Chromium(III)-Induced 8-Hydroxydeoxyguanosine in DNA and Its Reduction by Antioxidants: Comparative Effects of Melatonin, Ascorbate, and Vitamin E

Wenbo Qi, Russel J. Reiter, Dun-Xian Tan, Joaquin J. Garcia, Lucien C. Manchester, Malgorzata Karbownik, and Juan R. Calvo

Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, Texas, USA

Chromium compounds are well documented carcinogens. Cr(III) is more reactive than Cr(VI) toward DNA under *in vitro* conditions. In the present study, we investigated the ability of Cr(III) to induce oxidative DNA damage by examining the formation of 8-hydroxydeoxyguanosine (8-OH-dG) in calf thymus DNA incubated with $CrCl_3$ plus H_2O_2. We measured 8-OH-dG using HPLC with electrochemical detection. In the presence of H_2O_2, we observed that Cr(III)-induced formation of 8-OH-dG in isolated DNA was dose and time dependent. Melatonin, ascorbate, and vitamin E (Trolox), all of which are free radical scavengers, markedly inhibited the formation of 8-OH-dG in a concentration-dependent manner. The concentration that reduced DNA damage by 50% was 0.51, 30.4, and 36.2 μM for melatonin, ascorbate, and Trolox, respectively. The results show that melatonin is 60- and 70-fold more effective than ascorbate or vitamin E, respectively, in reducing oxidative DNA damage in this *in vitro* model. These findings also are consistent with the conclusion that the carcinogenic mechanism of Cr(III) is possibly due to Cr(III)-mediated Fenton-type reactions and that melatonin's highly protective effects against Cr(III) relate, at least in part, to its direct hydroxyl radical scavenging ability. *Key words*: ascorbate, chromium, 8-hydroxydeoxyguanosine, melatonin, vitamin E. *Environ Health Perspect* 108:399–402 (2000). [Online 16 March 2000]
http://ehpnet1.niehs.nih.gov/docs/2000/108p399-402qi/abstract.html

Chromium is a widely used industrial chemical, with uses in steel, alloy cast irons, chrome, paints, metal finishes, and wood treatments (*1*). Cr causes allergic dermatitis and has other toxic and carcinogenic effects in humans and animals (*2,3*). Epidemiologic studies have shown that industrial workers exposed to Cr have a higher incidence of respiratory cancer than does the unexposed population (*2–5*). Dermal, renal, and hepatic toxicity have been reported in Cr-exposed humans (*5,6*). Cr can also induce tumors in experimental animals and cause genotoxicity, i.e., chromosome aberrations, sister chromatid exchanges, cell transformations, and gene mutations in mammalian cell cultures (*7–10*).

Cr is found in the workplace primarily in the valence forms Cr(VI) and Cr(III) (*11*). Cr(VI) compounds are more toxic and carcinogenic than Cr(III) (*12,13*) because Cr(VI), in contrast to Cr(III), can readily cross cellular membranes via nonspecific anion carriers (*13–15*). However, once inside cells, Cr(VI) is reduced through reactive Cr intermediates such as Cr(V) and Cr(IV) to the ultimate kinetically stable Cr(III) by cellular reductants including glutathione and vitamin C (*13,16*). Therefore, Cr(III) or other intermediate oxidation states probably play an important role in Cr(VI)-induced toxicity (*16*).

Cr(III), which was initially thought to be relatively nontoxic, recently was found to be more effective than Cr(VI) in causing genotoxicity in cell-free systems (*11*). Cr(III) interacts with DNA to induce DNA strand breaks, DNA–protein cross-links, and oxidative DNA base modifications such as the formation of 8-hydroxydeoxyguanosine (8-OH-dG) (*17–21*). 8-OH-dG is a key biomarker relevant to carcinogenesis because the formation of 8-OH-dG in DNA causes misincorporation during replication and subsequently leads to G→T transversions (*22,23*). The carcinogenic mechanisms of Cr(III) relate to its ability to generate hydroxyl radicals (•OH) from H_2O_2 via a Fenton-type reaction (*20,24*). The highly toxic •OH then targets DNA, resulting in oxidative DNA base adducts such as 8-OH-dG.

Melatonin, an indoleamine product of the pineal gland, is an endogenous •OH scavenger and a highly effective antioxidant (*25,26*). *In vitro* melatonin is as effective or more effective than either glutathione and mannitol in reducing •OH toxicity (*25*) and is possibly more efficient than vitamin E in reducing the toxicity of the peroxyl radical (*27*). Moreover, melatonin is highly lipophilic (*28*) as well as somewhat hydrophilic (*29*); therefore, it easily passes all known morphophysiologic barriers and enters all subcellular compartments. Melatonin has a high affinity for cell nuclei in mammalian tissues, where its concentration can be 5 times higher than levels found in blood (*30*). By measuring a variety of oxidative indexes (including levels of 8-OH-dG), earlier studies have shown that melatonin effectively protects DNA from oxidative damage induced by a number of free-radical-generating agents including safrole, kainic acid, lipopolysaccharide, ferric nitrilotriacetate, ischemia/reperfusion, and ionizing radiation both *in vitro* and *in vivo* (*31–36*).

In the present study, we investigated the ability of melatonin to reduce Cr(III)-induced oxidative DNA damage *in vitro* and compared melatonin's efficacy to that of two well-known antioxidants, vitamins E and C. We examined the formation of 8-OH-dG in calf thymus DNA with Cr_3Cl plus H_2O_2 using HPLC with electrochemical detection.

Materials and Methods

Reagents. We purchased calf thymus DNA, $CrCl_3 \cdot 6H_2O$, H_2O_2, and ascorbate from Sigma (St. Louis, MO), and we obtained Trolox from Aldrich (Milwaukee, WI). Pure melatonin was a gift from Helssin Chemicals SA (Biasca, Switzerland). We purchased nuclease P_1 and alkaline phosphatase from Boehringer Mannheim (Indianapolis, IN). We used MilliQ-purified H_2O to prepare all solutions. All other chemicals were of the highest quality available.

Treatment. We dissolved calf thymus DNA (500 μg) in 10 mM potassium phosphate buffer (pH 7.4) at a final volume of 0.45 mL. In the first study, we incubated DNA with six concentrations of $CrCl_3$ (10, 50, 100, 250, 500, or 750 μM) in the presence of 0.5 mM H_2O_2 for 1 hr at 37°C in a water bath. This study was performed to establish the concentration of Cr(III) required to induce an appropriate amount of 8-OH-dG formation. In the second study, we selected 500 μM $CrCl_3$ for incubation with DNA in the presence of 0.5 mM H_2O_2 for 0, 20, 40, 60, 80, or 100 min to determine the optimal incubation time. In the final study, we used several concentrations of

Address correspondence to R.J. Reiter, Department of Cellular and Structural Biology, Mail Code 7762, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900 USA. Telephone: (210) 567-3859. Fax: (210) 567-6948. E-mail: reiter@uthscsa.edu

This study was supported in part by the Amoun Pharmaceutical Industries Company.

Received 27 August 1999; accepted 1 November 1999.

melatonin (0.25, 0.5, 1, 2.5, 5, or 10 μM), ascorbate (1, 10, 25, 50, 100, or 250 μM), or Trolox (1, 10, 25, 50, 100, or 250 μM) in combination with 500 μM $CrCl_3$ plus 0.5 mM H_2O_2 for 60 min to test the efficacy of these antioxidants in altering oxidative DNA damage.

Assay for 8-OH-dG. After incubation, we added 50 μL sodium acetate (3 M, pH 5.0) and two volumes of -20°C to each sample to terminate the reaction. DNA was precipitated and washed once with 70% ethanol. The DNA sample was dried and dissolved in 200-μL 20 mM sodium acetate (pH 5.0); the samples were denatured by heating at 95°C for 5 min and then cooled on ice. The DNA samples were digested to nucleotides by incubation with 8 U nuclease P_1 at 37°C for 30 min. Next, we added 20 μL 1-M Tris-HCl (pH 8.0) to the samples and they were treated with 4 U alkaline phosphatase at 37°C for 1 hr. We filtered the resulting deoxynucleoside mixture through a Millipore filter (0.22 μm; Millipore) and analyzed it using HPLC with an electrochemical detection system. We used an ESA HPLC system (ESA, Chelmsford, MA) equipped with an eight-channel CoulArray 5600 electrochemical detector: YMC-BD (4.6 mm × 250 mm, Partisil 5 μ OD53; Waters, Milford, MA) column (3 μm, 150 × 4.6 mm i.d.). The eluent was a 10% aqueous methanol containing 12.5 mM citric acid, 25 mM sodium acetic acid, 30 mM sodium hydroxide, and 10 mM acetic acid at a flow rate of 1 mL/min. We measured the quantities of 8-OH-dG and 2-deoxyguanosine (2-dG) using different channels and two oxidative potentials (300 and 900 mV, respectively). The level of 8-OH-dG in each sample was expressed as the ratio of 8-OH-dG to 10^5 2-dG (*37*).

Statistical analysis. We analyzed all data by a one-way analysis of variance followed by the Tukey test.

Results

The levels of 8-OH-dG increased in a dose-dependent manner with increasing concentrations of $CrCl_3$ (Figure 1). All concentrations of Cr(III) from 10 μM to 0.75 mM caused significant increases in 8-OH-dG levels in DNA. We selected a concentration of 0.5 mM Cr(III) for the subsequent studies because it yielded high levels of 8-OH-dG. In the second study, 8-OH-dG levels increased essentially in a linear manner during the incubation period when 0.5 mM $CrCl_3$ plus 0.5 mM H_2O_2 were incubated with DNA (Figure 2). We selected an intermediate time of 60 min for the subsequent studies because this incubation time produced optimal levels of 8-OH-dG. Figure 3 shows that melatonin inhibited Cr(III)-induced formation of 8-OH-dG in a dose-dependent manner. All melatonin concentrations > 0.25 μM significantly reduced 8-OH-dG formation in DNA induced by 0.5 mM Cr(III) plus 0.5 mM H_2O_2 ($p < 0.05$). Figure 4 shows that ascorbate inhibited Cr(III)-induced formation of 8-OH-dG in a dose-dependent manner. The effective concentrations of ascorbate against Cr(III)-induced formation of 8-OH-dG in DNA were between 1 and 250 μM. Figure 5 shows that the formation of 8-OH-dG in DNA was also inhibited by Trolox in a dose-dependent manner. The effective concentrations of Trolox ranged from 10 to 250 μM. To compare the efficacy of melatonin, ascorbate, and Trolox, we calculated the percentage-inhibition curves (Figure 6). The IC_{50} is the concentration of a particular agent that inhibits the formation of 8-OH-dG in DNA by 50%. The IC_{50} for melatonin was 0.51 μM; this value is much less than for ascorbate (IC_{50} = 30.4 μM) or Trolox (IC_{50} = 36.2 μM).

Discussion

H_2O_2 is a normal metabolite in the cell; its steady-state concentrations range from 10^{-9} to 10^{-8} M (*38*). The concentrations of H_2O_2 may markedly increase in tissues when they are subjected to ionizing radiation, during the metabolism of carcinogens, and at sites of inflammation (*39–41*). Although H_2O_2 may not cause DNA damage under physiologic conditions, it participates in the metal ion-catalyzed Haber-Weiss reaction and generates the highly reactive •OH, which can target DNA, resulting in oxidative DNA damage (*42*). Electron spin resonance spectroscopy studies have shown that •OH are generated in a DNA-free solution containing Cr(III) and H_2O_2 (*43*). The present study demonstrates that Cr(III) plus H_2O_2 is capable of inducing oxidative DNA damage. When we incubated calf thymus DNA with $CrCl_3$ + H_2O_2, the levels of 8-OH-dG detected were approximately 40 times higher than those in the untreated controls. Furthermore, the formation of 8-OH-dG increases in a dose- and time-dependent manner in the presence of 0.5 mM H_2O_2. Melatonin, ascorbate, and vitamin E (Trolox) all function as free radical scavengers and markedly inhibited the formation of 8-OH-dG in a concentration-dependent manner but, clearly, with different efficacies.

Vitamin E, a well-known antioxidant and inhibitor of lipid peroxidation in biologic membranes, has protective effects against the carcinogenic or mutagenic activity of chemical agents and ionizing radiation (*16*). We found that Trolox, a water-soluble vitamin E analogue, successfully inhibited the Cr(III)-induced formation of 8-OH-dG in isolated DNA in a concentration-dependent manner. Trolox concentrations > 10 mM significantly reduced 8-OH-dG levels. The IC_{50} value for Trolox was 36.2 μM.

In this *in vitro* system, we also found that ascorbate, a water-soluble physiologic antioxidant, had a protective effect, with an IC_{50} value of 30.4 μM calculated from its percent-inhibition curve. Vitamin C has antiviral, anticancer, and antimutagenic activity (*16*). However, under certain conditions, vitamin C acts as prooxidant, generating free radicals (*44*). In a number of studies, ascorbate

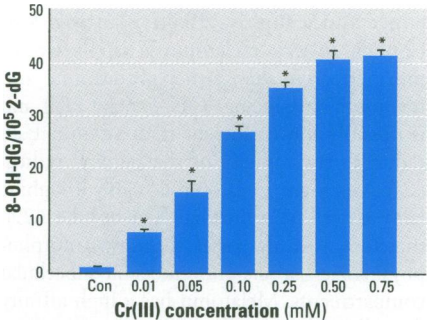

Figure 1. The effect of Cr(III) concentrations on the levels of 8-OH-dG in DNA incubated with 0.5 mM H_2O_2. Con, control. The incubation time was 60 min. Values are given as means ± SE ($n = 5$).
*$p < 0.05$ as compared to the Con group.

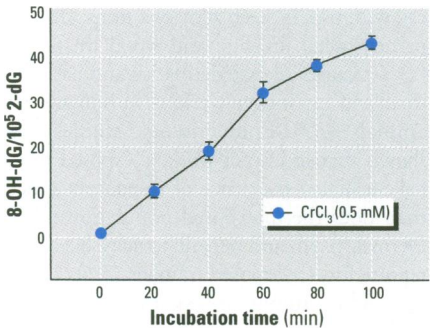

Figure 2. A time course of Cr(III)-induced formation of 8-OH-dG in DNA incubated with 0.5 mM H_2O_2. Results are given as means ± SE ($n = 5$). The concentration of Cr(III) was 0.5 mM.

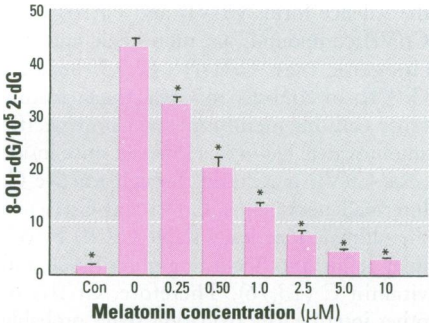

Figure 3. The effect of melatonin concentrations on Cr(III) (0.5 mM) plus H_2O_2 (0.5 mM)-induced 8-OH-dG levels in DNA. The incubation time was 60 min. Results are given as means ± SE ($n = 5$).
*$p < 0.05$ as compared to the Cr(III) + H_2O_2 treatment group.

potentiates the production of reactive oxygen species, DNA strand breaks, and 8-OH-dG formation induced by Cr(VI) plus H_2O_2 (*45–47*). Thus, although ascorbate functions as an free radical scavenger in the Cr(III) plus H_2O_2 system, the utility of ascorbate in Cr detoxification *in vivo* should be cautiously considered.

As compared to ascorbate (IC_{50} = 30.4 μM) and vitamin E (IC_{50} = 36.2 μM), melatonin was more effective in reducing the formation of 8-OH-dG in this system (IC_{50} = 0.51 μM). Thus, melatonin was roughly 60- and 70-fold more effective in reducing oxidative damage to DNA than ascorbate and vitamin E, respectively. Also, the minimal concentration of melatonin required to significantly reduce 8-OH-dG formation was much less than that of either vitamin. A melatonin concentration of 0.25 μM significantly reduced the 8-OH-dG formation, and a 10-μM concentration of the indole essentially reduced 8-OH-dG levels to control levels.

In the present study, melatonin's highly effective protection against Cr(III)-induced formation of 8-OH-dG in DNA may relate to several actions of the indoleamine. First, melatonin is a direct free radical scavenger and is a particularly efficient scavenger of the highly toxic •OH (*25,48,49*). Melatonin neutralizes two •OH for each melatonin molecule, resulting in the formation of the product cyclic 3-hydroxymelatonin (*50*). In the present study, the formation of 8-OH-dG was thought to be due to a Cr(III)-mediated Fenton-type reaction that generates •OH, which in turn attacked DNA, resulting in the accumulation of the oxidative DNA base adduct 8-OH-dG (*20,24*). Second, melatonin not only detoxifies the highly toxic •OH, but also scavenges its precursor, H_2O_2. We recently uncovered a new pathway in which melatonin interacts with H_2O_2 to yield N^1-acetyl-N^2-formyl-5-methoxykynuramine (*51*). The structure of the product was confirmed using mass spectrometry, proton nuclear magnetic resonance, and carbon nuclear magnetic resonance. By lowering the concentration of H_2O_2, •OH generation in this system would also be proportionally reduced. Such a dual strategy of antioxidant protection would be much more efficient than simply scavenging •OH. Third, because melatonin is highly lipophilic (*28*) as well as somewhat hydrophilic (*29*), it easily enters cells and sub-cellular compartments. Intracellularly, the highest radioimmuoassayable concentrations of melatonin are measured in the nuclei of brain cells after its peripheral administration to animals (*30*). Melatonin has a high affinity for the nucleus (and possibly DNA itself), which may contribute to its protective effect against Cr-induced formation of 8-OH-dG. Cr(III) accumulates in nuclei and has a high affinity for DNA (*52*). Melatonin may prevent the formation of 8-OH-dG by displacing Cr(III) from the Cr–DNA binding complex and thereby reduce H_2O_2-mediated •OH generation in the vicinity of DNA. Fourth, melatonin and its precursors reportedly have a high metal-binding affinity (*53*). Limson et al. (*53*) showed that melatonin chelated aluminum, cadmium, iron, copper, and lead, etc. Although the authors did not investigate Cr, melatonin may also chelate this transition metal ion to prevent the formation of the •OH via the Cr-mediated Fenton-type reaction: Cr(III) + H_2O_2 → Cr(IV) + •OH + OH^-.

Susa et al. (*54*) used different end points and cultured primary rat hepatocytes and found that melatonin markedly reduced nuclear DNA single-strand breaks induced by $K_2Cr_2O_7$ [Cr(VI)]. They speculated that melatonin protected cells from free radical toxicity by one of several means, including melatonin's ability to preserve intracellular levels of vitamins E and C, stimulation of catalase activity, and/or by directly scavenging the •OH. In current studies, two of the options proposed by Susa et al. (*54*), i.e., maintenance of Vitamin E and C levels and the stimulation of catalase activity, were clearly not involved in melatonin's protection of DNA from oxidative damage. Thus, the most likely explanation for the current findings is that melatonin's effects were a consequence of its ability to scavenge the •OH and possibly also H_2O_2.

In the current *in vitro* study, which used purified DNA, the curves for the inhibition of DNA damage by each of the three antioxidants, i.e., melatonin, ascorbate, and Trolox (Figure 6), were quite different. The relevance of these curves to the pharmacologic utility of these molecules in protecting nuclear DNA from oxidative damage *in vivo* remains to be investigated. However, in *in vivo* studies where other free-radical-generating agents were used, melatonin also proved highly effective in reducing DNA damage consistent with its ability to enter the nucleus with ease (*55,56*). There have been no *in vivo* studies where vitamin E, ascorbate, and melatonin were compared for their relative efficacies in protecting DNA from oxidative destruction.

Melatonin as an antioxidant is effective in protecting membrane lipids, nuclear DNA, and protein from oxidative damage induced by a variety of free-radical-generating agents and processes both *in vitro* as well as *in vivo* (*26,55–58*). Considering the apparent virtual absence of acute or chronic toxicity, melatonin's clinical application against Cr-induced genotoxicity in occupational and environmental situation where this metal is a problem should be considered.

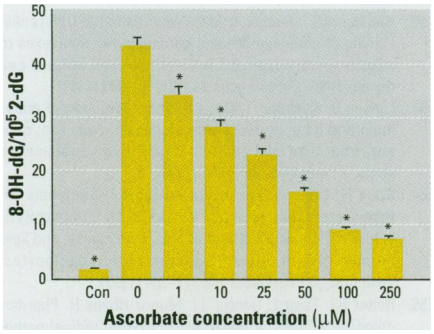

Figure 4. The effect of ascorbate on Cr(III) (0.5 mM) plus H_2O_2 (0.5 mM)-induced 8-OH-dG levels in DNA. The incubation time was 60 min. Results are given as means ± SE (n = 5).
*p < 0.05 as compared to the Cr(III) + H_2O_2 group.

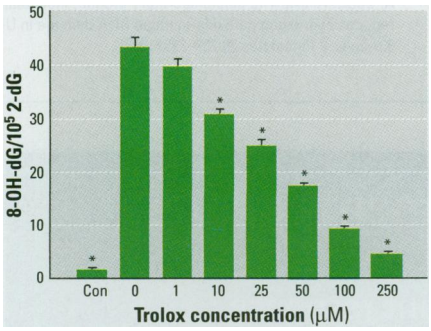

Figure 5. The effect of Trolox concentrations on Cr(III) (0.5 mM) plus H_2O_2 (0.5 mM)-induced 8-OH-dG levels in DNA. The incubation time was 60 min. Results are given as means ± SE (n = 5).
*p < 0.05 as compared to the Cr(III) plus H_2O_2 group.

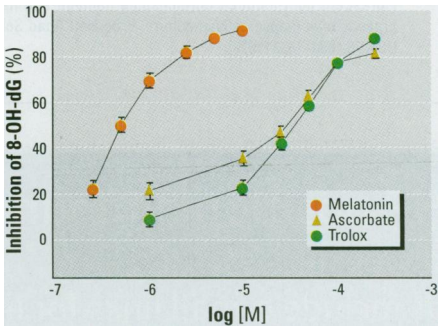

Figure 6. Percent-inhibition curves of concentrations of melatonin, ascorbate, and Trolox in reducing Cr(III)-induced 8-OH-dG formation in DNA. The incubation time was 60 min with 0.5 mM H_2O_2. Results are given as means ± SE (n = 5).

References and Notes

1. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 18:321–336 (1995).
2. Anttila S. Biological effects of occupational and environmental exposure to chromium. In: Metal Ions in Biology and Medicine (Collery P, ed). London:John Libbey, 1990;315–319.
3. Langard S. One hundred years of chromium and cancer: a review of epidemiological evidence and selected case reports. Am J Ind Med 17:189–215 (1990).

4. Norseth T. The carcinogenicity of chromium and its salts. Br J Ind Med 43:649–651 (1986).
5. Leonard A, Lawerys RR. Carcinogenicity and mutagenicity of chromium. Mutat Res 76:227–239 (1980).
6. Verschoor MA, Braget PC, Herber RFM, Zielhuis RL, Zwennis WCM. Renal function of chrom-plating workers and welders. Int Arch Occup Environ Health 60:67–70 (1988).
7. Sen P, Costa M. Incidence and localization of sister chromatid exchanges induced by nickel and chromium compounds. Carcinogenesis 7:1527–1533.
8. Yang JL, Hsieh Y-C, Wu C-W, Lee T-C. Mutational specificity of chromium(VI) compounds in the hprt locus of chinese hamster ovary-K1 cells. Carcinogenesis 13:2053–2057 (1992).
9. Manning FCR, Blankenship LJ, Wise JP, Xu J, Bridgewater LC, Patierno SR. Induction of internucleosomal DNA fragmentation by carcinogenic chromate: relationship to DNA damage, genotoxicity and inhibition of macromolecular synthesis. Environ Health Perspect 102(suppl 3):159–167 (1994).
10. Tsuda H, Kato K. Chromosomal aberrations and morphological transformation in hamster embryonic cells treated with potassium dichromate in vitro. Mutat Res 46:87–94 (1977).
11. De Flora S, Banasco M, Serra D, Zanacchi P. Genotoxicity of chromium compounds: a review. Mutat Res 238:99–112 (1990).
12. Bianchi V, Celotti L, Lanfranchi G, Majone F, Marin G, Montaldi A, Sponza G, Tamino G, Venier P, Zantedeschi A, et al. Genetic effects of chromium compounds. Mutat Res 117:279–300 (1983).
13. De Flora S, Wetterhahn KE. Mechanisms of chromium metabolism and genotoxicity. Life Chem Rep 7:168–244 (1989).
14. Danielsson BRG, Hassoun E, Dencker L. Embryo toxicity of chromium: distribution in pregnant mice and effects on embryonic cells in vitro. Arch Toxicol 51:233–245 (1982).
15. Lu Y-Y, Yang J-L. Long-term exposure to chromium(VI) oxide leads to defects in sulfate transport system in Chinese hamster ovary cells. J Cell Biochem 57:655–665 (1995).
16. Sugiyama M. Role of physiological antioxidants in chromium(VI)-induced cellular injury. Free Radic Biol Med 12:397–407 (1992).
17. Wolf TH, Kasemann R, Ottenwalder H. Molecular interaction of different chromium species with nucleotide and nucleic acids. Carcinogenesis 10:655–659 (1989).
18. Singh J, Carlisle DL, Pritchard DE, Paterno SR. Chromium-induced genotoxicity and apoptosis: relationship to chromium carcinogenesis. Oncol Rep 5:1307–1318 (1998).
19. Aiyar J, Berovits 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).
20. Tsou T-C, Chen C-L, Liu T-Y, Yang J-L. Induction of 8-hydroxydeoxyguanosione in DNA by chromium(III) plus hydrogen peroxide and its prevention by scavengers. Carcinogenesis 17:103–108 (1996).
21. Snow ET. A possible role for chromium(III) in genotoxicity. Environ Health Perspect 92:75–81 (1991).
22. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-hydroxydeoxyguanosione, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. J Biol Chem 267:166–172 (1992).
23. Shibutai S, Takeshita M, Grollman AP. Insertion of specific base during DNA synthesis past the oxidation-damaged base 8-oxo-dG. Nature 349:431–434 (1991).
24. Lloyd DR, Carmichael PL, Phillips DH. Comparison of the formation of 8-hydroxy-2-deoxyguanosione and single- and double-strand breaks in DNA mediated by Fenton reaction. Chem Res Toxicol 11:420–427 (1998).
25. Tan D-X, Chen L-D, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocrine J 1:57–60 (1993).
26. Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden LR, Chuang JI, Ortiz GG, Acuna-Castroviejo D. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 18:1–11 (1995).
27. Pieri C, Moroni F, Marra M, Recchioni R, Marcheselli F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci 55:271–276 (1994).
28. Costa EJX, Lopes RH, Lamy-Freund MT. Permeability of pure lipid bilayers to melatonin. J Pineal Res 16:123–126 (1994).
29. Shida CS, Castrucci AML, Lamy-Freund MT. High melatonin solubility in aqueous medium. J Pineal Res 16:198–201 (1994).
30. Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden L, Pablos MI, Tan DX. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J Cell Biochem 53:373–382 (1993).
31. Tan DX, Reiter RJ, Chen LD, Poeggeler B, Manchester LC, Barlow-Walden LR. Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis 15:215–218 (1994).
32. Tang L, Reiter RJ, Li Z-R, Ortiz GG, Yu BP, Garcia JJ. Melatonin reduces the increase in 8-hydroxydeoxyguanosine levels in the brain and liver of kainic acid-treated rats. Mol Cell Biochem 178:299–303 (1998).
33. Sewerynek E, Ortiz GG, Reiter JJ, Pablos MI, Melchiorri D, Daniels WMU. Lipopolysaccharide-induced DNA damage is greatly reduced in rats treated with the pineal hormone melatonin. Mol Cell Endocrinol 117:183–188 (1996).
34. Qi W, Reiter RJ, Tan D-X, Manchester LC, Kim SJ, Garcia JJ. Inhibitory effects of melatonin on ferric nitrilotriacetate-induced lipid peroxidation and oxidative DNA damage in rat kidney. Toxicology 139:81–91 (2000).
35. Vijayalaxmi, Reiter RJ, Herman TS, Meltz M. Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. Mutat Res 397:203–208 (297).
36. Wakatsuki A, Okatani Y, Izumiya C, Ikenoue N. Melatonin protects against ischemia and reperfusion-induced oxidative lipid and DNA damage in fetal rat brain. J Pineal Res 26:147–152 (1999).
37. Shigenaga MK, Aboujaoude EN, Chen Q, Ames BN. Assays of oxidative DNA damage biomarkers 8-oxo-2-deoxyguanosione and 8-oxoguanine in nuclear DNA and biological fluids by high-performance liquid chromatography with electrochemical detection. Methods Enzymol 234:16–33 (1994).
38. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide: general properties and effect of hyperbaric oxygen. Biochem J 134:707–716 (1973).
39. Parshad R, Sanford KK, Jones GM, Tarone RE. Florescent light-induced chromosome damage and its prevention in mouse cells in culture. Proc Natl Acad Sci USA 75:1830–1833 (1978).
40. Lorentzen RJ, Ts'o PO. Benzo[*a*]pyrenedione/benzo[*a*]pyrenediol oxidation-reduction couples and the generation of reactive reduced molecular oxygen. Biochemistry 16:1467–1473 (1977).
41. Simon RH, Scoggin CH, Patterson D. Hydrogen peroxide causes the fatal injury to human fibroblasts exposed to oxygen radicals. J Biol Chem 256:7181-7186 (1981).
42. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: its mechanism and measurement in mammalian systems. FEBS Lett 281:9–19 (1991).
43. Shi X, Dalal NS, Kasprzak KS. Generation of free radicals from hydrogen peroxide and lipid hydroperoxides in the presence of Cr(III). Arch Biochem Biophys 302:294–299 (1993).
44. Shamberger R. Genetic toxicology of ascorbic acid. Mutat Res 133:135–139 (1984).
45. 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).
46. Stearns DM, Courtney KD, Giangrande PH, Phieffer LS, Wetterhahn KE. Chromium(VI) reduction by ascorbate: role of reactive intermediates in DNA damage *in vitro*. Environ Health Perspect 102(suppl 3):21–25 (1994).
47. Shi X, Chiu A, Chen CT, Halliwell B, Castranova V, Vallyathan V. Reduction of chromium(VI) and its relationship to carcinogenesis. J Toxicol Environ Health B Crit Rev 2(1):87–104 (1999).
48. Stasica, P, Ulanski P, Rosiak JM. Melatonin as a hydroxyl radical scavenger. J Pineal Res 25:65–66 (1998).
49. Pahkla R, Zilmer M, Kullisaar T, Rago L. Comparison of the antioxidant activity of melatonin and pinoline in vitro. J Pineal Res 24:96–101 (1998).
50. Tan D-X, Manchester LC, Reiter RJ, Plummer BF, Hardies LJ, Weintraub ST, Vijayalaxmi, Shepherd AMM. A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. Biochem Biophys Res Commun 253:614–620 (1998).
51. Tan DX, Manchester LC, Reiter RJ, Qi W. Unpublished data.
52. Hneihen AS, Standeven AM, Wetterhahn KE. Differential binding of chromium(VI) and chromium(III) complexes to salmon sperm nuclei and nuclear DNA and isolated calf thymus DNA. Carcinogenesis 14:1795-1803 (1993).
53. Limson J, Nyokong T, Daya S. The interaction of melatonin and its precursors with aluminum, cadmium, copper, iron, lead and zinc: an adsorptive voltammetric study. J Pineal Res 24:15–21 (1998).
54. Susa N, Ueno S, Furukawa Y, Ueda J, Sugiyama M. Potent protective effect of melatonin on chromium(VI)-induced DNA single-strand breaks, cytotoxicity, and lipid peroxidation in primary cultures of rat hepatocytes. Toxicol Appl Pharmacol 144:377–384 (1997).
55. Reiter RJ, Tang L, Garcia JJ, Munoz-Hoyos H. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 60:2255–2271 (1997).
56. Reiter RJ, Tan DX, Qi W. Suppression of oxygen toxicity by melatonin. Acta Pharmacol Sinica 19:575–581 (1998).
57. Reiter RJ, Tan DX, Kim SJ, Qi W. Melatonin as a pharmacological agent against oxidative damage to lipids and DNA. Proc West Pharmacol Soc 41:229–236 (1998).
58. Romero P, Osuna C, Garcia-Perganeda A, Carrillo-Vico A, Guerrero JM. The pineal secretory product melatonin reduces hydroxgen peroxide-induced DNA damage in U-937 cells. J Pineal Res 26:227–235 (1999).