicals are very likely to be involved in the mechanism of Cr (VI)-induced toxicity and carcinogenesis. These studies also illustrate that *in vivo* EPR spectroscopy and magnetic resonance imaging can be very useful and powerful tools for studying paramagnetic metal ions in chemical and biochemical reactions occurring in intact animals. (Mol Cell Biochem **222**: 41–47, 2001)

Key words: EPR, chromium, free radical, MRI, reactive oxygen species

Introduction

Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases. Chromium can exist in several oxidative forms, most commonly as Cr (0), Cr (III), and Cr (VI). Cr (III) occurs naturally in the environment and is an essential nutrient in humans. Chromate (Cr (VI)) compounds, widely used in industry, have been shown to have serious toxic and carcinogenic effects on humans. Intensive epidemiological evidence has been published on the high incidence of respiratory tract cancers in workers occupationally exposed to Cr (VI) compounds [1, 2]. The mechanism of Cr (VI)-induced toxicity and carcinogenesis is not well understood, although recent results suggest the involvement of transient chromium intermediate states, such as Cr (V) and Cr (IV), and their related free radicals formation.

Cr (VI) has been demonstrated to induce a variety of DNA lesions such as single-strand breaks, alkali-labile sites, and DNA protein cross-links [3, 4]. In contrast, most Cr (III) compounds, the final product in the reduction of Cr (VI), are relatively nontoxic, noncarcinogenic, and nonmutagenic [3, 5]. Since Cr (VI) does not react with isolated DNA, the reduction of Cr (VI) to lower oxidation states after entering the human system has been considered an important step in Cr (VI)-induced carcinogenesis [5, 6]. While several studies have shown that the chromium-induced DNA damage is strongly dependent on the formation of Cr (V) intermediates, recent studies have suggested that free radicals generated by Cr (V) may also play an important role [7–9].

In this paper, we will review our current understanding of the *in vivo* reduction of Cr (VI), based mostly on our previously published results. New experiments have since been conducted, and are also included here. The goal of these studies is to demonstrate that upon administration into animals, Cr (VI) is reduced to Cr (V) that can mediate the formation of free radicals. These processes are likely important steps in the mechanism of Cr (VI)-induced carcinogenesis.

Address for offprints: K.J. Liu, College of Pharmacy, University of New Mexico, Albuquerque, NM 87131, USA

Materials and methods

Animal preparation

BALB/c mice weighing 18–20 g were obtained from Charles River Laboratories (Wilminton, MA, USA). After the animals were anesthetized with ketamine/xylazine (100/10 mg/kg body wt, im), 100 μl of sodium dichromate or other chemical solutions were introduced into the animal either intravenously, intraperitoneally, or intratracheally. Electron magnetic resonance (EPR) or magnetic resonance imaging (MRI) measurements followed immediately.

For the measurement of the reduction of Cr (VI) on the skin, Wistar rats from Charles River Laboratories were used. The hair on the back of the anesthetized rat was shaved, and aqueous sodium dichromate solution was applied topically to the exposed skin. The EPR spectra were collected using an extended loop resonator positioned over the area of interest.

In vivo *EPR measurement of Cr (V) in living mice*

In vivo EPR measurement were obtained using a custom-designed EPR spectrometer equipped with a low frequency (1.2 GHz, L-band) microwave bridge and a cylinder-shaped loop gap resonator. After administration of sodium dichromate, the animal was immediately inserted into the resonator, which was placed between the poles of the magnet. The thorax cavity of the mouse was placed in the center of the resonator. For kinetics studies, the EPR spectra were collected every 60 sec to record the formation and decay of the Cr (V) signal. Typical settings for the spectrometer included magnetic field, 425 G; incident microwave power, 50 mW; modulation frequency, 27 kHz; modulation amplitude, 1.6 G.

Magnetic resonance imaging of Cr (V) in mice

The MRI measurements were undertaken using a S.M.I.S.7.0 Tesla MR imager. Anesthetized mice were placed inside the 6 cm bird cage coil, and the tail vein was cannulated so that chromium compounds could be administered without moving the animal out of the magnet. A series of pre- and post-injection T1 weighted gradient echo MR image were collected with TR/TE/α = 100 msec/7 msec/70°, FOV = 4 cm, a slice thickness of 2 mm and a pixel resolution of 312 μm. There were four averages, resulting in an acquisition time of 51 sec.

Results and discussion

Detection of in vivo *formation of Cr (V) intermediate species by EPR*

When a mouse was intravenously injected with sodium dichromate (Cr (VI)), the *in vivo* EPR spectrometer recorded a signal from the thorax cavity of the mouse a few minutes after the administration (Fig. 1). Based on the spectral characteristics of the EPR spectra, including g-value (g = 1.9798), linewidth, splitting, and satellite peaks of Cr^{53} (Fig. 1D), the EPR signal in Fig. 1A was assigned to CR (V) [10, 11]. Administration of CR (VI) to mice by intraperitoneal or intratracheal injection produced identical EPR signal (Figs 1B and 1C), albeit at reduced signal intensity, due to delayed uptake and redistribution process. Since Cr (VI) is EPR silent, the observation of Cr (V) signal indicates that Cr (VI) is rapidly reduced to Cr (V) upon administration.

The formation of Cr (V) through the reduction of Cr (VI) is supported by conventional EPR experiments using isolated tissues after treatment *in vivo* [12]. In agreement with our *in vivo* experiments, liver and kidney have been reported to be the organs with the highest chromium accumulation in treated rats [13], and therefore is the target organ of many studies. Rats were intraperitoneally injected with Cr (VI), the liver and kidney tissue was collected immediately after sacrificing the animal, and then the EPR signals were recorded at liquid nitrogen temperature using conventional X-band EPR spectrometer. Again, Cr (V) species was found in both liver and kidney (Fig. 2). Here both of the in vivo EPR (L-band, 1.2 GHz) and conventional EPR (X-band, 9.6 GHz) are utilized to their full advantage. The *in vivo* EPR provides real time, localized formation of Cr (V) in living animals, while the conventional EPR, with higher detection sensitivity, can afford much lower administered concentrations of Cr (VI) to the animal, as well as more detailed spectral information about the Cr (V) species. Combination of the results from both types of EPR measurements shows unequivocal evidence that Cr (V) is formed through the reduction of Cr (VI) *in vivo*.

In addition to oral ingestion and inhalation as the main routes for occupational and accidental exposure, skin represents another possible route for chromium to enter into humans and exert its carcinogenecity. Application of an aqueous dichromate solution to the skin of a living rat resulted in an EPR signal which was identical to that of Cr (V) observed *in vivo* [14]. Removing the stratum corneum increased the rates of both formation and decay of Cr (V) (Fig. 3), indicating its important role as a protective skin barrier. Together with result from administration of Cr (VI) through intravenous, in-

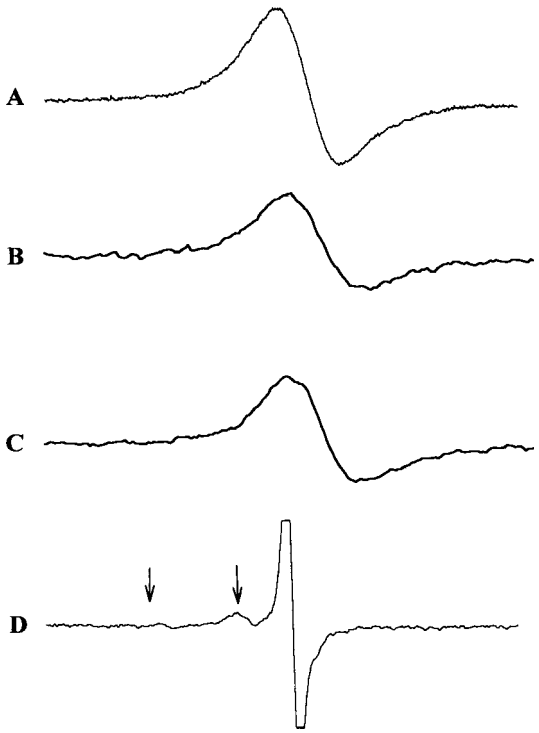

Fig. 1. In vivo EPR spectra of Cr (V) from a mouse. The L-band EPR spectra were obtained from mice 8 min after treatment with 100 µl of 100 mM sodium dichromate: (a) intravenous injection; (b) intraperitoneal injection; (c) intratracheal injection; and (d) 9.6 GHz X-band EPR spectrum of Cr (V) from the blood of a mouse. The blood sample was drawn from the tail vein of the same mouse as in (a). The arrows indicate Cr^{53} satellite peaks.

traperitoneal, and intratracheal injection, these studies lead to the conclusion that regardless of the route of administration, Cr (VI) will be reduced to Cr (V) once absorbed in the animal.

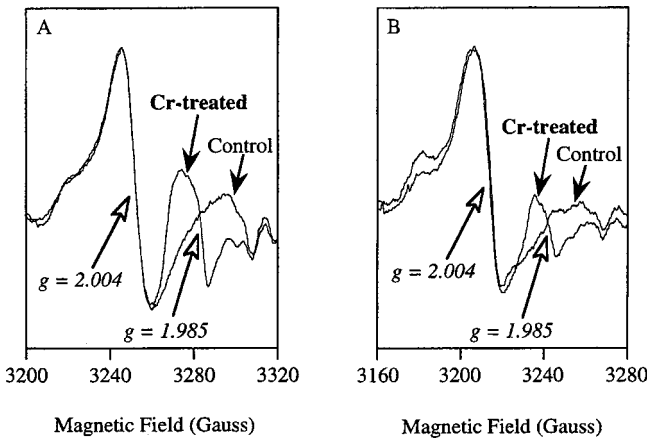

Fig. 2. X-band EPR spectra from liver and kidney of rats treated with Cr (VI). (A) Liver and (B) kidney was removed 30 min after intraperitoneal injection of sodium dichromate (10 mg/kg). Tissue Cr (V) was measured at 77 K in a finger Dewar fill with liquid nitrogen using a conventional X-band EPR spectrometer. From ref. [12] with permission.

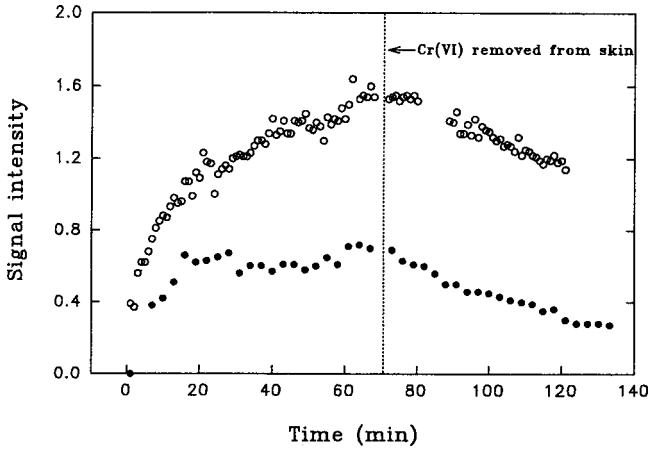

Fig. 3. The time course of the formation and decay of Cr (V) in the skin of a mouse after topical treatment with Cr (VI). Time zero is the time of application. (O) Skin was shaved and stripped 10 times with surgical tapes; (●) shaved skin without stripping. From ref. [14] with permission.

In all above studies, the intermediate species Cr (V) is relatively short lived, as shown in the case of skin (Fig. 3). The kinetics of the formation and decay of Cr (V) are well demonstrated in Fig. 4, where the signal intensity of Cr (V) reached a maximum approximately 10 min after intravenous injection of Cr (VI), and then decayed slowly with an observed lifetime of about 30 min. It is expected that Cr (V) will be further reduced to Cr (IV), and eventually to the stable Cr (III). Therefore, the time course in Fig. 4 represents the steady state concentration of Cr (V) over time, due to its continu-

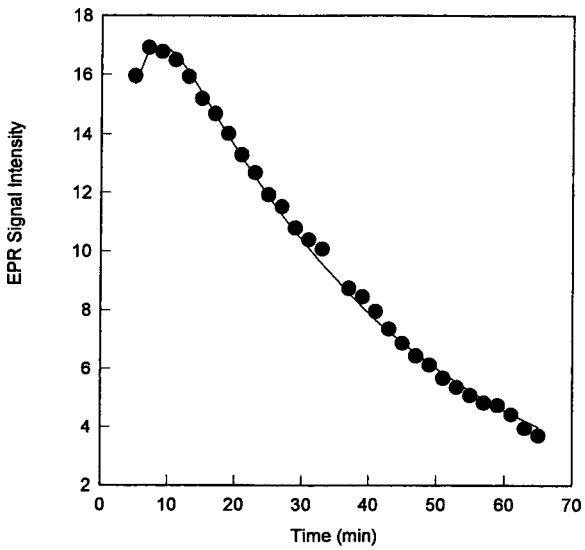

Fig. 4. The time course of the formation and decay of Cr (V). The EPR signal of Cr (V) was scanned every minute immediately following intravenous injection of 100 µl of 100 mM sodium dichromate into a mouse. Time zero is the time of injection. From ref. [10] with permission.

ous formation and decay. The actual lifetime of Cr (V) could be significantly shorter than shown in the figure. In order to estimate the real value, a Cr (V) model compound, K_3CrO_8, was synthesized [15, 16], and was intravenously injected to a mouse. The lifetime of the EPR signal of Cr (V) was found to be less than 1 min, suggesting that under in vivo conditions, Cr (V) is indeed very short lived [11].

Detection of in vivo *formation of Cr (V) and Cr (III) by MRI*

In spite of the transient nature of Cr (V), EPR is the technique of choice for characterizing the formation of Cr (V) species, and has been proved to be a valuable tool in chromium studies. On the other hand, the noninvasive magnetic resonance imaging (MRI) technique provides a much needed complimentary method on the *in vivo* localization and distribution of Cr (V). Because of their paramagnetic properties, Cr (V) and its final product Cr (III), can alter relaxation time of protons, hence Cr (V) and Cr (III) formed *in vivo* in the reduction of the non-paramagnetic Cr (VI) could act as contrast agents for proton MRI [17]. To test this idea, feasibility studies were carried out to record the MRI images of the liver and kidney of a mouse before and after administration of Cr (VI). High contrast was observed in the kidney cortex and in the liver (Fig. 5), indicating that Cr (VI) was rapidly reduced to Cr (V) and Cr (III) upon administration to a mouse, and that chromium accumulated at high concentrations in the liver and kidney. These results are consistent with, and further confirm, those findings obtained by EPR, suggesting that MRI could potentially be a very useful tool for chromium studies and produce certain information that are not obtainable by any existing methods. Experiments are currently underway to distinguish the contribution of contrast enhancement from each of the two chromium species, i.e. Cr (V) and Cr (III).

Effect of antioxidants and chelators on Cr (V) formation

Results presented above, and other evidence reported in the literature [18–25], indicate that the intermediate species Cr (V) is involved in the mechanism of chromium induced toxicity and carcinogenesis. Some of the approaches to attenuate the deleterious effects of Cr (V) include the treatment of the animal with agents that either prevent the reduction of Cr (VI) to Cr (V), or that would accelerate the reduction of Cr (VI) to the final product, Cr (III). Metal chelators are the first type of these agents [10, 23, 26, 27]. When the animals were pretreated with chelators EDTA, DETAPAC, or 1,10-phenanthroline before the administration of Cr (VI), a significant reduction in the yield of Cr (V) was observed (Fig. 6). The second type of agents are the antioxidants, or reductants, which can affect the reduction process of Cr (VI) metabolism [11, 20, 26–31]. Figure 7 shows the effect of glutathione, ascorbate, and NADH on the signal intensity of Cr (V). Pretreatment of the animal with ascorbate or glutathione significantly reduced the Cr (V) signal, while NADH increased the signal. Ascorbate and glutathione results can be easily understood because at higher concentrations, these cellular antioxidants would reduce Cr (VI) rapidly to the final product Cr (III), yielding lower steady state concentration of Cr (V). The increased EPR signal of Cr (V) when treated with NADH, however, is likely because NADH serves as a cofactor for the enzymes responsible for the one-electron reduction of Cr (VI), and the formation of a Cr (V)-NAD(P)H complex that enhance the stability of Cr (V) *in vivo* [11, 32–34]. Treatment with NADH therefore would increase production of Cr (V) and enhance Cr (V) stability, leading to higher steady state concentration of Cr (V)-NAD(P)H complex.

These results indicate that to prevent or attenuate Cr (VI) induced cellular damage, either proper chelation to inhibit the formation of Cr (V), or treatment with antioxidants, could be used as viable strategies.

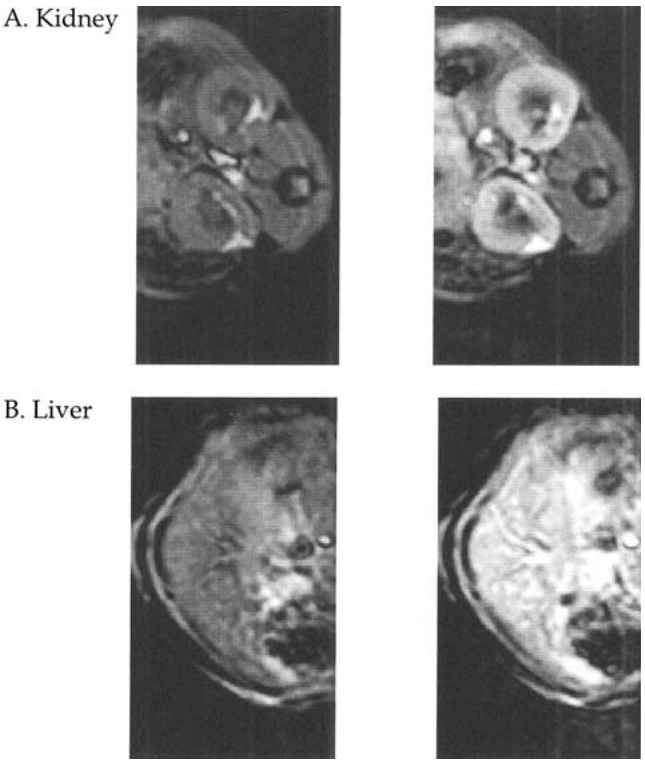

Fig. 5. Tissue localization of chromium based on T1 weighted MR imaging. Gradient echo images were collected before (left) and 10 min post (right) injection of 100 µl of 100 mM sodium dichromate. Increased intensity indicates an increased concentration of Cr (V) and Cr (III). Acquisition parameters are described in the Materials and methods.

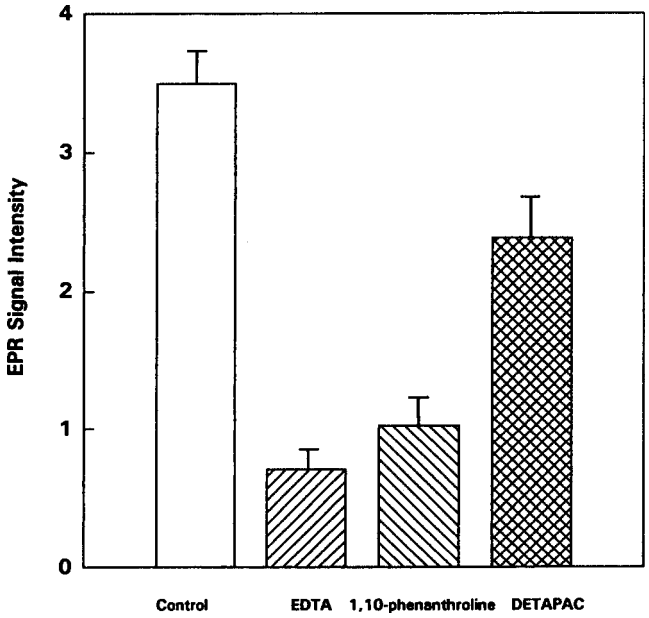

Fig. 6. Effect of metal ion chelators on the intensity of Cr (V) EPR signal. Three minutes before the intravenous injection of 100 µl of 100 mM sodium dichromate, the animals were injected intravenously with 200 µl of 200 mM EDTA, 100 µl of 200 mM DETAPAC, or 50 µl of 200 mM 1,10-phenanthroline, respectively. The values represent means ± S.E. (n = 3). From ref. [10] with permission.

Formation of Cr (V) and free radical in cells

The intermediate Cr (V) has been demonstrated in chemical solutions to be capable of generating free radicals, such as superoxide and hydroxyl radicals, commonly referred to reactive oxygen species (ROS) [7, 8, 20, 24, 26, 27, 35–41]. It is believed that these free radicals, mediated by Cr (V), are largely responsible for the observed Cr (VI) toxicity and carcinogenicity. Although attempts have been made to detect these Cr (V)-mediated free radicals directly in living animals by EPR techniques, to date, the effort has largely failed, due to the very short lifetime of the radicals, and low concentration of the radicals being formed. On the other hand, a significant amount of evidence, both direct and indirect, has been obtained in cellular studies, unambiguously demonstrating that free radicals are indeed formed through Cr (V)-mediated reactions. Figure 8 is a typical example of these studies in which characteristic EPR signal of Cr (V) was recorded when Jurkat cells were incubated with Cr (VI). Addition of glutathione reductase, which catalyzed the Cr (VI) reduction, enhanced the Cr (V) formation. If the Cr (VI) was incubated with the cells in the presence of the spin trap DMPO, the EPR signal of DMPO-OH is formed. At the same time, a reduc-

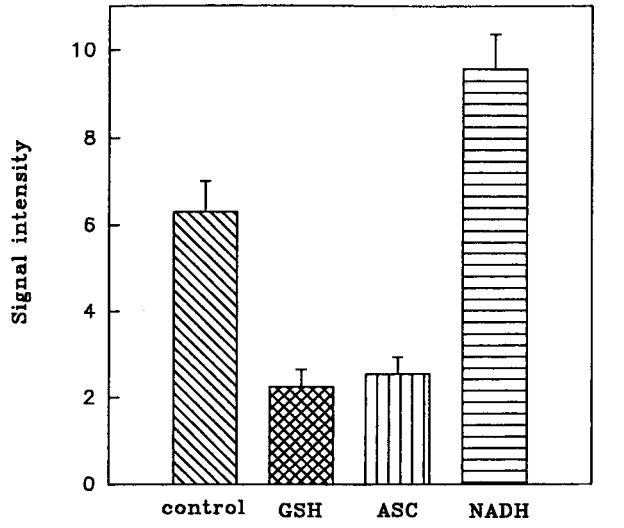

Fig. 7. Effect of glutathione, ascorbate, and NADH on the signal intensity of Cr (V). Three minutes before the intravenous injection of 100 µl of 100 mM sodium dichromate, the animals were injected intravenously with 100 µl of 250 mM glutathione, 250 mM ascorbate, or 500 mM NADH. The values represent means ± S.E. (n = 3). From ref. [11] with permission.

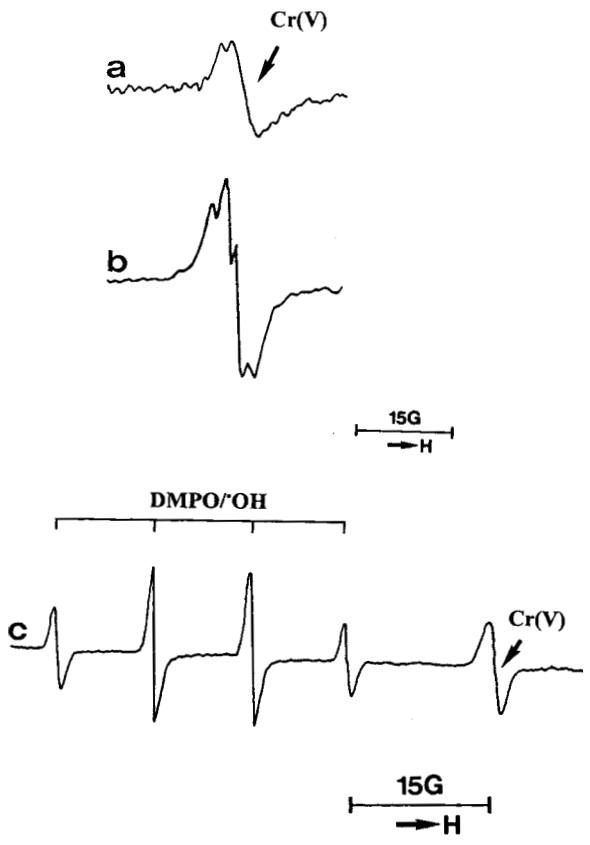

Fig. 8. Generation of hydroxyl radical through Fenton-like reaction. (a) An EPR spectrum recorded from a phosphate buffer solution containing 2 mM $K_2Cr_2O_7$ and 2×10^6/ml Jurkat cells. The spectrum was recorded 4 min after reaction initiation. (b) Same as (a) but with 2.5 units/ml glutathione reductase added. (c) Same as (a) but with 100 mM DMPO added. From ref. [26] with permission.

tion in the signal intensity of Cr (V) is noticed, as shown at the right side of Fig. 8c. These studies firmly establish that Cr (V) can generate hydroxyl radicals through a Fenton-like reaction, as in the following:

$$Cr\ (V) + H_2O_2 \rightarrow {}^{\bullet}OH + Cr\ (VI) + OH^{-}$$

The great advantage of EPR to study free radicals and Cr (V) is well demonstrated here. Both of these species are paramagnetic (i.e. EPR active) and can be recorded simultaneously in a single scan, making it possible to study the causal relationship of the two types of species. Perhaps because of the unique capability of EPR to both measure chromium intermediates and related free radicals, EPR has been one of the popular methods for chromium studies.

Extensive studies conducted recently have shown that the free radicals mediated by Cr (V) can cause DNA damages [8, 20, 24, 27, 39, 42–45], and play an important role in Cr (VI)-induced cell responses, including apoptosis, cell growth arrest, and activation of nuclear transcription factors, NF-κB, AP-1 and p53 [26, 46–49]. It has become increasingly clear that Cr (V) and its related free radicals are involved in the mechanism of Cr (VI)-induced toxicity and carcinogenesis.

Conclusion

The results presented above, and those from the literature lead to the following conclusions: (a) When entered into biological systems, Cr (VI) is reduced to the transient intermediate species Cr (V). Cr (V) is further reduced to the final stable product Cr (III); (b) Cr (V) is capable of generating hydroxyl radicals through a Fenton-like reaction; (c) The free radicals mediated by Cr (V) can cause DNA damage, as well as activate a variety of signal transduction pathways, leading to toxicity and carcinogenicity. These overall mechanism of Cr (VI) is summarized in Fig. 9; and (d) EPR is a valuable tool to study both Cr (V) and its related free radicals, while MRI could potentially provide the complementary results on the localization of chromium intermediates, and perhaps, with the right spin trap, even free radicals.

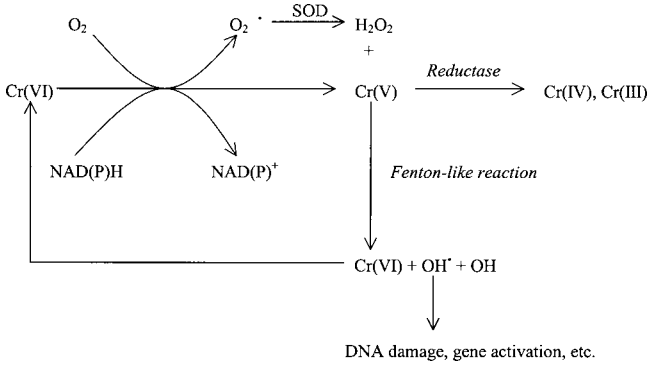

Fig. 9. The overall scheme for the reduction of Cr (VI) and its related free radical generation.

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References

1. Alcedo JA, Wetterhahn KE: Chromium toxicity and carcinogenesis. Int Rev Exp Pathol 31: 85–108, 1990
2. World Health Organization: IARC monographs on the evaluation of carcinogenic risks to humans, vol. 49. Lyon, France, 1990
3. Bartlett RJ: Chromium cycling in soils and water: Links, gaps, and methods. Environ Health Perspect 92: 17–24, 1991
4. De Flora S, Serra D, Basso C, Zanacchi P: Mechanistic aspects of chromium carcinogenicity. Arch Toxicol 13(suppl): 28–39, 1989
5. Connett PH, Wetterhahn KE: Metabolism of the carcinogen chromate by cellular constituents. Struct Bonding 54: 93–124, 1983
6. Jennette KE: Microsomal reduction of the carcinogen chromate produces chromium (V). J Am Chem Soc 104: 874–875, 1982
7. Shi X, Dalal NS: Generation of hydroxyl radical by chromate in biologically relevant systems: Role of Cr (V) complexes vs. etraperoxochromate (V). Environ Health Perspect 102(suppl 3): 231–236, 1994
8. Shi X, Mao Y, Knapton AD, Ding M, Rojanasakul Y, Gannett PM, Dalal N, Liu K: Reaction of Cr (VI) with ascorbate and hydrogen peroxide generates hydroxyl radicals and causes DNA damage: Role of a Cr (IV)-mediated Fenton-like reaction. Carcinogenesis 15: 2475–2478, 1994
9. Shi X, Dalal NS: The role of superoxide radical in chromium (VI)-generated hydroxyl radical: The Cr (VI) Haber-Weiss cycle. Arch Biochem Biophys 292: 323–327, 1992
10. Liu KJ, Jiang J, Swartz HM, Shi X. Low-frequency EPR detection of chromium (V) formation by chromium (VI) reduction in whole live mice. Arch Biochem Biophys 313: 248–252, 1994
11. Liu KJ, Shi X, Jiang JJ, Goda F, Dalal N, Swartz HM: Chromate-induced chromium (V) formation in live mice and its control by cellular antioxidants: An L-band electron paramagnetic resonance study. Arch Biochem Biophys 323: 33–9, 1995
12. Yuann JM, Liu KJ, Hamilton JW, Wetterhahn KE: *In vivo* effects of ascorbate and glutathione on the uptake of chromium, formation of chromium (V), chromium-DNA binding and 8-hydroxy-2′-deoxyguanosine in liver and kidney of osteogenic disorder shionogi rats following treatment with chromium (VI). Carcinogenesis 20: 1267–1275, 1999
13. Tandon SK, Behari JR, Kachru DN: Distribution of chromium in poisoned rats. Toxicology 13: 29–34, 1979
14. Liu KJ, Mader K, Shi X, Swartz HM: Reduction of carcinogenic chromium (VI) on the skin of living rats. Mag Res Med 38: 524–526, 1997
15. Dalal NS, Suryan MM, Seehra MS: Potassium perchromate standard for determination of paramagnetic spin concentration, g values, and magnetic moments of fossil fuels. Anal Chem 53: 938–940, 1981
16. Liebross RH, Wetterhahn KE: *In vivo* formation of chromium (V) in chick embryo liver and red blood cells. Carcinogenesis 13: 2113–2120, 1992

17. Fujii H, Wan X, Zhong J, Berliner LJ, Yoshikawa K: *In vivo* imaging of spin-trapped nitric oxide in rats with septic shock: MRI spin trapping. Mag Res Med 42: 235–239, 1999
18. Ueno S, Susa N, Furukawa Y, Sugiyama M: Formation of paramagnetic chromium in liver of mice treated with dichromate (VI). Toxicol Appl Pharmacol 135: 165–171, 1995
19. Sugiyama M, Tsuzuki K: Effect of glutathione depletion on formation of paramagnetic chromium in Chinese hamster V-79 cells. FEBS Lett 341: 273–276, 1994
20. Stearns DM, Kennedy LJ, Courtney KD, Giangrande PH, Phieffer LS, Wetterhahn KE: Reduction of chromium (VI) by ascorbate leads to chromium-DNA binding and DNA strand breaks *in vitro*. Biochemistry 34: 910–919, 1995
21. Rossi SC, Gorman N, Wetterhahn KE: Mitochondrial reduction of the carcinogen chromate: Formation of Cr (V), Chem Res Toxicol 1: 101–107, 1988
22. Micera G, Dessi A: Chromium adsorption by plant roots and formation of long-lived Cr (V) species: An ecological hazard? J Inorg Biochem 34: 157–166, 1988
23. Mao Y, Zang L, Shi X: Generation of free radicals by Cr (IV) from lipid hydroperoxides and its inhibition by chelators. Biochem Mol Biol Int 36: 327–337, 1995
24. Faux SP, Gao M, Chipman JK, Levy LS: Production of 8-hydroxydeoxyguanosine in isolated DNA by chromium (VI) and chromium (V). Carcinogenesis 13: 1667–1669, 1992
25. Barr-David G, Charara M, Codd R, Farrell R, Irwin J, Lay P, Bramley R, Brumby S, Ji JY, Hanson G: EPR characterization of the Cr (V) intermediates in the Cr (V/VI) oxidations of organic substrates and of relevance to Cr-induced cancers. J Chem Soc Faraday Trans 91: 1207–1216, 1995
26. Shi X, Dong Z, Huang C, Ma W, Liu K, Ye J, Chen F, Leonard SS, Ding M, Castranova V, Vallyathan V: The role of hydroxyl radical as a messenger in the activation of nuclear transcription factor NF-kappa B. Mol Cell Biochem 194: 63–70, 1999
27. Shi X, Ding M, Ye J, Wang S, Leonard SS, Zang L, Castranova V, Vallyathan V, Chiu A, Dalal N, Liu K: Cr (IV) causes activation of nuclear transcription factor-kappa B, DNA strand breaks and dG hydroxylation via free radical reactions. J Inorg Biochem 75: 37–44, 1999
28. Sugiyama M: Role of cellular antioxidants in metal-induced damage. Cell Biol Toxicol 10: 1–22, 1994
29. Standeven AM, Wetterhahn KE: Ascorbate is the principal reductant of chromium (VI) in rat lung ultrafiltrates and cytosols, and mediates chromium-DNA binding *in vitro*. Carcinogenesis 13: 1319–1324, 1992
30. Moghaddas S, Gelerinter E, Bose RN: Mechanisms of formation and decomposition of hypervalent chromium metabolites in the glutathione-chromium (VI) reaction. J Inorg Biochem 57: 135–146, 1995
31. Gannett PM, Dalal NS, Shi XL, Toth B: 8-Hydroxy-2′-deoxyguanosine formation during the catalytic oxidation of hydrazines in the presence of 2′-deoxyguanosine. Chem Biol Interact 80: 57–72, 1991
32. Shi XL, Dalal NS: NADPH-dependent flavoenzymes catalyze one electron reduction of metal ions and molecular oxygen and generate hydroxyl radicals. FEBS Lett 276: 189–91, 1990
33. Liu KJ, Shi X, Jiang J, Goda F, Dalal N, Swartz HM: Low frequency electron paramagnetic resonance investigation on metabolism of chromium (VI) by whole live mice. Ann Clinical Lab Sci 26: 176–184, 1996
34. Shi XL, Dalal NS: Chromium (V) and hydroxyl radical formation during the glutathione reductase-catalyzed reduction of chromium (VI). Biochem Biophys Res Commun 163: 627–634, 1989
35. Ueno S, Sugiyama M, Susa N, Furukawa Y: Effect of dimethylthiourea on chromium (VI)-induced DNA single-strand breaks in Chinese hamster V-79 cells. Mutat Res 346: 247–253, 1995
36. Sugiyama M, Tsuzuki K, Haramaki N: Influence of o-phenanthroline on DNA single-strand breaks, alkali-labile sites, glutathione reductase, and formation of chromium (V) in Chinese hamster V-79 cells treated with sodium chromate (VI). Arch Biochem Biophys 305: 261–266, 1993
37. Sugiyama M: Role of physiological antioxidants in chromium (VI)-induced cellular injury. Free Rad Biol Med 12: 397–407, 1992
38. Sugiyama M: Effects of vitamins on chromium (VI)-induced damage. Environ Health Perspect 92: 63–70, 1991
39. Kawanishi S, Inoue S, Yamamoto K: Active oxygen species in DNA damage induced by carcinogenic metal compounds. Environ Health Perspect 102(suppl 3): 17–20, 1994
40. Kadiiska MB, Xiang QH, Mason RP: *In vivo* free radical generation by chromium (VI): An electron spin resonance spin-trapping investigation. Chem Res Toxicol 7: 800–805, 1994
41. Aiyar J, Berkovits HJ, Floyd RA, Wetterhahn KE: Reaction of chromium (VI) with glutathione or with hydrogen peroxide: Identification of reactive intermediates and their role in chromium (VI)-induced DNA damage. Environ Health Perspect 92: 53–62, 1991
42. Aiyar J, Berkovits HJ, Floyd RA, Wetterhahn KE: Reaction of chromium (VI) with hydrogen peroxide in the presence of glutathione: Reactive intermediates and resulting DNA damage. Chem Res Toxicol 3: 595–603, 1990
43. Bagchi D, Hassoun EA, Bagchi M, Stohs SJ: Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague-Dawley rats. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 110: 177–187, 1995
44. Sugiyama M, Lin XH, Costa M: Protective effect of vitamin E against chromosomal aberrations and mutation induced by sodium chromate in Chinese hamster V79 cells. Mutat Res 260: 19–23, 1991
45. Tsuzuki K, Sugiyama M, Haramaki N: DNA single-strand breaks and cytotoxicity induced by chromate (VI), cadmium (II), and mercury (II) in hydrogen peroxide-resistant cell lines. Environ Health Perspect 102(suppl 3): 341–342, 1994
46. Chen F, Ding M, Lu Y, Leonard SS, Vallyathan V, Castranova V, Shi X: Participation of MAP kinase p38 and I-kappaB kinase in chromium (VI)-induced NF-kappaB and AP-1 activation. J Environ Pathol Toxicol Oncol 19: 231–238, 2000
47. Wang S, Leonard SS, Ye J, Ding M, Shi X: The role of hydroxyl radical as a messenger in Cr (VI)-induced p53 activation. Am J Physiol – Cell Physiol 279: C868–C875, 2000
48. Ye J, Wang S, Leonard SS, Sun Y, Butterworth L, Antonini J, Ding M, Rojanasakul Y, Vallyathan V, Castranova V, Shi X: Role of reactive oxygen species and p53 in chromium (VI)-induced apoptosis. J Biol Chem 274: 34974–34980, 1999
49. Ye J, Zhang X, Young HA, Mao Y, Shi X: Chromium (VI)-induced nuclear factor-kappa B activation in intact cells via free radical reactions. Carcinogenesis 16: 2401–2405, 1995