Distinct Mechanisms of Oxidative DNA Damage Induced by Carcinogenic Nickel Subsulfide and Nickel Oxides

Shosuke Kawanishi,[1] Shinji Oikawa,[1] Sumiko Inoue,[2] and Kohsuke Nishino[3]

[1]Department of Hygiene, Mie University School of Medicine, Mie, Japan; [2]Department of Public Health, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Department of Food Science and Nutrition, Faculty of Human Life and Science, Doshisha Women's College, Kyoto, Japan

The U.S. National Toxicology Program has shown clear evidence of carcinogenicity of nickel subsulfide (Ni_3S_2) and some evidence of carcinogenicity of NiO (green) in rats. In the present study, DNA damage in cultured cells and in lungs of rats induced by nickel compounds was investigated to clarify the mechanism of nickel carcinogenesis. In cultured HeLa cells, Ni_3S_2 induced a significant increase in 8-hydroxydeoxyguanosine (8-OH-dG) formation, whereas NiO (black), NiO (green), and $NiSO_4$ did not. On the other hand, in rats, intratracheal instillation of all these nickel compounds significantly increased 8-OH-dG content in the lungs. The disparities in DNA damage between cultured cells and animals could be accounted for by two different mechanisms for nickel-induced oxidative DNA damage in lungs of rats. One is direct oxidative DNA damage: Ni(II) enters the cells and then reacts with endogenous and/or nickel sulfide–produced hydrogen peroxide (H_2O_2) to give reactive oxygen species that cause DNA damage. This mechanism is supported by oxidative damage to isolated DNA treated with Ni(II) and H_2O_2. The other mechanism is indirect oxidative DNA damage due to inflammation. This double mechanism for DNA damage may explain the relatively high carcinogenic risk associated with Ni_3S_2. *Key words:* carcinogenesis, DNA damage, inflammation, nickel compounds, reactive oxygen species. *Environ Health Perspect* 110(suppl 5):789–791 (2002).
http://ehpnet1.niehs.nih.gov/docs/2002/suppl-5/789-791kawanishi/abstract.html

A number of metals have carcinogenic and co-carcinogenic potentials. Regarding mechanisms of metal carcinogenesis, we reported that carcinogenic chromate (VI) reacts with hydrogen peroxide (H_2O_2) to produce a hydroxyl free radical and singlet oxygen ($^1O_2$) that cause damage to isolated DNA (*1*). Furthermore, carcinogenic Fe(III) nitrilotriacetate, Co(II), and Ni(II) were shown to react with H_2O_2 to produce a hydroxyl free radical, $^1O_2$, and metal–oxygen complexes that cause site-specific DNA damage (*2–4*). On the basis of these findings, we have emphasized the role of reactive oxygen species (ROS) in metal carcinogenesis (*5*).

The early epidemiologic data indicated different carcinogenic risks from inhalation of different nickel compounds (*6*). The International Agency for Research on Cancer (IARC) has classified nickel compounds such as $NiSO_4$, NiO, nickel hydroxides, and crystalline nickel as agents carcinogenic to humans (Group 1) (*7*). Recently, the U.S. National Toxicology Program has shown clear evidence of carcinogenicity of Ni_3S_2 and some evidence of carcinogenicity of NiO (green) in rats (*8,9*). However, no evidence for carcinogenicity of water-soluble $NiSO_4$ in rats has been provided (*10*), and this result has been inconsistent with the evaluation by IARC.

In this study, to clarify the mechanism of nickel carcinogenesis, we investigate the formation of 8-hydroxydeoxyguanosine (8-OH-dG) in cultured cells and lungs of rats treated with nickel compounds by using electrochemical detector–coupled high-performance liquid chromatography (HPLC–ECD). A characteristic oxidative DNA lesion, 8-OH-dG, has attracted much attention in relation to mutagenesis and carcinogenesis (*11,12*). To clarify the participation of reactive oxygen species in nickel-induced DNA damage, we examined the DNA damage and site specificity of DNA cleavage induced by nickel compounds in the presence of H_2O_2, using ^{32}P–5′-end–labeled DNA fragments.

Materials and Methods

Analysis of 8-OH-dG Formation

Isolated DNA. Calf thymus DNA (100 μM) was incubated with 20 μM $NiSO_4$ and/or 100 μM H_2O_2 in 200 μL of 4 mM phosphate buffer (pH 7.8) at 37°C. After ethanol precipitation, DNA was digested to the nucleosides with nuclease P1 and alkaline phosphatase and measured by HPLC–ECD as described previously (*4*).

DNA of cultured cells. After the addition of nickel compounds suspended or in solution (10 μg/mL), cells were incubated at 37°C for 24 hr and harvested by centrifugation. For isolation of DNA, nucleic acid extraction system (model 341; Gene Pure, Applied Biosystems, Foster City, CA, USA) was used. DNA isolation steps were performed under helium. The content of 8-OH-dG was determined after enzyme digestion of DNA using an HPLC–ECD (*13*).

DNA of lungs. After cutting off the trachea, we gently homogenized half the defrosted lungs in 3 mL of phosphate-buffered saline (PBS) solution with a motor-driven Teflon–glass tissue homogenizer for 30 sec. The nuclei were obtained by centrifugation at 1,750×*g* for 5 min. DNA was isolated from 1/20 volume of suspended nuclei fraction in PBS by the same extraction system used for DNA isolation from cultured cells, and 8-OH-dG was analyzed as described previously (*4,13,14*).

Analysis of DNA Damage Induced by Ni(II) plus H_2O_2

Reaction mixtures containing the ^{32}P-5′-end–labeled DNA fragment, 20 μM/base of calf thymus DNA, 2.5 mM H_2O_2, and 50 μM $NiCl_2$ in 10 mM phosphate buffer (pH 7.8) containing 5 μM diethylenetriamine-*N*,*N*,*N*′,*N*′,*N*′-pentaacetic acid were incubated at 37°C for 60 min. The DNA fragments were treated with piperidine and electrophoresed on a polyacrylamide gel.

Electron Spin Resonance Measurements

The spin adducts were measured using 5,5-dimethylpyrroline-*N*-oxide as hydroxyl radical traps as previously described (*15*). Typical reaction mixture contained oligopeptides, $NiCl_2$, H_2O_2, and 292 mM 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) in 20 mM sodium phosphate buffer at pH 7.9. Spectra were recorded with a microwave power of 16 mW and a modulation amplitude of 1.0 G. Electron spin resonance (ESR) measurements were made at 25°C using a JES-FE-3XG spectrometer (JEOL, Tokyo, Japan) with 100 kHz field modulation.

Results

Formation of 8-OH-dG in Cultured Cells Treated with Nickel Compounds

Table 1 summarizes the amounts of 8-OH-dG in the DNA extracted from HeLa cells treated with nickel compounds. Incubation of cells

This article is part of the monograph *Molecular Mechanisms of Metal Toxicity and Carcinogenicity.*

Address correspondence to S. Kawanishi, Dept. of Hygiene, Mie University School of Medicine, Mie 514-8507, Japan. Telephone: 81 59 231 5011. Fax: 81 59 231 5011. E-mail: kawanisi@doc.medic.mie-u.ac.jp

This work was supported by a research grant from Nickel Producers Environmental Research Association and Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

Received 5 February 2002; accepted 29 May 2002.

with Ni_3S_2 for 24 hr significantly increased 8-OH-dG amounts. NiO (black), NiO (green), and $NiSO_4$ did not cause the increase of 8-OH-dG.

Formation of 8-OH-dG in DNA of Lungs of Rats Treated with Nickel Compounds

Table 1 also shows 8-OH-dG contents in the DNA extracted from lungs of rats treated with nickel compounds. A significant increase of 8-OH-dG was observed in lungs of rats treated with 1 mg of nickel compounds. The order of the increase was Ni_3S_2 > NiO (black) ≈ NiO (green) > $NiSO_4$. Even by $NiSO_4$, 8-OH-dG was increased to twice that of untreated rats. Treatment with 0.5 mg of nickel compounds also increased 8-OH-dG in lungs, with the exception of NiO (green) and $NiSO_4$.

Formation of 8-OH-dG in Calf Thymus DNA Treated with Ni(II) and H_2O_2

Incubation of calf thymus DNA with Ni(II) plus H_2O_2 induced a time-dependent 8-OH-dG increase. The amount of 8-OH-dG increased with increasing H_2O_2 concentration. In contrast H_2O_2 or Ni(II) alone induced little or no 8-OH-dG increase. These results suggest that Ni(II) reacts with H_2O_2 and produces ROS causing oxidative DNA damage.

Table 1. Comparison of formation of 8-OH-dG in HeLa cells and lung of rats treated with nickel compounds.

Nickel compounds	8-OH-dG/dG ×10^5 (exp. no.)	
	HeLa cells[a]	Rat lung[b]
Control	0.94 ± 0.25[c] (17)	0.78 ± 0.51 (9)
Ni_3S_2	1.33 ± 0.23* (6)	2.57 ± 0.87** (3)
NiO (black)	0.93 ± 0.34 (6)	2.33 ± 0.55** (4)
NiO (green)	0.78 ± 0.31 (6)	2.33 ± 0.61** (5)
$NiSO_4$	0.78 ± 0.25 (5)	1.65 ± 0.97* (4)

[a]HeLa cells were treated with nickel compounds (10 μg/mL) for 24 hr. [b]Wistar rats were treated with nickel compounds (1 mg) by intratracheal instillation and sacrified after 48 hr. [c]Mean ± SD. *$p < 0.05$, **$p < 0.01$, compared with control group.

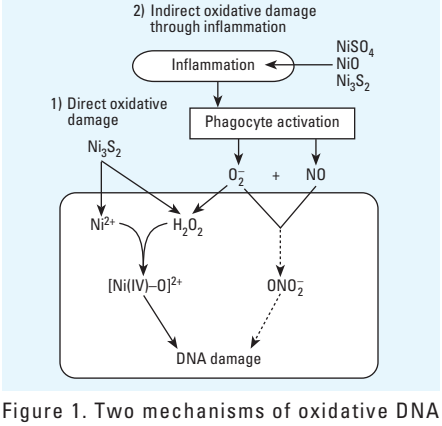

Figure 1. Two mechanisms of oxidative DNA damage induced by nickel compounds.

Effects of Scavengers on DNA Damage Induced by Ni(II) plus H_2O_2

To investigate whether active oxygen species were involved in the DNA damage, the effects of scavengers were examined. In experiments with hydroxyl radical scavengers, 1.4 M dimethyl sulfoxide (DMSO) and 0.4 M sodium formate inhibited the DNA damage considerably, whereas neither 1.4 M ethanol nor 0.4 M mannitol inhibited it. Addition of 0.1 M methional and 0.05 M methionine completely inhibited the DNA damage.

Site Specificity of DNA Damage Induced by the Treatment with Ni(II) plus H_2O_2

To estimate the site specificity of the DNA damage, ^{32}P–5′-end–labeled DNA fragments treated with Ni(II) plus H_2O_2, followed by the piperidine treatment, were electrophoresed and the autoradiogram was obtained. Autoradiograms were scanned with a laser densitometer. The DNA cleavage sites were determined by using the Maxam-Gilbert procedure (*16*). Ni(II) plus H_2O_2 induced piperidine-labile sites frequently at cytosine, thymine, and guanine residues and rarely at adenine residue.

ESR Spectra of the Spin Adducts of DMPO Generated during the Reaction of Ni(II) GlyGlyHis with H_2O_2

The hydroxyl radical adduct of DMPO was observed with 50 μM Ni(II) GlyGlyHis and 5 mM H_2O_2. With increasing concentrations of Ni(II) GlyGlyHis or H_2O_2, the spectrum of DMPO-OOH appeared in addition to that of DMPO-OH. The result suggests that superoxide, as well as hydroxyl radicals, was produced by the decomposition of H_2O_2 in the presence of Ni(II) GlyGlyHis. The coordination environment in Ni(II) GlyGlyHis may be suitable for the production of superoxide from H_2O_2.

Discussion

The present study and our previous data (*4*) have shown that Ni(II) ion binds to DNA and subsequently reacts with H_2O_2 to cause strong DNA damage. Although Ni(II) plus H_2O_2 could induce DNA damage at thymine and cytosine residues, there remains a possibility that certain base damage might be underrepresented depending on the base's sensitivity to piperidine. For example, 8-OH-dG-containing oligonucleotide is not cleaved by treatment with piperidine (*17*). It is concluded that Ni(II) plus H_2O_2 causes not only oxidation of thymine and cytosine residues but also 8-OH-dG formation. The oxidized nucleoside 8-OH-dG is a valid biomarker of the rate of oxidative DNA damage with a mechanistic relationship to carcinogenesis.

To examine what kind of ROS cause DNA damage, effects of various scavengers on DNA damage by Ni(II) plus H_2O_2 were examined. Among hydroxyl radical scavengers, DMSO, methionine, methional, and sodium formate inhibited DNA cleavage, whereas ethanol and mannitol did not. These observations suggest a possibility that hydroxyl free radicals are generated in close association with DNA such that ethanol and mannitol cannot remove them. Furthermore, ESR studies using spin traps revealed that hydroxyl radical adducts are produced by the decomposition of H_2O_2 in the presence of Ni(II) oligopeptides (*15*). These results support the speculation that reactive nickel–oxygen complexes participate in the DNA damage.

Treatment of cultured HeLa cells with Ni_3S_2 (10 μg/mL) induced large increases in 8-OH-dG, whereas NiO (black), NiO (green), and $NiSO_4$ did not enhance the production of 8-OH-dG. However, high concentrations of nickel in the lungs of rats via intratracheal instillation of Ni_3S_2, NiO (black), NiO (green), and $NiSO_4$ induced increases in 8-OH-dG. Also, light microscopy demonstrated that in addition to Ni_3S_2, NiO (black), NiO (green), and $NiSO_4$ induced inflammatory reactions. It is reported that some carcinogenic metal sulfide particles were found to stimulate polymorphonuclear leukocytes to produce ROS (*18*). Therefore, it is considered that the nickel compounds used in this study can induce indirect damage through inflammation.

Disparities in DNA damage between cultured cells and animals could be accounted for by two different mechanisms for nickel-induced oxidative DNA damage in lungs of rats (Figure 1). One is the direct oxidative DNA damage; that is, Ni(II) that has entered the cells reacts with endogenous and/or nickel sulfide-produced H_2O_2 to give ROS that cause DNA damage, as was seen in cultured cells. The reactive species may be an oxo-Ni(IV) complex or an Ni(III)–peroxide complex, which can release hydroxyl radicals. This idea is supported by our previous study and the studies of others (*15,19,20*).

The other mechanism is indirect oxidative DNA damage due to inflammation. The important sources of endogenous oxygen radicals are phagocytic cells such as neutrophils and macrophages (*21*). It has been proposed that ROS including nitrogen oxide generated in inflamed tissues can cause injury to target cells and also damage DNA, which contributes to carcinogenic processes (*22–24*).

Based on the above overall model, two mechanisms for nickel-induced oxidative DNA damage have been proposed as follows: all the nickel compounds tested induced indirect damage through inflammation, and Ni_3S_2 also showed direct oxidative DNA damage

through H_2O_2 formation. This double action may explain the relatively high carcinogenic risk associated with Ni_3S_2.

References and Notes

1. Kawanishi S, Inoue S, Sano S. Mechanism of DNA cleavage induced by sodium chromate(VI) in the presence of hydrogen peroxide. J Biol Chem 261:5952–5958 (1986).
2. Inoue S, Kawanishi S. Hydroxyl radical production and human DNA damage induced by ferric nitrilotriacetate and hydrogen peroxide. Cancer Res 47:6522–6527 (1987).
3. Yamamoto K, Inoue S, Yamazaki A, Yoshinaga T, Kawanishi S. Site-specific DNA damage induced by cobalt(II) ion and hydrogen peroxide: role of singlet oxygen. Chem Res Toxicol 2:234–239 (1989).
4. Kawanishi S, Inoue S, Yamamoto K. Site-specific DNA damage induced by nickel(II) ion and hydrogen peroxide. Carcinogenesis 10:2231–2235 (1989).
5. Kawanishi S, Inoue S, Yamamoto K. Hydroxyl radical and singlet oxygen production and DNA damage induced by carcinogenic metal compounds and hydrogen peroxide. Biol Trace Elem Res 21:367–372 (1989).
6. Costa M, Sutherland JE, Peng W, Salnikow K, Broday L, Kluz T. Molecular biology of nickel carcinogenesis. Mol Cell Biochem 222:205–211 (2001).
7. IARC Working Group. Nickel and nickel compounds. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 49. Lyon, France: International Agency for Research on Cancer, 1990;257–446.
8. NTP. Toxicology and Carcinogenesis Studies of Nickel Subsulfide in F344/N Rats and B6C3F_1 Mice. Tech Rep NTP Tech Rep 453. NTIS publ no. PB97–116784. Research Triangle Park, NC:National Toxicology Program, 1996.
9. NTP. Toxicology and Carcinogenesis Studies of Nickel Oxide in F344/N Rats and B6C3F_1 Mice. NTP Tech Rep 451. NTIS publ no. PB97–116701. Research Triangle Park, NC:National Toxicology Program, 1996.
10. NTP. Toxicology and Carcinogenesis Studies of Nickel Sulfate Hexahydrate in F344/N Rats and B6C3F_1 Mice. NTP Tech Rep 454. NTIS publ no. PB97–120216. Research Triangle Park, NC:National Toxicology Program, 1996.
11. Bruner SD, Norman DP, Verdine GL. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. Nature 403:859–866 (2000).
12. Miller H, Prasad R, Wilson SH, Johnson F, Grollman AP. 8-OH-dGTP incorporation by DNA polymerase beta is modified by active-site residue Asn279. Biochemistry 39:1029–1033 (2000).
13. Kawanishi S, Inoue S, Oikawa S, Yamashita N, Toyokuni S, Kawanishi M, Nishino K. Oxidative DNA damage in cultured cells and rat lungs by carcinogenic nickel compounds. Free Radic Biol Med 31:108–116 (2001).
14. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 387:147–163 (1997).
15. Inoue S, Kawanishi S. ESR evidence for superoxide, hydroxyl radicals and singlet oxygen produced from hydrogen peroxide and nickel(II) complex of glycylglycyl-L-histidine. Biochem Biophys Res Comm 15:445–451 (1989).
16. Maxam AM, Gilbert W. Sequencing end-labeled DNA with base specific chemical cleavages. Methods Enzymol 65:499–560 (1980).
17. Cullis PM, Malone ME, Merson-Davies LA. Guanine radical cations are precursors of 7,8-dihydro-8-oxo-2′-deoxyguanosine but are not precursors of immediate strand breaks in DNA. J Am Chem Soc 118:2775–2781 (1996).
18. Frenkel K. Carcinogen-mediated oxidant formation and oxidative DNA damage. Pharmacol Ther 53:127–166 (1992).
19. Bal W, Kozlowski H, Kasprzak KS. Molecular models in nickel carcinogenesis. J Inorg Biochem 79:213–218 (2000).
20. Chakrabarti SK, Bai C, Subramanian KS. DNA-protein crosslinks induced by nickel compounds in isolated rat lymphocytes: role of reactive oxygen species and specific amino acids. Toxicol Appl Pharmacol 170:153–165 (2001).
21. Grisham MB, Jourd'heuil D, Wink DA. Chronic inflammation and reactive oxygen and nitrogen metabolism—implications in DNA damage and mutagenesis [Review]. Aliment Pharmacol Ther 14 (suppl 1):3–9 (2000).
22. Ducrocq C, Blanchard B, Pignatelli B, Ohshima H. Peroxynitrite: an endogenous oxidizing and nitrating agent. Cell Mol Life Sci 55:1068–1077 (1999).
23. Chazotte-Aubert L, Oikawa S, Gilibert I, Bianchini F, Kawanishi S, Ohshima H. Cytotoxicity and site-specific DNA damage induced by nitroxyl anion (NO^-) in the presence of hydrogen peroxide. Implications for various pathophysiological conditions. J Biol Chem 274:20909–20915 (1999).
24. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van der Vliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. Nature 391:393–397 (1998).