Revue Roumaine de Chimie, **2006**, *51*(12), 1169–1173

EFFECTS OF NICKEL(II) ON HUMAN POLYMORPHONUCLEAR LEUKOCYTE FUNCTION *IN VITRO*

Raluca CIUBĂR, Anişoara CÎMPEAN and Dana IORDĂCHESCU*

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

Received April 4, 2006

A variety of heavy metals are recognized as environmental pollutants, and although a significant body of literature exists on the acute toxicity of these metals in various tissues, little is known about the effects of metals such as nickel on host defense. Therefore, the effect of nickel chloride ($NiCl_2$) on human polymorphonuclear leukocytes (PMN) function was evaluated in vitro. Cell viability was measured by MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide)) test and cytotoxicity after the treatment with nickel chloride by LDH (lactate dehydrogenase) assay. Concentrations of $NiCl_2$ between 1 and 10 mM decreased cell viability in a dose-dependent manner. The effects of $NiCl_2$ on human PMN functions involved in host defense were then evaluated. $NiCl_2$ consistently suppressed phagocytosis and respiratory burst of PMN at concentrations ≥ 1 mM. However, 0.1 mM $NiCl_2$ had a stimulatory action on PMN functions. *In vivo*, these effects would not only compromise host defense but also promote tissue injury via the local production of oxygen metabolites.

INTRODUCTION

Polymorphonuclear leukocytes (PMN) belong to the myeloid lineage, which multiplies and differentiates in the bone marrow. Mature neutrophils are stored in the bone marrow for several days and then released into the blood, where they remain for only a few hours before dying or migrating into tissues. Normal PMN turnover in humans is mediated by apoptosis, a process that presumably down-regulates proinflammatory and microbicidal functions and prepares these cells for removal from the tissue by macrophages.[1]

Polymorphonuclear neutrophils are the first line of defense against bacterial infections.[2] They are designed to phagocytose bacteria and kill them by releasing the contents of their cytoplasmic granules into the phagosome and by producing reactive oxygen intermediates. These bactericidal functions are essential for the host defense. However, overactivation of these cells contributes to destruction of host tissue.

In the past 20 years, there has been a growing awareness in industrial, academic, regulatory and public sectors concerning the health risks associated with the exposure to persistent toxic chemicals. These pollutants include compounds from various chemical classes (heavy metals, polychlorinated biphenyls, dioxins, bisphenols, etc.). There is a general consensus that these compounds may cause, directly or indirectly, detrimental effects on four main physiological systems: endocrine, reproductive, nervous and immune systems.[3] Exposure of adult animals to toxic environmental contaminants such as heavy metals can lead to immunosuppression. Using laboratory models such as rodents, this chemically induced immunosuppression has been associated with decreased humoral and cellular responses, by compromised lymphocyte proliferation, and by decreased thymic weight.[4-7] In addition, a number of *in vitro* tests, in which the toxic substances were in direct contacts with the cells, have shown that phagocytosis, mixed lymphocyte culture, lymphocyte proliferation in response to mitogens,[8-9] plaque forming cell and cytokine receptors were severely reduced,[10] indicating that both the afferent and the efferent phases of the immune response can be affected. For many chemical exposures, immunosuppression was correlated with decreased resistance to infections using parasite, bacterial, viral or tumoral models.[11]

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

The aim of the present article was to investigate *in vitro* nickel effects especially upon phagocytic activity and respiratory burst of PMN.

RESULTS AND DISCUSSION

Among environmental contaminants recognized for their toxicity and world-wide distribution, heavy metals are certainly of first concern. Epidemiological data on humans and studies on laboratory animals exposed to these chemicals indicate that heavy metals can affect several systems. They can also be responsible for a number of immunomodulatory effects, leading ultimately to an enhanced susceptibility of humans and animals to microbial agents.

Understanding the effects of nickel on human immune functions, could help in determining the risk associated with human exposure to this metal, at physiologically relevant concentrations. This would help in the design of strategies in preventing chronic toxicity to heavy metals. Acceptable safe environmental and human blood levels could then be evaluated and fixed for this metal.

To evaluate the toxic effects of nickel on human cells, a number of *in vitro* assays have been performed on human neutrophils.

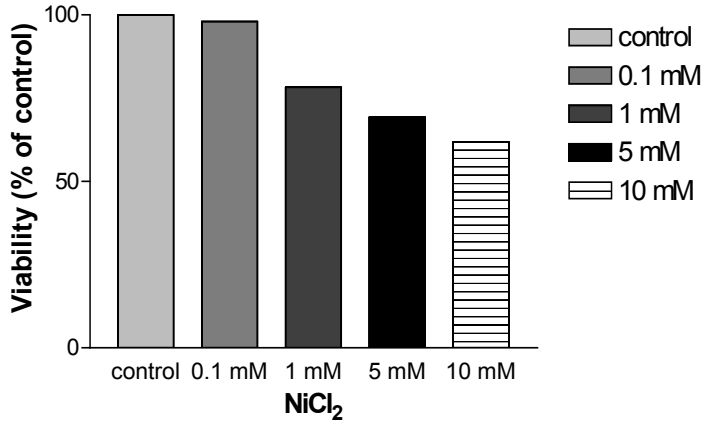

Fig. 1 – Effects of different concentrations of nickel chloride on the viability of human PMNs.

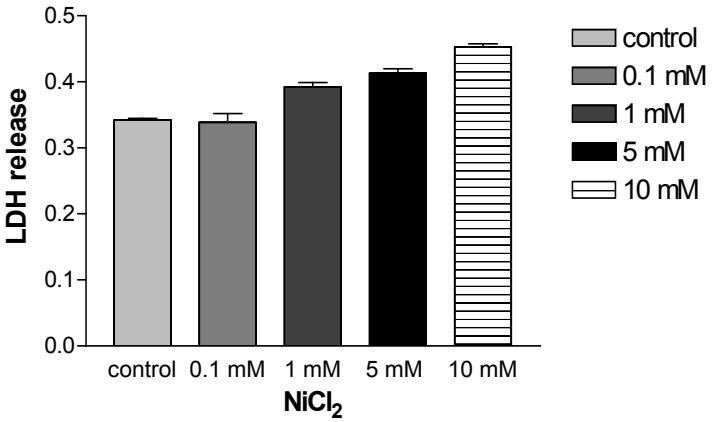

Fig. 2 – LDH release in the culture medium of human PMNs treated with different concentrations of nickel chloride.

The MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) and LDH (lactate dehydrogenase) assays have shown the cytotoxicity of higher doses of $NiCl_2$ (≥ 1 mM) after 24 h treatment noticed by the diminution of the cell viability and the increase of the LDH releasing in culture medium (Figure 1 and 2). We have not found the cytotoxic effects of low doses of $NiCl_2$ (0.1 mM) against PMN behaviour.

Exposure of neutrophils to a variety of agonists including soluble chemoattractant peptides and cytokines results in activation of the oxidative burst that is required for bacterial killing. Increased oxygen, consumed during this process, is reduced to superoxide (O_2^-) via membrane NADPH oxidase, a multicomponent system formed by the assembly of cytosolic and membrane proteins upon appropriate stimulation.[12] Superoxide can engender more harmful reactive oxygen species (ROS) when two molecules of O_2^- react together or enzymatically as a consequence of the activity of superoxide dismutase (SOD), to form dioxygen and hydrogen peroxide (H_2O_2) and finally hydroxyl radical ($OH\cdot$).[13]

To analyze wheather nickel chloride exerted an effect on the first function of neutrophils, the respiratory burst, we carried out a chemiluminiscence study on cells treated for 24 h with different concentrations of nickel and then stimulated with fMLP (formylmethionyl-leucyl-phenylalanine). The ROS production was monitored for 30 min by the luminol chemiluminescence reaction (data not shown). Our data show that the lowest dose of Ni (0.1 mM) stimulated the respiratory burst of PMN by the increase of ROS production while the higher levels of Ni (> 1 mM) inhibit it. If a 1 mM $NiCl_2$ dose decreased the intensity of the respiratory burst, the higher doses completely supressed this function of neutrophils. Our results are according to Ono et al.,[14] who showed that nickel suppresses the ROS generating capacity of neutrophils.

In vivo, these effects would not only compromise the mechanism of host defense but also promote tissue injury via the local production of toxic oxygen metabolites. Although production of antimicrobial molecules is essential in the processus of host defense, excessive ROS production can also cause injury to surrounding tissue.[12,13] For example, the ROS generated in respiratory burst plays a role in inducing pulmonary injury in some diseases such as acute respiratory distress syndrome. [15]

Zu et al.,[16] demonstrated that the activation of cellular p38 MAP kinase is indispensable for the fMLP-mediated cellular functions in human neutrophils. It is possible that nickel induced inhibition of respiratory burst might result from p38 MAP kinase inhibition. In order to elucidate the mechanism of respiratory burst inhibition by nickel further studies need to be performed.

The effect of nickel on phagocytosis of PMN is shown in Figure 3. Our results revealed that cells exposure to 1-10 mM $NiCl_2$ inhibits phagocytosis in a dose-dependent manner, while the lowest dose (0.1 mM) seems to slightly stimulate this function of neutrophils.

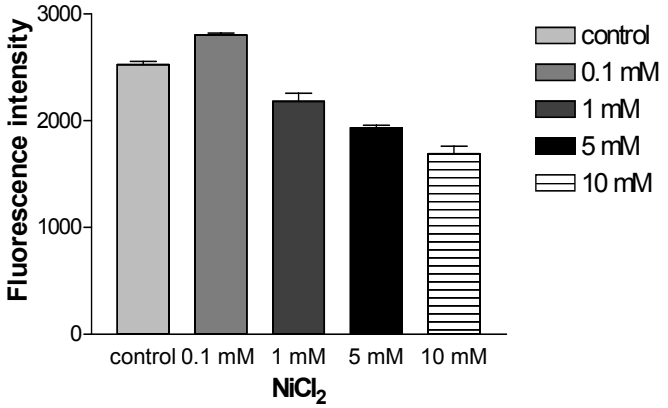

Fig. 3 – Effect of exposure to $NiCl_2$ on phagocytosis of human PMN.

The fact that nickel selectively inhibits phagocytosis at concentrations below those that caused lysis or cell death permits speculation upon its mechanism of action. The energy required for phagocytosis comes from the utilization of cellular adenosine 5'-triphosphate (ATP), and therefore any substance that reduces the availability of ATP would affect the cells' phagocytic activity. Since Ni binds with ATPase, thereby reducing ATP energy release [17] and with ATP itself to form a stable binary complex [18] that also inhibits ATP utilization, could be responsible for the effect of nickel on phagocytosis.

MATERIALS AND METHODS

Neutrophils isolation. Fresh human neutrophil granulocytes (PMN) were obtained from healthy donors. Venous blood was drawn into heparinized syringes. The PMN were separated by centrifugation on

Histopaque medium according to Boyum.[19] Remaining red blood cells were eliminated by hypotonic lysis. The purified neutrophils were washed and resuspended in Dulbecco's modified Eagle's medium (DMEM) and the cells were counted in a hemocytometer. Morphological analysis revealed that 90% of the cells were PMN. The cells were treated with nickel chloride in the range of 0.1-10 mM for 24 hours.

Viability test. Viability test was performed by the MTT dye-reduction assay. The tetrazolium dye 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced to a colored product by the activity of NADH-dependent dehydrogenases and this indicates the level of energy metabolism in cells. PMNs were treated in 24-well plates and incubated with 0.5 ml/well of MTT (1 mg/ml) for 3 hours at 37°C. After this time the formazan was released from the cells with dimethyl sulfoxide. Absorbance of the supernatant was measured at 550 nm with a Tecan reader. Viability was calculated as the percentage of formazan formation in cells treated with nickel chloride compared with control cells.

Cell cytotoxicity assay was carried out by measuring the release of lactate dehydrogenase from injured cells using a Sigma *in vitro* toxicology assay kit lactate dehydrogenase based, TOX-7 according to the manufacturer protocol.

Respiratory burst assay. ROS were quantified by measuring luminol-dependent chemiluminescence at 37°C using a Tecan reader at approximately one measurement per well every 25 s. Neutrophils ($2x10^4$ cells) subjected to nickel chloride treatment for 24 hours were washed with DMEM and then were suspended with 25 μM luminol, 4 μg/ml cytochalasin B, and DMEM for a final volume of 200 μl per well. The cells were stimulated with 1 μM fMLP, and ROS generation was measured by integrating photon counts for 30 min after agonist addition. The solvent's control, dimethyl sulfoxide (DMSO), did not stimulate ROS production.

***In vitro* phagocytosis assay.** In vitro phagocytosis assay was performed with Vybrant Phagocytosis Assay Kit (V-6694, Molecular Probes) according to manufacturer instructions. Briefly, PMNs were seeded in 96-well plate at a density of 10^5cells/well. After 24 h of treatment with $NiCl_2$ ranging from 0.1 to 10 mM, the medium was removed and 100 μl of fluorescein-labeled Escherichia coli (K-12 strain) BioParticles were added. The plate was transferred to incubator for 2 h. After this time, the BioParticle suspension was removed and 100 μl of trypan blue suspension were added for 1 min. at room temperature. The excess of trypan blue was removed and the samples were measured in the fluorescence plate reader (Tecan) using 480 nm for excitation and 520 nm emission wavelengths.

CONCLUSIONS

In summary, our studies illustrate the cellular toxicity produced by exposure to soluble nickel salts. Hence, they emphasize that in order to estimate the cytotoxicity of a substance it is necessary to evaluate besides the classical parameters (viability, LDH releasing) also some functional properties as the respiratory burst and phagocytosis for PMN. Additionally, the data suggest that nickel in high doses could profoundly deteriorate the main two functions of PMN and in this way the host response in a infectious disease.

REFERENCES

1. V. Witko-Sarsat, P. Rieu, B. Descamps-Latscha, P. Lesavre and L. Halbwachs-Mecarelli, *Lab. Invest*., **2000**, *80*, 617-653.
2. C. Akgul, D.A. Moulding and S.W. Edwards, *FEBS Lett.*, **2001**, *487*, 318-322.
3. B.C. Allen, R.J. Kavlock, C.A. Kimmel and E.M. Faustman, *Fund. Appl. Toxicol*., **1994,** *23*, 487-495.
4. J. Bernier, P. Brousseau, H. Tryphonas, K. Krystyniak and M. Fournier, *Env. Health Perspect*., **1995**, *103*, 23-34.
5. J.I. Kerkvliet, *Environ Health Perspect*., **1995**, *103*, 47-53.
6. K. Krzystyniak, H. Tryphonas and M. Fournier, *Env. Health Perspect*., **1995**, *103*, 17-22.
7. H. Tryphonas, *Environ Health Perspect*., **1995**, *103*, 35-46.
8. F. Omara, C. Brochu, D. Flipo and F. Denizeau, *Env. Tox. Chem*.,**1997**, *16*, 576-581.
9. F. Omara, D. Flipo, D.C. Brochu, F. Denizeau, P. Brousseau and E.F. Potworowski, *J. Tox. Env. Health*, **1998**, *54*, 101-117.
10. Y. Payette, M. Lachapelle, C. Daniel, J. Bernier, M. Fournier and K. Krzystyniak, *Int. J. Immunopharmac*., **1995**, *17*, 235-246.
11. M. Fournier, D.G. Cyr, P. Brousseau and H. Tryphonas, "Environmental endocrine disruptors", L. Guillette and D. Crain (eds.),Taylor and Francis Publishers, New York, 2000, p. 182-216.
12. G. Ricevuti, *Ann NY Acad Sci*, **1997**, *832*, 426–448.

13. P.A. Ward, *Ann NY Acad Sci*, **1997**, *832*, 304-310.
14. Y. Ono, S. Nakaji, K. Sugawara and T. Kumae, *Nippon Eiseigaku Zasshi*, **1994**, *49*, 645-653.
15. G. Bellingan, *Br Med Bull*, **1999**, *55*, 12-29.
16. Y.L. Zu, J. Qi, A. Gilchrist, G.A. Fernandez, D. Vazquez-Abad, D.L. Kreutzer, Chi-Kuang Huang, and R.I. Sha'afi, *The Journal of Immunology*, **1998,** *160*, 1982–1989.
17. M.G. Mustafa, E.C. Cross, R.J. Munn and J.A. Hardie, *J. Lab. Clin. Med*., **1971**, *77*, 563-571.
18. H. Sigel, K. Becker and D.B. Mccormick, *Biochim. Biophys. Acta*, **1967**, *148*, 655-664.
19. A. Boyum, *J. Clin. Lab. Invest*., **1968**, *21*, 77.