Preventive Effects of Nickel on Cadmium Hepatotoxicity and Nephrotoxicity*

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ABSTRACT

The effect of nickel on cadmium nephro-toxicity and hepatotoxicity in rats was investigated. The administration of nickel (6 mg per kg, i.p., three days) or cadmium (6 mg per kg, i.m., once) significantly enhanced the urinary excretion of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), amino acids, and proteins. In addition, it increased the activity of serum ALP, GOT, and glutamate pyruvate transaminase (GPT). These biochemical alterations in urine and serum were used as a measure of kidney and liver damage. Cadmium-induced enzymuria, proteinuria, amino aciduria and increase in the activity of serum enzymes were significantly less marked in animals pretreated with nickel than in controls. However, the accumulation of cadmium in kidneys and liver and its urinary excretion were unaffected by nickel pretreatment. The results suggest protection by nickel against cadmium nephro- and hepatotoxicity.

Introduction

Pretreatment of rats with low dosage of cadmium, mercury, lead, manganese or silver may be protected against their own acute toxicity; moreover, some of them may develop cross tolerance. Pretreatment with a sub-lethal dose of cadmium has been considered to be at least partly responsible for the protection against acute toxicity of mercuric chloride and cadmium chloride. The protection by pretreatment with a sub-lethal dose of cadmium against subsequent challenge with a normally lethal dose of the metal may be correlated with the induced amount of hepatic metallothionein. The prevention of cadmium toxicity by pretreatment with zinc has also been explained on the basis of induction of cadmium thionein.

Nickel has been shown, although to a lesser extent as compared to cadmium, mercury, and zinc, to induce hepatic and renal metallothionein-like proteins.
However, pretreatment with a small dose of nickel was not antidotal to a subsequent lethal dose of the metal. This suggests that nickel induced metallothionein like proteins do not play any significant role against acute nickel intoxication. It was, therefore, considered of interest to investigate whether or not pretreatment by nickel offered protection against acute toxicity of other heavy metals and, if so, the extent of protection. Thus, the effect of nickel pretreatment on hepato- and nephrotoxicity of cadmium in rats was investigated. The urinary excretion of enzymes, proteins, amino acids, and metals, the activity of serum enzymes, and the tissue levels of metals were used for the evaluation of damage and protection of kidneys and liver.

Materials and Methods

Forty eight female albino rats, weighing 180 ± 10 g, of ITRC colony, maintained on an ad libitum pellet diet,* were divided equally into two groups. The animals of group I were administered 6 mg per kg of Ni as NiSO₄ · 6H₂O dissolved in normal saline (4 ml per kg), intraperitoneally, daily for three days; those animals of group II received an equal volume of normal saline. Ten animals from each group were kept in cages with a provision to avoid faecal contamination (two per cage) for a 24 hour urine collection in ice cold tubes, consecutively for four days. The urine was centrifuged at 3000 × g for 10 min, and the supernatant dialysed against ice cold distilled water for three hours to remove enzyme inhibitors. Six animals from each group were sacrificed by decapitation on the fourth and the seventh day after the first injection of nickel. The kidneys and liver were removed and blood was obtained from the heart.

The remaining animals in group I and II were injected with a single dose of 6 mg per kg of Cd as CdCl₂ · H₂O dissolved in normal saline (4 ml per kg), intramuscularly seven days after the first injection of NiSO₄ · 6H₂O. Twenty-four hour urine collections were obtained for four days. Six animals from each group were decapitated on the fourth and seventh day after the Cd injection. The kidneys and liver were removed and blood was obtained from the heart.

Enzyme Assay

Determinations of enzyme activity were made at the predetermined pH optima and in the proportional range of activity with respect to the amount of enzyme and the period of incubation.

1. Alkaline phosphatase (ALP, E.C. 3.1.3.1): The enzyme activity was determined in serum and urine by the method of Wright et al²² by measuring p-nitrophenol liberation at 400 nm.

2. Glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), Glutamate pyruvate transaminase (GPT, E.C. 2.6.1.2): The activity of GOT in serum and urine and the activity of GPT in serum were determined following the method of Reitman and Frankel¹⁶ by measuring their respective hydrazones at 510 nm.

3. Lactate dehydrogenase (LDH, E.C. 1.1.1.27): The method of Leathwood et al⁸ was followed and the enzyme activity determined in urine by measuring the rate of oxidation of NADH at 340 nm.

Estimation of Total Proteins

The total proteins in urine were estimated by the method of Piscator and Pettersson.¹⁵ The proteins were precipitated by Tsuchiya's reagent (a mixture of phosphotungstic acid, hydrochloric acid and
Estimation of Amino Acids

The amino acids in urine were estimated by the method of Mack. The urine was deproteinized with ethanol-acetone and the free amino acids in supernatant reacted with ninhydrin. The aldehyde absorbance was read at 580 nm.

Estimation of Metals

The urinary, renal, and hepatic samples were wet ashed with acid mixture (HNO₃:H₂SO₄:HClO₄ 6:1:1). The resulting carbon free residue was dissolved in five ml of five percent HNO₃ and read at 232.0 nm for Ni and at 228.8 nm for Cd on a flame Atomic Absorption Spectrometer* using high intensity hollow

* Perkin Elmer Model 5000.
cathode lamps. The suitable standards were processed simultaneously.

Results

The administration of nickel enhanced urinary excretion of ALP, LDH, GOT, total proteins, and amino acids (figure 1) and increased the activities of serum ALP, GOT, and GPT on the fourth day (figure 2) significantly, indicating kidney and liver damage. The urinary excretion of enzymes tend to return to the normal range of value after the fourth day and the serum levels of enzymes returned to the normal range by the seventh day, suggesting regeneration of renal and hepatic tissues.

The level of nickel in kidneys and liver increased significantly on the fourth day after commencement of nickel treatment. While the renal nickel decreased, the hepatic nickel increased further by the seventh day (figure 3). The administration of cadmium seven days after the first injection of nickel caused a significantly less marked increase in the urinary excretion of ALP, LDH, GOT, total proteins, and amino acids in rats pretreated with nickel than
in animals pretreated with saline (figures 4 and 5). However, the urinary excretion of cadmium was practically unaffected by pretreatment with nickel (figure 5). Cadmium also caused an elevation of serum ALP, GOT, and GPT which was significantly less marked in rats pretreated with nickel than in controls (figure 6). The accumulation of cadmium in kidneys and liver was high in animals pretreated with both nickel and saline; however, no significant difference in the tissue cadmium levels could be observed between the two groups either at the fourth or seventh day after administration of cadmium (figure 7).

Discussion

The early signs of cadmium nephropathy are enzymuria and proteinuria, which precede functional disturbances.2,11 The increase in the levels of certain serum enzymes is an early indication of cadmium hepatotoxicity.5 Cadmium is a known inducer of renal and hepatic metallothionein in various species.1,7,21 Since all the cadmium does not bind to metallothionein, the toxicity is apparently due to binding of the cation at the functional sites.20

Pretreatment with nickel significantly reduced cadmium induced enzymuria, proteinuria, aminoaciduria, and the increased levels of serum enzymes showing protection by nickel against cadmium nephro- and hepatotoxicity.

Since kidney and liver levels of cadmium and its urinary excretion were not significantly different in experimental and control groups, the protective effect of nickel is apparently neither related to renal or hepatic accumulation of cadmium nor its elimination from the body. This suggests either the nickel induced tissue metallothionein-like proteins did not last
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Figure 7. Effect of the uptake of Cd in tissues of rats pretreated with Ni. Each bar represents mean ± S.E. of 6 values. *p < 0.001, compared to normal control (horizontal line).

until the seventh day when cadmium was administered or they had no affinity for cadmium. If nickel induced metalloproteins were cadmium binding and were still present in the tissues at the time of cadmium administration, the hepatic and renal levels of cadmium could be expected to be higher in animals pretreated with nickel than in ones pretreated with saline. A similar explanation could be offered for the failure of nickel pretreatment to protect against the lethal effect of nickel in mice. Furthermore, the induction of metallothionein alone could not explain the protection against mercury intoxication as male rats pretreated with cadmium have been shown to be protected against only the lowest dose of mercury, while the uptake of mercury by the kidneys at three dosage levels was essentially the same.

Since the hepatic and renal levels of nickel were still high on the seventh day after nickel injection and their levels of cadmium were significantly higher at the fourth and seventh days of its administration, it appears that nickel binds to vital functional sites of tissues and thus shields them from the subsequent action of cadmium. Alternatively, after initial damage by nickel, the regenerating tissue cells probably undergo a maturation process such as in neonatal animals and possess a protective mechanism against cadmium toxicity.

References

13. Piotrowski, J. K. and Syzmaska, J. A.: Influence of certain metals on the level of metallothionein-like proteins in the liver and kidneys of...


