Nickel-Induced Histone Hypoacetylation: The Role of Reactive Oxygen Species

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The carcinogenicity of specific insoluble nickel compounds is mainly due to their intracellular generation of Ni²⁺ ion and its suppression on gene transcription, while the inhibition of Ni²⁺ on histone acetylation plays an important role in the suppression or silencing of genes. Recent studies on Ni²⁺ and histone H4 acetylation suggest that Ni²⁺ inhibits the acetylation of histone H4 through binding with its N-terminal histidine-18. It is well known that bound Ni²⁺ readily produces reactive oxygen species (ROS) in vivo, a critical factor inversely related with the occurrence of resistance of mammalian cells to Ni²⁺. Thus, we tried to find the possible role of ROS in the induction of Ni²⁺ on histone acetylation in the present study. We found that a high concentration of Ni²⁺ (no less than 600 μM) caused a significant decrease of histone acetylation in human hepatoma cells. This inhibition was shown to result mainly from the influence of Ni²⁺ on the overall histone acetyltransferase (HAT) activity indicated by the histone acetylation assay with the presence of a specific histone deacetylase (HDAC) inhibitor, trichostatin A (TSA). The in vitro HAT and HDAC assays further confirmed this result. At the same time, we found that the exposure of hepatoma cells to Ni²⁺ generated ROS. Coadministration of hydrogen peroxide with Ni²⁺ generated more ROS and more histone acetylation inhibition. Addition of the antioxidants 2-mercaptoethanol (2-ME) at 2 mM or N-acetyl-cysteine (NAC) at 1 mM, with Ni²⁺ together, completely suppressed ROS generation and significantly diminished the induced histone hypoacetylation. The data presented here prove that the ROS generation plays a role in the inhibition of histone acetylation, and, hence, the gene suppression and carcinogenesis caused by Ni²⁺ exposure, providing a new door for us to continuously understand the mechanism of ROS in the carcinogenicity of Ni²⁺ and the resistance of mammalian cells to Ni²⁺.

Key Words: Nickel; carcinogenesis; histone acetylation; histone acetyltransferase (HAT); histone deacetylase (HDAC); reactive oxygen species (ROS).

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In order of abundance in the earth’s crust, nickel ranks as the 24th element. Due to its abundance in all types of food (Nielson, 1987; Sunderman and Oskarsson, 1999), the daily dietary intake of nickel is estimated at more than triple the possible requirement of daily nutrition (Anke et al., 1995; Barceloux, 1999; Kirchgessner et al., 1982; Nielson, 1987; Schnegg et al., 1975). Additionally, because of the wide usage of metallic nickel and its compounds in modern industry, the high consumption of nickel-containing products inevitably leads to more diseases by nickel and its by-products at all stages of production, recycling and disposal, as suggested by the epidemiological studies, which indicate an increased risk of respiratory tract and nasal cancers in miners and workers in nickel refineries (Anderson, 1992; Easton et al., 1992; Roberts et al., 1992). It is well known that exposure to nickel compounds has adverse effects on human health and causes serious illness related to the carcinogenic activity of specific insoluble nickel compounds. Based upon the epidemiological studies, all nickel compounds except for metallic nickel are classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC) in 1990 (IARC, 1990). Thus, the molecular mechanisms of the carcinogenicity of specific insoluble nickel compounds have been focused on for a long time.

Recent studies in this field found that, as human and rodent carcinogens, nickel compounds display a strong potential to inactivate gene expression in spite of their weak mutagenicity. Thus, not gene mutation, but gene silencing is critical in nickel-induced carcinogenesis (Beyersmann, 2002; Cangul et al., 2002). In the process of gene expression regulation, chromatin remodeling is proved to be crucial because, in eukaryotes, binding of genomic DNA with histones in the nucleosome and folding of the chromatin pose a barrier to transcription, i.e., the structure of chromatin prevents the transcription machinery from interacting with promoter DNA sequences (Fry and Peterson, 2002). To overcome this, chromatin remodeling is triggered during gene transcription initiation mainly by two classes of enzymes: those that covalently modify nucleosomal histone proteins through acetylation and those...
that alter chromatin structure through the hydrolysis of adenosine triphosphate (ATP) (Fry and Peterson, 2001, 2002; Grunstein, 1997). Among them, histone acetylation and its related enzymes are continuously needed during gene transcription initiation and elongation. Generally, histone hyperacetylation leads to some genes activation or up-regulation, while histone hypoacetylation leads to inactivation or down-regulation of genes (Archer and Hodin, 1999; Klochendler-Yeivin and Yaniv, 2001).

Ni\(^{2+}\) has been found to inactivate the antiangiogenic thrombospondin gene and a telomere marker gene by induction of histone H4 hypoacetylation and chromatin condensation (Broday et al., 1999; Salnikow et al., 1997). Further studies show that Ni\(^{2+}\) inhibits the acetylation of histone H4 through binding with its N-terminal histidine-18, which is located near the lysine residues for histone H4 acetylation (Broday et al., 2000; Zoroddu et al., 2000), i.e., histone H4 acetylation is inhibited by the histone H4-bound Ni\(^{2+}\). It is one well-known characteristic of bound-Ni\(^{2+}\) to produce reactive oxygen species (ROS) in vivo, and the generation of ROS by histone-bound Ni\(^{2+}\) has also been proved previously (Bal and Kasprzak, 2002). Since ROS are very reactive and react easily with surrounding protein, DNA, and other molecules in vivo, their generation likely plays important roles in Ni\(^{2+}\)-induced gene expression alterations, carcinogenesis, and especially resistance of mammalian cells to Ni\(^{2+}\) (Denkhaus and Salnikow, 2002; Qu, 2001; Salnikow et al., 1994). Hence, we are interested in whether the generation of ROS in vivo, especially those generated by histone-bound Ni\(^{2+}\), can influence the acetylation modification of histones through reacting with histones or molecules around them, such as histone acetyltransferases (HAT) or histone deacetylases (HDAC) (Archer and Hodin, 1999; Klochendler-Yeivin and Yaniv, 2001), two classes of enzymes involved in the control of the state of histone acetylation. Our knowledge of the relation between ROS generation and Ni\(^{2+}\)-induced histone hypoacetylation still remains unclear. To clarify this will provide a new approach to further understand the role of ROS generation in Ni\(^{2+}\)-induced gene silencing, toxicity, and the resistance of mammalian cells to Ni\(^{2+}\).

Here, we suppose that Ni\(^{2+}\)-induced generation of ROS may play a role in the carcinogenicity of specific nickel compounds by leading to the inhibition of histone acetylation. To address this hypothesis, effects of Ni\(^{2+}\) on histone acetylation alteration and ROS generation were studied in human hepatoma cells. Ni\(^{2+}\) treatment significantly inhibited histone acetylation through affecting the activity of HAT in cells, and the generation of ROS was induced by Ni\(^{2+}\) and partially involved in the induction of histone hypoacetylation. These results suggested a new approach, diminishing Ni\(^{2+}\)-generated ROS, to prevent and cure Ni\(^{2+}\)-related histone hypoacetylation, gene suppression, and diseases such as cancer.

MATERIALS AND METHODS

Cell culture and mycoplasma detection. Human hepatoma cells (Hep3B) were maintained in RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (equivalent to 100 units/ml and 100 mg/ml, respectively) at 37°C as monolayers in a humidified atmosphere containing 5% CO\(_2\). After culturing the cells (2 × 10\(^4\) cells/ml) for 24 h, the culture medium was aspirated and replaced with new medium containing Ni\(^{2+}\) and/or appropriate reagents where indicated. To ensure that the cells were free of mycoplasm, mycoplasmas in cultured cells were detected once every two weeks by staining of DNA with 4,6-diamidino-2-phenylindole (DAPI, Sigma, St. Louis, MO) (Russel et al., 1975).

Exposure of cells to Ni\(^{2+}\) and other agents. NiCl\(_2\) (Approx. 98%), H\(_2\)O\(_2\), 2-mercaptoethanol (2-ME) and N-acetyl-cysteine (NAC) were obtained from Sigma Chemical Co. (St. Louis, MO). NiCl\(_2\) and NAC stock solution (1 M) in distilled water were filtered through a sterile, pathogen-free nylon filter (pore size: 0.22 μm; MSI Inc., MA), the pH of stock solutions was carefully adjusted to 7.2 using 0.5 N HCl and 1 N NaOH before filtration. A freshly prepared stock solution was mixed with RPMI-1640 at indicated concentrations before use.

Histone purification. Preparation of histones from Hep3B cells was done according to Cousins et al. (1979) with the following modifications: the washed cells were suspended in lysis buffer [Cousens et al., 1979] containing trichostatin A (TSA) (100 ng/ml) and PMSF (1 mM). After pipetting up and down for 20 times, the nuclei were washed three times in the lysis buffer and then determined by liquid scintillation counting. The histones were extracted from the pellet in 0.4 N H\(_2\)SO\(_4\). After centrifugation, the histones in the supernatant were collected by cold-acetone precipitation, air-dried, then suspended in 4 M urea and stored at −20°C before use.

Western blotting analysis of histone H4 acetylation. Equal amounts of purified histones (30 mg/lane) were subjected to SDS-PAGE on 15% polyacrylamide gels and were electrophoretically transferred to a nitrocellulose membrane. Nitrocellulose blots were blocked with 5% milk in TTBS (Tris-buffered saline plus 0.05% Tween 20, pH 7.5) and incubated overnight at 4°C with a specific antibody for histone H4-acetyl-lysines in TTBS containing 5% milk. After incubation with horseradish peroxidase-conjugated secondary antibody, immunoreactivity was visualized by means of enhanced chemiluminescence (ECL, Amersham, UK).

Histone acetylation assay. Cells were plated at a density of 2 × 10\(^4\) cells/ml and exposed to 10 μM H\(_4\)-acetate (5.0 Ci/mmol, Amersham, UK). After incubation for 10 min at 37°C, the cells were stimulated with Ni\(^{2+}\) or and other agents for the indicated times in the absence or presence of TSA (Sigma, St. Louis, MO, USA). Then histones were purified and 3H-labelled histones were determined by liquid scintillation counting.

HDAC assay. An HDAC assay was performed as previously reported (Ito et al., 2000). Radiolabeled histones were prepared from Hep3B cells following incubation with TSA (100 ng/ml, 6 h) in the presence of 0.1 μCi/ml H\(_4\)-acetate. The histones were purified, dried, and resuspended in 50 mM Tris-HCl buffer (pH 8.0). Crude HDAC preparations were extracted from total cellular homogenates and then incubated with 0–1000 μM Ni\(^{2+}\) and the 3H-labelled histones in 50 mM Tris-HCl buffer (pH 8.0) for 30 min at 37°C before the reaction was terminated by the addition of 1 N HCl-0.4 N acetic acid. The released 3H-labelled acetic acid was extracted from the reaction mixture and then determined by liquid scintillation counting.

HAT assay. Crude HAT preparations were extracted from total cellular homogenates of Hep3B cells, and the in vitro acetylation assay was performed as described previously (Chicoine et al., 1987; Ito et al., 2000). A typical HAT assay was performed using a 50-μl reaction mixture containing: histone protein (20 mg of core histones extracted from Hep3B cells), 20 ml of crude HAT extract (from 1 × 10\(^4\) cells), 0–1000 μM Ni\(^{2+}\), 100 ng/ml TSA, 10 mM HEPES (pH 7.8), 4% glycerol and 0.1 μCi 3H-acetyl-CoA (5.6 Ci/mmol, Amersham, UK). Reactions were initiated by the addition of 3H-acetyl-CoA to the mixture, followed by incubation for 1 h at 30°C. After incubation, the
reaction mixture was spotted onto Whatman p81 phosphocellulose paper (Whatman), washed extensively with 0.2 M sodium carbonate buffer (pH 9.2), and then briefly washed with acetone. The dried filters were counted by liquid scintillation.

Measurement of intracellular ROS generation. The level of intracellular ROS was measured by the alteration of fluorescence resulting from oxidation of 29,79-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, OR) (LeBel, 1992). DCFH-DA was dissolved in DMSO to a final concentration of 20 μM before use. For the measurement of ROS, cells were incubated with different concentrations of Ni²⁺ for a time period indicated in the figure legends. The intensity of fluorescence was recorded using a flow cytometry (Becton Dickenson), with an excitation filter of 485 nm and an emission filter 535 nm. The ROS level was calculated as a ratio: ROS = mean intensity of exposed cells: mean intensity of unexposed cells.

Miscellaneous. Protein concentrations were determined with the BCA protein assay (Pierce, USA) using bovine serum albumin as a standard. Statistical analysis was performed by analysis of variance (ANOVA post-hoc Bonferroni), and p values less than 0.05 or 0.01 were denoted as * or **, respectively.

RESULTS

Effect of Ni²⁺ on Histone Acetylation in Hep3B Cells

Histone acetylation occurs in the lysine residues within the basic N-termini of core histones H2A, H2B, H3 and H4 (Klochendler-Yeivin and Yaniv, 2001; Salnikow et al., 1997), and the level of histone H4 acetylation has been reported to reflect the overall level of histone acetylation (Graham et al., 1991; Klochendler-Yeivin and Yaniv, 2001; Zoroddu et al., 2000). Thus, we first tested the effect of Ni²⁺ on histone H4 acetylation by Western blotting. As shown in Figure 1, Ni²⁺ exposure resulted in a dose- and time-dependent decrease in histone H4 acetylation. Then we quantitated the influence of Ni²⁺ on global histone acetylation by using the histone acetylation assay in the presence of H-acetate. Like those of Western blotting, both experiments, in Figures 2A and 2B, again indicated a dose- and time-dependent inhibitive effect of Ni²⁺ on histone acetylation. In both Western blotting and the histone acetylation assay, the reduction in acetylation of histone H4 and global histones showed a similar trend; thus we studied the links between Ni²⁺-generated ROS and histone hypoacetylation through quantitating the decrease of global histone acetylation. Simply, the reduction of acetylation was observed at concentrations no less than 600 μM, and a marked reduction was observed at a concentration of 1000 μM (the reduction rate is about 30% in Fig. 2A). The inhibition of histone acetylation was first apparent 2 h after exposure to 1000 mM Ni²⁺, and the hypoacetylation was sustained for 24 h.

Effect of Ni²⁺ on HAT and HDAC Activity

HAT and HDAC are two metabolic enzymes responsible for the status of histone acetylation. To determine which enzyme is involved in the histone hypoacetylation resulting from the Ni²⁺ treatment, histone acetylation levels in cells were first examined in the presence of an HDAC inhibitor, TSA. Compared with the data without the presence of TSA (Figs. 2A,B), Figures 2C and 2D indicated that Ni²⁺ similarly inhibited histone acetylation in the absence or presence of 0.3 μM TSA, demonstrating that deacetylation by HDAC is not involved in the Ni²⁺-mediated histone hypoacetylation. When in vitro HAT and HDAC assays were carried out with Ni²⁺, HDAC activity remained unaltered, but HAT activity was inhibited in a dose-dependent manner (Figs. 3A,B), providing an evidence for the direct inhibition of HAT activity in vivo by Ni²⁺. Collectively, these results suggest that Ni²⁺ induced histone hypoacetylation in vivo by the direct or indirect inhibition of HAT activity.

ROS Generation in Hep3B Cells Exposed to Ni²⁺

It has been previously shown that Ni²⁺ produced ROS in biological systems (Bal and Kasprzak, 2002; Denkhaus and Salnikow, 2002). To evaluate the induction of ROS in Ni²⁺-treated cells, cells preloaded with DCFH-DA were exposed to Ni²⁺ for the indicated times. DCFH-DA is commonly used to detect the generation of reactive oxygen intermediates in cells (LeBel et al., 1992). Figures 4A and 4B showed a dose- and time-dependent increase in ROS generation when Hep3B cells were incubated with Ni²⁺. Based on the Figure 4A, the exposure of cells to Ni²⁺ at a concentration of no less than 600 μM led to a very significant induction of ROS generation, and an about 2.6-fold increase of ROS compared with basal levels was found in cells exposed for 8 h to 1000 μM Ni²⁺.
To evaluate whether ROS participate in the induction of histone hypoacetylation, we used H$_2$O$_2$ or the antioxidants 2-ME and NAC to enhance or diminish the Ni$^{2+}$-induced ROS generation. Neither the addition of H$_2$O$_2$, with a concentration of 20 or 50 µM alone, nor the addition of 2-ME (1 or 2 mM) or NAC (0.5 mM or 1 mM) induced a significant ROS generation or histone acetylation alteration in Hep3B cells compared to the relative control group (data not shown). On the contrary, H$_2$O$_2$, 2-ME, or NAC was efficient at different concentrations in enhancing or diminishing the ROS generated by Ni$^{2+}$ (Fig. 5A). H$_2$O$_2$ at 50 µM, 2-ME at 2 mM, or NAC at 1 mM also efficiently enhanced or suppressed the inhibition of histone acetylation triggered by Ni$^{2+}$ (Fig. 5B). When effects of H$_2$O$_2$, 2-ME, or NAC on HAT activity were studied, the above result was confirmed by the finding that the inhibition of Ni$^{2+}$ on HAT activity was effectively enhanced or diminished by H$_2$O$_2$ (50 µM), 2-ME (2 mM), or NAC (1 mM) (Fig. 5C). Again, the addition of H$_2$O$_2$, 2-ME or NAC alone did not cause any significant alteration on the activity of HAT in our system (data not shown).

FIG. 2. Ni$^{2+}$ induced global histone hypoacetylation in Hep3B cells. Effect of Ni$^{2+}$ on histone acetylation in the presence of $^3$H-acetate was quantified by liquid scintillation. Cells (2 x 10$^6$) were exposed to the indicated concentrations of Ni$^{2+}$ for 8 h in the absence (A) or presence (C) of 0.3 µM TSA; or exposed to 1000 µM of Ni$^{2+}$ for the indicated times in the absence (B) or presence (D) of TSA. Means ± SD of three parallel experiments were indicated. n = 3 x 3 cultures per conditions, *p < 0.05, **p < 0.01 versus the control group.
These data herein indicate an important role of ROS in the induction of histone hypoacetylation caused by Ni\textsuperscript{2+}/H\textsubscript{11001}.

**DISCUSSION**

Recent studies suggest that through binding with histones, Ni\textsuperscript{2+}/H\textsubscript{11001} can promote ROS generation and induce histone hypoacetylation (Bal and Kasprzak, 2002; Broday et al., 2000; Zoroddu et al., 2000). Considering that ROS are active and easily react with surrounding molecules, there probably exists some linkage between Ni\textsuperscript{2+}-induced ROS generation and histone hypoacetylation, but to our knowledge, no information is currently available about that.

The carcinogenicity of specific insoluble nickel compounds, such as crystalline nickel sulfide (NiS) and subsulfide (Ni$_3$S$_2$), is believed due to the ability of cells to easily phagocytize and...
dissolve these compounds (Denkhaus et al., 2002; Kargacin et al., 1993). Accurately, it is the accumulation of the Ni^{2+} ion inside the cell that appears to determine the carcinogenic potencies of different nickel compounds. Although soluble nickel salts enter cells poorly, NiCl_{2} has been proved to be able to partially enter cells and concentration-dependently increase the Ni^{2+} ion accumulation inside cells, and hence NiCl_{2} is often used to study the carcinogenic mechanism of nickel compounds (Broday et al., 2000; Kargacin et al., 1993). In this study NiCl_{2} was chosen as a suitable agent for studying the role of ROS generation in Ni^{2+}-induced histone hypoacetylation.

Exposure of hepatoma cells to Ni^{2+} resulted in a decreased
histone acetylation in cells, as detected by Western blotting and 
*in vivo* histone acetylation assays. Two possible mechanisms 
can be proposed to explain the histone hypoacetylation resulting 
from Ni**2+**-exposure. One model postulates that Ni**2+** inhibits 
the acetylation of histones by the direct or indirect down-
regulation of overall HAT activity. The other model, on the 
contrary, involves the promoting of Ni**2+** on the deacetylation 
of acetylated histones through the up-regulation of HDAC 
activity. The present data show that HDAC activity is not 
required for the inhibition of Ni**2+** on histone acetylation, 
because TSA, an inhibitor of HDAC (Koyama et al., 2000), 
had no effect on the inhibition of histone acetylation triggered 
by Ni**2+**. Therefore we conclude that the decreased levels of 
histone acetylation must reflect the direct or indirect repression 
of HAT activity by Ni**2+**. This conclusion is confirmed by *in vitro* HAT and HDAC assays using an hepatoma cell extract, 
where the activity of HAT, but not that of HDAC, was inhibited 
by Ni**2+** in a dose-dependent manner. Although additional 
work is needed to clarify how Ni**2+** affects HAT activity, we 
proved that Ni**2+** induced histone hypoacetylation through 
inhibiting HAT activity in hepatoma cells.

Like in other systems (Bal and Kasprzak, 2002; Denkhaus 
and Salnikow, 2002; Salnikow et al., 2000), Ni**2+** treatment 
also led to an increased ROS generation in cells, and the 
addition of H**2**O**2** in Ni**2+**-treated cells significantly enhanced 
the ROS generation, whereas addition of 2-ME or NAC dimin-
ished that. A direct linkage between Ni**2+**-generated ROS and 
histone hypoacetylation was proved by the results that Ni**2+**-
triggered histone hypoacetylation was efficiently enhanced or 
suppressed by the enhancement or diminution of ROS generation. 
This linkage is also confirmed by the HAT assay, where the 
enhancement or diminution of Ni**2+**-generated ROS again 
displayed an efficient enhancing or suppressing effect on the 
HAT inhibition triggered by Ni**2+**. The mechanism underlying 
the inhibition of HAT activity by ROS is unclear; one hypoth-
esis is that the activity of HAT may be weakened by ROS 
through oxidative modification of critical cysteine or histidine 
residues in HAT proteins. This hypothesis seems extremely 
possible in Hep3B cells, since some of HAT proteins such as 
p300/CBP contain critical cysteine- and histidine-rich domains 
and have been found expressing in Hep3B cells (Arany et al., 
1996; Newton et al., 2000). Moreover, this hypothesis is sup-
ported by our unpublished data, where the addition of H**2**O**2** 
hindered the HAT activity of exogenously introduced p300 in 
Hep3B cells. Our study also suggests that the generation of 
ROS is not the whole cause of histone hypoacetylation trig-
gered by Ni**2+**, since H**2**O**2** at 20 μM, 2-ME at 1 mM, or NAC 
at 0.5 mM did not enhance or suppress the histone hypoacety-
lation induced by Ni**2+**, although they significantly affected the 
generation of ROS. It suggests that Ni**2+** inhibits histone acety-
lation through a multi-pathway, at least through inhibiting 
HAT activity by ROS generation (proved by us) and through 
affecting the access of HAT proteins to histones by the binding 
of Ni**2+** with histones (proved by Broday et al., 2000). Al-
though there exist other mechanisms, these data herein clearly 
prove that Ni**2+**-generated ROS plays a role in the inhibition of Ni**2+** on histone acetylation.

The toxicity of Ni**2+**, especially the carcinogenicity of spe-
cific insoluble nickel compounds, is believed mainly resulting 
from the suppression of gene transcription and expression 
(Beyersmann, 2002; Cangul et al., 2002), while the resistance 
of mammalian cells to Ni**2+** is closely allied to their defense 
capability against the generation of ROS triggered by Ni**2+** (Qu 
et al., 2001; Salnikow et al., 1994), i.e., mammalian cells 
diminish the effects of Ni**2+** through diminishing ROS gener-
ation. Thus, there must exist a linkage between Ni**2+**-induced 
gene suppression and ROS generation. Insofar as we are aware, 
this study constitutes the first report that Ni**2+**-induced ROS 
generation is involved in the inhibition of histone acetylation. 
The linkage between ROS generation and histone acetylation 
inhibition opens a new window for us to understand the role of 
ROS in the toxicity, especially the carcinogenicity of Ni**2+** and 
the resistance of mammalian cells to Ni**2+**. Presumably, Ni**2+** 
duces carcinogenesis through ROS generation, which inhib-
its histone acetylation and, hence, suppresses genes expression 
(Fig. 6). And whether a mammalian cell is sensitive or resistant 
to Ni**2+** is probably determined by its defense ability against 
Ni**2+**-induced gene suppression, while ROS generation plays 
an important role in this suppression through inhibiting histone 
acetylation.

Although as we have mentioned above, only high concen-
trations of NiCl**2** possessed the activities of generating ROS 
and inhibiting histone acetylation, i.e., at 600 or 1000 μM, our 
study can indeed be very relevant to the mechanisms of carci-
ogenesis induced by specific insoluble nickel compounds. 
This is because soluble nickel salts enters cells poorly. Kar-
gaicin et al. (1993) found that the cytotoxicity of 600 μM of 
soluble nickel chloride was only equivalent to approximately 
20 μM of nickel subsulfide.

In summary, we conclude that ROS production is increased 
during exposure of cells to Ni**2+**, and this production of ROS is 
involved in the inhibition of histone acetylation. Ni**2+** induces 
histone hypoacetylation through a multi-pathway, at least 
through affecting the HAT activity by ROS generation and 
influencing the access of HAT with histones. These data sug-
gest a possibly useful method to prevent nickel-induced histone 
hypoacetylation, gene silencing, and related diseases (cancer, 
etc.) through improving the antioxidative potential of people,

![FIG. 6. Schematic diagram of the possible role of ROS generation on the Ni**2+**-induced carcinogenesis.](https://academic.oup.com/toxsci/article-abstract/74/2/279/1716313)
especially those working in a high nickel-exposed environment.

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