Carcinogenesis vol.18 no.5 pp.1021–1026, 1997

Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells

Heike Dally and Andrea Hartwig[1]

University of Bremen, Department of Biology and Chemistry, Postfach 330440, D-28334 Bremen, Germany

[1]To whom correspondence should be addressed

Compounds of nickel(II) and cadmium(II) are carcinogenic to humans and to experimental animals. One frequently discussed mechanism involved in tumor formation is an increase in reactive oxygen species by both metals with the subsequent generation of oxidative DNA damage. In the present study we used human HeLa cells to investigate the potential of nickel(II) and cadmium(II) to induce DNA lesions typical for oxygen free radicals in intact cells and the effect on their repair. As indicators of oxidative DNA damage, we determined the frequencies of DNA strand breaks and of lesions recognized by the bacterial formamidopyrimidine–DNA glycosylase (Fpg protein), including 7,8-dihydro-8-oxoguanine (8-hydroxyguanine), a pre-mutagenic DNA base modification. Nickel(II) caused a slight increase in DNA strand breaks at 250 μM and higher, while the frequency of Fpg-sensitive sites was enhanced only at the cytotoxic concentration of 750 μM. The repair of oxidative DNA lesions induced by visible light was reduced at 50 μM and at 100 μM nickel(II) for Fpg-sensitive sites and DNA strand breaks, respectively; the removal of both types of lesions was blocked nearly completely at 250 μM nickel(II). In the case of cadmium(II), DNA strand breaks occurred at 10 μM and no Fpg-sensitive sites were detected. However, the repair of Fpg-sensitive DNA lesions induced by visible light was reduced at 0.5 μM cadmium(II) and higher, while the closure of DNA strand breaks was not affected. Since oxidative DNA damage is continuously induced during aerobic metabolism, an impaired repair of these lesions might well explain the carcinogenic action of nickel(II) and cadmium(II).

Introduction

Compounds of nickel and cadmium are well known carcinogens to humans and to experimental animals (1,2). Due to the widespread occurrence of nickel and cadmium compounds at workplaces and in the environment, much effort has been made to elucidate the molecular basis for their carcinogenic potentials, but so far no mechanism has been unequivocally established for either metal. Nickel(II) is mostly non-mutagenic in bacterial test systems and only weakly mutagenic in mammalian cell lines; however, it induces DNA strand breaks as well as DNA–protein cross-links and chromosomal aberrations in mainly heterochromatic regions (1). One aspect frequently discussed is the role of oxidative processes catalyzed by nickel(II) (for reviews see 3–5). In subcellular test systems, oxidative DNA base damage, which is typical of hydroxyl radical attack, has been observed after treatment of isolated calf thymus DNA with nickel(II) and H_2O_2 (6) and of isolated human chromatin with nickel(II) in the absence or presence of H_2O_2 (7). The formation of 7,8-dihydro-8-oxoguanine (8-hydroxyguanine*) was further enhanced when nickel(II) was complexed to heterochromatic proteins (8). An increase in oxidative DNA damage by nickel(II) has also been detected in experimental animals. An elevated level of 8-hydroxyguanine was induced in rat kidney cells after a single i.p. injection of nickel acetate (9), and the extent was considerably higher after the administration of nickel(II)-histidine (10). Nevertheless, data supporting the induction of oxidative DNA damage in mammalian cells in culture are still missing.

Like nickel(II), cadmium(II) is mostly non-mutagenic in bacterial test systems. In V79 Chinese hamster cells, it has been demonstrated that $CdCl_2$ induces DNA single strand breaks, DNA–protein crosslinks and chromosomal aberrations. Additionally, it enhances the number of mutations at the thymidine kinase (*tk*) locus in mouse lymphoma L51784/TK$^{+/-}$ cells and at the *hprt* locus in V79 cells (for reviews see 11,12). Despite the fact that cadmium(II) is unable to catalyze Fenton-type reactions directly, it has been shown to enhance lipid peroxidation in cultured mammalian cells and in animal studies. Furthermore, protective effects of various radical scavengers and antioxidants point towards the involvement of reactive oxygen species in the generation of different types of cellular damage including DNA strand breaks and chromosomal aberrations. Even though the underlying mechanisms are not fully understood, one possible reason is the interference with cellular defense systems against reactive oxygen species (for recent reviews see 5,13,14). Nevertheless, the induction of DNA base modifications typical for reactive oxygen species has not been demonstrated in mammalian cells in culture.

Besides the induction of cellular damage via reactive oxygen species, another line of evidence supports the interference with DNA repair processes as a potential mechanism in nickel- and cadmium-induced genotoxicity and carcinogenicity (for review see 15). In support of this theory, nickel(II) has been shown to enhance the UV-induced mutation frequency and to inhibit the removal of UV-induced DNA damage by interfering with the incision and ligation step of the nucleotide excision repair process (16–19). Similarly, cadmium(II) increased the number of UV-induced mutations (20) and inhibited the repair of UV-, benz[*a*]pyrene- and methyl methanesulfonate-induced DNA damage (21–23).

In the present study we investigated the potentials of nickel(II) and cadmium(II) to (i) induce oxidative DNA damage in intact human HeLa cells, and (ii) to interfere with the repair of oxidative DNA damage. As sensitive indicators of oxidative DNA damage, we determined the frequencies of DNA strand breaks and of DNA base modifications recognized by the

***Abbreviations:** AP, apurinic/apyrimidinic; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; Fpg protein, formamidopyrimidine-DNA glycosylase; Hoechst 33258, bisbenzimidtrihydrochloride; 8-hydroxyguanine, 7,8-dihydro-8-oxoguanine; MEM, minimal essential medium; SDS, sodium dodecylsulfate.

bacterial formamidopyrimidine–DNA glycosylase (Fpg protein) (24). The Fpg protein is a repair glycosylase derived from *Escherichia coli*, which specifically removes 8-hydroxyguanine as well as imidazol ring-opened forms of guanine and adenine, and the resulting abasic sites are converted into single strand breaks by the associated apurinic/apyrimidinic (AP) endonuclease activity (25). The frequency of enzyme-sensitive sites was quantified by the determination of DNA strand breaks measured by the alkaline unwinding method. To assess the effect of nickel(II) and cadmium(II) on the repair of oxidative DNA damage, HeLa cells were irradiated with visible light, which results in the predominant induction of Fpg-sensitive sites and, to a lesser extent, DNA strand breaks and the removal of both types of lesions were investigated in the absence and presence of nickel(II) and cadmium(II).

Our results demonstrate that even though the induction of DNA damage is either not detectable or restricted to comparatively high concentrations of nickel(II) and cadmium(II), the repair of the respective DNA lesions is impaired at much lower concentrations and might therefore give rise to an elevated level of oxidative DNA damage *in vivo*.

Materials and methods

Materials

Minimal essential medium (MEM), fetal calf serum, as well as the trypsin and penicillin–streptomycin solutions are products of Gibco, Karlsruhe. Bisbenzimidtrihydrochloride (Hoechst 33258) was obtained from Sigma (Munich). Triton X-100 was bought from Pierce, Oud-Beijerland, Netherlands, and sodium dodecylsulfate (SDS) as well as hydroxyapatite from Calbiochem (Bad Soden, Germany). All other chemicals, including $NiCl_2 \times 6\ H_2O$ and $CdCl_2 \times H_2O$, were of p.a. grade and were from Merck, Darmstadt. The culture dishes were supplied by Nunc (Wiesbaden, Germany).

Cell culture

HeLa cells were grown as monolayers in minimal essential medium (MEM), which contained 5% fetal bovine serum, 100 units penicillin/ml and 100 μg streptomycin/ml. The cultures were incubated at 37°C with 5% CO_2 in air and 100% humidification.

Cytotoxicity

Logarithmically growing HeLa cells were incubated with $NiCl_2$ or $CdCl_2$ for 24 h or 5 h, respectively. 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 the control. Untreated controls exhibited a colony forming ability of 75%.

DNA strand breaks and Fpg-sensitive sites

DNA strand breaks and Fpg-sensitive sites were determined as described elsewhere (24). Briefly, 2×10^5 HeLa cells were seeded and allowed to attach for at least 5 h before treatment with the test chemicals. At the end of treatment, the culture medium was removed and a lysis buffer was added containing 0.006 M Na_2HPO_4, 0.001 M KH_2PO_4, 0.137 M NaCl, 0.003 M KCl and 0.1% Triton X-100. After 5 min on ice, the solution was removed by aspiration 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 Fpg protein (1 μg/ml) in enzyme buffer (0.05 M sodium phosphate, pH 7.5, 0.01 M EDTA, 0.1 M NaCl) for 30 min at 37°C. For the detection of DNA strand breaks, the Fpg protein was omitted. At the end of incubation, an alkaline solution was added to yield a final concentration of 0.07 N NaOH, 0.013 M EDTA, 0.37 M NaCl, pH 12.3, and the DNA was allowed to unwind for 30 min in the dark. The further steps of unwinding, neutralization and separation of single- and double-stranded DNA were performed as described previously (26). Briefly, the solution was neutralized with HCl, sonicated and SDS was added to a final concentration of 0.05%. Separation of single- and double-stranded DNA was performed on 1 ml hydroxyapatite columns (Calbiochem, high resolution) at 60°C, where single- and double-stranded DNA were eluted with 3 ml of 0.15 M and 0.35 M potassium phosphate buffer, respectively.

The DNA content of both fractions was determined by adding Hoechst 33258 (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 (26).

In order to quantitate the lesion frequencies, the fraction of double-stranded DNA was correlated with the amount of DNA strand breaks by calibration with X-rays (24).

Irradiation with visible light

Irradiation with visible light was carried out essentially as described by Pflaum *et al.* (27). The cells were illuminated as monolayers in cell culture dishes covered with phosphate buffered saline (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 0.1% glucose) on ice, by applying a 1000 W halogen lamp (Philips; 400 - 800 nm) for 20 min at a distance of 33 cm, which corresponded to 450 kJ/m^2. To avoid overheating, the dishes were gently shaken during the entire procedure.

Table I. Cytotoxicity of nickel(II) in HeLa cells

Nickel(II) [μM]	CFA[a] (% of control) ± SD[b]
0	100 ± 6.8
100	99.8 ± 9.6
250	87.8 ± 8.0
500	83.0 ± 3.2
600	90.8 ± 6.5
750	45.7 ± 6.0
1000	19.9 ± 2.6

Logarithmically growing cells were treated with $NiCl_2$ for 24 h, trypsinized and reseeded for the determination of the colony forming ability. Shown are mean values from six determinations.
[a]Colony forming ability.
[b]Standard deviation.

Results

Incubation conditions and cytotoxicity

One important prerequisite to elucidate the effect of nickel(II) on the extent of oxidative DNA damage is the intracellular bioavailability of nickel ions. Since the uptake of soluble nickel(II) is comparatively slow and reaches a maximum after 16–24 h of incubation with $NiCl_2$ (19), the cells were incubated for at least 18 h in the subsequent experiments to insure sufficient uptake. The cytotoxicity of $NiCl_2$ after 24 h of incubation is shown in Table I. The colony forming ability is reduced at concentrations higher than 600 μM, leaving ~20% of the cells viable after 24 h of incubation with 1 mM $NiCl_2$.

In contrast to nickel(II), cadmium(II) is taken up very efficiently by mammalian cells in culture. As determined by atomic absorption spectrometry, an ~10-fold intracellular accumulation of cadmium, as compared with the extracellular concentration, is reached after 2 h of incubation with a further linear increase up to 5 h (data not shown). Therefore, the direct DNA damage has been determined after 5 h of incubation, and pre-incubation periods of 2 h were applied in the repair studies. The cytotoxicity of $CdCl_2$ in HeLa cells after 5 h incubation is shown in Table II. The colony forming ability is only slightly decreased at concentrations of up to 25 μM $CdCl_2$; thereafter, it drops to 23.9% at 50 μM and to 2.1% at 75 μM.

Induction of oxidative DNA damage by nickel(II) and cadmium(II)

As an indicator of oxidative DNA damage, the frequencies of DNA strand breaks and Fpg-sensitive sites were quantified as described previously (24).

To assess the induction of oxidative DNA damage by nickel(II), HeLa cells were incubated with $NiCl_2$ for 18 h. DNA strand breaks were induced in a dose-dependent manner at concentrations of 250 μM and higher (Figure 1A). However,

Table II. Cytotoxicity of cadmium(II) in HeLa cells

Cadmium(II) [μM]	CFA[a] (% of control) ± SD[b]
0	100 ± 1.9
1	106.2 ± 3.3
5	109.2 ± 8.1
10	108.0 ± 4.9
25	72.7 ± 3.2
50	23.9 ± 1.0
75	2.1 ± 0.7

Logarithmically growing cells were treated with $CdCl_2$ for 5 h, trypsinized and reseeded for the determination of the colony forming. Shown are mean values from six determinations.
[a]Colony forming ability.
[b]Standard deviation.

the spontaneously occurring frequency of Fpg-sensitive sites is not significantly enhanced at non-cytotoxic concentrations of up to 500 μM nickel(II); about 1.1 Fpg-sensitive DNA lesions per 10^6 base pairs are detected at 750 μM (Figure 1B).

The induction of DNA damage by cadmium(II) was determined after 5 h of incubation. As shown in Figure 2, a considerable amount of DNA strand breaks were generated at 10 and 50 μM; in contrast, no increase in Fpg-sensitive sites was observed at concentrations between 1 and 100 μM cadmium(II) (data not shown).

Effect of nickel(II) and cadmium(II) on the repair of oxidative DNA damage

To elucidate the effect of the metal compounds on the repair of oxidative DNA lesions, HeLa cells were irradiated with visible light. This treatment yields predominantly Fpg-sensitive sites and to a lesser extent DNA strand breaks, presumably due to the reaction of singlet oxygen or excited intracellular photosensitizer molecules with DNA (27). Most probably, the Fpg-sensitive DNA modifications are mainly 8-hydroxyguanine, the prevailing DNA lesion induced by both types of reactive species (25,28).

To induce the DNA damage, HeLa cells were illuminated as monolayers in cell culture dishes with 450 kJ/m^2 visible light, yielding about 1.7 Fpg-sensitive sites per 10^6 base pairs and 0.8 DNA strand breaks per 10^6 base pairs. To investigate the repair of the respective DNA lesions, the cells were post-incubated for different times and the remaining DNA damage was quantified. The repair kinetics of Fpg-sensitive sites and of DNA strand breaks induced by visible light in the absence of nickel(II) or cadmium(II) are shown in Figure 3. About 55% and 65% Fpg-sensitive sites were removed within 4 and 6 h respectively; within 24 h, no increase above the spontaneous level was detected, indicating complete repair within this time period (data not shown). The DNA strand breaks were closed faster and their repair was almost completed within 2 h after irradiation.

To elucidate the effect of nickel(II) on the repair of oxidative DNA damage, the cells were pre-incubated with $NiCl_2$ for 18 h, irradiated with visible light as described before and post-incubated with $NiCl_2$ for 4 h. While nickel(II) had no effect on the initial extent of DNA damage, the repair of both types of DNA lesions was affected in a dose-dependent manner in the presence of the metal (Figure 4). The removal of Fpg-sensitive sites was reduced to ~68% at 50 μM $NiCl_2$ and blocked completely at 250 μM $NiCl_2$. The closure of DNA single strand breaks was not affected at 50 μM nickel(II);

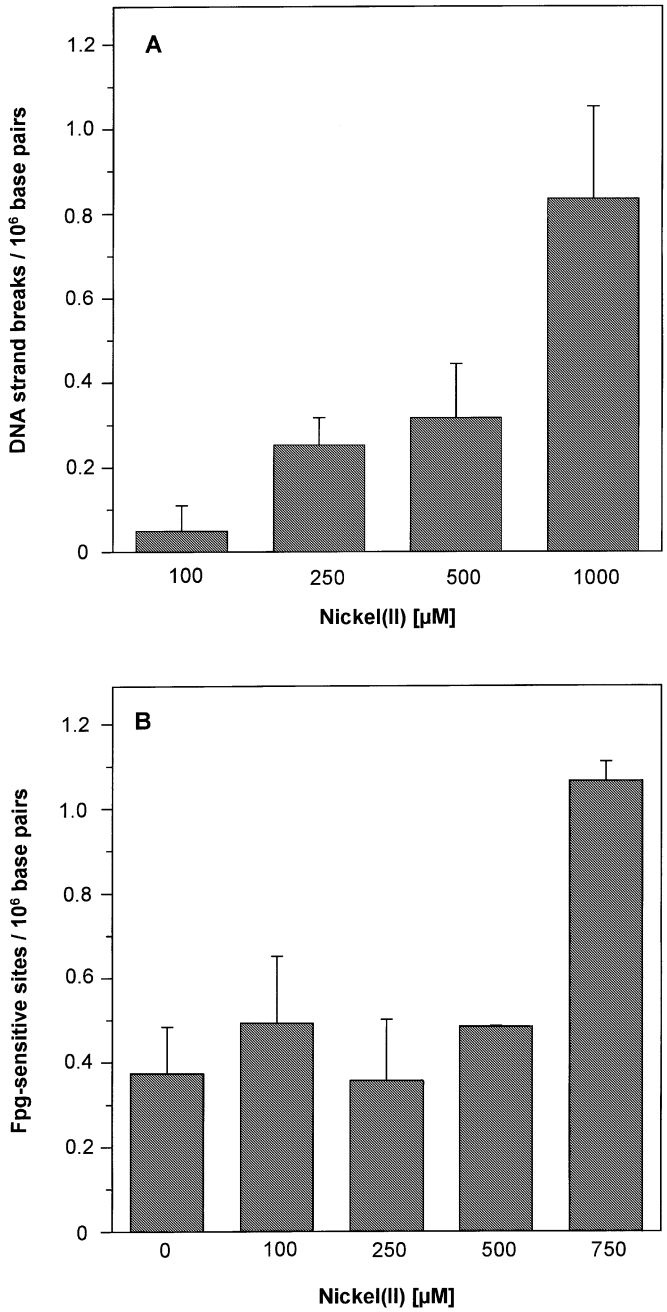

Fig. 1. Induction of DNA strand breaks (**A**) and Fpg-sensitive DNA base modifications (**B**) by nickel(II) in HeLa cells. Logarithmically growing cells were treated with $NiCl_2$ for 18 h and the frequency of the respective lesions was determined by alkaline unwinding as described in Materials and methods (mean values of triplicate determinations ± SD).

however, at concentrations of 100 μM and higher, the inhibition was quite similar when compared to the Fpg-sensitive DNA lesions, yielding an almost complete impairment of repair at 250 μM.

To investigate the effect of cadmium(II) on the removal of both types of lesions, HeLa cells were pre-incubated with $CdCl_2$ for 2 h, irradiated with visible light as described before and post-incubated with $CdCl_2$ for 4 h (Figure 5). In contrast to the results obtained with nickel(II), no significant repair inhibition occurred with respect to DNA strand breaks at concentrations of up to 50 μM cadmium(II). However, the removal of Fpg-sensitive sites was inhibited in a dose-depend-

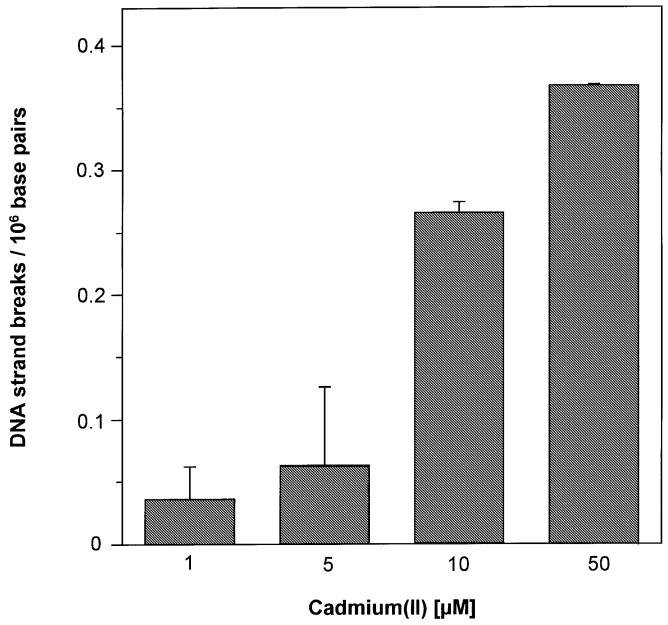

Fig. 2. Induction of DNA strand breaks by cadmium(II) in HeLa cells. Logarithmically growing cells were treated with $CdCl_2$ for 5 h and the frequency of DNA strand breaks was determined by alkaline unwinding as described in Materials and methods (mean values of triplicate determinations ± SD).

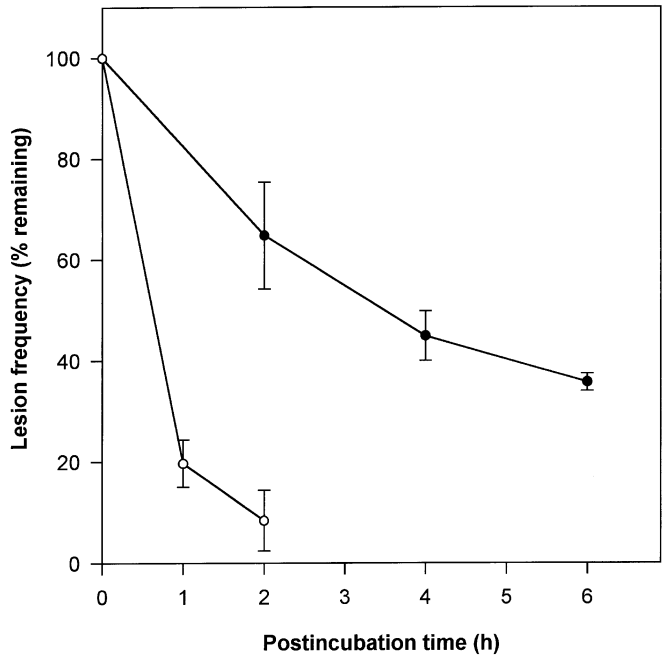

Fig. 3. Repair kinetics of DNA strand breaks (○–○) and Fpg-sensitive sites (●–●) in HeLa cells. Logarithmically growing cells were irradiated with 450 kJ/m^2 visible light as described in Materials and methods and the lesion frequencies were quantified after post-incubation in complete medium, times as indicated (mean values of triplicate determinations ± SD).

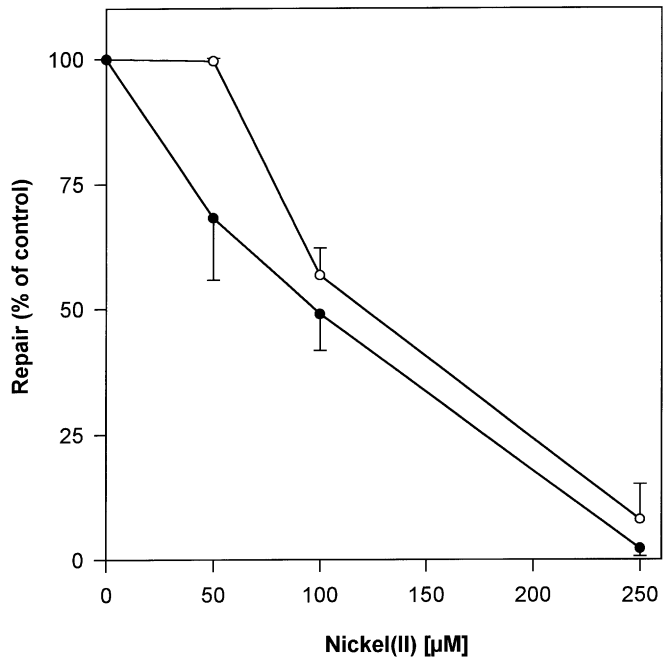

Fig. 4. Effect of nickel(II) on the repair of DNA strand breaks (○–○) and Fpg-sensitive DNA lesions (●–●) induced by visible light. Logarithmically growing HeLa cells were pre-incubated with $NiCl_2$ for 18 h, irradiated with 450 kJ/m^2 visible light and post-incubated in the presence of $NiCl_2$ for 4 h (mean values of triplicate determinations ± SD).

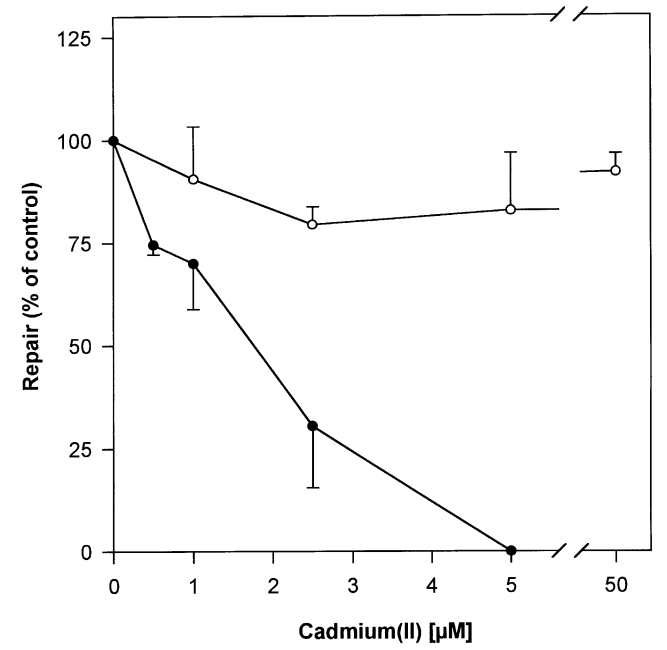

Fig. 5. Effect of cadmium(II) on the repair of DNA strand breaks (○–○) and Fpg-sensitive DNA lesions (●–●) induced by visible light. Logarithmically growing HeLa cells were pre-incubated with $CdCl_2$ for 2 h, irradiated with 450 kJ/m^2 visible light and post-incubated in the presence of $CdCl_2$ for 4 h (mean values of triplicate determinations ± SD).

ent manner at concentrations as low as 0.5 µM cadmium(II); a complete block of the repair reaction was observed at 5 µM.

Discussion

The results presented in this study demonstrate that nickel(II) and cadmium(II) induce oxidative DNA damage and, at much lower concentrations, interfere with the repair of DNA strand breaks and oxidative DNA base modifications induced by visible light. Oxidative DNA base modifications were quantified by the alkaline unwinding technique in combination with the purified Fpg protein derived from *E.coli*. The spectrum of DNA lesions detected by this approach corresponds to the substrate specificity of this bacterial repair enzyme. By applying γ-irradiated DNA, the Fpg protein has been shown to recognize

and remove ring-opened forms of guanine and adenine like 2,6-diamino-4-hydroxy-5-formamido-pyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde) as well as small amounts of 8-hydroxyadenine. The biologically most relevant lesion recognized by the Fpg protein, however, is 8-hydroxyguanine, which is induced very efficiently by ionizing radiation and singlet oxygen (25) and which is considered to be an important physiological substrate of this enzyme (29). If not repaired, this lesion has miscoding and mutagenic properties, and is suspected to play a predominant role in carcinogenesis (30).

While the induction of DNA strand breaks by nickel(II) has been described previously (31), the generation of oxidative DNA base modifications in intact cells in culture is demonstrated for the first time in this study. However, the effect is restricted to the cytotoxic concentration of 750 μM nickel(II), where the colony forming ability is reduced to <50%. In principle, this observation confirms results described from experiments with isolated chromatin, where nickel(II) in the presence of H_2O_2 generated a broad spectrum of DNA base modifications typical for •OH radical attack on the DNA; this effect required also comparatively high concentrations of nickel(II) (100 μM) and long incubation times (24 h) (7). The inefficiency of nickel(II) to induce appreciable amounts of Fpg-sensitive DNA lesions in intact cells might be due to the fact that •OH radicals are very short-lived and react at the site of their generation. This would require high concentrations of nickel(II) in close proximity or bound to the DNA. Since nickel ions have a higher affinity to certain amino acids as compared with DNA (32), this might explain its ability to damage DNA-associated proteins via oxidative mechanisms (33), while the potential to induce oxidative DNA base modifications in intact cells is rather low.

The generation of DNA strand breaks by cadmium(II) is in agreement with data presented by Ochi and Ohsawa (34) and Snyder (35) and others (2). However, no oxidative DNA base modifications were detected in the present study. Even though cadmium has been shown repeatedly to induce lipid peroxidation (5) and isolated lipid peroxidation products have been shown to induce 8-hydroxyguanine (36), this effect might be too weak to lead to appreciable amounts of DNA base modifications in intact cells.

In addition to the induction of DNA damage, both nickel(II) and cadmium(II) efficiently inhibit the repair of oxidative DNA damage at low, non-cytotoxic concentrations. In the absence of either metal compound, the time course of the removal of Fpg-sensitive sites and of DNA strand breaks induced by visible light, closely resembles observations described previously (27). In the presence of 50 μM and 100 μM nickel(II), respectively, the repair of both types of lesions is markedly delayed and blocked completely at 250 μM nickel(II). Concerning the ligation of DNA strand breaks, the results are in agreement with data presented by Snyder *et al.* (17), where nickel(II) inhibited the repair of DNA strand breaks induced by ionizing radiation. As stated above, current evidence suggests that the prevailing oxidative DNA base modification induced by visible light, is 8-hydroxyguanine (25,27,28); the finding that nickel(II) prevents the removal of this and possibly other Fpg-sensitive DNA base modifications is new and might also in part explain results obtained in experimental animals. While after i.p. injection of soluble nickel compounds the nickel concentrations in the blood and kidney reach their maximum level within 15–30 min and thereafter diminish rapidly (37), enhanced levels of 8-hydroxyguanine and other oxidatively modified DNA bases in renal and hepatic chromatin were detected only 48 h after the same route of exposure (38). Since oxidative DNA lesions are continuously generated as a consequence of the cellular oxygen metabolism, their increase *in vivo* after exposure to nickel(II) could be in part due to an impaired repair of the respective endogenously induced DNA modifications.

The repair of oxidative DNA base modifications induced by visible light is inhibited by very low concentrations of cadmium(II) as well. The repair capacity of the cells towards Fpg-sensitive DNA lesions is reduced to 75% at 0.5 μM cadmium(II), which is 50-fold below the concentration above which the colony forming ability starts to decline: the repair is abolished completely at 5 μM. In contrast to nickel(II), no inhibitory effect of up to 50 μM cadmium(II) was observed with respect to the closure of DNA strand breaks. This is in agreement with observations made previously (17), where the ligation of DNA strand breaks after ionizing radiation was not affected by cadmium(II) and may be due to differences in the processing of both types of lesions by cellular repair enzymes.

The underlying mechanisms of repair inhibition by nickel(II) and cadmium(II) are still unclear. Concerning the removal of 8-hydroxyguanine from DNA, the main repair pathway is the base excision repair system, where the first steps consist in the recognition and excision of the modified DNA base by a specific DNA glycosylase and the incision at abasic sites by an AP-endonuclease. In *E.coli*, these events are all mediated by the Fpg protein described above. In mammalian cells, some repair activities against DNA containing 8-hydroxyguanine have been discovered recently, including a DNA glycosylase and, as separate enzymes, endonucleases specific for 8-hydroxyguanine (39,40). However, the biochemical properties of these enzymes are less well characterized and their respective contributions to the repair of 8-hydroxyguanine remain unclear. Our data indicate that the first steps of the base excision repair pathway are affected by nickel(II) and cadmium(II) because the remaining DNA lesions after the repair incubation are still substrate for the Fpg protein. Since the Fpg protein recognizes the unexcised damaged DNA bases by its glycosylase activity as well as abasic sites by its AP endonuclease activity, nickel(II) and cadmium(II) disrupt either the recognition of the damage by the respective glycosylase, the incision in the DNA backbone by the AP endonuclease and/or a direct acting endonuclease specific for 8-hydroxyguanine. Regarding possible mechanisms of repair inhibition, nickel(II) and cadmium(II) may either inactivate the enzyme(s) directly, for example by reaction with histidine or cysteine, or compete with and displace essential metal ions. In the case of nickel(II), the latter possibility is supported by the observation that the inhibition of the incision step in nucleotide excision repair and the disturbance of DNA–protein interactions involved in the damage recognition of the repair process by nickel(II) were partly reversible by the addition of magnesium(II) (19,23). Similarly, the endonuclease activity specific for 8-hydroxyguanine in human polymorphonuclear neutrophils as well as various AP endonucleases depend on divalent metal ions as cofactors (41). In the case of cadmium(II), the interaction with essential zinc ions may be relevant. For example, there are several studies describing the displacement of zinc by cadmium in 'zinc finger' structures, a frequently occurring protein motif involved in DNA binding (e.g. 42,43). However, even though the bacterial Fpg protein contains a zinc finger structure

essential for its activity, none of the mammalian enzymes involved in the removal of 8-hydroxyguanine identified so far, has been shown to depend on zinc as a cofactor. Therefore, the exact mechanisms of repair inhibition will have to be elucidated in future studies.

Taken together, the results presented in this study demonstrate a rather weak or missing potential of nickel(II) and cadmium(II), respectively, to induce oxidative DNA base modifications in cultured mammalian cells and a more pronounced effect on the repair of the respective DNA lesions. The data add further evidence that the inhibition of DNA repair processes is an important mechanism in nickel- and cadmium-induced genotoxicity, especially, because these effects are observed at low, non-cytotoxic concentrations. Since oxidative DNA damage is continuously induced during aerobic metabolism, an impaired repair of these lesions might explain the carcinogenic action of nickel(II) and cadmium(II).

Acknowledgements

The Fpg protein was a kind gift of Dr Serge Boiteux, Commisariat Energie Atomique, Fontanay aux Roses, France. This work was supported by the Deutsche Forschungsgemeinschaft, grant no. Ha 2372/1-1 and by the University of Bremen.

References

1. IARC (1990) *Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 49: Nickel and Nickel Compounds*. IARC, Lyon, France.
2. IARC (1993) *Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 58: Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry*. IARC, Lyon, France.
3. Klein,C.B., Frenkel,K. and Costa,M. (1991) The role of oxidative processes in metal carcinogenesis. *Chem. Res. Toxicol*., **4**, 592–604.
4. Sunderman,F.W. Jr (1993) Search for molecular mechanisms in the genotoxicity of nickel. *Scand. J. Work Environ. Health*, **19**(Suppl. 1), 75–80.
5. Kasprzak,K.S. (1995) Possible role of oxidative damage in metal-induced carcinogenesis. *Cancer Invest*., **13**, 411–430.
6. Datta,A.K., Misra,M., North,S.L. and Kasprzak,K.S. (1992) Enhancement by nickel(II) and L-histidine of 2′-deoxyguanosine oxidation with hydrogen peroxide. *Carcinogenesis*, **13**, 283–287.
7. Nakerdien,Z., Kasprzak,K.S., Rao,G., Halliwell,B. and Dizdaroglu,M. (1991) Nickel(II)- and cobalt(II)-dependent damage by hydrogen peroxide to the DNA bases in isolated human chromatin. *Cancer Res*., **51**, 5837–5842.
8. Huang,X., Kitahara,J., Zhitkovich,A., Dowjat,K. and Costa,M. (1995) Heterochromatic proteins specifically enhance nickel-induced 8-oxo-dG formation. *Carcinogenesis*, **16**, 1753–1759.
9. Kasprzak,K.S., Diwan,B.A., Konishi,N., Misra,M. and Rice,J.M. (1990) Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. *Carcinogenesis*, **11**, 647–652.
10. Misra,M., Olinski,R., Dizdaroglu,M. and Kasprzak,K.S. (1993) Enhancement by L-histidine of nickel(II)-induced DNA–protein cross-linking and oxidative DNA base damage in the rat kidney. *Chem. Res. Toxicol*., **6**, 33–37.
11. Rossman,T.G., Roy,N.K. and Lin,W. (1992) Is cadmium genotoxic? In Nordberg,G.F., Alessio,L. and Herber,R.F.M. (eds) *Cadmium in the Human Environment: Toxicity and Carcinogenicity*. IARC, Lyon,France, pp. 367–375.
12. Hartwig,A. (1994) Role of DNA repair inhibition in lead- and cadmium-induced genotoxicity: A review. *Environ. Health Perspect*,, **102**(Suppl. 3), 45–50.
13. Sugiyama,M. (1994) Role of cellular antioxidants in metal-induced damage. *Cell Biol. Toxicol*., **10**, 1–22.
14. Stohs,S.J. and Bagchi,D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med*., **18**, 321–336.
15. Hartwig,A. (1995) Current aspects in metal genotoxicity, *Bio Metals*, **8**, 3–11.
16. Hartwig,A. and Beyersmann,D. (1989) Enhancement of UV-induced mutagenesis and sister-chromatid exchanges by nickel ions in V79 cells: evidence for inhibition of DNA repair. *Mutat. Res*., **217**, 65–73.
17. Snyder,R.D., Davis,G.F. and Lachmann,P.J. (1989) Inhibition by metals of X-ray and ultraviolet-induced DNA repair in human cells. *Biol. Trace Elem. Res*., **21**, 389–398.
18. Lee-Chen,S.F., Wang,M.C., Yu,C.T., Wu,D.R. and Jan,K.Y. (1993) Nickel chloride inhibits the DNA repair of UV-treated but not methyl methanesulfonate-treated Chinese hamster ovary cells. *Biol. Trace Elem. Res*., **37**, 39–50.
19. Hartwig,A., Mullenders,L.H.F., Schlepegrell,R., Kasten,U. and Beyersmann,D. (1994) Nickel(II) interferes with the incision step in nucleotide excision repair. *Cancer Res*., **54**, 4045–4051.
20. Hartwig,A. and Beyersmann,D. (1989) Comutagenicity and inhibition of DNA repair by metal ions in mammalian cells. *Biol. Trace Elem. Res*., **21**, 359–365.
21. Nocentini,S. (1987) Inhibition of DNA replication and repair by cadmium in mammalian cells. Protective interaction of zinc. *Nucleic Acids Res*., **15**, 4211–4225.
22. Hartmann,A. and Speit,G. (1996) Effect of arsenic and cadmium on the persistence of mutagen-induced DNA lesions in human cells. *Environ. Mol. Mutagen*., **27**, 98–104.
23. Hartwig,A., Schlepegrell,R., Dally,H. and Hartmann,M. (1996) Interaction of carcinogenic metal compounds with deoxyribonucleic acid repair processes. *Ann. Clin. Lab. Sci*., **26**, 31–38.
24. Hartwig,A., Dally,H. and Schlepegrell,R. (1996) Sensitive analysis of oxidative DNA damage in mammalian cells: Use of the bacterial Fpg protein in combination with alkaline unwinding. *Toxicol. Lett*., **88**, 85–90.
25. Boiteux,S., Gajewski,E., Laval,J. and Dizdaroglu,M. (1992) Substrate specificity of the *Escherichia coli* Fpg protein (Formamidopyrimidine–DNA glycosylase): Excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry*, **31**, 106–110.
26. Hartwig,A., Klyszcz-Nasko,H., Schlepegrell,R. and Beyersmann,D. (1993) Cellular damage by ferric nitrilotriacetate and ferric citrate in V79 cells: interrelationship between lipid peroxidation, DNA strand breaks and sister-chromatid exchanges. *Carcinogenesis*, **14**, 107–112.
27. Pflaum,M., Boiteux,S. and Epe,B. (1994) Visible light generates oxidative DNA base modifications in high excess of strand breaks in mammalian cells. *Carcinogenesis*, **15**, 297–300.
28. Schneider,J.E., Price,S., Maidt,M.L., Gutteridge,J.M.C. and Floyd,R.A. (1990) Methylene blue plus light mediates 8-hydroxy-2′-deoxyguanosine formation in DNA preferentially over strand breakage. *Nucleic Acids Res*., **18**, 631–635.
29. Tchou,J., Kasai,H., Shibutani,S., Chung,M.-H., Laval,J., Grollman,A.P. and Nishimura,S. (1991) 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc. Natl Acad. Sci., USA*, **88**, 4690–4694.
30. Floyd,R.A. (1990) The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis*, **11**, 1447–1450.
31. Robison,S.H., Cantoni,O. and Costa,M. (1982) Strand breakage and decreased molecular weight of DNA induced by specific metal compounds. *Carcinogenesis*, **5**, 657–662.
32. The Chemical Society (1971) *Stability Constants of Metal-ion Complexes*. Supplement No. 1, Special Publication 25, The Chemical Society, London.
33. Zhuang,Z., Huang,X. and Costa,M. (1994) Protein oxidation and amino acid-DNA crosslinking by nickel compounds in intact cultured cells. *Toxicol. Appl. Pharmacol*., **126**, 319–325.
34. Ochi,T. and Ohsawa,M. (1983) Induction of 6-thioguanine-resistant mutants and single strand scissions of DNA by cadmium chloride in cultured Chinese hamster cells. *Mutation Res*., **111**, 69–78.
35. Snyder,R.D. (1988) Role of active oxygen species in metal-induced DNA strand breakage in human diploid fibroblasts. *Mutat. Res*., **193**, 237–246.
36. Park,J.-W. and Floyd,R.A. (1992) Lipid peroxidation products mediate the formation of 8-hydroxydeoxyguanosine in DNA. *Free Rad. Biol. Med*., **12**, 245–250.
37. Sunderman,F.W. Jr and Selin,C.E. (1968) The metabolism of nickel-63 carbonyl. *Toxicol. Appl. Pharmacol*., **12**, 207–218.
38. Kasprzak,K.S, Diwan,B.A., Rice,J.M., Misra,M., Riggs,C.W., Olinski,R. and Dizdaroglu,M. (1992) Nickel(II)-mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. *Chem. Res. Toxicol*., **5**, 809–815.
39. Chung,M-H., Kim,H.-S., Ohtsuka,E., Kasai,H., Yamamoto,F. and Nishimura,S. (1991) An endonuclease activity in human polymorphonuclear neutrophils that removes 8-hydroxyguanine residues from DNA. *Biochem. Biophys. Res. Comm*., **178**, 1472–1478.
40. Bessho,T. , Tano,K., Kasai,H., Ohtsuka,E. and Nishimura,S. (1993) Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J. Biol. Chem*., **268**, 19416–19421.
41. Demple,B. and Harrison,L. (1994) Repair of oxidative damage to DNA: Enzymology and Biology. *Annu. Rev. Biochem*., **63**, 915–948.
42. Predki,P.F. and Sarkar,B. (1992) Effect of replacement of 'zinc finger' zinc on estrogen receptor DNA interactions. *J. Biol. Chem*., **267**, 5842–5846.
43. Hanas,J.S. and Gunn,C.G. (1996) Inhibition of transcription factor IIIA-DNA interactions by xenobiotic metal ions. *Nucleic Acids Res*., **24**, 924–930.

Received on December 3, 1996; revised on February 10, 1997; accepted on February 11, 1997