The Role of Nickel and Nickel-mediated Reactive Oxygen Species in the Mechanism of Nickel Carcinogenesis

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Increasing evidence demonstrates that reactive oxygen species (ROS) are implicated in metal carcinogenesis. Exposure of cultured Chinese hamster ovary (CHO) cells to several nickel compounds, i.e., NiS, Ni₃S₂, NiO (black and green), and NiCl₂ has been shown to increase oxidation of 2',7'-dichlorofluorescein to the fluorescent 2',7'-dichlorofluorescein (DCF), suggesting that nickel compounds increased the concentration of oxidants in CHO cells. This fluorescence can be attenuated by addition of exogenous catalase to the extracellular media, indicating that H₂O₂ is one of the formed oxidants in this system. Fluorimetric measurements of chromogens following thiobarbituric acid reaction showed that nickel compounds also induce lipid peroxidation with a decreasing potency NiS, Ni₃S₂>black NiO>green NiO>NiCl₂. These results suggest that lipid hydroperoxides may also be produced through the action of nickel in intact cells. MgCl₂, an antagonist of Ni-induced DNA strand breaks and cell transformation, has no effect on the formation of DCF fluorescence induced in CHO cells by nickel. The results suggest that nickel is an active inducer of ROS in intact mammalian cells and that the molecular mechanism of nickel carcinogenesis may involve multiple steps of nickel-mediated ROS. — Environ Health Perspect 102(Suppl 3):281–284 (1994).

Key words: nickel, reactive oxygen species, dichlorofluorescein, lipid peroxidation, fluorescence, magnesium

Introduction

It is becoming apparent that reactive oxygen species (ROS) play an important role in the etiology of diverse human pathologies such as carcinogenesis (1,2), irradiation injury (3), and tumor promotion (4) as well as the normal process of aging (5). ROS, such as hydroxyl radical or metal-oxo, and their subsequent reaction products, may be responsible for single-strand breaks in cellular DNA, as well as oxidation of DNA bases, chromosomal aberrations, and DNA-protein cross-links (2,6).

Increasing experimental evidence suggests that nickel-induced genotoxicity may also be mediated by oxygen radical intermediates (7–10). Ni compounds have been shown, for example, to produce reactive oxygen species through the interactions of nickel ions with protein ligands, such as the imidazole nitrogen of histidine (11).

Incubation of calf thymus DNA with Ni₃S₂ or NiCl₂ in the presence of H₂O₂ or ascorbate leads to the formation of oxidative base damage (8-OHdG) and depurination of the DNA (12). When isolated human chromatin was treated with Ni(II) in the presence of H₂O₂, several oxidative DNA base modifications were demonstrated (13). Lipid peroxidation has also been reported to occur in lung tissue due to activation of alveolar macrophages after parenteral injection of NiCl₂ in rats (14).

Although Ni can lead to oxidation of DNA bases in vitro, direct evidence for Ni-induced oxidation in intact cells has only recently been reported (15). We found that Ni₃S₂ and NiCl₂ caused increased oxidation of nonfluorescent 2',7'-dichlorofluorescein (DCFH) that resulted in formation of a measurable fluorescent product in intact Chinese hamster ovary (CHO) cells (15). Only very strong oxidants, such as H₂O₂ and organic hydroperoxides can oxidize DCFH, suggesting that Ni elevated the level of these oxidants in intact mammalian cells. In the present study, we compared the effect of exogenous catalase or MgCl₂ on the capacity of several different Ni compounds to induce oxidation of DCFH. The capacity of these Ni compounds to induce lipid peroxidation was also studied.

Figure 1. The effect of catalase (Cat) on formation of dichlorofluorescein (DCF) induced by Ni₃S₂. CHO cells were simultaneously treated with Ni₃S₂ (10 μg/cm²) and Cat (130 U/ml) for 6 hr. The data were obtained from three independent experiments and are expressed as the mean ± SE.
Materials and Methods

Assay for Dichlorofluorescein

The principle of the assay is based upon the cellular uptake of nonpolar dichlorofluorescein diacetate (DCF-dAc, Kodak, Rochester, NY), which is then hydrolyzed to a polar product (DCFH) that is trapped within the cells (16). The optimal conditions for this assay with intact CHO cells were previously reported (15). Briefly, CHO cells were seeded at 3 x 10^4 cells per 60 mm dish in 5 ml of complete a-minimum essential medium (α-MEM, JRH Biosciences, Lenexa, KS) containing 10% fetal bovine serum (Sigma, St. Louis, MO) and grown in an atmosphere of 95% air, 5% CO_{2} at 37°C. After 2 days, the cells were pretreated with NiO (INCO, Ontario, Canada) or NiS (Alfa, Johnson-Mathey, Danver, MA) for 4 hr, then incubated for an additional hour with DCF-dAc. Ni particle doses were expressed as microgram per surface area of the culture dish (cm²) since at these exposure conditions the particles exhibited limited dissolution. Following treatment, cells were washed twice with ice-cold phosphate buffer solution (PBS), then collected with a rubber policeman. The cells were resuspended for measurements of fluorescence in warmed PBS at a final density of 1 x 10^6 cells/ml. The fluorescence was monitored on a Spex Fluorolog (Edison, NJ), with an excitation wavelength of 502 nm (bandwidth 1.8 nm) and an emission wavelength of 522 nm (bandwidth 4.5 nm). Fluorescence intensity is presented as the actual meter reading divided by 1000.

To study the effects of catalase (Sigma), CHO cells were treated with catalase plus NiS_{2} (INCO); (Alfa, Johnson-Mathey). Two treatment protocols were followed for the MgCl_{2} studies. CHO cells were either pretreated with MgCl_{2} (Fisher, Fair Lawn, NJ), then incubated with NiCl_{2}, or were simultaneously treated with MgCl_{2} and NiCl_{2}. In all cases, DCF-dAc was added to the medium 1 hr before the end of the treatment (see above).

Lipid Peroxidation

The levels of lipid peroxide in the nickel-treated CHO cells were measured by thiobarbituric acid (TBA) reactions, as previously described by Ohkawa et al. (17). CHO cells were seeded at 5 x 10^5 cells in 100 mm dishes in 10 ml of complete α-MEM medium. The next day, cells were treated with several Ni compounds for 24 hr, washed twice in ice-cold PBS and scraped with a rubber policeman. Cells were resuspended in PBS at 10^7 cells/ml and frozen at -70°C overnight.

After thawing 0.5 ml of the frozen cell suspension was mixed with 0.16 ml of 10% SDS (Bio Rad, Richmond, CA) and 3 ml of 0.4% TBA (Sigma) in 10% acetic acid (Fisher), pH 5. The mixture was adjusted to 4.0 ml with distilled water, and then heated to 90°C for 60 min. After cooling, 4 ml of butanol were added and the mixture was shaken vigorously. After centrifugation at 1500 rpm for 15 min, the fluorescence of TBA-related chromogens in the butanol phase was measured at an excitation wavelength of 515 nm (bandwidth 4.5 nm), and an emission wavelength of 553 nm (bandwidth 9.0 nm).

Results and Discussion

Effects of NiO and NiS on the Formation of DCF in Intact CHO Cells

Table 1 shows the effects of NiO (black and green) and NiS on the formation of DCF in intact CHO cells. Similar effects with NiS_{2} and NiCl_{2} were previously reported (15). The increased DCF fluorescence intensities induced by NiO and NiS were dose dependent. The fluorescence increase induced by green NiO was less than that induced by the same dose range of black NiO. At higher doses of NiO (green) the fluorescence intensity decreased. Longer treatment (18 hr) of CHO cells with green NiO (doses ranging from 4-16 Mg/cm²) did not induce any increase of the observed fluorescence (data not shown). The difference between the capacity of different doses of black and green NiO to induce DCF fluorescence in intact CHO cells is not completely understood but may be related to differences in

Table 1. The effect of NiO and NiS on formation of DCF in intact CHO cells.

| Dose, Mg/cm² | NiS | NiO black | NiO green |
|-------------|-----|-----------|-----------|
| 5           | 129.8 ± 5.6 | 104.1 ± 3.9 | 130.7 ± 7.2 |
| 10          | 146.7 ± 7.8 | 122.8 ± 2.6 | 126.5 ± 4.2 |
| 15          | 189.3 ± 5.6 | 133.2 ± 4.7 | 121.1 ± 4.4 |
| 20          | 172.4 ± 9.9 | 148.1 ± 5.3 | 96.7 ± 5.0 |

CHO cells were treated with NiO (black and green) and NiS for 5 hr, then incubated with DCF-dAc for 1 hr. Control cells received everything except nickel. The data are obtained from three independent experiments and are expressed as the mean ± SE.
Effects of Catalase and MgCl$_2$ on Oxidation of DCFH by NiCl$_2$ or Ni$_3$S$_2$$^2$.

The effects of catalase on the formation of fluorescent DCFH induced by Ni$_3$S$_2$ are shown in Figure 1. Incubation with 10 µg/cm$^2$ of Ni$_3$S$_2$ (6 hr) increased the level of oxidants in the intact CHO cells as previously reported (15). These doses were not very toxic as measured by plating efficiency (20). Catalase attenuated the fluorescence induced by Ni$_3$S$_2$ but had no effect on the control fluorescence level. These results show that H$_2$O$_2$ was one of the oxidants induced by nickel compounds. Although catalase cannot enter CHO cells, intracellular H$_2$O$_2$ can easily escape through the cell membrane and subsequently be decomposed by the catalase present in the extracellular medium.

Magnesium has been shown to be a potent inhibitor of nickel-induced cell transformation in vitro and reduces nickel-induced carcinogenesis in mice (21,22). Figure 2 shows that MgCl$_2$ had no effect on the formation of oxidized DCF in intact CHO cells, even at a concentration of 20 mM for 5 hr. Magnesium only slightly inhibited nickel uptake by lung tissue (22,23).

Effects of Nickel Compounds on Lipid Peroxidation

Although strong oxidants such as hydroxyl radicals or metal oxo have been implicated as genotoxic, lipid peroxidation products such as malondialdehyde may also be mutagenic and carcinogenic (24). The effects of NiS, Ni$_3$S$_2$, and NiCl$_2$ on the formation of TBA-related chromogens in intact CHO cells is shown in Figure 3. After 24-hr treatments, relatively water insoluble NiS and Ni$_3$S$_2$ induced lipid peroxidation whereas NiCl$_2$ did not. Figure 4 shows that black and green NiO induced a dose-dependent formation of TBA-related chromogens. The capacity of nickel compounds to induce lipid peroxidation can be classified by decreasing potency: NiS, Ni$_3$S$_2$>black NiO>green NiO>NiCl$_2$. This classification of the nickel compounds is also consistent within the results of cell transformation studies (6). The results indicate that lipid peroxides may play an important role in nickel carcinogenesis, although other studies in rat liver showed that lipid peroxidation was not causally related to genetic damage, as detected by DNA strand breakage (25).

Summary

In summary, the exposure of intact CHO cells to different Ni compounds resulted in increased formation of oxidants as detected by DCF formation. Both H$_2$O$_2$ and lipid hydroperoxides are induced by these Ni compounds. The increase of DCF fluorescence required only 3-hr exposures and the fluorescence induced by Ni$_3$S$_2$ could be attenuated by catalase. Lipid peroxidation was studied after 24-hr treatments with the Ni compounds. In contrast to insoluble nickel, highly water-soluble NiCl$_2$ did not induce lipid peroxidation in CHO cells. Our results show that Ni is an active inducer of ROS in intact mammalian cells and that nickel carcinogenesis may involve multiple types of oxidative damage.

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