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Effect of Some Nickel Compounds on Red Blood Cell Characteristics

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ABSTRACT

The effect of certain inorganic and coordinated nickel compounds on the resistance to different destructive substances, rheological properties, and functional activity of healthy human red blood cells (RBC), was investigated. It is shown that nickel compounds affect the erythrocyte membrane lipid bilayer, as well as membrane proteins to various extents, depending on the type of compounds used. In general, the acceleration of erythrocyte aging was observed to be more pronounced in young erythrocytes. The observed results suggest that nickel compounds decrease water permeability across erythrocyte membranes. Almost all the investigated nickel compounds decrease erythrocyte thermostability, deformability, and the rate of O_2 release by erythrocytes.

Index Entries: Effect of nickel compounds; erythrocyte membrane stability; erythrocyte deformability; effect of different detergents.

INTRODUCTION

Nickel is generally known as a toxic and carcinogenic element in human and many animal species (*1–5*); however, there is some evidence for nickel stimulation of erythropoiesis (*6–9*), and for the negative influence of nickel deficiency on growth and development (*10–11*). In spite of con-

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siderable progress in nickel studies, the mechanism of nickel carcinogenicity and toxicity is poorly understood as yet.

This paper presents data on one of our investigations devoted to the study of the effect of nickel on the structure and function of cells as well as macromolecules.

MATERIALS AND METHODS

The effect of $NiCl_2$, $NiSO_4$, $NiEn_2Cl_2$, and $NiEnSO_2$ was investigated (En denotes ethylendiamine, which forms a stable coordination bond with nickel and undoubtedly affects the Ni^{2+} properties). In $NiEnSO_4$, two water molecules are replaced by one molecule of ethylendiamine, whereas in $NiEn_2Cl_2$, four molecules of water are replaced by two molecules of ethylendiamine.

Erythrocytes were isolated by centrifugation from healthy donors' fresh blood, using 20 units/mL heparin, and then washed three times with saline. Isolated erythrocytes were suspended in a solution containing nickel compounds (adjusted optical density of $d = 0.7$ at $\lambda = 670$ nm). After 30 min incubation with gentle shaking (5 min for H_2O), the investigation was carried out by the method described in Ref. *12*.

We have examined the effects of nickel compounds on erythrocyte membranes from the data on erythrocyte stability changes with different hemolytic agents. H_2O, Triton X-100, or SDS were used after erythrocyte incubation in a solution containing 10^{-4} *M* $NiCl_2$, $NiSO_4$, $NiEn_2Cl_2$, or $NiEnSO_4$. In the case of H_2O, these compounds were used in concentrations of 10^{-6} *M*. We obtained hemolysis differential curves that reflected the rate of decrease in the number of RBCs in the suspension. In each case, control samples of RBCs were incubated under the same conditions without addition of nickel compounds. Moreover, we studied the rate of oxygen output using the method described in Ref. *13*, erythrocyte thermostability and elasticity, as well as the distribution of erythrocytes by volume and RBC deformability (*14,15*).

RESULTS AND DISCUSSION

As a result of our investigation, it was established that the data on RBC membrane resistance to the different detergents proved to be the most informative (Fig. 1A–C).

First, it should be noted that the effect of nickel, as well as that of other metals, depends considerably on the electronic configuration of the cation structure formed in solution. In aqueous medium, nickel occurs mostly as the $(Ni(H_2O)6)^{2+}$ cation, which has an octahedral configuration. The bond between water and metal is sufficiently weak for water to be easily replaced by other ligands. It was established that $(Ni(H_2O)6)^{2+}$ proved to be more aggressive, whereas the presence of ethylendiamine

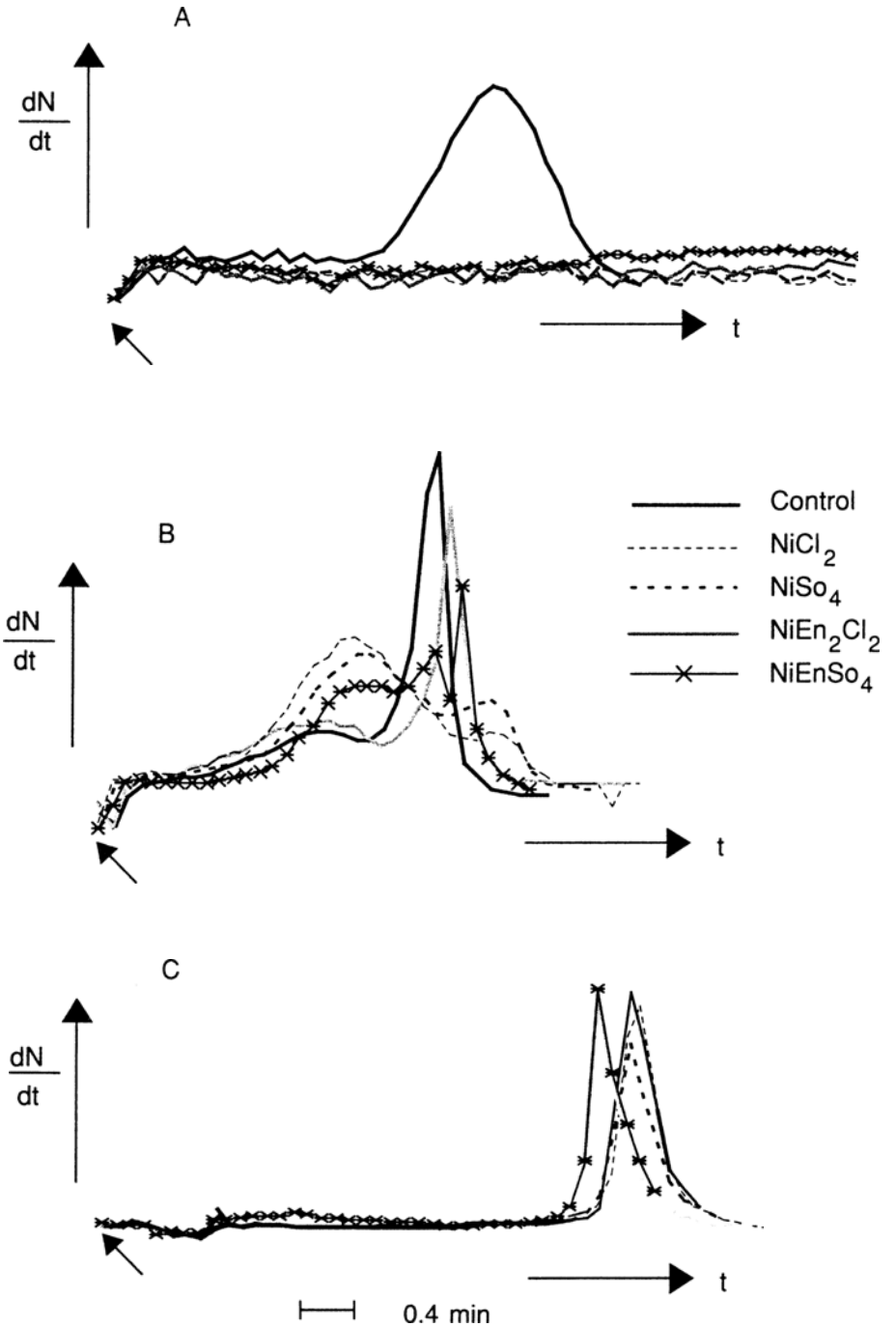

Fig. 1. Differential curves of RBC hemolysis with, H_2O (A), SDS (B) and Triton X-100 (C). Rate of addition of H_2O - 1.3 mL/min, others 0.45 mL/min. Hemolytics initial concentration: Triton X-100 - 0.07%, SDS - 0.8 m*M*. The solvent in all cases was 0.9% NaCl. *X* denotes time in min, *Y* denotes dN/dt, where N = number of erythrocytes. Arrow shows time of addition of hemolytic agents.

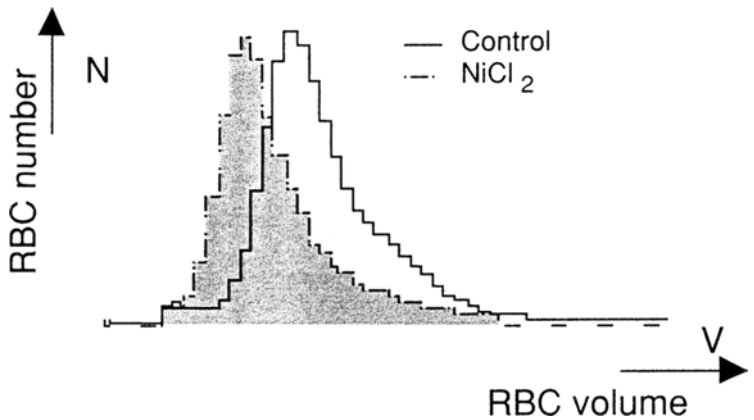

Fig. 2. RBCs distribution is shown according to their size.

in $(NiEn_2(H_2O)_2)^{2+}$ and $(NiEn(H_2O)4)^{2+}$ markedly reduces the toxic effect of nickel.

The most interesting results were obtained with water (Fig. 1A). After incubating in 10^{-6} *M* nickel complexes for 5 min, osmotic resistance changes were observed. After more prolonged incubation, there is a significant increase of RBC stability in the absence of spherulation.

As seen in the histogram of erythrocyte distribution according to volume before and after addition of water to the erythrocyte suspension incubated in $NiCl_2$ (Fig. 2), the erythrocyte volume, instead of enlarging, was somewhat condensed. This observation indicates that in a neutral medium nickel ions cause changes leading to the closure of erythrocyte membranes' water channels. Moreover, investigation of erythrocyte deformability showed a decrease in the elasticity of RBC. This data once more shows that hypotonic stability is not owing to increased elasticity, but rather to the fact that water does not permeate the cell.

It appears that nickel action takes place after its penetration across the cell membrane. It must be noted that other divalent metal ions that are proximal in the periodical system of elements (Co, Cu, Zn) do not elicit the same effect. Therefore, it is possible that water channel closure is characteristic only for nickel ions, but not for divalent metals in general.

These distinct changes were also observed during sodium hydroxide hemolysis (data not shown). It is known that alkaline hemolysis is osmotic in character (*16*). Apparently, water channel closure does not occur in the alkaline solution. However, a decrease of membrane elasticity is manifested in this case by an instantaneous hemolysis of the incubated erythrocytes followed by aggregation of their components. This aggregation may be explained by the formation of $Ni(OH)_2$. Ethylendiamine present in the complex serves as good protection. It should also be stated that nickel cations do not firmly bind with the membranes, and the cation complexes in the presence of erythrocytes stay the same as they are in aqueous solution.

The existence of two peaks on the differential curves of SDS hemolysis (Fig. 1B) could be explained by assuming that the erythrocyte populations in humans consist of two groups of different resistance—a fact that probably reflects age differences. Furthermore, it appears that we are not dealing here with a gradual change in the structure of the erythrocytes as they age, but rather with a distinct age "phase transition" peculiar to some developmental stage. It follows from our data that, under the effect of nickel complexes, a reconstruction takes place similar to that that occurs during the aging of cells—the peak of lesser resistance increases, whereas the peak of greater resistance decreases. In comparison to control, the peaks change in both young as well as old erythrocytes, but it is more evident in the old erythrocytes. Summing up the data obtained during the erythrocyte hemolysis by different hemolyzing agents, the following assumptions may be made.

Nickel cations do not form stable bonds with erythrocyte membranes. Their effect is mainly related to Ni^{2+} membrane penetration, where they may associate with the negatively charged lipid heads, for example, phosphatidylserine and phosphoinositide. This weakens the bond between the water phase and lipid layers of the membrane, leading to destabilization of the membrane structure. In addition, nickel cations diminish the total negative charge of membranes, an additional destabilizing factor.

Because nickel complexes do not affect resistance relative to Triton X-100 (Fig. 1C), we may assume that the reconstruction of the membranes during aging, as well as under the effect of nickel complexes, does not apply to the hydrophobic lipids and proteins. The nickel complexes also decrease the thermostability of the erythrocytes, a condition that also indicates that the water phase of the membranes is destroyed.

The changes observed in the properties of the membranes are reflected in the function of the erythrocytes. We find, for example, a decrease of O_2 release by the erythrocytes, which showed more distinctly in comparison to the control in the case of $(Ni(H_2O)6)^{2+}$ than in $(NiEn_2(H_2O)^{2+}$ and $(NiEn(H_2O)4)^{2+}$.

CONCLUSION

On the basis of results obtained, we may conclude that one pathway of toxic effects of nickel ions on living organisms consists in structural changes to cell membranes. In the case of erythrocytes in particular, these lead to a decrease in cell resistance. These structural changes affect all the components of the membranes, with the exception of the hydrophobic proteins and lipids. In neutral medium, water channel closure takes place. Structural changes occurring in the membranes of erythrocytes affect their physiological function. The harmful effect of nickel varies, depending on the type of ligand to which it is bound. Specifi-

cally, ethylendiamine decreases the toxic effect of nickel to a considerable degree.

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