Interference by Toxic Metal Ions with DNA Repair Processes and Cell Cycle Control: Molecular Mechanisms

A. Hartwig,[1] M. Asmuss,[1,2] I. Ehleben,[1] U. Herzer,[1] D. Kostelac,[1] A. Pelzer,[1] T. Schwerdtle,[1] and A. Bürkle[3]

[1]Institut für Lebensmittelchemie und Toxikologie, Universität Karlsruhe, Karlsruhe, Germany; [2] Bundesamt für Strahlenschutz, Institut für Strahlenhygiene, Neuherberg, Germany; [3]Department of Gerontology, University of Newcastle, Newcastle upon Tyne, United Kingdom

Nickel, cadmium, cobalt, and arsenic compounds are well-known carcinogens to humans and experimental animals. Even though their DNA-damaging potentials are rather weak, they interfere with the nucleotide and base excision repair at low, noncytotoxic concentrations. For example, both water-soluble Ni(II) and particulate black NiO greatly reduced the repair of DNA adducts induced by benzo[*a*]pyrene, an important environmental pollutant. Furthermore, Ni(II), As(III), and Co(II) interfered with cell cycle progression and cell cycle control in response to ultraviolet C radiation. As potential molecular targets, interactions with so-called zinc finger proteins involved in DNA repair and/or DNA damage signaling were investigated. We observed an inactivation of the bacterial formamidopyrimidine–DNA glycosylase (Fpg), the mammalian xeroderma pigmentosum group A protein (XPA), and the poly(adenosine diphosphate–ribose)polymerase (PARP). Although all proteins were inhibited by Cd(II) and Cu(II), XPA and PARP but not Fpg were inhibited by Co(II) and Ni(II). As(III) deserves special attention, as it inactivated only PARP, but did so at very low concentrations starting from 10 nM. Because DNA is permanently damaged by endogenous and environmental factors, functioning processing of DNA lesions is an important prerequisite for maintaining genomic integrity; its inactivation by metal compounds may therefore constitute an important mechanism of metal-related carcinogenicity. *Key words:* cell cycle control, DNA repair, Fpg, XPA, PARP, metal ions. *Environ Health Perspect* 110(suppl 5):797–799 (2002).
http://ehpnet1.niehs.nih.gov/docs/2002/suppl-5/797-799hartwig/abstract.html

Nickel, cobalt, arsenic, and cadmium compounds are well-known carcinogens to humans and/or to experimental animals. Several types of cellular damage have been identified that may contribute to their carcinogenic potentials, such as the induction of oxidative DNA damage (*1,2*) and epigenetic alterations such as gene silencing by changes in DNA methylation patterns, as shown for particulate nickel compounds and arsenite (*3,4*). Furthermore, As(III), Co(II), Ni(II), and Cd(II) exerted pronounced comutagenic activities in bacteria as well as in mammalian cells (*5*), and recently cocarcinogenicity of arsenite in combination with ultraviolet C (UVC) radiation was demonstrated in hairless mice (*6*). These enhancing effects may be explained by interference with distinct steps of different DNA repair systems [summarized in Hartwig (*7*)]. Thus, during the last years, detailed studies have been conducted to elucidate potential molecular mechanisms leading to DNA repair inhibitions. Furthermore, more recently the question has been addressed whether metal ions also interfere with other cellular responses to DNA damage, such as cell cycle progression and control.

DNA Damage, Repair, and Interference by Toxic Metal Ions

Maintenance of genetic information, and thus the correct sequence of nucleotides in DNA, is essential for replication, gene expression, and protein synthesis; DNA lesions at critical sites like oncogenes or tumor suppressor genes may lead to cell cycle arrest, programmed cell death, mutagenesis, genomic instability, and cancer. Surprisingly, in addition to replication errors, DNA is not only damaged by environmental mutagens including UV light and polycyclic aromatic hydrocarbons (PAHs), but also permanently by reactive oxygen species (ROS) generated endogenously due to oxygen metabolism (*8*). To minimize adverse consequences, a complex network of different repair systems has evolved to maintain genomic integrity (Figure 1). Thus, replication errors are repaired by the mismatch repair pathway, and double-strand breaks are repaired by homologous or illegitimate recombination processes. The major pathway eliminating DNA base damage and helix distortions is the excision repair pathway, subdivided into nucleotide excision repair (NER) and base excision repair (BER) (*9*). There has been accumulating evidence that diverse DNA repair systems including BER and NER are inhibited at low, noncytotoxic concentrations of Ni(II), Co(II), Cd(II), and As(III). Thus, arsenite was shown to inhibit the ligation step during the removal of *N*-methyl-*N*-nitrosourea–induced DNA damage during BER (*10*). With respect to oxidative DNA damage, even though neither Ni(II) nor Cd(II) increased the overall frequency of oxidative DNA base damage at biologically relevant concentrations in HeLa cells, both compounds inhibited the removal of oxidative DNA base modifications induced by visible light at low, noncytotoxic concentrations (*11*). Regarding NER, detailed investigations revealed that different steps of the repair process were affected and different mechanisms were identified to account for the observed inhibitions (*12*). For example, Ni(II) and Cd(II) disturbed the very first step of nucleotide excision repair, namely, the recognition of DNA damage (*13*), whereas Co(II) affected both the incision as well as the polymerization of repair patches (*14*). Arsenite impaired the incision step at low concentrations and the ligation at higher concentrations (*15*).

Recently we investigated the effects of $NiCl_2$ and particulate black NiO on the induction and removal of stable DNA adducts generated by the carcinogen benzo[*a*]pyrene (B[*a*]P). B[*a*]P belongs to the class of PAHs generated by incomplete combustion of organic matter and therefore is present not only at many workplaces, but also in the ambient air. Its carcinogenic activity is attributed to the formation of DNA adducts, resulting from electrophilic attack predominantly at guanine residues by metabolically activated intermediates formed from the parent hydrocarbon. For carcinogenicity, the most relevant metabolic pathway is probably connected to the action of cytochromes P450 1A1 and P4501B1 and epoxide hydrolases, yielding *syn*- and *anti*-B[*a*]P-7,8-diol 9,10-epoxides (BPDEs), which form stable adducts at the N^2 position of guanine and, less frequently, of adenine. When replicated prior to repair, these adducts can lead to mutations and cancer (*16*). Thus, the induction and removal of the latter adducts generated by the active metabolite (+)-*anti*-BPDE in the absence and presence of nickel compounds was quantified by a very sensitive high-performance liquid chromatography/fluorescence method in A549 human lung cancer cells

This article is part of the monograph *Molecular Mechanisms of Metal Toxicity and Carcinogenicity.*

Address correspondence to A. Hartwig, Institute of Food Chemistry and Toxicology, University of Karlsruhe, Postfach 6980, D-76128 Karlsruhe, Germany. Telephone: 49 721 608 2936. Fax: 49 721 608 7254. E-mail: andrea.hartwig@chemie.uni-karlsruhe.de

This work was supported by Deutsche Forschungsgemeinschaft grant Ha 2372/1-2, European Union grant QLK4-CT-1999-01142, and Programm Lebensgrundlage Umwelt und ihre Sicherung (BW-PLUS) grant BW BG 99012.

Received 7 February 2002; accepted 29 May 2002.

(*17,18*). Both nickel compounds inhibited the removal of these lesions in a dose-dependent manner. Importantly, these inhibitions occurred in both cases at noncytotoxic concentrations and were slightly more pronounced in the case of NiO, where lesion removal was reduced to about 20% of the control (*18*). Regarding these findings, repair inhibition by Ni(II) appears to be largely independent of the compound applied, and at first sight these observations do not provide an explanation for the marked differences in carcinogenic potencies between water-soluble and particulate nickel compounds (*19*). However, when considering the 1,000-fold higher persistent nickel burden in rat lung after exposure toward NiO compared with water-soluble nickel sulfate (*19*), exposure to particulate nickel compounds may give rise to continuous DNA repair impairment, and thus the biological consequences may be far more severe.

Interactions with Cell Cycle Progression and Control

In addition to DNA repair processes, one other response to DNA damage is the regulation of cell cycle progression by activation of DNA damage checkpoints. In general, these checkpoints control the ability of cells to arrest the cell cycle in response to DNA damage; furthermore, they control the activation of DNA repair pathways, the movement of DNA repair proteins to sites of DNA damage, the activation of transcriptional programs, and in some cell types, the induction of cell death by apoptosis in case of heavily damaged DNA [Figure 2; for recent review see Zhou and Elledge (*20*)]. These control mechanisms operate throughout the cell cycle, and three DNA damage-response checkpoints appear to be important. Thus, DNA strand breaks appear to induce a G_1 arrest, increasing the probability that replication does not occur until repair is completed. Other damage-responsive checkpoints can be observed in the S and G_2 phases, where the initiation of still inactive replicon clusters is delayed, and the entry of damaged cells into mitosis is prevented, respectively, until DNA damage is repaired [Figure 3; reviewed in Kaufmann and Paules (*21*)]. When investigating the effects of carcinogenic metal compounds on cell cycle progression by flow cytometry, our results obtained in A549 human lung tumor cells revealed a G_1 arrest by Co(II) and Ni(II), whereas arsenite caused a G_2 arrest. Whether these metal compounds also interfere with cell cycle control in response to DNA damage was studied by applying UVC radiation as primary DNA-damaging agent. Whereas UVC alone caused a pronounced S-phase arrest in this cell line, a normal cell cycle distribution was obtained in the presence of Ni(II) and As(III), suggesting that cell cycle control mechanisms are disturbed. With respect to Co(II), the G_1 arrest was still present in combination with UVC radiation also. All these effects were observed at higher but still noncytotoxic concentrations of the respective metal compounds (*22*).

Potential Molecular Mechanisms

Because DNA repair inhibitions in particular, as well as altered cell cycle progression and/or diminished cell cycle control, have been observed at low, noncytotoxic concentrations of the metal compounds, this raises the question of why these DNA damage-response pathways are particularly sensitive toward toxic metal ions. Some toxic metal ions exert high affinities toward sulfhydryl groups; therefore, potential targets are so-called zinc finger proteins. They comprise a family of proteins where zinc is complexed through four invariant cysteine and/or histidine residues forming a zinc finger domain, which is mostly involved in DNA binding but also in protein–protein interactions (*23*). Even though most zinc finger structures have been described as DNA-binding motifs in transcription factors, they have also been identified in several DNA repair enzymes, including the mammalian xeroderma pigmentosum group A protein (XPA) essential for DNA damage recognition during NER, and the bacterial formamidopyrimidine–DNA glycosylase (Fpg) involved in the removal of oxidative DNA base modifications. The results concerning the interaction of carcinogenic metal compounds with zinc finger DNA repair enzymes have been summarized recently: Fpg was inhibited by Cd(II), Cu(II), and Hg(II), whereas Co(II), As(III), Pb(II), and Ni(II) had no effect. Nevertheless, in addition to Cd(II) and Cu(II), both Ni(II) and Co(II) inhibited DNA binding of XPA, whereas Hg(II), Pb(II), and As(III) were ineffective (*24,25*). One other zinc finger protein activated in response to DNA damage is the poly(adenosine diphosphate [ADP]–ribose)polymerase (PARP)-1. Poly(ADP-ribosyl)ation of various proteins is one of the earliest nuclear events following DNA strand break induction. These modifications are believed to promote changes in the nuclear structure and to direct repair enzymes to sites of damage. Furthermore, PARP-1 seems to play a complex role in drug-induced and spontaneous apoptosis, which, however, is not yet fully understood [for review, see Bürkle (*26*)]. It contains two zinc fingers of the Cys_3His_1-type, which are involved in the recognition of DNA breaks and the subsequent formation of poly(ADP-ribose) (*27*). With respect to toxic metal compounds, PARP activity was decreased in a human T-cell lymphoma-derived cell line by As(III) (*28*), and recent results from our group demonstrate an inhibition of hydrogen peroxide–induced PARP activity in intact cells by Ni(II), Co(II), Cd(II), Cu(II) and concentrations as low as 10 nM As(III) in HeLa cells, whereas no effect was seen with Pb(II) and Hg(II) (*29*). Whether inhibitions are due to interactions with the zinc finger structures must be further investigated. Interestingly, one other zinc-dependent transcription factor regulating cell cycle control and apoptosis by several coordinated pathways (and thus with great impact on the processing of DNA damage and genomic stability) is the p53 tumor suppressor protein [for review, see Hainaut and Hollstein (*30*)]. DNA binding is mediated by a rather complex structure brought together by tetrahedral coordination of zinc to three cysteines and one histidine, and exposure of either the isolated p53 protein or human breast cancer MCF7 cells to Cd(II) resulted

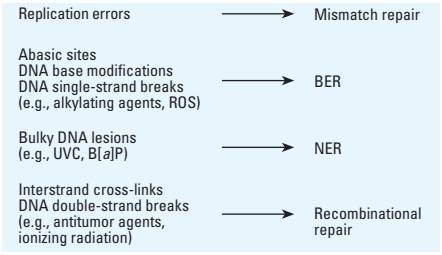

Figure 1. DNA repair systems in response to different types of DNA damage.

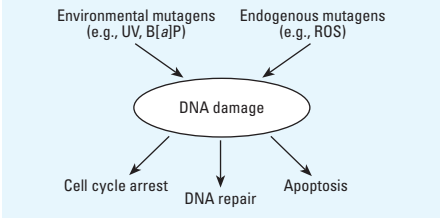

Figure 2. Schematic outline of responses to DNA damage in mammalian cells. Cell cycle arrest prevents damaged DNA to be replicated or cells to enter mitosis. In case of heavily damaged DNA, the apoptotic pathway is activated, depending on the cell type [for details, see text and Zhou and Elledge (*20*)].

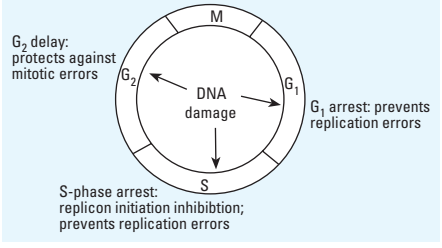

Figure 3. Cell cycle control in response to DNA damage. Figure modified from Kaufmann and Paules (*21*).

in disruption of native p53 conformation and inhibition of DNA binding. Furthermore, suppression of the p53-mediated cell cycle arrest in response to DNA damage by Cd(II) was observed in the cellular system (*31*). Metal ions Ni(II) and Co(II) inhibit its DNA-binding activity as well (*32*).

Conclusions and Perspectives

In summary, there is accumulating evidence that different carcinogenic metal compounds disturb DNA repair systems and cell cycle control by diverse mechanisms. Zinc finger proteins appear to be sensitive toward toxic metal compounds, but each zinc finger protein exerts its unique sensitivities. Perhaps most remarkable is the inhibition of poly(ADP-ribosyl)ation by nanomolar concentrations of arsenite, as no isolated DNA repair enzyme has been identified to date that is inhibited below millimolar concentrations. This finding provides further evidence that arsenite may rather indirectly cause genomic instability via interference with DNA damage response pathways such as poly(ADP-ribosyl)ation, even though contrasting results have been reported by different groups for different cell lines. For example, arsenite at submicromolar to low micromolar concentrations has been reported recently to increase (*33–35*) or decrease cellular p53 levels (*36*), or to elevate cytosine methylation of the *p53* promotor region (*37*).

Taking into account that DNA is continuously damaged by environmental and endogenous processes, cell cycle control and the efficient repair of these lesions are important prerequisites to maintain DNA integrity. Impairment of these protective mechanisms by carcinogenic metal compounds may lead to increased risk of cancer, evident, for example, by high tumor incidences of patients suffering from rare nucleotide excision repair deficiency syndromes like xeroderma pigmentosum (*9*) or defects in mismatch repair involved in the correction of DNA replication errors, which have been associated with increased susceptibility to hereditary nonpolyposis colon cancer (*38*).

References and Notes

1. Kasprzak KS. Possible role of oxidative damage in metal-induced carcinogenesis. Cancer Invest 13:411–430 (1995).
2. Bal W, Kozlowski H, Kasprzak KS. Molecular models in nickel carcinogenesis. J Inorg Biochem 79:213–218 (2000).
3. Klein CB, Costa M. DNA methylation, heterochromatin and epigenetic carcinogens. Mutat Res 386:163–180 (1997).
4. Mass MJ, Wang L. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. Mutat Res 386:263–277 (1997).
5. Hartwig A. Current aspects in metal genotoxicity. Biometals 8:3–11 (1995).
6. Rossman TG, Uddin AN, Burns FJ, Bosland MC. Arsenite is a cocarcinogen with solar ultraviolet radiation for mouse skin: an animal model for arsenic carcinogenesis. Toxicol Appl Pharmacol 176:64–71 (2001).
7. Hartwig A. Recent advances in metal carcinogenicity. Pure Appl Chem 72:1007–1014 (2000).
8. Lindahl T. Instability and decay of the primary structure of DNA. Nature 362:709–715 (1993).
9. de Boer J, Hoeijmakers JH. Nucleotide excision repair and human syndromes. Carcinogenesis 21:453–460 (2000).
10. Li JH, Rossman TG. Inhibition of DNA ligase activity by arsenite: a possible mechanism of its comutagenesis. Mol Toxicol 2:1–9 (1989).
11. Dally H, Hartwig A. Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 18:1021–1026 (1997).
12. Hartwig A. Carcinogenicity of metal compounds: possible role of DNA repair inhibition. Toxicol Lett 102–103:235–239 (1998).
13. Hartmann M, Hartwig A. Disturbance of DNA damage recognition after UV-irradiation by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 19:617–621 (1998).
14. Kasten U, Mullenders LH, Hartwig A. Cobalt(II) inhibits the incision and the polymerization step of nucleotide excision repair in human fibroblasts. Mutat Res 383:81–89 (1997).
15. Hartwig A, Groblinghoff UD, Beyersmann D, Natarajan AT, Filon R, Mullenders LH. Interaction of arsenic(III) with nucleotide excision repair in UV-irradiated human fibroblasts. Carcinogenesis 18:399–405 (1997).
16. Melendez-Colon VJ, Luch A, Seidel A, Baird WM. Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. Carcinogenesis 20:1885–1891 (1999).
17. Alexandrov K, Rojas M, Geneste O, Castegnaro M, Camus AM, Petruzzelli S, Giuntini C, Bartsch H. An improved fluorometric assay for dosimetry of benzo[*a*]pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. Cancer Res 52:6248–6253 (1992).
18. Schwerdtle T, Seidel A, Hartwig A. Effect of soluble and particulate nickel compounds on the formation and repair of stable benzo[*a*]pyrene DNA adducts in human lung cells. Carcinogenesis 23:47–53 (2002).
19. Dunnick JK, Elwell MR, Radovsky AE, Benson JM, Hahn FF, Nikula KJ, Barr EB, Hobbs CH. Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. Cancer Res 55:5251–5256 (1995).
20. Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. Nature 408:433–439 (2000).
21. Kaufmann WK, Paules RS. DNA damage and cell cycle checkpoints. FASEB J 10:238–247 (1996).
22. Ehleben I, Herzer U, Kostelac D, Hartwig A. Unpublished data.
23. Mackay JP, Crossley M. Zinc fingers are sticking together. Trends Biochem Sci 23:1–4 (1998).
24. Asmuss M, Mullenders LHF, Eker A, Hartwig A. Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. Carcinogenesis 21:2097–2104 (2000).
25. Hartwig A. Zinc finger proteins as potential targets for toxic metal ions: differential effects on structure and function. Antioxid Redox Signal 3:625–634 (2001).
26. Bürkle A. Physiology and pathophysiology of poly(ADP-ribosyl)ation. Bioessays 23:795–806 (2001).
27. Gradwohl G, Menissier de Murcia JM, Molinete M, Simonin F, Koken M, Hoeijmakers JH, de Murcia G. The second zinc-finger domain of poly(ADP-ribose) polymerase determines specificity for single-stranded breaks in DNA. Proc Natl Acad Sci USA 87:2990–2994 (1990).
28. Yager JW, Wiencke JK. Inhibition of poly(ADP-ribose) polymerase by arsenite. Mutat Res 386:345–351 (1997).
29. Asmuss M, Jahnke G, Pelzer A, Khandelwal S, Bürkle A, Hartwig A. Unpublished data.
30. Hainaut P, Hollstein M. p53 and human cancer: the first ten thousand mutations. Adv Cancer Res 77:81–137 (2000).
31. Meplan C, Mann K, Hainaut P. Cadmium induces conformational modifications of wild-type p53 and suppresses p53 response to DNA damage in cultured cells. J Biol Chem 274:31663–31670 (1999).
32. Palecek E, Brazdova M, Cernocka H, Litsen D, Brazda V, Vojtesek B. Effect of transition metals on binding of p53 protein to supercoiled DNA and to consensus sequence in DNA fragments. Oncogene 18:3617–3625 (1999).
33. Yih LH, Lee TC. Arsenite induces p53 accumulation through an ATM-dependent pathway in human fibroblasts. Cancer Res 60:6346–6352 (2000).
34. Menendez D, Mora G, Salazar AM, Ostrosky-Wegman P. ATM status confers sensitivity to arsenic cytotoxic effects. Mutagenesis 16:443–448 (2001).
35. Vogt BL, Rossman TG. Effects of arsenite on p53, p21 and cyclin D expression in normal human fibroblasts - a possible mechanism for arsenite's comutagenicity. Mutat Res 478:159–168 (2001).
36. Hamadeh HK, Vargas M, Lee E, Menzel DB. Arsenic disrupts cellular levels of p53 and mdm2: a potential mechanism of carcinogenesis. Biochem Biophys Res Commun 263:446–449 (1999).
37. Mass MJ, Wang L. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. Mutat Res 386:263–277 (1997).
38. Peltomaki P. Deficient DNA mismatch repair: a common etiologic factor for colon cancer. Hum Mol Genet 10:735–740 (2001).