

Molecular targets of nickel and chromium in human and experimental systems

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COSTA M. Molecular targets of nickel and chromium in human and experimental systems. *Scand J Work Environ Health* 1993;19 suppl 1:71—4. Water-insoluble nickel compounds tend to be carcinogenic because they enter cells through phagocytosis. Highly water-soluble nickel salts, such as nickel chloride, do not enter cells well and thus are not carcinogenic in vivo. Carcinogenic nickel compounds produce selective damage to heterochromatic regions in Chinese hamster chromosomes, probably because of the high concentration of proteins and amino acids in this highly condensed genetically inactive chromatin region. Bivalent nickel ions are highly affinitive to amino acids as compared with deoxyribonucleic acid (DNA). The interaction with proteins is believed to cause this effect. In addition, when nickel binds to proteins, it can be oxidized, and DNA protein damage ensues. A new method detects DNA-protein cross-links induced by various agents, such as nickel, chromium and *cis*-platinum. It has demonstrated an increase in DNA-protein cross-links in cultured cells from animals treated with chromate and welders exposed to welding fumes.

Key terms: biomarker of exposure, DNA-protein cross-links, oxidative damage.

Nickel (Ni) and chromium (Cr) compounds have been associated with an increased incidence of cancer in humans (1). The molecular mechanisms of nickel and chromium carcinogenesis have received considerable research attention over the last several years, and many advances have been made in the understanding of the genetic lesions produced by these compounds (2—5). A very potent class of nickel compounds is the crystalline nickel sulfide compounds that, although they do dissolve, exhibit a relatively low level of water solubility as compared with nickel sulfate and nickel chloride (6). However, the water-soluble nickel salts are generally not carcinogenic, probably because they cannot enter cells (4). Extremely water-insoluble nickel compounds, such as nickel oxide, also exhibit carcinogenic potential even though the dissolution of nickel from nickel oxide is likely to be very low, even inside cells, (6).

The mechanism by which water-insoluble nickel compounds, such as crystalline nickel sulfide and nickel oxide, gain entry into cells to produce their carcinogenic response seems to be phagocytosis (reviewed in reference 4). In the case of crystalline nickel sulfide and nickel subsulfide phagocytized particles are contained in cytoplasmic vacuoles, and nickel ions are released at a high rate due to the acidification of these vacuoles (7, 8). Nickel ions are released into the cell and subsequently reach the nu-

cleus, where they can interact with chromatin to produce a variety of genetic effects that have been described and reviewed in the literature (2—5). However, one of the most interesting and striking genetic effects produced by nickel compounds is the selective damage that they induce in heterochromatin (9, 10). Figure 1 shows an X chromosome of Chinese hamster ovary (CHO) that has been damaged in intact cells treated with increasing concentrations of crystalline nickel sulfide. The selective damage produced in the heterochromatic long arm of the X chromosome is in marked contrast to the lack of damage observed in the euchromatic short arm of this chromosome.

My co-workers and I have examined the significance of nickel interaction with heterochromatin in terms of its carcinogenic activity using primary cultures of male and female CHO embryo cells (12, 13). We found that nickel induced a very high incidence of transformation among male cells as compared with female ones, and more than half the nickel-transformed male cells had a complete or partial deletion of the long arm of the X chromosome (the heterochromatin region that nickel originally damaged; *vide supra*) (figure 1) (13). To study the role of these deletions, we introduced a normal intact X chromosome back into nickel-transformed male cells in which some or all of the X chromosome heterochromatin was missing, and observed that, when the X chromosome was successfully transferred to these cells, they senesced (12). We subsequently found that the senescing activity was dependent upon the deoxyribonucleic acid (DNA) methylation status of the X chromosome (12). Thus we had identified a putative senescence gene that was deleted during nickel

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Figure 1. Photographs of the X chromosome from the Chinese hamster ovary cell after cells had been treated with crystalline nickel sulfide particles. Cells were treated with crystalline nickel sulfide particles ranging in concentration from 10 to 20 mg · ml⁻¹ for 24–48 h. The figure shows the increasing degree of fragmentation of the long arm of the X chromosome with higher dosages and longer exposure times (a–i). Note the absence of fragmentation of the short arm. [Reprinted, with permission, from Sen & Costa (11)]

carcinogenesis and could be restored by the introduction of a normal hypomethylated X chromosome into the transformed cells. A similar senescing activity was observed with the human X chromosome as well, although it was less active in transformed Chinese hamster cells, probably because of problems related to species homology, as well as the fact that the human X chromosome is much less stable in the hybrid mouse cell line than the Chinese hamster X chromosome is (14).

It is somewhat surprising to find such selectivity in damage induced by bivalent nickel ions (Ni²⁺), since nickel ions are rather nonspecific in their interactions with most cellular ligands. However, if one examines the binding constant of Ni²⁺ for DNA or individual DNA bases and compares it with that of nickel for amino acids, such as cysteine and histidine, one finds a very high binding constant for amino acids (eg, cysteine $4.4 \cdot 10^9 \cdot \text{mol}^{-1}$, histidine $1.9 \cdot 10^9 \cdot \text{mol}^{-1}$), peptides, and proteins, whereas the binding constant of Ni²⁺ for DNA is very low ($7.3 \cdot 10^2 \cdot \text{mol}^{-1}$) (15). Binding constants of Ni²⁺ for many other amino acids are still higher (eg, $10^5 \cdot \text{mol}^{-1}$) than for DNA bases ($10^2 \cdot \text{mol}^{-1}$) (15). Thus, according to these chemical predictions, one would conclude that the chromosome damage produced by nickel in heterochromatin is due to its interaction with proteins and small peptides.

Another interesting result with regard to nickel-induced genotoxicity is the fact that, when nickel binds to certain ligands, such as the imidazole nitrogen of histidine, its coordination allows it to be oxi-

dized more readily by various intracellular oxidants (ie, oxidative potential – peptides = 1.04 V; + peptides = 0.79 – 0.96 V) (2,16). Preliminary results in our laboratory indicate that nickel chloride, perhaps through the inhibition of catalase or some other mechanism, can increase the concentration of hydrogen peroxide in the cell. Thus nickel binds with a high affinity to the nitrogen of histidine and can be oxidized, presumably to trivalent nickel (Ni³⁺) by hydrogen peroxide (16). However, since very little oxidation to Ni³⁺ occurs, it is difficult to measure Ni³⁺ by techniques such as electron spin resonance. The formation of Ni³⁺ leads to the generation of hydroxyl, and other oxygen radicals can produce oxidized proteins that react directly with DNA. Alternatively, oxidized DNA bases form, or oxygen radicals can induce, lipid peroxidation and subsequent malonyldialdehyde formation to produce DNA-protein cross-links, as well as other types of genetic damage (2, 16). The oxidation of Ni²⁺ to Ni³⁺ is a rather subtle reaction in the cell and is likely to be localized to specific ligands. It may, however, be a very important reaction. While not producing overt cytotoxicity, since it occurs at relatively low levels in cells, its localized toxicity is precisely the type of mechanism that would be expected with a dangerous carcinogen such as nickel sulfide or nickel subsulfide.

For several years our laboratory has been interested in studying DNA-protein cross-links induced by nickel and chromium compounds (3–5). We have analyzed the proteins involved in chromate- and nickel-induced DNA-protein cross-links, but it has

been difficult to study these proteins because of a high background problem (3, 17). Consequently, we have recently shifted our focus to examining amino acids complexed to DNA (18). We have found that both nickel and chromium complex certain amino acids to DNA, histidine and cysteine being among several that are complexed by both these metals (18). However, the complexing of histidine and cysteine to DNA by Ni^{2+} does not appear to be directly mediated by the metal (18). In fact, nickel is catalytic in its ability to cause the formation of residual complexes of amino acid to DNA (18). Thus amino acids still remain complexed to DNA when nickel is removed, a phenomenon suggesting that metal ions catalyzed the formation of such complexes but did not directly participate in the complex (18).

In contrast to nickel, chromate-induced DNA-protein cross-links appear to involve substantial participation of trivalent chromium in the DNA-protein cross-links (3,17). The chemistry of trivalent chromium is such that it forms very tight complexes with DNA and protein, unlike Ni^{2+} , which is primarily catalytic in its ability to form DNA-protein cross-links. Chromate treatment of intact cells has been shown to complex a number of proteins to DNA (3, 17). One of the major proteins complexed to DNA by hexavalent chromate has been shown to be actin, and trivalent chromium has been shown to be the ultimate reductant participating in this DNA-protein complex formation (19).

In subsequent work focusing upon the study of the amino acids involved in DNA-protein complexes induced by chromium, we found that cysteine and histidine, as well as serine, threonine and tyrosine, were complexed to DNA by chromium (18). Residual cysteine seemed to be derived primarily from glutathione, whereas the other amino acids were derived mostly from protein components of DNA-protein complexes (18). Additional work demonstrated that chromium appeared to bind to the phosphate backbone of DNA rather than to any particular DNA base and, through the binding of trivalent chromium to the phosphate backbone, coordinated to form the DNA-protein complex lesion through certain amino acids (20).

We have recently developed a very simple method for assaying DNA-protein cross-links which exploits the selective precipitation of such cross-links by potassium sodium dodecyl sulfate (21). This method has been utilized to detect DNA-protein cross-links formed in cultured cells by chromate, formaldehyde, and *cis*-platinum (21). We have also used this assay to detect DNA-protein cross-links in animals exposed to chromate, and preliminary human evidence suggests that it can be utilized to detect a higher level of such cross-links in the peripheral blood lymphocytes of welders as compared with the blood the peripheral lymphocytes of referents (22, 23).

Acknowledgments

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