IN VITRO EFFECTS OF NICKEL ON HUMAN EMBRIONARY
LUNG FIBROBLASTS

Raluca CIUBĂR, Valentina MITRAN, Anişoara CÎMPEAN, Dana IORDĂCHESCU[*]

Research Center for Biochemistry and Molecular Biology, University of Bucharest, 93 Spl. Independenţei, Sector 5, Bucharest
050095, Roumania

Occupational and environmental exposure to nickel compounds is an increasing problem because it may cause chronic active inflammation and lung fibrosis. In the present study, the effects of nickel chloride on human embrionary lung fibroblasts (HELF) were investigated. In dose-dependent experiments, HELF were incubated for 24 h with various concentrations of $NiCl_2$ (100 μM – 20 mM). HELF treatment with $NiCl_2$ doses higher than 1 mM showed a significant decrease in cell viability and an increased intracellular and extracellular ROS production, suggesting that cytotoxicity is due to ROS formation. High doses of nickel also induced cell apoptosis as seen with annexin V-FITC and propidium iodide staining.

INTRODUCTION

Nickel is a metallic element that is naturally present in the earth's crust. Due to unique physical and chemical properties, metallic nickel and its compounds are widely used in modern industry. The high consumption of nickel-containing products inevitably leads to environmental pollution by nickel and its by-products at all stages of production, recycling and disposal. Human exposure to nickel occurs primarily *via* inhalation and ingestion. Significant amounts of nickel in different forms may be deposited in the human body through occupational exposure and diet over a lifetime. Since nickel has not been recognized as an essential element in humans it is not clear how nickel compounds are metabolized. It is known, however, that exposure to nickel compounds can have adverse effects on human health. Inhalation of metal dusts and fumes can cause a variety of pathophysiological responses including inflammation, airway hypersensitivity reactions, parenchymal diseases and cancer. The cellular and molecular mechanisms of action of metals in the lung are unresolved and involve complex pleiothropic effects. These effects are mediated by direct reaction of the metals with cellular macromolecules and indirect effects of reactive oxygen species generated when cells are exposed to metals. This article focuses on nickel which remains on the list of the top 33 hazardous air pollutants of greatest concern, associated with high incidence of noncancerous respiratory diseases such as asthma, fibrosis and chronic obstructive pulmonary disease.[1]

Similar to other metals nickel can affect multiple pathways. The action of nickel is complicated by the species of the metal interacting with cells.[2, 3] Particulate nickel is taken into cells and induces prolonged gene expression, whereas soluble nickel requires chronic exposure to produce similar changes.[2-4] Nickel enhances the intracellular oxidative state and can deplete intracellular glutathione.[5] Nickel stimulates signaling cascades that increase the expression of profibrotic proteins, inflammatory cytokines and genes involved in hypoxic responses. It also supresses the expression of the tumor suppressor thrombospondin-1.[2, 4-7] The signaling mechanisms for these changes appear to involve both ROS-dependent and -independent pathways.

The aim of the present report was to obtain a deeper insight into the effects of nickel on HELFs by study of viability, citotoxicity, intracellular production of ROS, and cell apoptosis.

---

[*] Corresponding author: dior@bio.bio.unibuc.ro

MATERIALS AND METHODS

HELFs were obtained in our laboratory by the explant technique from lung biopsies. Informed consent was obtained from each patient, and approval was received from the local committee for research on human subjects in accordance with the Declaration of Helsinki. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were incubated at 37°C in a humidified 5% CO_2/air-mix incubator. All experiments were performed with cells in the 5th passage, immediately after nickel treatment.

After the culture reached 60% confluence, the monolayers were rinsed with PBS and incubated at 37°C for 24 h in DMEM without FBS plus various concentrations of $NiCl_2$ (100 μM – 20 mM). Controls for these experiments consisted of similar cells incubated in DMEM alone   (basal condition).

The morphological changes were examined with an inverted phase-contrast microscope Nikon Eclipse TS 100 at 24 hours after addition of nickel chloride in the culture medium.

Viability test

Viability test was performed by the MTT dye-reduction assay. The tetrazolium dye 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (MTT) is reduced to a colored product by the activity of NAD(P)H-dependent dehydrogenases and this indicates the level of energy metabolism in cells. Fibroblasts were treated in six-well plates and incubated with 1 mL/well of MTT (1 mg/mL) for 3 hours at 37°C. After this time the formazan was released from the cells with DMSO. Absorbance of the supernatant was measured at 550 nm. Viability was calculated as the percentage of formazan formation in cells treated with nickel chloride compared with control cells.

Chemiluminiscence detection of ROS production

HELFs were treated as mentioned above. After 24 hours the medium was removed. 50 μL of sample (culture medium), 200 μL 10^{-5} M luminol, 700 μL 0.2 M Tris-HCl pH 8.6 and 50 μL 10^{-5} M H_2O_2 were mixed and placed in a luminometer and the chemiluminiscence(CL) emissions were registered. For the intracellular detection of ROS after 24 hours of treatment HELFs were lysed by multiple cycles of freezing and thawing.

Apoptosis detection

For apoptosis detection a kit (from Sigma) containing annexin V-FITC (SIGMA Chemical Co) and propidium iodide was used. Cells treated in a 24-well plate were washed twice with PBS and then covered with 200 μL binding buffer 1X provided by the kit. In each well 2.5 μL of annexin and PI were added and the plate was incubated for 15 min at room temperature in the dark. After this time the cells were monitorised with the fluorescence microscope and photographed with a digital camera.

RESULTS AND DISCUSSION

Exposure of HELFs at 37°C for 24 h to different doses of nickel chloride (100 μM, 1 mM, 10 mM and 20 mM) resulted in dose-dependent morphological changes of cells (Fig. 1). HELFs, grown as monolayers, for 24 h in serum-free DMEM medium, have an acicular shape with slender lamellar expansions. The treatment with doses higher than 1mM $NiCl_2$ for 24 h showed cytotoxic effects, characterized by a refractile halo at cell border, loss of matrix attachment and their death, in a dose-dependent manner.

control   100 microM NiCl$_2$ 1 mM NiCl$_2$

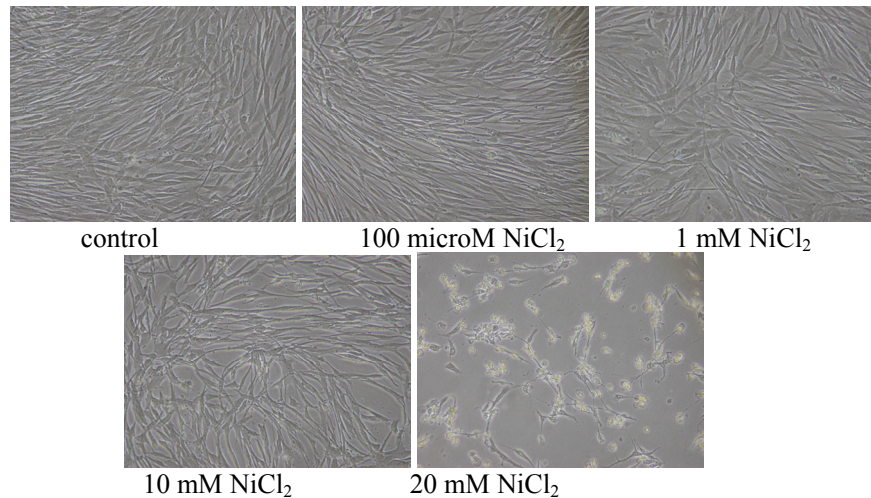10 mM NiCl$_2$ 20 mM NiCl$_2$

Fig. 1 – Morphology alteration of cultured HELFs treated with different levels
of nickel as viewed by phase contrast microscopy

Fig. 2 shows the results of the cell viability studies, measured by the MTT test, after 24 h of treatment with nickel that confirm the morphological observations.
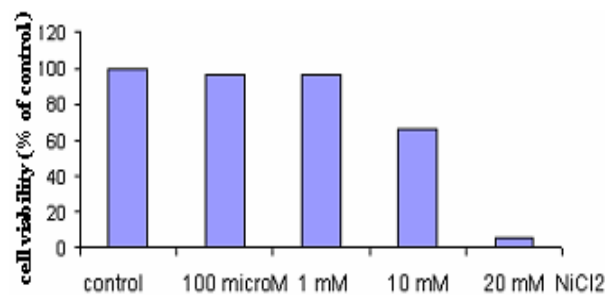

Fig. 2 – Effects of different concentrations of nickel chloride on the viability of human embrionary lung fibroblasts.

The incubation of HELF with 100 μM and 1 mM NiCl$_2$, for 24 h, resulted in a very small decrease in the number of metabolically active cells at 96.81%, respectively 96.23%, compared with control considered 100%. Nevertheless, increasing the concentration of nickel up to 20 mM caused a concentration-dependent decrease in cell survival, which was significant.

The mechanism of nickel related cellular damage is not fully understood, but increasing evidence indicates that ROS may play an important role.

To confirm the possible role of oxidative stress in the nickel induced citotoxicity in HELFs, ROS formation has been studied. The incubation of cells with nickel induced a dose-dependent increase in the ROS formation (Figs. 3 and 4).

Apoptosis is a highly conserved process that can be triggered by a wide range of physiological and pathological conditions. Nickel-induced apoptosis was first reported in Chinese hamster ovary cells.[8] Kim *et al.*[9] using nickel(II) acetate showed a rapid induction of apoptosis in T cell hybridoma cells. In these cells, there was an increase in FasL protein levels and transient activation of caspase-3, a downstream effector of Fas/FasL-induced apoptosis.[9] Since nickel induces oxidative damage resulting in an increase of ROS production,[10] it is possible that nickel-induced ROS are involved in apoptosis.

We studied cell apoptosis in an experiment in which the HELFs were incubated with NiCl$_2$ at a dose ranging from 100 μM to 20 mM. Fig. 5 shows the effects of different treatments on the HELFs apoptosis assessed by annexin V-FITC and propidium iodide. In control and at doses of 100 μM, 1 mM nickel chloride (data not shown) there were no apoptotic cells as seen by phase-contrast and fluorescence microscopy. Doses of 10 and 20 mM of nickel in exchange induced cell apoptosis. HELFs lost their normal morphology and apoptotic cells stained with annexin V-FITC and PI became visible at fluorescence microscope
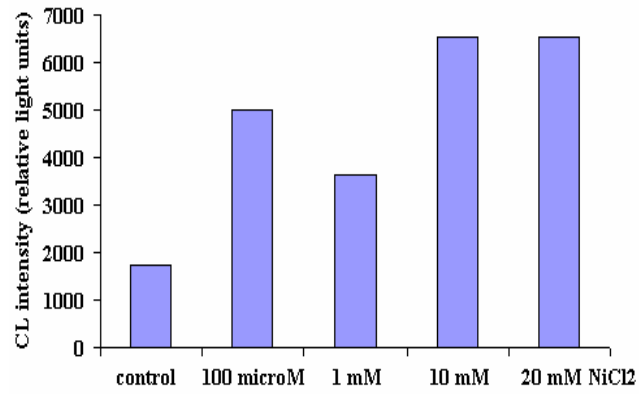
Fig. 3 – ROS levels in culture medium of HELFs treated with different concentrations of nickel in the range 100 μM -20mM NiCl$_2$.
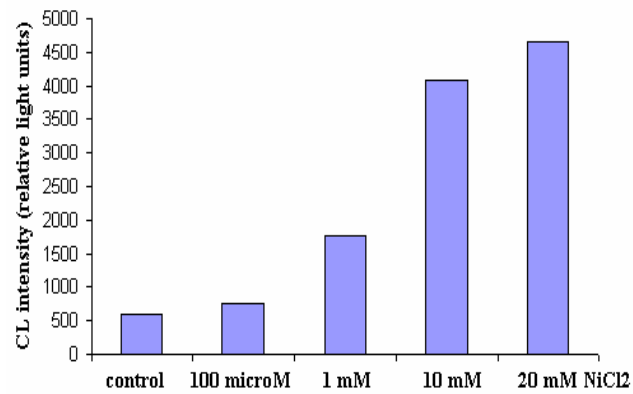

Fig. 4 – Intracellular ROS production in cells treated with different levels of nickel chloride.
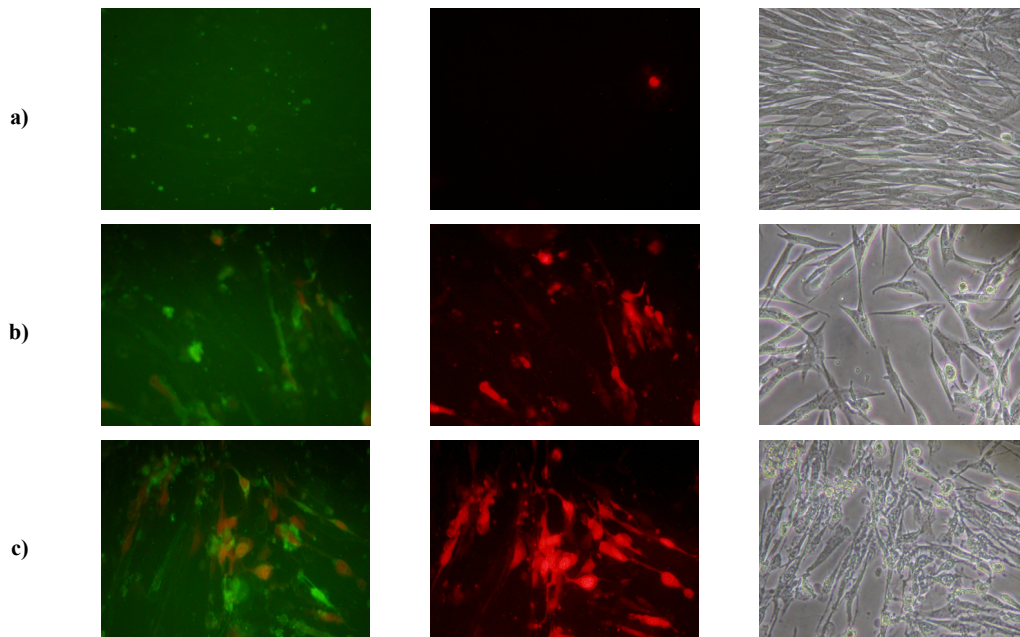

Fig. 5 – Annexin V-FITC, propidium iodide staining and phase-contrast images of HELFs treated with nickel chloride for 24 hours: a) control; b) 10 mM NiCl$_2$; c) 20 mM NiCl$_2$.

In summary, the results presented in this study demonstrated that the effects of nickel upon the oxidative status and apoptosis of HELFs are strongly dependent on their concentrations. It is clear that high concentrations of nickel produce cell death by increasing the accumulation of ROS.

REFERENCES

1. G. D. Leikauf, *Environ. Health Perspect*, **2002**, *110*, 505-526.
2. E. Denkhaus and K. Salnikow, *Crit. Rev. Oncol. Hematol.*, **2002**, *42*, 35-56.
3. A. R. Oller, M. Costa and G. Oberdorster, *Toxicol. Appl. Pharmacol.*, **1997**, *143*, 152-166.
4. A. Barchowsky, N. V. Soucy, K. A. O'Hara, J. Hwa, T. L. Noreault and A. S. Andrew, *J. Biol. Chem.*, **2002**, *277*, 24225-24231.
5. A. S. Andrew, L. R. Klei and A. Barchowsky, *Am. J. Physiol.*, **2001**, *281*, L607-L615.
6. K. Salnikow, W. Su, M. V. Blagosklonny and M. Costa, *Cancer Res.*, **2000**, *60*, 3375-3378.
7.   A. S. Andrew, L. R. Klei and A. Barchowsky, *Am. J. Physiol.*, **2001**, *281*, L616-L623.
8. Y. H. Shiao, S. H. Lee and K. S. Kasprzak, *Carcinogenesis*, **1998**, *19*, 1203–1207.
9. K. Kim, S. H. Lee, Y. R. Seo, S. N. Perkins and K. S. Kasprzak, *Toxicol. Appl. Pharmacol.*, **2002**, *185*, 41–47.
10. H. Dally and A. Hartwig, *Carcinogenesis*, **1997**, *18*, 1021–1026.