Induction of genetic instability and chromosomal instability by nickel sulfate in V79 Chinese hamster cells

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Nickel compounds are known to be carcinogenic to humans and show genotoxicity, including the ability to induce chromosome aberrations and neoplastic transformation in vitro. The mutagenicity of nickel compounds is, however, equivocal and the mechanisms of carcinogenesis are still not clear. In this study, the possibility that nickel compounds induce genetic or chromosomal instability was examined, because recent studies in cancer research show that these conditions are critically involved in carcinogenesis. V79 Chinese hamster cells were treated with 320 µM nickel sulfate for 24 h at low cell density (100 cells/100 mm diameter dish) and clones derived from single cells surviving Ni treatment were isolated. When cells grew up to 23–25 population doublings post-treatment, mutation frequency at the Hprt locus and the chromosome aberration frequency of each clone were examined. Five out of 37 clones (13.5%) derived from Ni-treated cells showed a remarkably increased frequency of Hprt mutations (≥1 × 10^−5), while only one out of 37 control clones (2.7%) showed this high mutation rate. In addition, 17 out of 37 clones (45.9%) from Ni-treated cells showed structural chromosomal aberrations in 10% or more of cells (up to 45.5%), while only three out of 31 control clones (9.7%) showed this high aberration rate. Out of 37 clones derived from Ni-treated cells, eight (21.6%) and 11 (29.7%) clones showed an increased frequency (≥5%) of aneuploid and polyploid cells, respectively, while only a few control clones showed such an increase in aneuploid and polyploid cells. These results indicate that nickel sulfate can induce genetic and chromosomal instability in V79 cells.

Introduction

It is well known that some nickel compounds are carcinogenic to humans and animals (ICNMC, 1990; Oller et al., 1997). In vitro studies have shown that nickel compounds induce morphological and neoplastic transformation in mammalian cells (Costa and Mollenhauer, 1980; Miura et al., 1989; Kerckhaert et al., 1996; Ramnath et al., 1998). In addition, many studies have shown the ability of nickel compounds to induce chromosome aberrations (Nishimura and Umeda, 1979; Sen and Costa, 1985; Ohshima, 2001), DNA strand breaks (Sunderman, 1989), DNA–protein crosslinking (Chakrabarti et al., 1999) and oxidative DNA damage (Huang et al., 1995). Effects on DNA repair (Hartwig et al., 1994) and DNA methylation (Lee et al., 1995) have also been reported. In contrast to these various genotoxicities, however, it is also reported that nickel compounds are not effective in causing gene mutations in regular mutagenicity assays using bacterial or mammalian cells (Heck and Costa, 1982; Miura et al., 1989). This lack of significant mutagenicity suggests that other mechanisms may play an important role in nickel carcinogenesis. In a previous report, the ability of nickel sulfate (NiSO₄) to induce aneuploidy in V79 Chinese hamster cells was shown and the involvement of aneuploidy in nickel carcinogenesis was suggested (Ohshima, 2001). Aneuploidy may contribute to carcinogenesis through changes in dosage of many genes (Duesberg, 1999).

Another hypothesis is that nickel compounds induce genetic instability or chromosome instability in exposed cells, which could result in multiple genetic alterations. Recent research suggests that genetic instability or chromosome instability induced by radiation or carcinogens is an important mechanism in carcinogenesis. For example, several studies have shown that ionizing radiation can induce mutation at the hypoxanthine phosphoribosyltransferase (Hprt) locus in the progeny of exposed cells many generations post-irradiation (Chang and Little, 1992; Harper et al., 1997; Little, 1999). Others have shown high levels of non-clonal chromosome aberrations in the clonal descendants of exposed cells many generations post-irradiation (Kadhim et al., 1992, 1994; Sabatier et al., 1992). Recently, one report indicated that exposure to specific carcinogens could select for tumor cells with distinct forms of genetic instability, chromosome instability and microsatellite instability (Bardelli et al., 2001).

In the present study, the frequency of Hprt mutations and chromosome aberrations in the clonal cells derived from single cells treated with NiSO₄ was examined. The results indicate that NiSO₄ can induce genetic instability and chromosomal instability in V79 cells.

Materials and methods

Cell culture and chemical treatment

V79 Chinese hamster cells were grown in Eagle’s minimum essential medium supplemented with 2 mM l-glutamine and heat-inactivated 10% fetal bovine serum. Cells were incubated in 5% CO₂ in air at 37°C. Nickel sulfate hexahydrate (Sigma–Aldrich, St Louis, MO) was dissolved in distilled water and sterilized with a syringe filter.

Cells were seeded at low density (100 cells/dish) into several 100 mm diameter dishes and incubated overnight before treatment. Then cells were incubated with medium containing 320 µM nickel sulfate for 24 h, after which they were maintained in fresh medium in the absence of the compound. Cell survival with this treatment, which was determined as cloning efficiency, was ~15%.

Isolation of clones

Approximately 10 days after treatment, colonies developed from single cells surviving Ni treatment were isolated with cloning rings and seeded into new dishes. Cells were allowed to grow up to 23–25 population doublings (PDL) (2–3 weeks after treatment) to obtain a sufficient number of cells for Hprt mutation assay and chromosome analysis. Colonies developed from non-treated cells (seeded at 20 cells/100 mm diameter dish) were also isolated and grown to obtain control clones.

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Cells from each clone were seeded into mutation Measurement of frequency. number of clones with high (hypoxanthine-guanine phosphoribosyltransferase) (HPRT) gene mutation in V79 clonal cells derived from control and Ni-treated cultures. Clones derived from single cells treated with 320 µM NiSO₄ for 24 h were grown up to 23–25 PDL and frequencies of 6-TO-resistant cells were measured. Clones derived from non-treated cells were also examined. Figures in parentheses indicate number of clones with high (>1 × 10⁻⁴) and low (<1 × 10⁻⁵) mutation frequency.

Measurement of HPRT (hypoxanthine-guanine phosphoribosyltransferase) mutation

Cells from each clone were seeded into five dishes at a density of 2 × 10⁵ cells/100 mm diameter dish and incubated for 7 days in selection medium containing 10 µM 6-thioguanine (6-TG). Mutation frequency for each clone was calculated from number of colonies developed in selection medium and cloning efficiency in regular medium. Several clones that showed a notably high mutation frequency were grown up to 10 passages (~69 PDL) after treatment to examine whether an increased frequency of gene mutation persisted. In addition, separate dishes from these clones were treated with medium containing glycine, hypoxanthine, aminopterin and thymidine (GHAT) to eliminate mutant cells and allowed to grow further in regular medium without GHAT to examine whether HPRT mutation in these clones relapsed.

Chromosome analysis

Chromosome slides were prepared for each clone by standard procedures and 50 metaphase cells per clone were scored to determine the frequency of structural aberrations and chromosome number per cell. Chromosome analysis was performed on 31 out of 37 clones derived from the control culture and all 37 clones derived from the Ni-treated culture.

Statistical analysis

The differences in the distributions of mutation frequencies and chromosome aberration frequencies in clones from control and Ni-treated cultures were statistically significant by the Wilcoxon rank sum test (P = 0.001). When two of these clones were cultured further to examine whether the increased mutation frequencies persisted, they maintained the increased mutation frequencies up to 10 passages (~69 PDL) after treatment, as shown in Figure 2. In addition, these two clones were treated with GHAT for 3 days (between passages 5 and 6) and cultured further without GHAT, the mutation frequencies fell back to control levels with GHAT treatment and increased again to >1 × 10⁻⁴, as shown in Figure 2.

Frequency of cells with structural chromosome aberrations in V79 clonal cells

The data on structural chromosome aberrations obtained in this study are shown in Figure 3. The frequency of cells with structural chromosome aberrations in 31 control clones ranged from 0 to 12.5% and the mean frequency was 3.3%. The frequency in 37 clones derived from Ni-treated cells ranged from <1 × 10⁻⁶ to 3.8 × 10⁻³ and the mean frequency was 1.6 × 10⁻⁴. Five out of 37 clones (13.5%) derived from Ni-treated cells showed a remarkably increased frequency of mutations (≥1 × 10⁻⁴), while only one out of 37 control clones (2.7%) showed this high mutation rate. The differences in distributions of mutation frequencies in clones from control and Ni-treated cultures were statistically significant by the Wilcoxon rank sum test (P = 0.008). Three out of five clones that showed a high mutation frequency of the HPRT gene in clones from the treated culture also showed a high frequency of chromosome aberration (>10% of clonal cells), while two clones did not. The frequency of cells with chromatid or chromosome gaps and dicentric chromosomes (Figure 4A)
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Fig. 3. Frequency of cells with structural chromosome aberrations in the clones shown in Figure 1. Chromosome aberrations were scored on 50 metaphase cells per clone. Figures in parentheses indicate number of clones with high (≥10%) and low (<10%) frequency of cells showing chromosome aberration. Asterisks indicate clones with a high mutation frequency (≥1 × 10⁻⁴) of the HPRT gene.

especially were higher in clones from Ni-treated cells than in control clones (Table I). In addition, ring chromosomes (Figure 4B) were observed in three clones from Ni-treated cells but were not observed in control clones at all.

Frequency of aneuploid and polyploid cells in V79 clonal cells
The data on aneuploid and polyploid cell frequencies obtained in this study are shown in Figures 5 and 6. Eight out of 37 clones (21.6%) derived from Ni-treated cells showed an increased frequency (5% or more) of aneuploid cells (cells with 24–41 chromosomes), while only one out of 31 control clones (3.2%) showed such an increase in aneuploid cells. The difference in the distributions of aneuploid cell frequencies between control and treated clones was nearly significant by the Wilcoxon rank sum test \( P = 0.056 \). Eleven out of 37 clones (29.7%) derived from Ni-treated cells showed an increased frequency (≥5%) of polyploid cells (cells with ≥42 chromosomes), while only three out of 31 control clones (9.7%) showed such an increase in polyploid cells. The difference in distributions of polyploid cell frequencies between control and treated clones was statistically significant \( P = 0.017 \) by the Wilcoxon rank sum test. Two clones each from control and treated cultures were polyploid clones, i.e. the modal chromosome number was more than 42 in those clones. Three out of the five clones that showed a high mutation frequency of the HPRT gene in clones from the treated culture also showed a high frequency of aneuploid or polyploid cells (>5% of clonal cells), while the other two clones did not.

Discussion
Regarding mutagenicity of nickel compounds, both positive (Miyaki et al., 1979) and negative (Heck and Costa, 1982; Miura et al., 1989) results have been reported so far and the ability of the compounds to induce gene mutation is equivocal. In this study, a prominent increase in HPRT mutation frequency in V79 clonal cells was observed long after treatment with NiSO₄ in five out of 37 clones derived from treated cells, although one of 37 control clones also showed a high frequency of mutation. This result suggests that the compound induced a mutator phenotype in the treated cells, although a small fraction of non-treated cells intrinsically exhibit a mutator phenotype. Because the clonal cells were derived from single cells that survived exposure to the compound, the observed HPRT mutations were not caused directly by the compound but occurred during cell proliferation after treatment. Persistence of the mutator phenotype of these clones was confirmed by the fact that the high frequency of mutation relapsed during proliferation after elimination of mutants by treatment of cells with GHAT.

The ability of nickel compounds to induce chromosome aberrations has been clearly shown by many investigators (Nishimura and Umeda, 1979; Sen and Costa, 1985; Ohshima, 2001). The frequency of cells with chromosome aberrations,
Fig. 5. Frequency of aneuploid cells (cells with 24–41 chromosomes) in the clones derived from control and Ni-treated cultures. Chromosome counts were scored on 50 metaphase cells per clone. Figures in parentheses indicate number of clones with high (≥5%) and low (<5%) frequency of aneuploid cells. Asterisks indicate clones with a high mutation frequency (>1 × 10⁻⁴) of the HPRT gene.

Table I. Mean frequency of cells with chromosome aberrations in clones derived from control and Ni-treated cultures

<table>
<thead>
<tr>
<th></th>
<th>Chromatid gap</th>
<th>Chromatid break</th>
<th>Chromosome gap</th>
<th>Chromosome break</th>
<th>Exchange</th>
<th>Ring</th>
<th>Dicentric</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (31 clones)</td>
<td>0.9</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
<td>0.0</td>
<td>0.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Ni-treated (37 clones)</td>
<td>2.9</td>
<td>0.3</td>
<td>1.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>3.2</td>
<td>10.4</td>
</tr>
<tr>
<td>t-test</td>
<td>0.05 &lt; P &lt; 0.1</td>
<td>*</td>
<td>0.05 &lt; P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
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however, seems to decrease with a lapse of time after treatment (Ohshima, 2001). In this study, high levels of non-clonal chromosome aberrations in the clonal descendants of Ni-treated cells many generations post-treatment were observed in nearly half of the clones. This result suggests that the compound induced chromosomal instability in the treated cells. Although it is not clear how and when the treated cells acquired chromosomal instability, it is likely that this occurred immediately after treatment and that it persisted for many generations up to the time of observation. In particular, the high frequency of dicentric chromosomes was noteworthy because this type of aberration is not transmissible (an unstable-type chromosome aberration). In addition, ring chromosomes, which are also not transmissible, were observed in three clones of Ni-treated cells, whereas these were not observed in control clones. These unstable-type chromosome aberrations are considered a hallmark of chromosomal instability and their appearance in cells exposed to radiation long after irradiation has also been reported (Sabatier et al., 1992; Martins et al., 1993). These aberrations can be formed by telomeric fusions (Riboni et al., 1997) and may cause breakage–fusion–bridge cycles that may play an important role in carcinogenesis (Gieelesson et al., 2001). In addition to structural aberrations, high frequencies of aneuploid cells in clonal descendants of Ni-treated cells were also observed in about one third of the clones. The link between these chromosomal instabilities and the occurrence of clones with a mutator phenotype is not clear, because the number of clones were too few to discuss at this point.

Many studies have shown that radiation and certain carcinogens induce genetic or chromosomal instability. In the case of X-irradiation, it is reported that ~10% of the clones derived from exposed cells exhibit a high mutation frequency of the HPRT gene (Chang and Little, 1992; Little, 1999). Induction of chromosomal instability by radiation is now being widely investigated (Sabatier et al., 1992; Grosovsky et al., 1996; Wright, 1999) since the induction of a high frequency of non-clonal aberrations in the clonal descendants of hemopoietic cells exposed to α-particles was reported (Kadhim et al., 1992). Another group also showed that mutagens such as ethyl methane sulfonate or UV light induces delayed mutational events at the G6PD or HPRT locus, which occur at 12–14 cell divisions after treatment (Stamato and Perez, 1998). Induction of microsatellite instability by various factors has also been shown. One study reported that NiSO₄ induces microsatellite...
mutations in human cells (Zienolddiny et al., 2000). Other studies also showed that genotoxic stresses, such as oxidative DNA damage or X-ray exposure, induce microsatellite instability in vitro (Jackson et al., 1998; Romney et al., 2001). Even non-DNA-damaging stress exposures, such as heat treatment or serum starvation, were found to induce genetic instability (Li et al., 2001). Therefore, induction of genetic or chromosomal instability might be a common pathway to carcinogenesis but little is understood of the underlying mechanisms. One possible mechanism is that mutation in a gene or genes that contribute to genome or chromosome stability is responsible for the observed phenotype. This is unlikely, however, because the frequency of such a mutation should be very low (Loeb, 2001) and cannot account for the significant percentage of the clones with a high frequency of gene mutations and chromosome aberrations. Other mechanisms that could affect a large fraction of exposed cells and, at the same time, is inheritable by daughter cells should be considered. Such mechanisms might involve elements other than the primary structure of DNA, such as the DNA methylation status. The last point is worth examining because some studies have shown a link between change in DNA methylation status and genomic instability (Chen et al., 1998; Breivik and Gaudernack, 1999; Rizwana and Hahn, 1999).

References


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