Active Oxygen Species in DNA Damage Induced by Carcinogenic Metal Compounds

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Some carcinogenic metal compounds [chromate(VI), Fe(III) nitrilotriacetate, cobalt(II), and nickel(II)] induced formation of various oxygen radical species in the presence of hydrogen peroxide. These oxygen radicals were suggested to give different kinds of site-specific DNA damage; 8-hydroxyl-2'-deoxyguanosine formation is included in the DNA damage. Using pulsed-field gel electrophoresis, nickel sulfide was shown to induce oxidative DNA cleavage in cultured cells. On the basis of these findings, we have emphasized the role of oxygen radicals in metal carcinogenesis. — Environ Health Perspect 102(Suppl 3):17–20 (1994).

Key words: carcinogenic metal, chromate(VI), Fe(III) nitrilotriacetate, cobalt(II), nickel(II), DNA damage, hydrogen peroxide, hydroxyl radical, singlet oxygen, metal-oxygen complex

Introduction

In 1986, we reported that carcinogenic chromate(VI) reacts with (H_2O_2) to produce hydroxyl free radicals (•OH) and singlet oxygen ($^1O_2$), which cause DNA damage (*1*) Since then, we have demonstrated that carcinogenic Fe(III) nitrilotriacetate, cobalt(II), and nickel(II) react with H_2O_2 to produce hydroxy radical, $^1O_2$, and metal-oxygen complexes, which cause site-specific DNA damage (*2–5*). On the basis of these findings, we have proposed that oxygen radicals may contribute to metal carcinogenesis (*6*). In 1989, Sugiyama et al. suggested that chromate(VI) induced DNA single-strand breaks in cultured cells via •OH formation (*7,8*). In addition, Kasprzak et al. reported 8-hydroxyl-2'-deoxyguanosine (8-OH-dG) formation in the kidney of rats treated with nickel acetate (*9*). Some recent studies in our laboratory of the important role of oxygen radicals in metal carcinogenesis are described here.

Materials and Methods

DNA damage was analyzed by the DNA sequencing technique using ^{32}P 5'-end-labeled DNA fragments obtained from the human c-Ha-*ras*-1 protooncogene as previously described (*3,10*). DNA strand breaks in cultured cells were detected by using pulsed-field gel electrophoresis according to the method described previously (*11*). 8-OH-dG formation in DNA was analyzed by high-pressure liquid chromatography-electron capture detector (HPLC-ECD) (*12*). Electron spin resonance (ESR) spectra were measured at room temperature using a JES-FE-3XG spectrometer (JEOL, Tokyo, Japan)(*10*). Chemiluminescence was measured by using the Luminescence Reader (Aloka, Tokyo, Japan) with fluorescein.

This paper was presented at the Second International Meeting on Molecular Mechanisms of Metal Toxiciity and Carcinogeniciity held 10–17 January 1993 in Madonna di Campiglio, Italy.

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Results and Discussion

Chromium(VI)

Carcinogenic chromium(VI) [Cr(VI)] has been reported to induce DNA lesions *in vivo* and in culture (*13*). We investigated reactivities of Cr compounds with DNA by the DNA sequencing technique using ^{32}P 5'-end-labeled DNA fragments. Figure 1 shows piperidine-labile sites of the DNA fragment treated with sodium chromate(VI) plus H_2O_2. Cleavage occurred at every base residue but the cleavage at the guanine positions was more dominant than at the other three bases. ESR studies using 5,5-dimethylpyrroline-*N*-oxide (DMPO) and (4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4-POBN) as •OH traps demonstrated that •OH is generated during the reaction of chromate(VI) with H_2O_2 (*1*). ESR studies using 2,2,6,6-tetramethyl-4-piperidone demonstrated that $^1O_2$ is also generated during the reaction, and reacts specifically with deoxyguanosine monophosphate (dGMP) (*1*). These results indicate that sodium chromate(VI) reacts with H_2O_2 to produce •OH and $^1O_2$; •OH causes every base alteration and deoxyribosephosphate backbone breakage, and $^1O_2$ oxidizes the guanine residues resulting in the formation of alkali-labile sites (*1*).

Aiyar et al. reported the enhancing effect of glutathione (GSH) on Cr(VI)-induced •OH formation (*14*). Shi et al. showed that •OH generated by the Cr(VI)/flavoenzyme/NAD(P)H enzymatic system reacts with 2'-deoxyguanosine to form 8-OH-dG (*15*).

Ferric Nitrilotriacetate

In 1986, Ebina et al. reported the induction of the renal adenocarcinoma in rats by Fe-nitrilotriacetate (Fe-NTA) (*16*). We showed that Fe(III)-NTA catalyzes the decomposition of H_2O_2 to produce •OH, which subsequently causes DNA base alterations and backbone breakages. The DNA damage was without marked site specificity. Dizdaroglu et al. reported that Fe(III)-NTA plus H_2O_2 caused 8-OH-dG formation in isolated chromatin (*17*). Umemura et al. reported formation of 8-OH-dG in rat kidney DNA after IP administration of Fe-NTA (*12*). These studies suggest that Fe-NTA-induced

Table 1. Activities of Fe(III)-chelates of aminopolycarboxylic acids for H_2O_2-dependent DNA damage and the •OH formation from H_2O_2.

Chelator	H_2O_2-dependent DNA damage	Relative catalytic activity in •OH formation, %
NTA	++	100
HEDTA	-	97
EGTA	-	17
EDTA	-	10
CDTA	-	10
DTPA	-	0

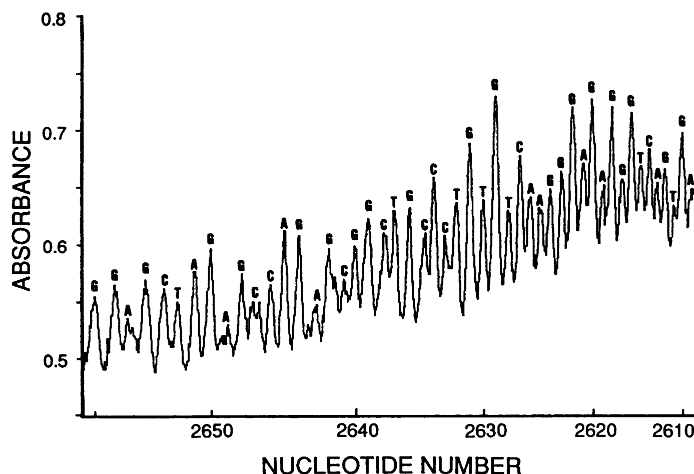

Figure 1. Piperidine-labile sites in DNA fragments treated with chromate(VI) in the presence of H_2O_2. The ^{32}P 5'-end-labeled 337 base-pair fragment of the human c-Ha-*ras*-1 sequence (Pst I 2345 - Ava I* 2681) in 200 µl of 50 mM Tris-HCl buffer, pH 8.0, was incubated with 2.5 mM sodium chromate(VI) in the presence of 25 mM H_2O_2 for 30 min at 37°C. After the piperidine treatment at 90°C for 20 min, DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel. The relative amounts of oligonucleotides produced were measured by a laser densitometer. The alkali-labile sites of the treated DNA were determined by direct comparison with the same DNA fragment after undergoing DNA sequence reaction according to the Maxam-Gilbert procedure. The nucleotide number of the human c-Ha-*ras*-1 protooncogene is shown starting with the BamHI site.

DNA damage *in vivo* is induced by oxygen radicals.

Table 1 summarizes the activities of Fe(III)-chelates of aminopolycarboxylic acids for H_2O_2-dependent DNA damage and for •OH formation from H_2O_2 (*2*). Fe(III)-NTA induced DNA cleavage in the presence of H_2O_2 , whereas Fe(III)-chelates of other aminopolycarboxylic acids did not induce that response under the conditions used. Fe(III)-HEDTA/ H_2O_2 system did not cause DNA damage, although it produced as much •OH as the Fe(III)-NTA/ H_2O_2 system. These results may be interpreted by structural considerations. Fe(III)-NTA is supposed to approach the groove of the DNA double helix readily, whereas Fe(III)-HEDTA may not. Since •OH is short-lived, it damages DNA only when produced in the vicinity of the DNA.

Cobalt(II)

In 1992, The International Agency for Research on Cancer (IARC) estimated that cobalt and cobalt compounds are possibly carcinogenic to humans (group 2B) (*18*). Previously, Costa et al. reported that cobalt sulfide caused DNA single-strand breaks in animal cells in culture (*19*). With isolated DNA, we demonstrated that cobalt(II) [Co(II)] ions caused extensive site-specific damage (G>T~C>A) in the presence of H_2O_2. ESR experiments suggested that reactive oxygen species (probably $^1O_2$ and/or cobalt-oxygen complex) were involved in the DNA damage (*3*).

We measured fluorescein-dependent chemiluminescence induced by Co(II) and H_2O_2. Figure 2 shows that the Co(II)-induced chemiluminescence increased with increasing concentrations of H_2O_2. The intensity was enhanced about 3-fold in D_2O in which the lifetime of singlet oxygen is at least 10 times that in H_2O. These results indicate that $^1O_2$ is generated during the reaction of Co(II) with H_2O_2.

Nickel(II)

Nickel compounds have been shown to have seriously toxic and carcinogenic effects on humans (*20*). Costa and Mollenhauer reported that carcinogenic activity of particulate nickel compounds was proportional to their cellular uptake (*21*). Nickel salts have been shown to cause DNA single strand breaks in cultured cells. Our experiments (*4*) with isolated DNA showed that nickel(II) [Ni(II)] ion caused extensive site-specific damage (C~T~G>A) in the presence of H_2O_2. The incubation of calf thymus DNA with Ni(II) plus H_2O_2 for 6 hr increased the 8-OH-dG level about 10-fold. The result shows that Ni(II) ion reacts with H_2O_2 to produce active species causing oxidative DNA damage. Kasprzak and Hernandez (*22*) reported that addition of Ni(II) doubled the 8-OH-dG formation from double-stranded DNA by H_2O_2 in the presence of ascorbic acid.

We examined by ESR spectroscopy whether activated oxygen species are produced by the reaction of Ni(II) oligopeptides with H_2O_2. Figure 3 shows that the •OH adduct of DMPO was formed by the decomposition of H_2O_2 in the presence of Ni(II) GlyGlyHis. Adducts of DMPO were not observed with either H_2O_2 (Figure 3) or Ni(II) GlyGlyHis (data not shown). It is known that •OH reacts with ethanol and formate to produce α-hydroxyethyl radicals and •CO_2^- radicals, respectively. However, in the case of Ni(II) oligopeptides and H_2O_2, the spin adducts of α-hydroxyethyl radicals and •CO_2^- radicals were scarcely observed although ethanol and formate had inhibitory effects (Figure 3). With mannitol, the spin adducts of mannitol-derived radicals were not observed. In contrast, with sulfur compounds (methional), which can scavenge active species with less reactivity than •OH, the spin adducts of methional-derived radi-

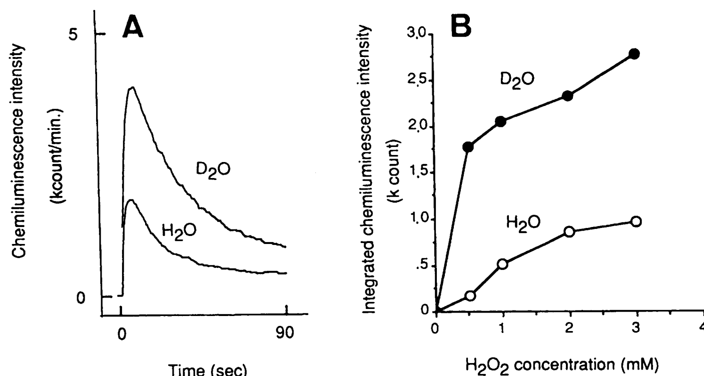

Figure 2. Fluorescein-dependent chemiluminescence induced by Co(II)-H_2O_2 systems. The reaction mixture contained 0.2 mM $CoCl_2$ and 0.1 mM fluorescein in 1 ml of 8 mM sodium phosphate buffer, pH 7.9, in H_2O or D_2O. The reaction was initiated by rapid injection of 2 mM H_2O_2 (A) or indicated concentrations of H_2O_2 (B). The chemiluminescence intensity in (B) was obtained by integration for 90 sec.

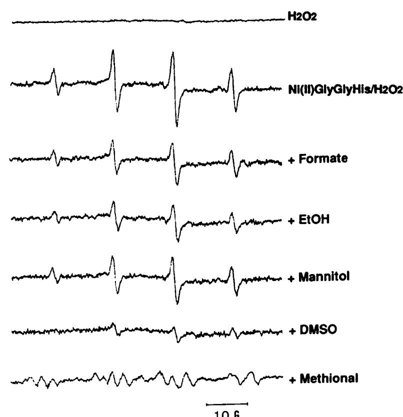

Figure 3. ESR spectra of the •OH adduct of DMPO produced during the incubation of Ni(II)GlyGlyHis with H_2O_2 in the presence of •OH scavengers. Sample (200 µl) contained 0.1 mM Ni(II)GlyGlyHis and 146 mM DMPO in 20 mM sodium phosphate buffer, pH 7.9, containing 5 µM DTPA. Where indicated, 0.1 M sodium formate, 1 M ethanol, 50 mM mannitol, 0.6 M DMSO, or 0.1 M methional was added. After 5 mM H_2O_2 was added, aliquots of the solutions were taken in calibrated capillaries, and ESR spectra were measured at room temperature.

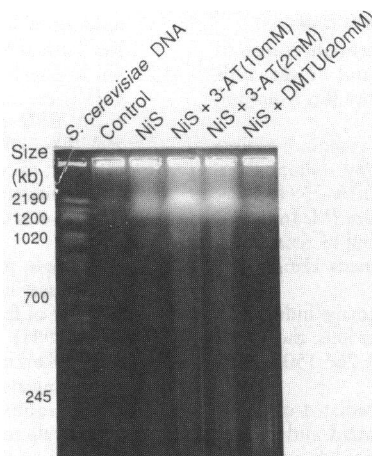

Figure 4. Effects of catalase inhibitor and H_2O_2 scavenger on nickel sulfide-induced cellular DNA damage. Raji cells were treated with 3-AT or DMTU for 1 hr and then exposed to nickel sulfide (10 µg/ml) in RPMI 1640 containing 6% fetal calf serum. After incubation at 37°C for 24 hr, the medium was removed and the cells were washed three times with PBS and prepared into agarose plugs and lysed. Electrophoresis was performed in TBE buffer, pH 8.3, by pulsed field (CHEF-DRII DNA megabase electrophoresis system, Bio-Rad) at 200 volts at 14°C. Switch time was 60 sec for 15 hr followed by a 90-sec switch time for 9 hr. The DNA in the gels was visualized in ethidium bromide.

cals were observed. This result led us to the idea that the •OH adduct is formed in the reaction of nickel-oxygen complex and DMPO (*5*).

In recent years, pulsed-field gel electrophoresis has emerged as a powerful tool for detection of DNA strand breaks in cultured cells. Figure 4 reveals that nickel sulfide induced cellular DNA cleavage. To clarify whether H_2O_2 participates in cellular DNA damage, we examined the effects of 3-aminotriazol (3-AT, a catalase inhibitor) and dimethylthiourea (DMTU, a highly permeable scavenger of H_2O_2) on the DNA double-strand breaks. 3-AT enhanced nickel sulfide-induced DNA damage whereas DMTU inhibited it (Figure 4). These results suggest that H_2O_2 participates in DNA damage induced by nickel sulfide *in vivo.*

In summary, Table 2 outlines the DNA damage induced by some carcinogenic metal compounds via active oxygen species formation *in vitro* and *in vivo.* The metal compounds produced various types of oxygen radicals from H_2O_2. These oxygen radicals seem to be responsible for the metal carcinogenesis.

Table 2. Summary of active oxygen species-mediated DNA damage by carcinogenic metal compounds.

Metal	Reaction		DNA reaction	Reference
Cr	$Cr(VI) + H_2O_2 \rightarrow \cdot OH, {}^1O_2$	→	base oxidation (G>T~C~A)	Kawanishi et al. (*1*)
FeNTA	$Fe(III)NTA + H_2O_2 \rightarrow \cdot OH$	→	base oxidation (G~T~C~A)	Inoue and Kawanishi (*2*)
Ni	$Ni(II)\ GlyGlyHis + H_2O_2$	→	$O_2^{\cdot -}$, crypt-•OH and ${}^1O_2$	Inoue and Kawanishi (*5*)
Co	$Co(II) + H_2O_2 \rightarrow {}^1O_2, [Co^{IV}\text{-}O]^{2+}$	→	base oxidation (G>T~C>A)	Yamamoto et al. (*3*)
Ni	$Ni(II) + H_2O_2 + VC$	→	8-OH-dG	Kasprzak and Hernandez (*22*)
NI	$Ni(II) + H_2O_2 \rightarrow [Ni^{IV}\text{-}O]^{2+}$	→	base oxidation (C~T~G>A)	Kawanishi et al. (*4*)
Cr[a]	VB_2 enhanced Cr(VI)-induced DNA single strand breaks in cells.			Sugiyama et al. (*7*)
Ni[a]	8-OH-dG formation in rat kidney by nickel acetate			Kasprzak et al. (*9*)
FeNTA[a]	8-OH-dG formation in rat kidney by Fe-NTA.			Umemura et al. (*12*)
Cr[a]	$Cr(VI) + GSH/H_2O_2 \rightarrow \cdot OH$	→	8-OH-dG	Aiyar et al. (*14*)
FeNTA[a]	$Fe(III)NTA + H_2O_2$	→	oxidized products in chromatin	Dizdaroglu et al. (*17*)
Cr	$Cr(VI)$ + flavoenzymes/ $NAD(P)H \rightarrow \cdot OH$	→	8-OH-dG	Shi et al. (*15*)

[a] In tissue culture or animal experiment.

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