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Disturbance of Cellular Iron Homeostasis by Soluble Nickel

Abstract

Nickel compounds have been identified as human carcinogens/cocarcinogens using both *in vivo* studies and epidemiological evaluations. Exposure of cells to soluble nickel turns on the hypoxic reponse pathway by stabilizing hypoxia inducible factor-1α (HIF-1α). The stability of the HIF-1α protein is directly controlled by a family of prolyl hydroxylases, whose activity is dependent on iron, ascorbic acid, and 2-oxoglutarate. Here, we study the ability of nickel to affect iron homeostasis by competing with iron for entry into the cell and inhibiting the activity of iron dependent enzymes. Using a human cell line (HEK) with a tetracycline inducible expression vector of the divalent metal ion transporter-1 (DMT1), we show that nickel is transported into the cell via DMT1. In addition, we demonstrate that soluble nickel can compete with iron at DMT1 for entry into the cell. Levels of total iron were then analyzed using graphite furnace atomic absorption after exposure to soluble nickel. Exposure to soluble nickel decreased total iron levels in a dose and time dependent manner. Since total iron levels were lower, we expected that the activity of iron dependent enzymes would also be affected. The enzyme activity of aconitase, catalase and prolyl hydroxylase were all decreased by exposure to soluble nickel. Iron dependent processes are a requirement for life and interference with these processes may be involved in nickel-induced carcinogenesis. These data may give new insight into the mechanisms of nickel induced carcinogenesis, as well as, contribute important information for the treatment and prevention of occupational diseases.

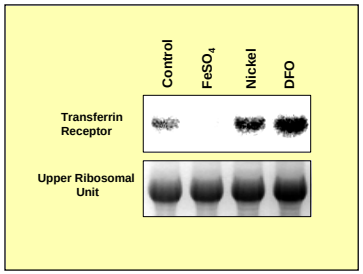

Figure 1. Effect of Nickel on Transferrin Receptor Expression. A549 cells were treated with 500μM $FeSO_4$, 1mM $NiCl_2$, or 200μM DFO for 24 hours. RNA (15μg) was hybridized to a probe for the human transferrin receptor.

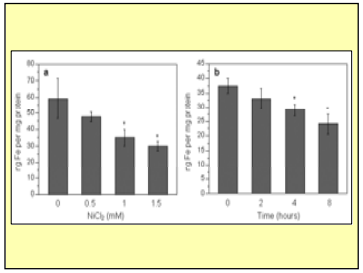

Figure 2. Nickel treatment decreases total cellular iron levels. A549 cells were exposed to nickel chloride at various doses and time points. Cells were collected in ice cold PBS and subjected to graphite furnace atomic absorption. Total cellular iron levels were expressed in nanograms of iron per milligram of protein.

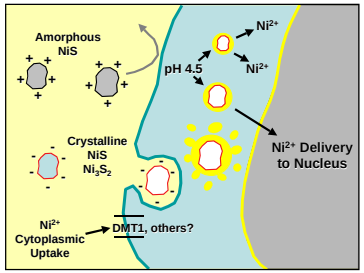

Figure 3. Model of cellular nickel uptake.

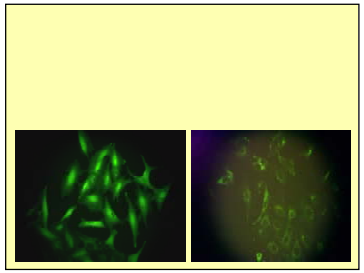

Figure 4 Intracellular Distribution of Nickel in A549 Cells after Nickel Exposure. (A). Structure of Newport Green DCF. (B). A549 cells exposed to nickel sulfide particles (10ug/cm²) for 72 hours. (C). A549 cells exposed to nickel chloride (1mM) for 24 hours.

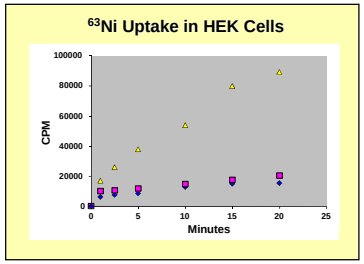

Figure 5. Uptake of nickel chloride into wild type HEK cells (blue squares), HEK cells containing an inducible vector containing DMT1 (non-induced) (pink squares), and HEK cells containing an inducible vector containing DMT1 (induced with tetracycline) (yellow triangles).

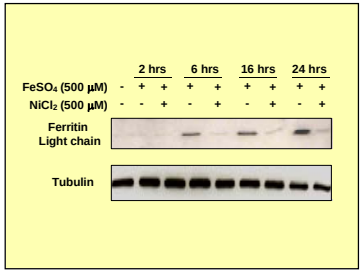

Figure 6. Effect of nickel on iron(II) inducible expression of ferritin. A549 cells were exposed to either 500μM $FeSO_4$, or 500μM $FeSO_4$ plus 500μM $NiCl_2$ at various time points as indicated.

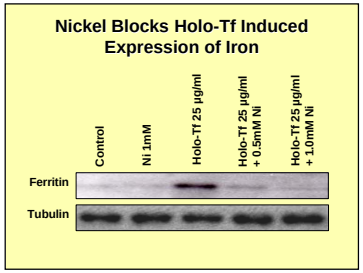

Figure 7. Effect of nickel on Holo-tranferrin induced expression of ferritin.

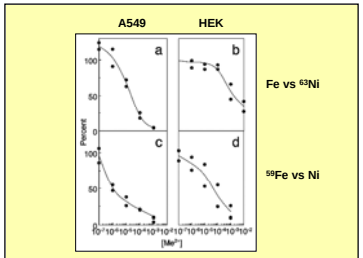

Figure 8. Nickel Competes with Iron for Entry into Cells at DMT1. (a, c) HEK293 cells overexpressing a doxycycline induced rat DMT1 construct or (b, d) A549 cells expressing endogenous transporters were incubated with (a,b) $^{59}FeSO_4$ and selected concentrations of $NiCl_2$ or (c, d) $^{63}NiCl_2$ and selected concentrations of $FeSO_4$. Incorporation into cells is shown on the ordinate as percent of control incubations with (a,b) no $NiCl_2$ or (c, d) no $FeSO_4$; while the molar concentration of competing divalent metal ion ($[Me^{2+}]$) is plotted logarithmically on the abcissa with (a, b) = $[Ni^{2+}]$ and (c, d) = $[Fe^{2+}]$.

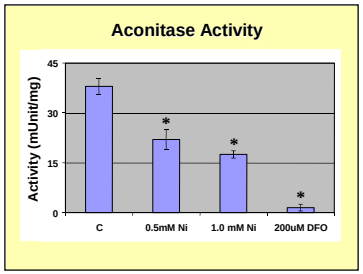

Figure 9. Nickel treatment decrease cellular aconitase activity in A549 cells. A549 cells were treated with 0.5 $NiCl_2$, 1mM $NiCl_2$ or 200μM DFO for 24 hours. Aconitase activity is expressed as activity per mg of protein.

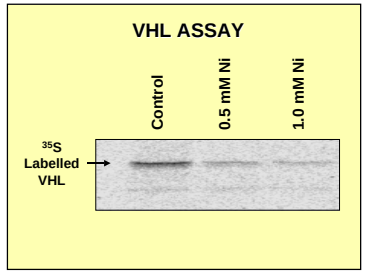

Figure 10. Nickel inhibits protein hydroxylation of HIF-1α. This assay measures the binding of VHL to the ODD domain of HIF-1α.

Highlights

- **Total cellular iron levels were lower in A549 cells after exposure to soluble nickel.**
- **Soluble nickel can enter the cell via the DMT1 transporter, but does not enter the cell nucleus after 24 hours of exposure.**
- **Nickel competes with iron for entry into the cell.**
- **Exposure of A549 cells to soluble nickel results in the inhibition of iron dependent enzymes.**

Impact

- **This project will provide valuable information on the mechanisms of nickel induced toxicity and carcinogenesis.**
- **May lead to preventative strategies for protecting workers in nickel related industries.**

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