Perspectives On The Mechanism Of Nickel Carcinogenesis Gained From Models of *In Vitro* Carcinogenesis

by Max Costa*

This article briefly reviews the approach taken to understand the mechanism of nickel-induced neoplastic transformation. The initial phases of the studies were focused on particulate nickel compounds and on the regulation of phagocytosis of nickel compounds by cells undergoing transformation. The particulate nickel compounds most potent in inducing cell transformation were selectively phagocytized by cells, whereas those that were not active were not phagocytized. The intracellular fate of phagocytized nickel sulfide particles is discussed as well as the interaction of nickel with chromatin. Phagocytized nickel sulfide particles were dissolved in the cytoplasm of cells by the acidification of vacuoles containing phagocytized particles. Nickel ions released from the phagocytized particles produced selective damage in heterochromatin. The selective effects of nickel on heterochromatin are also discussed and related to its mechanism of carcinogenesis.

There is an old Chinese saying that when you drink the water from the well, remember who built it. Although I have only been at the Institute of Environmental Medicine for 2 years, I am very appreciative of the efforts expended by Norton Nelson in establishing the Institute as a leading center of environmental research. It continues to flourish under the directorship of Arthur Upton. As I drink the water here, I will always remember all that Nelson has done. The environment here is conducive to continuing the type of research I will describe below.

There are conclusive epidemiological data implicating nickel compounds in the etiology of human respiratory cancers (*1*). In experimental animals there are substantial differences in the carcinogenic potencies of specific nickel compounds (*2*). An example is the case of crystalline and amorphous nickel sulfide (NiS): The former compound is a very potent carcinogen, while the latter lacks carcinogenic activity (*2*). Carcinogenic nickel compounds induce a wide variety of tumors in many different animal species (*3-5*). For example, crystalline nickel subsulfide (Ni$_3$S$_2$) is a broad-acting carcinogen that has recently been shown to induce tumors in the Japanese newt, an amphibian that exhibits some resistance to chemical carcinogenesis (*6*).

Work in this laboratory has focused on understanding the cellular basis for the differences in carcinogenic activity of specific water-insoluble nickel compounds.

Water-soluble nickel compounds are generally not carcinogenic *in vivo* (*7*). One of the reasons for this is the poor bioavailability of nickel ions *in vivo* (*7*). However, *in vitro*, water-soluble nickel compounds display cell-transforming activity, probably because the extracellular concentration of nickel can be maintained at a high level, making the nickel ions considerably more bioavailable to the cell. The results from our studies demonstrate that the bioavailability of nickel is the key to understanding carcinogenic potency of specific nickel compounds. Using the Syrian hamster embryo cell-transformation assay, we demonstrated that crystalline Ni$_3$S$_2$ induced neoplastic transformation of these cells, while amorphous NiS lacked cell-transforming activity (*8*). We found that crystalline NiS particles were selectively phagocytized by the cells that become transformed, while amorphous NiS particles were not phagocytized. Substantial effort was expended to understand the cellular basis for this difference in phagocytosis. Two hypotheses were set forth to explain why crystalline NiS particles were phagocytized more than amorphous NiS particles: a) Amorphous NiS particles had a slightly faster dissolution rate than crystalline NiS particles. This faster dissolution rate may have resulted in more nickel ions binding to the cell membrane, which could be inhibitory to the phagocytic process (*9*). b) There were differences in the surface charge of amorphous and crystalline NiS (*9*).

A number of experiments were conducted to test whether the first hypothesis was true, but all of these indicated that while nickel ions were inhibitory to phagocytosis, the differential dissolution rates were probably not

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sufficient to account for any substantial differences in phagocytosis activity (9). One type of experiment that was used to test this hypothesis involved a study of the effect of tissue culture media volume in phagocytosis of crystalline or amorphous NiS particles. Presumably, the greater the culture media volume, the more diluted dissolving nickel ions would be, resulting in less inhibition of phagocytosis (9). However, dilution of tissue culture media volume had no effect on amorphous NiS particle phagocytosis.

The surface charge of amorphous or crystalline NiS particles was examined by measurement of the zeta potential (measure of surface charge) or, alternatively, by studying the binding of the particles to filter paper discs offering different charged surfaces (5,9). These studies demonstrated that crystalline NiS particles have a considerably greater negative surface charge than amorphous NiS particles. Zeta potential measurement indicated that amorphous NiS particles have a slightly positive potential, whereas crystalline NiS particles have a -27 mV potential. The significance of the difference in surface charge in terms of phagocytosis of the particles was examined further by altering the charge on the surface of amorphous NiS particles by treatment of the particles with $LiAlH_4$ (10). This resulted in the active phagocytosis of amorphous NiS particles producing an enhanced biological response in cells (10).

NiS particle phagocytosis was studied using video intensification microscopy and electron microscopy (8,11). NiS particles were contained in cytoplasmic vacuoles, and lysosomes repeatedly interacted with these vacuoles (11). The vacuoles containing phagocytized NiS particles became acidified, as evidenced by enhanced acridine orange fluorescence (11). The acidified vacuoles were proposed to greatly accelerate the crystalline NiS dissolution rate, compared to its extracellular dissolution at physiological pH (11). With time, the NiS particles aggregated around the cell nucleus (11). They became relatively stationary at this site and also exhibited highly intense acridine orange fluorescence, indicating even greater acidification of the vacuoles. Ni(II) ions are thought to be the ultimate carcinogen of most, if not all, nickel compounds. Nickel ions were released from phagocytized NiS particles into the nucleus of cells (12). Our more recent efforts to study the mechanisms of nickel carcinogenesis have focused on the interaction of nickel ions with chromatin.

Nickel(II) ions bind poorly to DNA in comparison to their relatively high affinity for certain amino acids of cellular protein. The binding constant for DNA is about six to seven orders of magnitude lower than that for cysteine and histidine, and its ability to bind tightly to protein is probably an important determinant of the nickel effects on chromatin. Our first attempt to examine the effect of nickel on chromatin used alkaline elution to investigate the type of DNA lesions induced by nickel. Based upon alkaline elution, both nickel chloride ($NiCl_2$) and crystalline NiS particles induced DNA strand breaks and DNA-protein cross-links (13,14). However, the extent of these lesions was not striking. Further studies with nickel re-

vealed that the DNA-protein complexes were not stable to sodium dodecyl sulfate at neutral pH (13). In fact, recent studies in this laboratory suggest that DNA-protein complexes induced by nickel may be more stable at alkaline pH than at physiological pH (15). Almost all of the DNA damage induced by nickel ions was specific to the late S phase of the cell cycle (13). This is the time period when heterochromatic DNA is known to replicate.

At the same time we were studying DNA lesions by alkaline elution, we also were investigating DNA damage induced by nickel at the level of the whole chromosome. Nickel ions produced highly selective chromosome damage in heterochromatic regions of mouse and Chinese hamster ovary (CHO) cell chromosomes. Excess magnesium ions are capable of inhibiting nickel-induced carcinogenesis and nickel-induced cell transformation (16,17). Excess magnesium ions also inhibit the DNA lesions induced by nickel ions. At the chromosome level, the DNA lesions induced by nickel in heterochromatin are inhibited to a greater extent by excess magnesium than those induced in euchromatin (17). This was one of the first direct demonstrations that DNA damage induced in heterochromatin was related to nickel carcinogenesis. The striking effect that nickel ions had on heterochromatin was surprising in view of the fact that Ni(II) is a divalent metal ion and would be expected to interact with many proteins that compose chromatin. Thus, its selective effect on heterochromatin is interesting even if it is not involved in carcinogenesis.

Unfortunately, the heterochromatic region of the long arm of the X-chromosome of CHO cells does not consist of highly repetitive DNA, and it was difficult to obtain cloned DNA sequences that could be used to study the effect of nickel on specific DNA-protein interactions in vitro. However, nickel ions also had selective effects on the heterochromatin regions of mouse chromosomes (18), and we were able to obtain a mouse satellite DNA probe that represents DNA localized in centromere heterochromatin. Currently, we are examining the effect of nickel as well as a number of other metal ions on specific DNA-protein binding (19). Preliminary evidence suggests that nickel ions have a greater effect on specific protein binding to the mouse satellite DNA than on other DNA-protein binding sites such as the metallothionein promoter (19). Specific DNA-protein interactions are being examined by band shift analysis and by footprinting.

There is additional evidence accumulating to suggest that nickel interaction with heterochromatin may be important in its carcinogenesis. For example, in preliminary experiments conducted in this laboratory, it has been observed that male Chinese hamster embryo cells transform at a much higher frequency than the female Chinese hamster embryo cells. In male Chinese hamster cells, there is only one X-chromosome and the long arm of this chromosome is entirely heterochromatic, while the female cells have two X chromosomes. As discussed earlier, the long arm of the X-chromosome in the cell has been a selective site for the effects of nickel. In five out of six of the male nickel-transformed Chinese hamster cell lines,
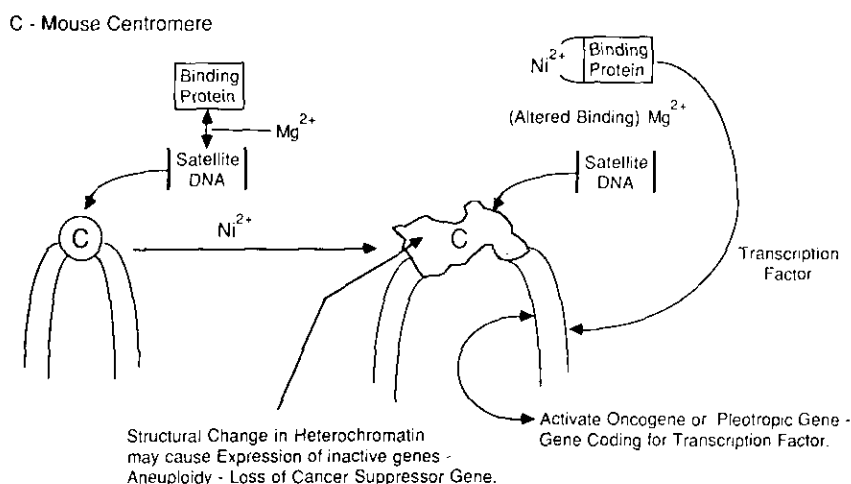
C - Mouse Centromere

FIGURE 1. Proposed mechanisms of nickel carcinogenesis in mouse cells.

there is a deletion of the long arm of the X-chromosome as the primary chromosome aberration associated with nickel-induced neoplastic transformation. These results suggest that there might be a tumor suppressor gene located on the long arm of the X-chromosome that is deleted during nickel-induced transformation. Another indication that nickel interaction with heterochromatin can be important in genetic diseases such as cancer comes from studies with nickel-resistant mouse cells that exhibit a very high degree of centromeric fusion (Robertsonian fusion) (20).

The initial interaction of nickel with heterochromatin may cause a change in chromatin organization at this site. This change in organization may have effects during replication of heterochromatic DNA. This may come about in two ways: a) the disruption of chromatin structure caused by the initial effects of nickel ions leads to errors during DNA replication, i.e., the template does not interact normally with polymerase; b) nickel ions are present at high concentrations in heterochromatic regions and cause inhibition of DNA polymerase by interacting with magnesium sites on the enzyme as it replicates heterochromatic DNA (1). It has recently been shown, with temperature-sensitive mutants, that inhibition of DNA polymerase is sufficient to induce substantial chromosome damage and sister chromatid exchange (22). Thus, the selective effect of nickel on DNA polymerase copying of heterochromatic DNA could account for the lesions at this site. Such a change in the DNA sequence by these mechanisms is probably an obligatory step in understanding chemical carcinogenesis by nickel compounds.

Figure 1 illustrates some of the inital changes that may be induced in heterochromatin by nickel ions. A major effect of nickel ions may be to release proteins from the DNA and inhibit their binding to heterochromatic DNA by interacting with magnesium binding sites on the protein. Alternatively, by a similar mechanism, proteins may bind more tightly to DNA. These effects probably lead to the observed damage in heterochromatin. The proteins that bind to heterochromatic yeast centromeres have been shown to be similar to transcription factors (23). If nickel inhibits the ability of these proteins to bind to satellite DNA, they may still be active as transcription factors, as shown in the figure, and activate important genes. Alternatively, the structural change in heterochromatin may lead to expression of inactive genes in neighboring euchromatin.

At the present time, it is hard to imagine how such changes could be inherited from cell to cell except for alterations in the way DNA polymerase interacts with the heterochromatic DNA template. However, if damage in heterochromatin leads to aneuploidy and loss of a cancer suppressor gene (24), then these effects could be inherited. We have evidence that nickel induces aneuploidy (25) and that it leads to deletions of heterochromatin during neoplastic transformation.

Another interesting mechanism by which a nongene mutagenic agent could produce a heritable change in DNA is by alteration of DNA methylation. We have investigated whether nickel ions affect DNA methylation of whole genomic DNA in collaboration with F. Becker and R. Imbra and thus far have found that nickel does not alter DNA methylation.

Recent studies have shown that constitutive heterochromatin may in certain circumstances exhibit transcriptional activity equivalent to euchromatin, and this switched-on transcriptional activity may be inherited (26). Perhaps nickel ions are capable of activating transcriptional activity in heterochromatin.

REFERENCES

1. Sunderman, F. W., Jr. Carcinogenicity and mutagenicity of some metals and their compounds. In: Environmental Carcinogens: Selected Methods of Analysis, Vol. 8 (I. K. O'Neill, P. Schuller, and L. Fishbein, Eds.), International Agency for Research on Cancer, Lyon, 1986, pp. 17–43.
2. Sunderman, F. W., Jr., and Maenza, R. M. Comparisons of carcinogenicities of nickel compounds in rats. Res. Commun. Chem. Pathol. Pharmacol. 14: 319–330 (1976).
3. Sunderman, F. W., Jr. Recent Progess in nickel carcinogenesis. Toxicol. Environ. Chem. 8: 235–252 (1984).
4. Sunderman, F. W., Jr., Ed. Nickel in the Human Environment. Oxford University Press, Oxford, 1984.
5. Brown, S. S., and Sunderman, F. W., Jr., Eds. Progress in Nickel Toxicology. Blackwell Scientific Publications, Oxford, 1985.
6. Okamoto, M. Induction of ocular tumor by nickel subsulfide in the Japanese common newt, *Cynops pyrrhogaster*. Cancer Res. 47: 5213–5217 (1987).
7. Costa, M., and Heck, J. D. Perspectives on the mechanism of nickel carcinogenesis. Adv. Inorg. Biochem. 6: 285–309 (1985).
8. Costa, M., and Mollenhauer, H. M. Carcinogenic activity of particulate nickel compounds is proportional to their cellular uptake. Science 209: 515–517 (1980).
9. Heck, J. D., and Costa, M. Influence of surface charge and dissolution on the selective phagocytosis of potentially carcinogenic particulate metal compounds. Cancer Res. 43: 5652–5656 (1983).
10. Abbracchio, M. P., Heck, J. D., and Costa, M. The phagocytosis and transforming activity of crystalline metal sulfide particles are related to their negative surface charge. Carcinogenesis 3: 175–180 (1982).
11. Evans, R. M., Davies, P. J. A., and Costa, M. Video time-lapse microscopy of phagocytosis and intracellular fate of crystalline nickel sulfide particles in cultured mammalian cells. Cancer Res. 42: 2729–2735 (1982).
12. Costa, M., Simmons-Hansen, J., Bedrossian, C. W. M., Bonura, J., and Caprioli, R. M. Phagocytosis, cellular distribution, and carcinogenic activity of particulate nickel compounds in tissue culture. Cancer Res. 41: 2868–2876 (1981).
13. Patierno, S. R., Sugiyama, M., Basilion, J. P., and Costa, M. Preferential DNA-protein cross-linking by $NiCl_2$ in magnesium-insoluble regions of fractionated Chinese hamster ovary cell chromatin. Cancer Res. 45: 5787–5794 (1985).
14. Patierno, S. R., and Costa, M. DNA-protein cross-links induced by nickel compounds in intact cultured mammalian cells. Chem.-Biol. Interact. 55: 75–91 (1985).
15. Coogan, T. P., and Costa, M. A filter binding assay to detect chromium-induced DNA-protein crosslinks in isolated nuclei. Toxicologist 9(1): 105 (1988).
16. Kasprzak, K. S., Quander, R. V., and Poirier, L. A. Effects of calcium and magnesium salts on nickel subsulfide carcinogenicity in Fischer rats. Carcinogenesis 6: 1161–1166 (1985).
17. Conway, K., Wang, X. W., Xu, L., and Costa, M. Effect of magnesium on nickel-induced genotoxicity and cell transformation. Carcinogenesis 8(8): 1115–1121 (1987).
18. Sen, P., Conway, K., and Costa, M. Comparison of the localization of chromosome damage induced by calcium chromate and nickel compounds. Cancer Res. 47: 2142–2147 (1987).
19. Imbra, R. J., Latta, D. M., and Costa, M. Studies on the mechanism of nickel-induced heterochromatin damage; effect on specific DNA-protein interactions. In: Proceedings of the Third Hans Wolfgang Nurnberg Memorial Workshop on Toxic Metal Compounds, Follonica, Italy. Int. J. Environ. Anal. Chem., in press.
20. Wang, X. W., Imbra, R. J., and Costa, M. Characterization of nickel chloride-resistant BALB/c-3T3 mouse fibroblast cells (abstract). Toxicologist 8(1): 15 (1988).
21. Sirover, M. A., and Loeb, L. A. Metal activation of DNA synthesis. Biochem. Biophys. Res. Commun. 10: 812–817 (1976).
22. Eki, T., Enomoto, T., Murakami, Y., Hanaoka, F., and Yamada, M. Characterization of chromosome aberrations induced by incubation at a restrictive temperature in the mouse temperature-sensitive mutant tsFT20 strain containing heat-labile DNA polymerase α. Cancer Res. 47: 5162–5170 (1987).
23. Bram, R. J., and Kornberg, R. D. Isolation of a *Saccharomyces cerevisiae* centromere DNA-binding protein, its human homolog, and its possible role as a transcription factor. Mol. Cell. Biol. 7(1): 403–409 (1987).
24. Oshimura, M., and Barrett, J. C. Chemically induced aneuploidy in mammalian cells: Mechanisms and biological significance in cancer. Environ. Mutagen. 8: 129–159 (1986).
25. Conway, K., Athwal, R. S., and Costa, M. Induction of aneuploidy by Ni(II) and Cr(VI) in a human/mouse hybrid cell system. Toxicologist 8(1): 105 (1988).
26. Sperling, K., Kalscheuer, V., and Neitzel, H. Transcriptional activity of constitutive heterochromatin in the mammal *Microtus agretis* (Rodentia, Cricetidae). Exp. Cell. Res. 173: 463–472 (1987).