Nickel(II) Interferes with the Incision Step in Nucleotide Excision Repair in Mammalian Cells

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ABSTRACT

Nickel compounds are carcinogenic to humans and experimental animals. However, the mechanisms leading to tumor formation are still not understood since the mutagenic potential is rather weak. In contrast, nickel(II) enhances the cytotoxicity and genotoxicity in combination with several other DNA-damaging agents. To elucidate possible interactions with DNA repair processes, the effect of nickel(II) on the nucleotide excision repair pathway has been investigated after UV irradiation in HeLa cells. Nickel(II) blocks the removal of cyclobutane pyrimidine dimers as determined by T4 endonuclease V-sensitive sites. When the alkaline unwinding technique was applied, significantly less transient DNA strand breaks after UV irradiation were detected in the presence of nickel(II) compared to UV alone, suggesting an inhibition of the incision step of nucleotide excision repair. Once incisions are made, the ligation of repair patches is delayed as well in nickel-treated cells, as observed by the alkaline unwinding and nucleoid sedimentation techniques. This inhibition of DNA repair is partly reversible by the addition of magnesium(II), indicating that the competition between Ni^{2+} and Mg^{2+} may provide an important mechanism for the disturbance of DNA-protein interactions involved in the repair process. Since the repair inhibition is observed at nontoxic concentrations of nickel(II), it may well be relevant for its carcinogenic action.

INTRODUCTION

Epidemiological studies identified compounds of nickel as carcinogenic to humans, causing nasal and lung cancer in exposed workers, predominantly in the nickel refining industry. In experimental animals, compounds of nickel induced lung tumors and local sarcomas in different species after various routes of exposure. The carcinogenic potency depends largely on properties such as solubility and toxicity of the respective compound, which affect its bioavailability (reviewed in Ref. 1). However, the mechanisms involved in tumor formation are still not understood. In mammalian cells in culture, compounds of nickel have been shown to induce DNA strand breaks (2), as well as DNA-protein cross-links and chromosomal aberrations, both generated in mainly heterochromatic DNA regions (3, 4). The production of reactive oxygen species has been suggested as one possible mechanism for DNA damage induction, presumably by Fenton-type reactions. In support of this model, oxidative DNA damage induced by nickel(II) has been observed in isolated DNA and chromatin in the presence of H_2O_2, as well as in rats after i.p. administration of nickel acetate (reviewed in Ref. 5). Recently, nickel(II) complexed to the tripeptide Gly-Gly-His in combination with H_2O_2 has been shown to induce mutations in vitro on single-stranded M13 mp2 DNA as scored by a forward mutation assay in Escherichia coli, including tandem double CC→TT mutations typical for damage by oxygen free radicals (6). However, in intact cells in culture, nickel(II) is mostly nonmutagenic in bacterial test systems (1) and only weakly mutagenic in mammalian cell lines (7—10). Therefore, the carcinogenic potential of nickel compounds is not readily explained by its mutagenic action.

In contrast to its weak mutagenic potential, nickel(II) has been shown to enhance the cytotoxicity and mutagenicity of several other DNA-damaging agents in a more pronounced manner. In E. coli, nickel(II) increased the mutation frequency in combination with methyl methanesulfonate (11). In mammalian cells in culture, nickel(II) enhanced the UV-induced cytotoxicity, mutagenicity, and sister chromatid exchanges in V79 Chinese hamster cells (9); a comutagenic activity in combination with UV light was also detected in G12 cells, a transgenic V79 cell line carrying a bacterial guanine phosphoribosyltransferase (gpt) gene (12). In combination with benzo(a)pyrene, nickel(II) enhanced the frequencies of mutations and cell transformations in Syrian hamster embryo cells (13). These findings suggest that interactions with enzymes or proteins involved in DNA replication and/or DNA repair might be relevant; there are several indications that nickel(II) interferes with the repair of UV- and X-ray-induced DNA damage (12, 14—16). However, the mechanism of repair inhibition is still unclear.

In the present study, UVC light was used as a well characterized DNA-damaging agent to study the interaction of nickel(II) with DNA repair processes. After irradiation, two major DNA photoproducts are generated, the cyclobutane pyrimidine dimer as the most frequent one and the pyrimidine-(6-4)-pyrimidone photoprodut. Both types of damage cause structural distortions of the DNA and are removed by the nucleotide excision repair pathway (17).

We investigated the effect of nickel(II) on this repair pathway in HeLa cells by different approaches: (a) the induction and removal of pyrimidine dimers as determined by the frequency of T4 endonuclease V-sensitive sites after low, biologically relevant doses of UV irradiation; and (b) the detection of incision events measured by the nucleoid sedimentation technique and the alkaline unwinding method. By comparative studies using these different end points, we were able to show that the damage recognition/incision step is disrupted by nontoxic concentrations of nickel(II), leading to a significantly reduced number of repair events in the presence of the metal with a subsequent delay in postincision steps. This interference with DNA repair is partly reversible by the addition of magnesium(II), providing further evidence that the competition with this essential metal ion may be an important mechanism for the toxic action of nickel(II).

MATERIALS AND METHODS

Materials. MEM and fetal calf serum, as well as the trypsin and penicillin-streptomycin solutions, are products of GIBCO (Karlsruhe, Germany). Hoechst 33258 was obtained from Sigma Chemical Co. (Munich, Germany). Triton X-100 was bought from Pierce Chemical Co. (Oud-Beijerland, the Netherlands). Sodium dodecyl sulfate and hydroxyapatite were from Calbiochem (Bad Soden, Germany). All other chemicals, including NiCl_2·6H_2O, were of p.a. grade from Merck (Darmstadt, Germany). The culture dishes were supplied by Nunc (Wiesbaden, Germany).

Cell Culture. HeLa cells were grown as monolayers in MEM containing 5% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

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2. To whom requests for reprints should be addressed.
The cultures were incubated at 37°C with 5% CO₂ in air and 100% humidity.

**Uv Irradiation.** UV irradiation of cells was carried out in dishes where cells had been washed and from which all media had been removed with a General Electric germicidal lamp (Bioblock Scientific) delivering 0.025 l/m²/s at a wavelength of 254 nm from a distance of 117 cm or 0.125 J/m²/s from a distance of 52 cm.

**Atomic Absorption Spectrometry.** Logarithmically growing HeLa cells were incubated with NiCl₂ for different lengths of time. At the end of treatment, the cells were washed three times with ice-cold MEM, trypsinized, counted, and mineralized with 65% (v/v) HNO₃ and 30% (v/v) H₂O₂ (1:1). Uptake was measured with a Perkin Elmer Model 2380 atomic absorption spectrophotometer equipped with a HGA 400 graphite furnace following the manufacturer's instructions. To calculate the intracellular nickel concentration, the cell volume was determined to be 1.69 × 10⁻¹² liter as described previously (18).

**Trypan Blue Exclusion.** HeLa cells (5 × 10⁶) were allowed to attach for 24 h and were incubated with NiCl₂ for 24 h. After trypan blue exclusion, the cells were diluted with phosphate-buffered saline and incubated with trypan blue solution (0.9% NaCl-0.5% trypan blue, yielding a final concentration of 0.18% of the dye). Viable cells were calculated by the unstained:total cells counted ratio. Control cells exhibited 93% dye exclusion. Statistical evaluation was done using the t test.

**Colony-forming Ability.** HeLa cells (5 × 10⁶) were allowed to attach for 24 h and were subsequently incubated with NiCl₂ for 24 h. Thereafter, they were trypsinized and 300 cells/dish were seeded for colony-forming ability. After 9 days of incubation, colonies were fixed with ethanol, stained with Giemsa (25% in ethanol), counted, and calculated as a percentage of control. Untreated controls exhibited a colony-forming ability of 75%. During the investigation of the effect of nickel(II) on UV-induced cytotoxicity, 24 h after seeding, the cells were preincubated with NiCl₂, UV irradiated, and postincubated for 5 h in the presence of NiCl₂. Thereafter, the cells were trypsinized and seeded for colony-forming ability.

**Nucleoid Sedimentation.** The procedure is based on the method of Cook and Brazell (19) with modifications described by Yew and Johnson (20). HeLa cells (5 × 10⁶) were lysed in the presence of 2 M NaCl, 0.01 M EDTA (pH 8.0), and 0.5% Triton X-100 and centrifuged down a 15–30% sucrose gradient containing 2 M NaCl, 0.01 M EDTA, and 1 µg/ml Hoechst 33258 dye at 9000 rpm for 30 min. The position of the DNA nucleoid band was determined under UV light and calculated as distance sedimented compared to control cells. Statistical evaluation of the data was performed using the t test.

**Alkaline Unwinding.** DNA strand breaks were determined according to the method of Ahnström and Erixon (21) with modifications as described before (22). Briefly, 2 × 10⁶ HeLa cells were allowed to attach for 24 h, preincubated with nickel(II), UV irradiated, and postincubated for different times. Afterwards, the medium was removed and an alkaline solution was added containing 0.03 M NaOH, 0.02 M Na₂HPO₄, and 0.9 M NaCl. Separation of single- and double-stranded DNA was performed on 1-ml hydroxyapatite columns (Calbiochem, high resolution) at 60°C. Single- and double-stranded DNA were eluted with 3 ml of 0.1 M and 0.35 M potassium phosphate buffer, respectively.

The DNA content of both fractions was determined by adding Hoechst 33258 dye to a final concentration of 7.5 × 10⁻⁷ M to 1 ml of each sample and measuring the fluorescence with a spectrophotofluorometer (Aminco-Bowman) at an excitation wavelength of 360 nm and an emission wavelength of 450 nm. The fraction of double-stranded DNA was calculated as described before (22). Statistical evaluation of all data was performed using the t test.

**T4 Endonuclease V-sensitive Sites.** The T4 endonuclease V was isolated as described previously (23). For the detection of T4 endonuclease V-sensitive sites, 2 × 10⁶ HeLa cells were seeded and allowed to attach for 24 h before preincubation with NiCl₂. At the end of treatment, lysis of the cells was conducted essentially by the method of Föré and Dikomey. The culture medium was removed and a lysis buffer was added containing 0.006 M Na₂HPO₄, 0.001 M KH₂PO₄, 0.137 M NaCl, 0.003 M KCl, and 0.1% Triton X-100. After 5 min on ice, the solution was sucked off and the cells were treated with a high salt solution containing 2 M NaCl, 0.01 M EDTA, and 0.002 M Tris (pH 8.0) for 2 min on ice, whereafter the cells were left on ice for additional 8 min. The nucleoids were then incubated with the T4 endonuclease V (5.7 µg/ml) in endonuclease buffer, pH 6.7 (0.05 M sodium phosphate, pH 6.7-0.01 M EDTA-0.01 M β-mercaptoethanol), for 30 min at 37°C. At the end of incubation, the alkaline solution was added, yielding a final concentration of 0.07 M NaOH, 0.013 M EDTA, and 0.37 M NaCl. The further steps of unwinding, neutralization, and separation of single- and double-stranded DNA were performed as described above.

**RESULTS**

**Uptake of Nickel(II) in HeLa Cells**

To establish appropriate incubation conditions, the uptake of nickel(II) in HeLa cells was determined by atomic absorption spectrometry after treatment with 500 µM NiCl₂ (Fig. 1). The uptake is time dependent and the intracellular concentration reaches a maximum after 16 h of treatment. After this time span, a nearly 2-fold intracellular accumulation is observed, with no further increase up to 24 h. Therefore, to ensure sufficient uptake of the metal, the cells were preincubated with nickel(II) for 18–20 h during the investigation of its effect on UV-induced cytotoxicity or repair.

**Cytotoxicity of Nickel(II)**

The cytotoxicity of NiCl₂ in HeLa cells has been tested by applying two different end points. Immediate toxic effects after incubation have been determined by trypan blue exclusion as a measure of membrane integrity. No significant loss of dye exclusion was observed after 24 h of incubation with up to 1 µM nickel(II). As a second parameter of rather long-term cytotoxic effects, the colony-forming ability has been investigated. While the colony-forming ability is not considerably reduced up to 600 µM nickel(II), it drops thereafter, leaving about 20% cells viable after 24 h incubation with 1 mM NiCl₂ (Fig. 2).

**Effect of Nickel(II) on UV-induced Cytotoxicity**

The cytotoxicity of UV light in the presence and absence of nickel(II) has been investigated by determination of the colony-
forming ability (Fig. 3). HeLa cells were preincubated for 18 h with NiCl₂, UV irradiated, and postincubated with NiCl₂ for 5 h. Regarding UV light alone, a cytotoxic response is observed only at doses higher than 5 J/m², leading to about 55% colony-forming ability after irradiation with 10 J/m² UV. In the presence of 500 or 750 μM nickel(II), however, the UV-induced cytotoxicity is increased in a dose-dependent manner, and the colony-forming ability is reduced after UV doses as low as 1 J/m².

Interaction with DNA Repair Processes

To investigate the effect of nickel(II) on DNA repair, different end points have been applied: (a) the induction and removal of specific photolesions (cyclobutane pyrimidine dimers) as determined by T4 endonuclease V-sensitive sites; and (b) the measurement of incision rates as determined by nucleoid sedimentation and the alkaline unwinding method.

Effect of Nickel(II) on the Induction and Removal of T4 Endonuclease V-Sensitive Sites

The quantification of DNA lesions by using damage-specific enzymes in combination with DNA strand break assays has been widely applied. Concerning UV-induced DNA damage, the T4 endonuclease V specifically incises at cyclobutane pyrimidine dimers; since this enzyme possesses both a glycosylase and an endonuclease activity (23), sites of pyrimidine dimers are converted into single-strand breaks, which can be analyzed by the alkaline unwinding technique. This combination allows the sensitive detection of DNA lesions after low, biologically relevant doses of UV irradiation from 0.1 to 1.0 J/m² UV, which do not affect the cell viability. With the alkaline unwinding conditions applied, the fraction of double-stranded DNA decreases linearly on a log scale with an increasing number of DNA strand breaks, as determined by calibration with an X-ray source (data not shown). A linear relationship on a log scale between the fraction of double-stranded DNA and UV dose is also seen after UV irradiation (Fig. 4), indicating that the number of T4 endonuclease V-sensitive sites is proportional to the UV dose applied. Concerning the repair of UV-induced pyrimidine dimers (Fig. 5), after irradiation with 0.5 J/m² UVC, 34% of pyrimidine dimers were removed within 2 h and 62% were removed within 5 h postincubation, as calculated from the linear regression of the dose-response curve shown in Fig. 4. With these unwinding conditions and due to the low UV dose, no DNA strand breaks were detected in the same time span in UV-irradiated cells in the absence of the T4 endonuclease V.

To investigate the effect of nickel on the removal of pyrimidine dimers, HeLa cells were preincubated with 750 μM NiCl₂ for 18 h, UV irradiated, and postincubated with NiCl₂ for the respective repair period. No decrease in T4 endonuclease V-sensitive sites was observed within 5 h in nickel-treated cells. Therefore, nickel(II)
completely blocks the removal of T4 endonuclease V-sensitive sites; this effect is specific, since no increase in DNA strand breaks was observed after UV irradiation of nickel-treated cells in the absence of the T4 endonuclease V (data not shown). Furthermore, concentrations up to 1 mM nickel(II) do not affect the initial amount of T4 endonuclease V-sensitive sites (Table 1).

These results indicate that no detectable amount of incisions by cellular repair enzymes takes place at the sites of pyrimidine dimers in the presence of nickel(II); it is not clear, however, whether this is due to an actual inhibition of the incision itself or whether a postincision event is affected which prevents further incisions to occur.

Nucleoid Sedimentation. To further elucidate which step in the nucleotide excision repair pathway is inhibited by nickel(II), the nucleoid sedimentation technique has been applied. This assay detects structural DNA changes (24) as well as the occurrence and ligation of DNA strand breaks during excision repair (25). To investigate the effect of nickel(II) on UV-induced DNA repair, HeLa cells were preincubated with 500 μM nickel(II), UV irradiated, and postincubated in the absence of nickel(II) for the respective repair periods (Fig. 6). In the case of UV alone, the sedimentation distance dropped shortly after treatment, indicating a decrease of supercoiling due to incision events. About 1–3 h after irradiation, initial repair events were ligated and repair was diminished, leading to the restoration of the topological structure of the DNA. Nickel(II) alone decreases the sedimentation distance only slightly to 93% of the control value. However, it significantly (P < 0.001) changes the sedimentation behavior after UV irradiation. While the initial decrease in the sedimentation distance is observed in the presence of nickel(II), it does not return to control values within the time of observation. These data suggest that incisions occur in the presence of nickel(II) but DNA strand breaks accumulate due to an inhibition of either the polymerization or the ligation step. However, since the nucleoid sedimentation technique not only detects DNA strand breaks but also changes in the superhelicity of the DNA occurring during excision repair, the interaction by nickel(II) with the repair process was further investigated by the alkaline unwinding method.

Alkaline Unwinding. To discriminate between structural changes of the DNA and DNA strand breaks, we applied the alkaline unwinding technique to follow the effect of nickel(II) on the transient appearance of DNA strand breaks after UV irradiation (Fig. 7). With respect to UV light alone, shortly after irradiation with 5 J/m² UV, DNA strand breaks were generated, which were no longer detectable at 3 h after irradiation, indicating that early repair events were completed. Nickel(II) alone does not induce DNA strand breaks at this concentration. Regarding the repair kinetics in nickel-loaded cells after UV irradiation, the number of transient strand breaks was reduced significantly (P < 0.05), indicating that nickel(II) disrupted the incision step in the repair of UV-induced DNA damage. The postincision steps of those repair events that still occur in the presence of nickel(II) also appeared to be slowed down.

Protective Effect of Magnesium(II). One possible reason for repair inhibition might be the competition with essential metal ions like magnesium(II). Magnesium is important for the structural integrity of the DNA (26) but also serves as a cofactor in repair enzymes like DNA polymerases (27). Furthermore, magnesium(II) has been shown to protect from toxic effects of nickel(II) and it diminishes the carcinogenic action of nickel(II) (28). To investigate whether the repair inhibition is reversible by the addition of magnesium(II), the nucleoid sedimentation was applied. Since the uptake of nickel(II) into cultured cells is inhibited in the presence of magnesium(II) (29), incubation conditions were chosen that prevented simultaneous treatment with nickel(II) and magnesium(II). Nickel(II) alone inhibits DNA repair, and magnesium(II) provides protection (30).

Table 1 Effect of nickel(II) on the induction of T4 endonuclease V-sensitive sites by UV irradiation

<table>
<thead>
<tr>
<th>Nickel(II) (μM)</th>
<th>% of double-stranded DNA</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>27.7 ± 3.7</td>
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<tr>
<td>500</td>
<td>32.3 ± 4.7</td>
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<tr>
<td>750</td>
<td>24.7 ± 4.7</td>
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<tr>
<td>1000</td>
<td>29.3 ± 5.2</td>
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Fig. 6. Nucleoid sedimentation after single and combined treatment of HeLa cells with 500 μM NiCl₂ and 5 J/m² UV. The cells were preincubated with NiCl₂ for 20 h, irradiated with UV, and postincubated in the absence of the metal compound for the respective repair periods. Points, means from 4 to 8 determinations; bars, SD. •, UV; O, UV + NiCl₂.
INHIBITION OF NUCLEOTIDE EXCISION REPAIR BY NICKEL(II)

Both metal compounds. HeLa cells were incubated with 500 μM NiCl₂, irradiated with 5 J/m² UV, and postincubated with 10 mM MgCl₂ for the respective repair period (Fig. 8). The results show that the inhibitory effect of nickel(II) is significantly (P < 0.01) reduced by the subsequent incubation with magnesium(II). Under the same conditions, magnesium(II) protected from the cytotoxicity-enhancing effect of nickel(II) in combination with UV light (data not shown).

DISCUSSION

The results presented in this paper demonstrate that nickel(II) interferes with the repair of UV-induced photoproducts in HeLa cells, thereby enhancing the cytotoxicity after UV irradiation. The persistence of T4 endonuclease V-sensitive sites is in agreement with data presented by Snyder et al. (14), who showed that nickel(II) blocks the removal of thymine-thymine dimers after irradiation with 24 J/m² UVC as determined by high pressure liquid chromatography. Due to the high sensitivity of the applied test system in the present study as compared to the high pressure liquid chromatography method, the inhibition of adduct removal by nickel(II) was shown to occur after irradiation with the low, biologically relevant UV dose of 0.5 J/m². This raises the question of which step in the nucleotide excision repair pathway is affected by nickel(II). The fact that the cyclobutane pyrimidine dimers in nickel-loaded cells are still substrates for the T4 endonuclease V indicates that these lesions are not incised by cellular repair enzymes, suggesting that the incision step is inhibited. It cannot be excluded, however, that this inhibition is due to an interference with any postincision event like the polymerization or ligation, which in turn prevents further incisions to occur. Some extended information about the time course of early repair events is provided by the nucleoid sedimentation behavior after UV irradiation. The initial decrease in the sedimentation distance is due to a relaxation of DNA mediated by incision events. The original nucleoid sedimentation distance is achieved only if repair patches are ligated and the topological structure of the DNA is restored (25). While the sedimentation behavior of UV-irradiated cells presented in this paper resembles very closely the data reported before (25), nickel(II) changes this profile. Even though repair events start in nickel-preloaded cells, they are not complete within the first 5 h, indicating a delayed restoration of superhelicity due to an inhibition of the polymerization and/or ligation step. However, a comparison with our data obtained with the alkaline unwinding technique also points toward an interference with the incision step since significantly less incisions occur in nickel-loaded cells. Once incisions are made, the repair patches remain unligated for prolonged times, indicating that postincision events are affected as well.

With respect to the interference with the incision step by nickel(II), these results contradict those reported by Lee-Chen et al. (16). When investigating the effect of nickel(II) on different steps in nucleotide excision repair in CHO-K1 cells, they observed no effect on the accumulation of DNA strand breaks after UV irradiation when postincubated with ara-C and hydroxyurea for 1 h, indicating that incisions still occur. When UV-irradiated cells were allowed to accumulate DNA strand breaks in the presence of ara-C and hydroxyurea, the ligation during the subsequent 2-h incubation in drug-free medium was retarded by nickel(II). The main reason for this discrepancy as compared to our study might be different incubation conditions since the CHO cells were either pretreated in the first experiment or postincubated in the second experiment with nickel(II) for only 2 h, which does not seem to be long enough for sufficient uptake of soluble nickel compounds into cultured cells (Fig. 1), and might also account for differences in intracellular distributions.

Concerning the interaction of nickel(II) with postincision events, an inhibition of DNA strand break resealing after X-irradiation has been demonstrated in HeLa and CHO cells (12, 14). Furthermore, the interference by nickel(II) with DNA polymerization and replicative DNA synthesis has been observed by different approaches. With synthetic poly nucleotides, nickel(II) was partly able to substitute for magnesium(II) in the activation of polymerases derived from different sources, thereby decreasing the fidelity of polymerization (30, 31). This was confirmed by more recent investigations where nickel(II) led...
to an initial stimulation of DNA synthesis followed by an inhibition of the incorporation of DNA precursors when using partly single-stranded calf thymus DNA and a HeLa cell extract as polymerase source (32). Finally, nickel(II) increased the fraction of CHO cells arrested in S phase, indicating an interaction with the DNA replication process (33).

One further question which remains to be answered is whether the repair inhibition is restricted to UV-induced cyclobutane pyrimidine dimers as demonstrated by the persistence of T4 endonuclease V-sensitive sites. Since pyrimidine-(6-4)-pyrimidone photoproducts are repaired more efficiently after UV irradiation, early repair events observed by the nucleoid sedimentation and alkaline unwinding technique refer very likely to the repair of pyrimidine-(6-4)-pyrimidone photoproducts (34), indicating that the incision at these lesions is inhibited by nickel(II) as well. Some further evidence that the nucleotide excision repair system is affected in general stems from the observation that nickel(II) not only enhances the cytotoxicity and genotoxicity of UV light but also decreases the colony-forming ability in combination with cis-dichlorodiammineplatinum(II) (9), trans-dichlorodiammineplatinum(II),5 and mitomycin C (35).

Concerning the applied concentrations of nickel(II), a complete repair inhibition as determined by the persistence of T4 endonuclease V-sensitive sites was observed after incubation with 750 μM nickel(II), a concentration which does not affect dye exclusion as a measure of membrane integrity but which reduces the colony-forming ability to about 46% of the control. However, the interference with the incision step in nucleotide excision repair observed by the nucleoid sedimentation technique and by alkaline unwinding occurred at concentrations where no cytotoxic effect was observed in either test system.

**Possible Mechanisms of Repair Inhibition.** Several reasons might account for the disruption of the incision step by nickel(II) in nucleotide excision repair. In general, the first steps in this repair pathway, including the damage recognition/incision events, are very complex in eukaryotic cells. This complexity is evident from the existence of multiple genes involved in repair-deficient disorders in human cell lines derived from UV-sensitive patients like those with xeroderma pigmentosum (at least seven complementation groups), all of which are deficient in the early steps of nucleotide excision repair; even more human DNA repair genes have been identified by the correction of repair-deficient rodent mutants. Among these, several proteins from cloned human genes possess DNA-binding activities; some have been shown or suspected to function as helicases, either with or without DNA-binding activity (36); and recently at least one repair protein has been shown to be also part of the transcription factor TFIIH (37).

Regarding possible interactions of nickel(II) with the incision step in the nucleotide excision repair pathway, one mechanism could be the disruption of repair proteins binding to DNA, either by distortions of DNA structures or by modifications of the respective repair proteins. In support of this theory, a decrease in specific DNA binding of proteins derived from a nuclear extract of human hepatoma (Hep G2) cells in the presence of 100 μM nickel(II) has been reported, presumably due to modifications of nuclear proteins by nickel(II) (38). The preferential binding of nickel(II) to the protein fraction as compared to the DNA and RNA fractions was also reported for CHO cells after incubation with 65NiCl2 (39), and in vitro, a higher binding affinity of nickel(II) to amino acids as compared to isolated DNA was found as well (40).

The protective effect of magnesium(II) observed in the nucleoid sedimentation assay suggests that the competition of both metal ions for either protein- or DNA-binding sites provides one important mechanism of repair inhibition and could account for the interference with the incision step, as well as with postincision events. Besides being a cofactor in DNA polymerases, Mg2+-binding sites in the functional domain have been described for the repair enzymes ERCC2 and XPBC (36). Furthermore, magnesium(II) decreased the inhibitory effect of nickel(II) on the DNA-protein interactions described above (38) and antagonized the binding of nickel(II) to DNA (40).

In addition to the competition with magnesium(II), nickel(II) might also interact with zinc in DNA-binding motifs of DNA repair proteins. In several *in vitro* studies, nickel(II) was able to displace zinc from "zinc finger" structures as shown for the bovine estrogen receptor (41) and the human transcription factor SP1 (42, 43). Since the DNA binding of at least one eucaryotic repair protein, XPAC, is mediated by zinc finger structures (44), this mechanism might also be relevant for the disruption of the incision step in nucleotide excision repair by nickel(II).

Concerning possible interactions by nickel(II) with the DNA template, nickel(II) has been repeatedly found to be very efficient in causing structural changes of the DNA, both by promoting B → Z transitions and by stabilizing preformed Z-DNA (45); several DNA repair proteins like the G–methylguanine-DNA methyltransferase are unable to use Z-DNA as substrate (46, 47).

Taken together, our results indicate that nickel(II) interferes with the nucleotide excision repair pathway and may well explain its enhancing effects in combination with other DNA-damaging agents. Since the ability of DNA repair enzymes and/or proteins to maintain the integrity of the genomic information is of major importance for the prevention of cancer, the interference with this process by nickel(II) may well be relevant for its carcinogenic action. The fact that this inhibition is observed at basically noncytotoxic concentrations adds further to its potential relevance.

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