Nickel(II) Affects Poly(ADP-ribose) Polymerase-Mediated DNA Repair in Normal and Cancer Cells

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Nickel(II) can be genotoxic, but the mechanism of its genotoxicity is not fully understood and the process of DNA repair may be considered as its potential target. We studied the effect of nickel chloride on the poly(ADP-ribose) polymerase (PARP)-mediated repair of DNA damaged by γ-radiation and idarubicin with the alkaline comet assay in normal and cancer cells. Our results indicate that nickel chloride at very low, non-cytotoxic concentration of 1 μM can affect PARP-mediated DNA repair of lesions evoked by idarubicin and γ-radiation. We also suggest that in the quiescent lymphocytes treated with γ-radiation, nickel(II) could interfere with DNA repair process independent of PARP.

Key words: DNA Repair, Nickel, Poly(ADP-ribose) Polymerase

Introduction

DNA repair plays the main role in protection of the genome against mutations, which can be lethal or can lead to the development of malignant transformation. In cancer cells, the activity of DNA repair proteins may contribute to their resistance to chemo- or/and radiotherapy, which may be overcome by the use of DNA repair inhibitors. It was shown that metal ions can inhibit DNA repair by several mechanisms, including free radical formation, substitution of other metals indispensable in repair process or by lowering the level of GSH (Chao, 1996; Hartwig and Schwerdtle, 2002). It is postulated that the inhibition of DNA repair by some metals, including Cr, Cd, Ni, Co and As, is one of the main mechanisms underlying their carcinogenic properties.

Nickel(II) has been shown to interfere with the DNA repair mechanisms involved in removing of UV, platinum, mitomycin C, γ-radiation, MNNG and benzo[*a*]pyrene-induced DNA damage (Au *et al.*, 1994; Dally and Hartwig, 1997; Hartmann and Hartwig, 1998; Krueger *et al.*, 1999; Takahashi *et al.*, 2000; Schwerdtle *et al.*, 2002; Wozniak and Blasiak, 2004). Nickel(II) can impair DNA repair by base excision (BER), that can result in decreased efficacy of removing oxidative DNA base modifications, abasic sites as well as the closure of DNA strand breaks. Some compounds of nickel have been also shown to inhibit the nucleotides excision DNA repair (NER). In consequence, the removing of (6–4)-photoproducts, cyclobutane pyrimidine dimers and bulky DNA adducts is incomplete. It is also possible that nickel can interfere with recombination repair involved in removing such DNA damage as interstrand cross-links and DNA double-strand breaks, induced by γ-radiation and some antitumour drugs including cisplatin and mitomycin C (Krueger *et al.*, 1999; Takahashi *et al.*, 2000).

Poly(ADP-ribose) polymerase (PARP) is an abundant nuclear enzyme involved in DNA repair (Satoh and Lindahl, 1992; Burkle, 2001; Ziegler and Oei, 2001). It is a specific detector of DNA strand breaks generated as a consequence of the action of alkylating agents, oxidants or ionizing radiation. The enzyme recognises and rapidly binds to both single- and double-strand breaks in DNA. According to current models, PARP catalyses the attachment of ADP-ribose units provided by nicotinamide adenine dinucleotide (NAD^+) to suitable protein acceptors including topoisomerase, histones and PARP itself. This process results in the synthesis of large negatively charged poly(ADP-ribose) polymers, which facilitate dissociation of the modified PARP protein from DNA, allowing other enzymes to access and repair DNA strand breaks.

In the present work, we investigated the effect of nickel chloride at a very low concentration, 1 μM, on the repair of DNA damage induced by two agents used in cancer therapy – idarubicin

and γ-radiation. We used 1 μM concentration of nickel because it was the lowest concentration of this metal by which we observed inhibition of DNA repair and activity of repair enzymes in our previous experiments (Wozniak and Blasiak, 2004). Additionally, we did not employ higher concentrations of nickel because its physiological concentration in serum is much lower (2.5 nM) (Christensen *et al.*, 1999). We were interested in the minimum concentration of nickel, which could give an effect in DNA repair inhibition. Idarubicin is used mainly in acute myelogenous leukaemia. It is a member of the anthracycline group, whose anti-proliferative action origins from its ability to diffuse across the cell membrane and to intercalate between DNA base pairs and target topoisomerase II. Idarubicin is not free from adverse side effects, the main of which is free radicals-based cardiotoxicity (Horenstein *et al.*, 2000). Recently, we have shown that idarubicin can also induce free radicals-mediated DNA damage in normal lymphocytes and leukemic cells (Blasiak *et al.*, 2002). γ-Radiation induces various oxidative changes in DNA bases as well as single- and double-strand breaks. We used the comet assay at pH > 13 to detect DNA strand breaks introduced by above agents and those arising during DNA repair. The experiments were performed on human normal lymphocytes and two types of cancer cells, HeLa and K562 cells. We used cancer cells because they differ from normal cells in their morphology and genetic constitution, including different expression pattern for genes involved in the reaction of the cells to damaging stimuli. In order to identify the involvement of nickel in the DNA repair process mediated by PARP, we compared its action with the effect exerted by the specific inhibitor of PARP, 3-aminobenzamide (3-AB). We also performed the MTT assay in order to evaluate the cytotoxic potential of the single compounds used in the present work and their combined treatments.

Materials and Methods

Chemicals

Nickel chloride ($NiCl_2$), 3-aminobenzamide (3-AB), DMEM medium, RPMI 1640 medium without L-glutamine, fetal bovine serum (FBS), penicillin, streptomycin, low melting point (LMP) and normal melting point (NMP) agarose, phosphate buffered saline (PBS), 4′,6-diamidino-2-phenylindole (DAPI) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phytohemagglutinin (PHA) were purchased from Sigma. Idarubicin (4-demethoxy-daunorubicin) was obtained from Pharmacia and Upjohn (Milan, Italy). All other chemicals were of the highest grade commercially available.

Cells

Human normal lymphocytes were isolated from peripheral blood obtained from healthy donors by centrifugation in a density gradient of Gradisol L (15 min, 280 × *g*, 4 °C). The viability of the cells was measured by the trypan blue exclusion assay and was found to be about 98%. Human chronic myelogenous leukemia cells (K562) were obtained from Professor Jacques Robert, Department of Biochemistry, Institute Bergonié, Bordeaux, France. They were grown in RPMI 1640 medium with L-glutamine supplemented with 10% FBS, 100 IU/ml penicillin and 100 μg/ml streptomycin in the presence of a 5% CO_2 atmosphere at 37 °C. Human cervical cancer cells (HeLa cells) were grown in Dulbecco's modified essential medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin in 5% CO_2 at 37 °C.

Cell treatment

The final density of the cells in each sample was adjusted to $1-3 \times 10^5$ cells/ml by adding RPMI 1640 medium to the single cell suspension. The cells were pre-incubated with nickel chloride at 1 μM or 3-AB at 200 μM or with a combination of both agents for 20 h at 37 °C. Subsequently, the cells were washed and resuspended in fresh RPMI 1640 medium preheated to 37 °C. Idarubicin was added to the suspension of the cells to give a final concentration of 0.5 μM and a 1-h incubation at 37 °C was carried out. In experiments with ionizing radiation the cells were irradiated at 3 Gy at 25 °C at a dose rate of 1.904 Gy/min using a γ-^{60}Co source (Technical University of Lodz, Lodz, Poland). Control samples were incubated with nickel chloride at 1 μM or 3-AB at 200 μM or with both agents for 20 h at 37 °C without any further treatment. Such incubation did not introduce significant DNA damage, so the results for these cells were not presented for the sake of clarity and only results for cells untreated with any agent (negative control) were shown.

MTT assay

We performed the MTT assay after incubation of the lymphocytes, HeLa cells and K562 cells for 3 d with single or combined treatments of nickel chloride (1 μM), 3-AB (200 μM) and idarubicin (0.5 μM) or γ-radiation (3 Gy) (data not shown). We added PHA at 0.1 mg/ml in the case of the lymphocytes. Briefly, the cells were plated onto 96-well plates in 100 μl of growth medium and 10 μl of 5 mg/ml MTT were added to each well. After incubation at 37 °C for 3 h, the supernatant was removed and 100 μl of a solution containing 10% SDS and 0.04 M HCl were added to dissolve the water-insoluble formazan salt. The difference ($OD_{650\,nm} - OD_{570\,nm}$) was measured next day with an ELISA microplate reader (Bio-Rad, Hercules, CA, USA).

DNA repair

The cells after the treatment with idarubicin or γ-radiation, as well as control samples, were washed and resuspended in fresh RPMI 1640 medium pre-heated to 37 °C. Aliquots of the suspension were taken immediately and 120 min later. Further steps were as described in the next section.

Comet assay

The comet assay was performed under alkaline conditions essentially to the procedure of Singh *et al.* (1988) with some modification (Klaude *et al.*, 1996) as described previously (Blasiak and Kowalik, 2000). A freshly prepared suspension of the cells in 0.75% LMP agarose dissolved in PBS was spread onto microscope slides (Superior, Germany) pre-coated with 0.5% NMP agarose. The cells were then lysed for 1 h at 4 °C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After the lysis, the slides were placed in an electrophoresis unit, DNA was allowed to unwind for 20 min in the solution consisting of 300 mM NaOH and 1 mM EDTA, pH > 13. Electrophoresis was conducted in the electrophoretic solution consisting of 30 mM NaOH and 1 mM EDTA, pH > 13 at ambient temperature of 4 °C (the temperature of the running buffer did not exceed 12 °C) for 20 min at an electric field strength of 0.73 V/cm (28 mA). The slides were then washed in water, drained and stained with 2 μg/ml DAPI and covered with cover slips. To prevent additional DNA damage, all the steps described above were conducted under dimmed light or in the dark.

The comets were observed at 200 × magnification in an Eclipse fluorescence microscope (Nikon, Japan) attached to a COHU 4910 video camera (Cohu, Inc., San Diego, CA, USA) equipped with a UV-1 A filter block and connected to a personal computer-based image analysis system Lucia-Comet v. 4.51 (Laboratory Imaging, Praha, Czech Republic). 100 images were randomly selected from each sample and the tail DNA (%) was measured. The tail DNA is positively correlated with the level of DNA breakage in a cell. The mean value of the tail DNA in a particular sample was taken as an index of DNA damage.

Data analysis

The data from the comet assay in this study were expressed as mean ± S.E.M. from three independent experiments, *i.e.*, data from three experiments were pooled and the statistical parameters were calculated. If no significant differences between variations were found, as assessed by the Snedecor-Fisher test, the differences between means were evaluated by applying Student's *t*-test. The data were analysed using STATISTICA (StatSoft, Tulsa, OK) statistical package.

Results and Discussion

We noticed a decrease in cell viability after a 3-d incubation in the case of idarubicin-treated lymphocytes and K562 cells ($P < 0.01$) and γ-radiation-treated lymphocytes ($P < 0.05$), HeLa cells ($P < 0.05$) and K562 cells ($P < 0.01$) (data not shown). Co-incubation of idarubicin-treated lymphocytes with nickel chloride or 3-AB also decreased their viability ($P < 0.05$ and $P < 0.01$, respectively). In K562 cells we observed a decrease in cell viability only in the case of co-incubation with nickel chloride ($P < 0.01$). Combined incubation of γ-radiation-treated normal and cancer cells with nickel chloride or 3-AB also evoked a significant decrease of the cell viability. Fig. 1 (I) shows the kinetics of DNA repair in lymphocytes (A), HeLa cells (B) and K562 cells (C) exposed to γ-radiation. About 60% of DNA damage, measured as the comet tail DNA was removed during a 120-min repair incubation in the case of lymphocytes and HeLa cells and about 70% in the case of K562 cells. In lymphocytes pre-incubated with 3-AB similar results were observed – majority of DNA

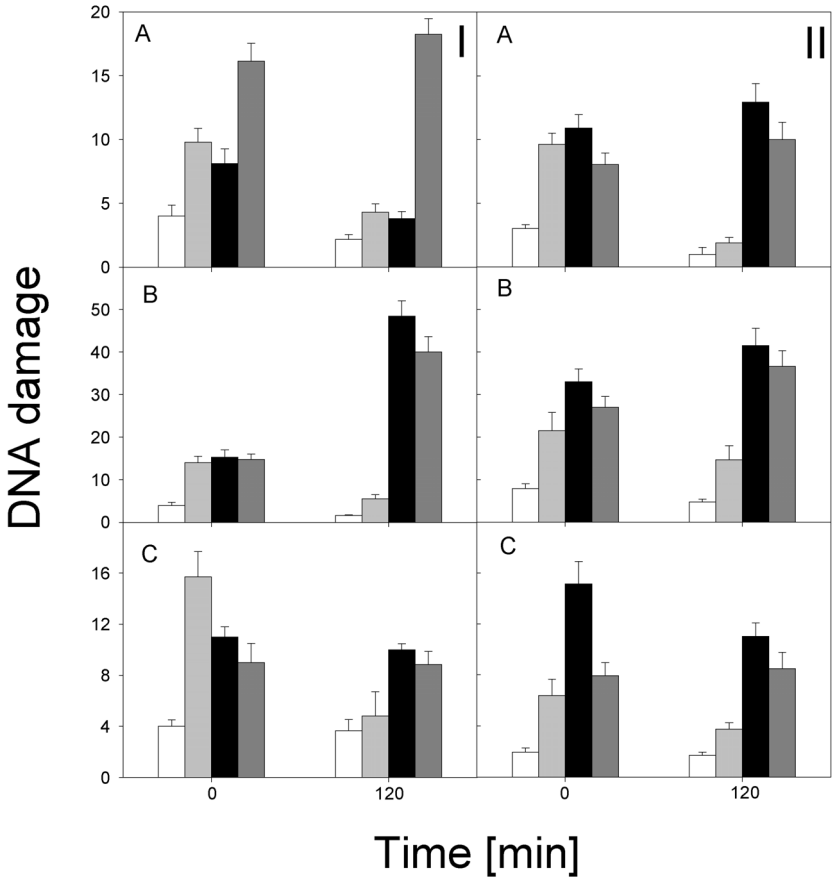

Fig. 1. The kinetics of DNA repair in (A) lymphocytes, (B) HeLa cells and (C) K562 cells exposed to γ-radiation at 3 Gy (I) and exposed to idarubicin at 0.5 μM (II) after a 20 h-incubation at 37 °C in the absence of nickel chloride or 3-AB (light grey cols.) or with nickel chloride at 1 μM (dark grey cols.) or with 3-AB at 200 μM (black cols.) as compared with untreated control (empty cols). The number of cells scored for each treatment was 100. Presented data are means of three independent experiments ± S.E.M.

damage was repaired in the same period. In lymphocytes pre-incubated with nickel chloride at 1 μM we observed a high constant level of DNA damage ($P < 0.001$). In HeLa cells that were pre-incubated with nickel chloride and 3-AB, we did not detect DNA repair. After 120 min of post-incubation, we observed a significant increase ($P < 0.001$) in the % tail DNA of comets originating from the cells pre-incubated with nickel chloride or 3-AB. In the case of K562 cells pre-incubated with nickel chloride or 3-AB we also did not detect DNA repair. We observed constant level of DNA damage in these cells. After 120 min of post-incubation, the level of DNA damage was higher in cells pre-incubated with these agents than in control and irradiated cells ($P < 0.001$).

Fig. 1 (II) displays the kinetics of DNA repair in lymphocytes, HeLa cells and K562 cells after incubation with idarubicin at 0.5 μM. DNA damage induced by this drug was effectively removed during repair incubation in lymphocytes (about 80% of DNA damage). In the case of HeLa cells and K562 cells about 30% and 40%, respectively, of DNA lesions were removed. Lymphocytes pre-incubated with nickel chloride or 3-AB did not remove DNA damage evoked by idarubicin. In HeLa cells pre-incubated with nickel chloride or 3-AB we observed a higher level of DNA damage compared with the cells pre-incubated without nickel chloride or 3-AB ($P < 0.001$). In the case of K562 cells pre-incubated with 3-AB we observed less effective DNA repair than in the cells pre-incubated in the absence of 3-AB (about 30% of DNA damage was removed). In these cells pre-incubated with nickel chloride, we observed a constant level of DNA damage at the beginning and at the end of repair incubation.

The molecular mechanism of DNA repair inhibition by nickel and other metals is still poorly understood. Nickel may bind to DNA repair en-

zymes and generates oxygen-free radicals to cause protein degradation *in situ* (Lynn *et al.*, 1997). On the other hand, a competition of toxic metals with essential ions appears to be an important mechanism of their genotoxicity (Asmuss *et al.*, 2000; Hartwig *et al.*, 2002; Bal *et al.*, 2003). Potentially sensitive targets for the toxic action of nickel are zinc finger structures in DNA binding motifs present in several DNA repair enzymes, including the bacterial Fpg protein, the mammalian xeroderma pigmentosum group A protein (XPA) and DNA ligase III. It was shown that nickel(II) reduced the DNA-binding ability of XPA which plays a central role in the first step of nucleotide excision repair (NER) (Asmuss *et al.*, 2000). The nickel(II) ion can form a square planar complex with the sulphur atoms of XPA zinc fingers, opposed to the tetrahedral structure of the native zinc(II) complex. Consequently, the overall zinc finger structure may be lost in the nickel(II)-substituted peptide (Bal *et al.*, 2003). The effects of interference with DNA repair by nickel were reported to be reversible by the addition of magnesium, indicating that the inactivation of DNA repair indeed occurred at the protein level by the displacement of the respective essential metal ions, like zinc(II), rather than by an interference with the expression of the repair proteins (Hartmann and Hartwig, 1998). In the light of these findings we can see that the negative influence of the nickel ions on DNA repair is very specific, and such ions as Mg^{2+} or Ca^{2+}, that physiologically occur in the cell at higher concentrations, as they are co-factors of many enzymes, and play a special role in many processes, may play a rather opposite role and protect DNA repair proteins against the toxic effect of nickel. Poly(ADP-ribose) polymerase contains two zinc finger motifs, which are located in the *N*-terminal DNA-binding domain. The role of PARP in DNA base excision repair may be connected with its direct or indirect association with the repair proteins: XRCC1, DNA polymerase β and DNA ligase III in a repair complex (Caldecott *et al.*, 1996). PARP-deficient cells or organisms are more sensitive to ionizing radiation, monofunctional alkylating agents and free radicals.

In the present work we used two DNA-damaging agents, ionizing radiation and idarubicin, which induce oxidative DNA damage. PARP was shown to play a central role in the repair of such DNA lesions (Schraufstatter *et al.*, 1986; Decker and Muller, 2002). In order to identify DNA repair mediated by PARP we used 3-aminobenzamide, an inhibitor of PARP activity. Benzamides and their derivatives act as alternative substrates to NAD^+ for PARP activity. 3-AB was shown to delay the rejoining step of DNA strands breaks induced by mono-alkylating agents. Benzamides may potentate the cytotoxicity of a wide range of anti-tumour agents *in vitro*, such as bleomycin and methylating drugs. A number of benzamide derivatives have also been shown to have the potential to act as tumour cell radiosensitisers *in vitro* and *in vivo* (Berthet *et al.*, 1999; Virag and Szabo, 2002).

Fig. 1 (I) shows the repair of DNA damage induced by γ-radiation in lymphocytes, HeLa cells and K562 cells. We speculate that in lymphocytes DNA repair proceeds without PARP involvement. This is in agreement with a report suggesting that PARP is not required for X-ray-induced damage repair in quiescent cells (Chatterjee and Berger, 2000). Therefore, our studies indicate the differential sensitivity of normal cells [Fig. 1 (IA)] and tumour cells [Figs. 1 (IB, C)] towards the radiosensitizing effect of PARP inhibition by 3-AB. It was shown that nicotinamide apparently enhanced tumour radiation damage while it had minimal effects in normal tissues (Horsman *et al.*, 1997). It seems that nickel cannot be considered as a specific radiosensitizing agent because it inhibited the repair of radiation-induced DNA damage in normal [Fig. 1 (IA)] and cancer cells [Figs. 1 (IB, C)]. However, the level of DNA strand breaks arisen during repair incubation in HeLa cells pre-incubated with nickel chloride was higher than in the lymphocytes [Figs. 1 (IA, B)]. Further experiments are needed to assess whether nickel could be used as a radiotherapy sensitizer. In HeLa cells pre-incubated with nickel chloride at 1 μM we observed, similarly to the case of 3-AB, an increase of % tail DNA during repair incubation [Fig. 1 (IB)]. Our results indicate that nickel(II) affects DNA repair by inhibition of PARP. Hartwig *et al.* (2002) showed that this metal could inhibit the activity of PARP. It is possible that nickel inhibited other repair enzymes under our experimental conditions. In leukemic K562 cells, nickel similarly to 3-AB inhibited the formation of transient DNA breaks brought by the repair process after exposure to γ-radiation, what might follow from the interfering with the recognition/incision step of DNA repair [Fig. 1 (IC)]. In the cells incubated with idarubicin,

pre-incubation with nickel caused an increase in comet tail DNA after 120 min of repair incubation compared to the cells pre-incubated in the absence of this metal [Figs. 1 [IIA, B and C)]. This increase is similar to the increase of DNA damage caused by 3-AB.

In our experiments, the respective treatments showed different levels of DNA damage in different cell lines. We suggest that it could result from different genetic constitutions of employed cell lines. We used the cells with different expression of proteins participating in cell response to genotoxic stress and DNA repair. We chose K562 cells because they have endogenous expression of BCR/ABL fusion kinase, which contributes to the genetic instability responsible for leukemia progression (Laneuville *et al.*, 1992). Our recent studies revealed a novel mechanism of resistance in BCR/ABL-positive cells: stimulation of DNA double-strand break repair by homologous recombination (Slupianek *et al.*, 2002). Moreover, K562 cells lack the p53 protein activity (Usuda *et al.*, 2003).

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