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-hydroxy-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.

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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 chromium(VI) reacts with (H₂O₂) to produce hydroxyl free radicals (·OH) and singlet oxygen (O₂), which cause DNA damage (1). Since then, we have demonstrated that carcinogenic Fe(III) nitrilotriacetate, cobalt(II), and nickel(II) react with H₂O₂ to produce hydroxy radical, ·OH, 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-hydroxy-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 ³²P 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-3XG spectrometer (JEOL, Tokyo, Japan) (10). Chemiluminescence was measured by using the Luminescence Reader (Aloka, Tokyo, Japan) with fluorescence.

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 ³²P 5'-end-labeled DNA fragments. Figure 1 shows piperidine-labile sites of the DNA fragment treated with sodium chromate(VI) plus H₂O₂. 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-butyl-nitronate (4-POBN) as ·OH traps demonstrated that ·OH is generated during the reaction of chromate(VI) with H₂O₂ (1). ESR studies using 2,2,6,6-tetramethyl-4-piperidone demonstrated that O₂ is also generated during the reaction, and reacts specifically with deoxyguanosine monophosphate (dGMP) (1). These results indicate that sodium chromate(VI) reacts with H₂O₂ to produce ·OH and O₂. ·OH causes every base alteration and deoxyribosephosphate backbone breakage, and ¹⁰⁰

| Chelator | H₂O₂-dependent DNA damage | Relative catalytic activity in ·OH formation, % |
|----------|---------------------------|-----------------------------------------------|
| NTA      | ++                        | 100                                          |
| HEDTA    | -                         | 97                                           |
| EGTA     | -                         | 17                                           |
| EDTA     | -                         | 10                                           |
| CDTA     | -                         | 10                                           |
| DTPA     | -                         | 0                                            |

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DNA damage in vivo is induced by oxygen radicals.

Table 1 summarizes the activities of Fe(III)-chelates of aminopolycarboxylic acids for H$_2$O$_2$-dependent DNA damage and for ·OH formation from H$_2$O$_2$ (2). Fe(III)-NTA induced DNA cleavage in the presence of H$_2$O$_2$, whereas Fe(III)-chelates of other aminopolycarboxylic acids did not induce that response under the conditions used. Fe(III)-HEDTA/H$_2$O$_2$ system did not cause DNA damage, although it produced as much ·OH as the Fe(III)-NTA/H$_2$O$_2$ 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$_2$O$_2$. ESR experiments suggested that reactive oxygen species (probably ·O$_2$ and/or cobalt-oxygen complex) were involved in the DNA damage (3).

We measured fluorescein-dependent chemiluminescence induced by Co(II) and H$_2$O$_2$. Figure 2 shows that the Co(II)-induced chemiluminescence increased with increasing concentrations of H$_2$O$_2$. The intensity was enhanced about 3-fold in D$_2$O in which the lifetime of singlet oxygen is at least 10 times that in H$_2$O. These results indicate that ·O$_2$ is generated during the reaction of Co(II) with H$_2$O$_2$.

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$_2$O$_2$. The incubation of calf thymus DNA with Ni(II) plus H$_2$O$_2$ for 6 hr increased the 8-OH-dG level about 10-fold. The result shows that Ni(II) ions react with H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$. Figure 3 shows that the ·OH adduct of DMPO was formed by the decomposition of H$_2$O$_2$ in the presence of Ni(II) GlyGlyHis$. Adducts of DMPO were not observed with either H$_2$O$_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$_2$H radicals, respectively. However, in the case of Ni(II) oligopeptides and H$_2$O$_2$, the spin adducts of α-hydroxyethyl radicals and ·CO$_2$H 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 3. ESR spectra of the •OH adduct of DMPO produced during the incubation of Ni(II)GlyGlyHis with H$_2$O$_2$ in the presence of •OH scavengers. Sample (200 µl) contained 0.1 mM Ni(II)GlyGlyHis and 148 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 methionol was added. After 5 mM H$_2$O$_2$ 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$_2$O$_2$ 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-soc 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 damage. To clarify whether H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$. 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$_2$O$_2$ → OH, O$_2$              | base oxidation   | Kawanishi et al. (1) |
| FeNTA  | Fe(III)NTA + H$_2$O$_2$ → OH                | base oxidation   | Inoue and Kawanishi (2) |
| Ni     | Ni(II)GlyGlyHis + H$_2$O$_2$ → O$_2$, crypt-OH and $^1$O$_2$ | base oxidation (G-T-C-A) | Inoue and Kawanishi (5) |
| Co     | Co(III) + H$_2$O$_2$ → [O$_2$ (C$_2$N$_2$)O]   | base oxidation   | Yamamoto et al. (3) |
| Ni     | Ni(III) + H$_2$O$_2$ + VC → 8-OH-dG         | base oxidation   | Kasprzak and Hernandez (22) |
| Ni     | Ni(III) + H$_2$O$_2$ → [N$_2$O$_2$]          | base oxidation   | Kawanishi et al. (4) |
| Cr$^a$ | VB$_3$ 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 FeNTA. |                  | Umemura et al. (12) |
| Cr$^a$ | Cr(VI) + GSH/H$_2$O$_2$ → OH                | 8-OH-dG          | Ayar et al. (14)   |
| FeNTA$^a$ | Fe(III)NTA + H$_2$O$_2$ → oxidized products in chromatin | | Dizdaroglu et al. (17) |
| Cr     | Cr(VI) + flavoenzymes/ NAD(P)H → OH        | 8-OH-dG          | Shi et al. (15)    |

$^a$ In tissue culture or animal experiment.

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