

# Toxicological Interactions between Nickel and Radiation on Chromosome Damage and Repair

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Carcinogenic nickel compounds are usually found to be weak mutagens; therefore these compounds may not exert their carcinogenic activity through conventional genotoxic mechanisms. On the other hand, the activities of many nickel compounds have not been adequately investigated. We evaluated the genotoxic activities of nickel acetate using conventional chromosome aberration and sister chromatid exchange assays and found that there was no increase of chromosome aberrations or sister chromatid exchanges, although the highest dose (1000  $\mu$ M) caused mitotic inhibition. In addition, we investigated its effect on DNA repair using our challenge assay. In this assay, lymphocytes were exposed to 0.1 to 100  $\mu$ M nickel acetate for 1 hr during the G0 phase of the cell cycle. The cells were washed free of the chemical and, 1.5 hr later, were irradiated with two doses of  $\gamma$ -rays (75 cGy per dose separated by 60 min). A significant dose-dependent increase of chromosome translocations was observed ( $p < 0.05$ ). The increase is more than expected based on additive effects from exposure to nickel or  $\gamma$ -rays individually. In contrast to the increase of chromosome translocations, there was no increase in chromosome deletions, although there was a nickel dose-dependent reduction of mitotic indices. Our data suggest that pretreatment with nickel interferes with the repair of radiation-induced DNA damage and potentially cause mistakes in DNA repair. Furthermore, we suggest that nickel-induced abnormal DNA repair may be a mechanism for its carcinogenic properties. The DNA repair problems that we observed after exposure to low doses of nickel may be viewed as a type of adaptive response. Contrary to some investigators who showed that adaptive responses may be beneficial, our data indicated that some responses may cause more problems than expected. — Environ Health Perspect 102(Suppl 9):73–77 (1994)

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## Introduction

It is well recognized by scientists and by knowledgeable lay people that many of our long-term health problems are due to chronic exposure to low doses of varying hazardous agents, including cigarette smoke and occupational exposures to known carcinogens (1,2). These exposures may induce detectable genetic alterations in the exposed populations, e.g., chromosome aberrations (3), which are indicative of long-term consequences. On the other

hand, the exposure may not cause detectable genetic damage although the exposed populations may be at risk for adverse health outcomes. The latter phenomenon requires special attention and requires studies to elucidate further the mechanisms for induction of health effects. One such mechanism is the induction of DNA repair problems. Defects in DNA repair may cause cells to make serious mistakes from repairing innocuous DNA lesions to generating multiple genetic damage, such as chromosome instability. Such DNA repair errors allow genetic alterations relevant to the development of cancer to evolve. Therefore, defects in DNA repair can cause genetic instability and subsequently health effects.

Carcinogenesis is a complicated process involving multiple and, often, sequential genetic alterations (4,5) so that errors in DNA repair may lead ultimately to carcinogenesis. Using an *in vivo/in vitro* mouse mammary tumor model, we have documented that the following sequence of events occurred before the cells became tumorigenic: chromosome instability, evolution of cells with stabilized but altered karyotypes, inactivation of the RB tumor suppressor gene and amplification of the *c-myc* oncogene (6–8). We hypothesize that the induced chromosome instability is due

to induction of DNA repair problems and we have recently developed a challenge assay to detect this phenomenon (9). In this report, the activity of a nongenotoxic carcinogen, nickel, is presented.

Workers exposed to nickel compounds have a significantly increased risk for the development of lung cancers; therefore, these compounds are classified as human carcinogens (10–12). The properties of some of these compounds have been studied extensively and these findings have been summarized in several recent reviews (13–15). Among the different kinds of nickel compounds, insoluble compounds are usually more carcinogenic than the soluble ones. Although their carcinogenic properties are well documented, the mechanisms for these activities are not established yet. Due to the problems of establishing dosimetry for insoluble compounds, many of the genotoxic studies have been conducted with soluble ones. These reports indicate that most of the carcinogenic nickel compounds are inactive or weakly mutagenic in standard genotoxic assays.

Biochemical studies have documented that nickel can bind to DNA and protein in cells *in vitro* (16,17) and to chromatin *in vivo* (18). Such binding to cellular macromolecules is correlated with the compounds' ability to interfere with DNA syn-

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thesis (16,19). Nickel compounds can induce slight increases in chromosome aberrations (16,20) and sister chromatid exchanges (21,22), and tend to be weak mutagens (21,23).

Since nickel compounds are not strong mutagens by themselves, some investigators feel that they may be potent co-mutagens. In fact, nickel compounds can enhance the induction of mutations by methylmethane sulfonate in bacteria (24). In mammalian cells, they can enhance the induction of mutation by ultraviolet light (21), single stranded DNA breaks by X-rays, and cell transformation by benzo[*a*]pyrene (25). This enhancement activity of nickel compounds may be due to their effect on DNA repair. On the other hand, no enhancement effect was detected in a CHO/HGPRT gene mutation assay (26). Nevertheless, the mechanisms for the potential synergistic effects need to be elucidated.

We have systematically investigated the cytogenetic effects of a soluble nickel compound, nickel acetate. In addition, we have used our challenge assay to elucidate cytogenetically its effects on DNA repair. We have chosen to study nickel acetate because it is a soluble carcinogenic compound (27-30) and its clastogenic effects have not been adequately investigated.

The challenge assay is based on our hypothesis that chemicals that can bind to cellular macromolecules are able to interfere with normal cellular functions such as DNA repair processes and can cause mistakes in DNA repair (9). The assay is conducted by exposing cells first to the target chemical (pulse-treatment) and then to ionizing radiation. These cells are therefore challenged to repair the radiation-induced DNA lesions after chemical exposure so that repair occurs in the absence of the chemical. If pretreatment with the target chemical causes errors in the repair process, mistakes in repair would occur. One type of such mistakes is the rejoining of DNA fragments to the wrong DNA molecules which leads to the formation of rearranged chromosomes. Since radiation-induced DNA damage is repaired within a very short time (during the G0 phase of the cell cycle in our protocol), the observed abnormal chromosomes are, therefore, not caused by replication errors. Thus, we interpret our assay data to indicate DNA repair problems.

The feasibility of the challenge assay has been tested with lymphocytes from cigarette smokers. We observed that cells from smokers have significantly more dicentric chromosomes than those from nonsmokers after the challenge with X-rays (31). The

most effective protocol for detecting the difference was found to be the exposure of lymphocytes to a double dose of 100 cGy of X-rays each separated by 60 min. Therefore, the double exposure protocol was used in this study. Furthermore, the exposure doses were reduced from 100 cGy to 75 cGy per dose since the detection of effects using low doses is more relevant than those with higher doses. In this study, we found that nickel acetate does not cause chromosome aberrations or sister chromatid exchanges in human lymphocytes even when cytotoxic doses are used. On the other hand, this compound does induce abnormal DNA repair.

## Materials and Methods

### Source of Lymphocytes and Blood Culture Procedures

Blood samples are obtained from normal volunteers who are healthy and not occupationally exposed to known hazardous agents. Most of the experiments are conducted with blood samples from two male donors. The samples are collected by venipuncture in the presence of sodium heparin as anticoagulant. After collection of samples, whole blood cultures are set up using phytohemagglutinin as mitogen and RPMI 1640 culture medium according to the techniques established in our laboratory (31). The only exception is that autologous plasma from each donor is added to each culture to enhance cell growth (0.25 ml per 10 ml culture).

### Chemicals

Nickel acetate is purchased from Alfa Products (Danvers, MA). An appropriate amount of the compound is weighed out for use in each experiment. The compound is dissolved in distilled water, filter sterilized and diluted with sterile distilled water to the desired concentrations.

### Irradiation Conditions

Blood cultures are irradiated in a Mark I Cesium-137 Pneumatic Irradiator which is located in the Department of Radiation Therapy. The dose rate is set at 80 cGy/min.

For irradiation, tubes containing blood cultures are loaded onto a styrofoam-type holder which can receive six tubes for irradiation at one time. The holder is placed in the center of the cavity of the irradiator and on top of a rotating platform turning with a speed of approximately 10 revolutions/min.

### Treatment Conditions

Cells are either treated during the G0 or the G1/S phases of the cell cycle. For treat-

ment during the G0 phase, cells are treated with chemicals and/or radiation before cultures are set up. For treatment at the G1/S phases, cells are treated at 24 hr after initiation of cell cultures. Each culture is treated with 0.1 ml of the appropriate concentrations of nickel acetate.

**Chromosome Aberrations and Sister Chromatid Exchanges.** For treatment at the G0 phase of the cell cycle, cells are treated with chemicals for 1 hr, washed free of the chemicals (twice with excess amount of Hank's Ca<sup>++</sup> and Mg<sup>++</sup> free balanced salt solution), and then cultures are initiated. At 16 to 24 hr after initiation, bromodeoxyuridine (BrdU) is added to each culture to achieve a final concentration of 5  $\mu$ M. At 52 hr after initiation of cultures, cells are harvested, stained with fluorescent-plus-Giemsa technique and analyzed for the presence of chromosome aberrations according to our standardized technique (31). Two experiments are performed and 100 cells per experiment are analyzed for the presence of chromosome aberrations.

For treatment at the G1/S phases of the cell cycle, cells are exposed to different concentrations of the chemical from 28 hr until harvest time. BrdU is added to cultures at the same time. At 52 hr after initiation of cell cultures, cells are harvested for chromosome aberration and at 72 hr for sister chromatid exchange analyses. Three experiments are performed and 50 cells per experiment are analyzed for chromosome aberrations and for sister chromatid exchanges.

### Challenge Assay for DNA Repair.

Cells are treated with different concentrations of the chemical at the G0 phase of the cell cycle for 1 hr and washed free of the chemical as described earlier. At 1.5 hr after termination of treatment with chemical, cells are irradiated with two doses of  $\gamma$ -rays (75 cGy per dose and separated by 60 min). The radiation doses for this experiment are less than those of our earlier experiment (31) because our emphasis is to identify biological responses using as reduced doses of chemical and radiation as possible. Furthermore,  $\gamma$ -rays instead of X-rays are used because the former machine is more readily available for us to conduct studies. Furthermore, the biological effects of these two kinds of radiation are very similar. Cell cultures are initiated after the completion of irradiation. At 52 hr after initiation, cells are harvested for chromosome aberration analyses. Four experiments are performed and approximately 50 cells are analyzed from each experiment for a total of 200 cells for each treatment condition. Due to the demand for many cultures

**Table 1.** Induction of chromosome abnormalities after exposure to nickel acetate in human lymphocytes.

Concentration, (μM)	G0 exposure <sup>a</sup>			G1/S exposure <sup>a</sup>		
	No. cells analyzed	% chromatid breaks, ± SEM	No. cells analyzed	% chromatid breaks, ± SEM	No. cells analyzed	Mean SCE per cell, ± SD
0	200	0.5(0.4)	150	0	150	5.2(0.5)
0.1	NA	—	150	0	150	4.9(0.3)
1.0	200	1.0(0.6)	150	0.3(0.3)	150	4.9(0.2)
10	200	1.0(0.6)	150	1.0(0.3)	150	5.0(0.4)
100	200	0	150	0	150	4.9(0.1)
1000	NA	—	0	MI	0	MI

Abbreviations: NA, not analyzed; MI, mitotic inhibition. <sup>a</sup>Lymphocytes were exposed to the chemical for 1 hr before initiation of culture (G0) or from 28 hr until harvest time (G1/S). Chromosome-type abnormalities are rarely observed.

**Table 2.** Induction of chromosome translocations by a combination of nickel acetate followed by γ-rays.<sup>a</sup>

Concentration, μM	γ-rays, cGy	Dicentric frequencies per 100 cells (no. cells analyzed)			
		Experiment 1	Experiment 2	Experiment 3	Experiment 4
0	75 + 75	14.0 (50)	14.0 (50)	16.0 (50)	22.0 (50)
0.1	75 + 75	12.0 (50)	13.0 (70)	10.0 (30)	20.0 (50)
1.0	75 + 75	20.0 (50)	17.6 (34)	22.7 (66)	26.0 (50)
10	75 + 75	25.0 (64)	19.4 (36)	32.5 (40)	20.0 (60)
100	75 + 75	23.7 (59)	12.2 (41)	23.8 (21)	19.0 (79)

<sup>a</sup>Lymphocytes were exposed to the chemical for 1 hr at G0, washed and irradiated 1.5 hr later with two doses of γ-rays separated by 60 min. Cultures were initiated after the treatment.

**Table 3.** Induction of infidelity of DNA repair by nickel acetate in human lymphocytes.<sup>a</sup>

Concentration, μM	γ-rays, cGy	MI <sup>b</sup>	No. cells analyzed	% chromosome delet., ± SD	% chromosome transl., ± SD	% changes from expected
0	75 + 75	17	200	9.8 (2.6)	16.5 (3.8) <sup>c,d</sup>	—
0.1	75 + 75	13	200	9.8 (2.2)	13.8 (4.3) <sup>c,d</sup>	-16.4
1.0	75 + 75	7	200	10.5 (2.6)	21.6 (3.6) <sup>c,d</sup>	30.9
10	75 + 75	8	200	9.5 (2.6)	24.2 (6.1) <sup>c,d,e</sup>	46.7
100	75 + 75	6	200	9.0 (3.2)	19.8 (5.5) <sup>c</sup>	20.0

<sup>a</sup>Treatment conditions are similar to those of Table 2. <sup>b</sup>MI = mitotic index (number of metaphase cells from 10,000 cells analyzed). <sup>c</sup>Significant dose-dependent increase ( $p < 0.05$ ; analysis of variance). <sup>d</sup>Significantly different from each other ( $p < 0.05$ ; contrast analysis). <sup>e</sup>Significantly different from each other ( $p < 0.05$ ; contrast analysis).

for each experiment, one tube of lymphocyte culture is used for each treatment in each experiment. For the challenge assay, the cells are treated with chemicals and radiation, and washed and centrifuged many times. Many cells are lost from the extensive manipulation. Sometimes not enough cells are recovered for analysis of 50 well-spread metaphase cells. In these cases, additional cells are scored from other experiments in order to fill the deficiencies.

## Results

A summary of the frequencies of chromosome aberrations and sister chromatid exchanges is presented in Table 1. The cytogenetic effects of nickel acetate are tested with concentrations ranging from 0.1 to 1000 μM for 1 to 48 hr of exposure. As shown in the table, none of the exposure conditions induced any increase of

chromosome aberrations nor sister chromatid exchanges. At 1000 μM concentration, nickel acetate is cytotoxic as indicated by the inhibition of mitosis.

Our challenge assay is used to investigate whether exposure to nickel acetate can cause mistakes in DNA repair and lead to the formation of abnormal chromosomes. The frequencies of dicentric chromosomes obtained from four independently conducted studies are summarized in Table 2. As shown in the table, the collected data are rather consistent from one experiment to another. Additional data from these four experiments are summarized in Table 3. As shown in the table, the chemical induces a significant dose-dependent increase of chromosome-type rearrangements ( $p < 0.05$ ) as determined by the analysis of variance procedure although the response falls off at high doses of nickel acetate (100 μM). In

addition, the translocation frequencies in cells treated with 0.1, 1.0, and 10 μM nickel acetate are significantly different from each other ( $p < 0.05$ ; contrast analysis). The frequencies for those cells treated with 10 μM of the chemical is significantly different from the control ( $p < 0.05$ ; contrast analysis). The observed phenomenon is induced by nickel acetate with concentrations that are not clastogenic by themselves. On the other hand, no change in chromosome-type deletions is observed. In addition, there is a nickel dose-dependent reduction of mitotic indices.

## Discussion

Carcinogenic nickel compounds are found to be weakly mutagenic in a variety of short-term assays, therefore, these compounds may not exert their carcinogenic activities using conventional genotoxic mechanisms.

A unique observation is that these compounds appear to affect heterochromatin preferentially. Nickel-transformed Chinese hamster embryo cells have nonrandom deletion of the heterochromatin in the X-chromosome (32). On the other hand, other investigators have shown that nickel compounds can enhance the activities of mutagenic agents (21,24,25). However, the mechanisms for expression of these phenomena have not yet been elucidated.

Since nickel compounds are capable of binding to cellular DNA and proteins (16-18), they can cause abnormal expression of cellular functions. We hypothesize that one of those functions is fidelity of DNA repair. Using a challenge assay, we have shown that noncytotoxic and nonclastogenic doses of nickel acetate induce significant increase of rearrangement-type chromosome aberrations ( $p < 0.05$ ; Table 3). On the other hand, the frequency of deletion-type chromosome aberrations is unchanged after exposure to different combinations of nickel and  $\gamma$ -rays. The latter observation suggests that the amount of DNA strand breaks induced by  $\gamma$ -rays may not have been increased significantly by prior exposure to different concentrations of nickel. The increased frequency of rearranged chromosomes is therefore caused by problems in repair of radiation-induced DNA damage. One of the causes for the problem is the inability to rejoin radiation-induced DNA fragments so as to reform the original DNA molecules. This can be caused by interference from complexes formed between nickel and DNA and between nickel and repair enzymes. Another possibility is due to nickel-induced delay of DNA repair. Delay in repair may allow a significant amount of damage induced by the first dose of radiation to interact with damage induced by the second dose of radiation (60 min later). The phenomenon is well documented in experiments which showed that inhibition of DNA repair by *ara-C* after exposure to X-rays causes significant increase of rearrangement-type chromosome aberrations (33,34). Both mechanisms may exist for nickel under our experimental conditions. Therefore, we interpret our data to be indicative of infidelity of DNA repair caused by exposure to nickel.

A wide range of concentration of nickel acetate (from noncytotoxic to cytotoxic doses) is tested in our assay. The shape of the dose-response curve (last two columns of Table 3) reflects the biological effects from exposure to these different doses of nickel. Although we have not conducted

mechanistic studies to elucidate the cause of the response, there are data from the literature to explain our observed phenomenon. It is possible that nickel induces an adaptive cellular response (35). The study shows that after exposure of cells to a low dose of a DNA damaging agent, cells become resistant to damage from exposure to a high dose of another agent. Induction of DNA repair enzymes is a possible explanation for this phenomenon. Our data indicate that the induced adaptive response may not always be beneficial.

Exposure to the highest dose of nickel leads to a translocation frequency lower than that from the second highest dose. This response may be due to toxicity of nickel to lymphocytes. It may, on the other hand, be due to reduced intranuclear concentration of nickel at this extracellular dose. For example, in an *in vivo* study, the tissue concentrations of nickel increase with time, as expected, after exposure to increasing doses of nickel carbonate (18). However, at certain tissue concentrations of nickel, the concentrations in cell nuclei are actually reduced. This study suggests that the nucleus has specific mechanisms to remove nickel after certain extracellular concentrations of nickel are reached. The same mechanism may exist in lymphocytes in our study.

Infidelity of DNA repair is probably an important causal mechanism in the development of cancer. Mistakes in the repair of spontaneous or induced DNA lesions may convert generally innocuous DNA damage into a major defect that can cause long-term health effects. It is conceivable that infidelity of DNA repair creates instability of the genome which permits genetic alterations significant in the development of cancer to occur. In fact, the observation of altered *p53* genes in cells exposed to nongenotoxic nickel is consistent with our suggested activity of nickel (36). The well documented increase of cancer risk among patients with DNA repair defects and/or chromosome instability syndromes is also consistent with the hypothesis. Therefore, infidelity of DNA repair may contribute to the production of multiple and sequential genetic alterations within the same cell for clonal adaptation and progression of abnormal cells. This is the hallmark for development of cancer (4,5,37,38). Our data suggest that nickel acetate, and perhaps other carcinogenic nickel compounds, can cause DNA repair problems and that this activity may be one of the mechanisms responsible for their carcinogenic properties. Our suggestion is also consistent with

the observation of chromosome abnormalities in mouse tumors induced by nickel compounds which are not clastogenic (39).

Our challenge assay is designed with the intention of using it in population monitoring studies. Therefore, cells are treated with chemicals during the G<sub>0</sub> phase of the cell cycle in order to mimic *in vivo* exposure to chemicals. As part of our *in vitro* assay, cells are then exposed to radiation in the absence of chemicals. As demonstrated by our study with lymphocytes from cigarette smokers (31), these lymphocytes are exposed to cigarette smoke *in vivo* and then to radiation *in vitro*. In that study, our data indicate that exposure to cigarette smoke causes DNA repair problems. After the challenge assay is better characterized, it may be used to detect DNA repair problems in cells from workers who are occupationally exposed to potentially hazardous agents.

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