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# Effect of Pre-Exposure to Cadmium and Silver on Nickel Induced Toxic Manifestations in Mice: Possible Role of Ceruloplasmin and Metallothionein

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Recent studies from our laboratory and elsewhere have provided strong evidence for the involvement of reactive oxygen species (ROS) in nickel (Ni) toxicity (Athar et al. 1987; Srivastava et al. 1990; Xianglin et al. 1992; Huang et al. 1993; Misra et al. 1993). The toxic manifestations of Ni are further evident from animal studies where it caused significant depletion in serum ceruloplasmin (CP) activity (Misra et al. 1988). CP has been implicated in a variety of crucially important cellular functions including its role in the protection against oxidative damage and as a carrier for the transport of essential metals (Ashby et al. 1980; Sugawara and Sugawara 1984; Lovstad 1987; Samokyszyn et al. 1989; Halliwel and Gutteridge 1990). Since there is a lack of information about its role in Ni toxicity, an attempt was made to evaluate its implication(s) during Ni exposure. Administration of cadmium chloride ($CdCl_2$) or silver nitrate ($AgNO_3$) in mice respectively enhance or deplete the activity of serum CP (Sugawara and Sugawara 1984). Besides, both the salts induce metallothioneins (MT), which also serve as an efficient scavenger of ROS (Ashby et al. 1980; Sugawara and Sugawara 1984; Thomas et al. 1986). We therefore, studied the effect of Cd and Ag under various predisposing conditions on the protection against Ni-mediated toxic manifestations by evaluating (i) hepatic lipid peroxidation (ii) Ni content in target tissues and (iii) mortality in mice. Our results demonstrate that Cd pretreatment(s) provided significant protection against Ni-mediated toxic responses and reflect the possible interplay of the induced levels of CP and MT.

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## MATERIALS AND METHODS

All chemicals used in the experiment were either analytical reagent or of highest purity grade. Nickel(II) chloride hexahydrate ($NiCl_2.6H_2O$) was purchased from Fluka (Buchs, Switzerland). Thiobarbituric acid (TBA) and $CdCl_2$ were purchased from Sigma Chemical Co. (St. Louis, USA), and $AgNO_3$ from E. Merck India Ltd. (Bombay). The experimental animals were 8 wk old female albino mice from the Industrial Toxicology Research Centre, breeding colony, weighing 30±2g. They were housed in an air conditioned room and had free access to pellet diet (Lipton India Ltd. Bombay) and water. The studies were conducted under three different experimental conditions. Low doses of Cd or Ag were selected which would only alter the levels of CP and MT without producing any enhancement in lipid peroxidation or mortality.

In the first experiment, animals were divided into three groups consisting of 36 mice in each. The first two groups received two sub-cutaneous (sc) injections of $CdCl_2$ or $AgNO_3$ (0.75 mg/kg/10ml as Cd or Ag in water) at a gap of 24 hrs, while the third group received same volume of normal saline. All the treated animals were equally divided into two subgroups. The first three subgroups, 48 hrs later received a single intraperitoneal (ip) injection of $NiCl_2$ (11.8 mg/kg/10 ml as Ni in water) while the animals of the remaining three sub groups received only normal saline.

In the second experiment, a similar protocol was followed except that animals received single injection of $CdCl_2$ or $AgNO_3$ (1.5 mg/kg/10ml as Cd or Ag). The treatment and dose schedule of Ni was same as in experiment 1.

In the third experiment animals were divided into four groups consisting of 18 mice in each. The first three groups received single ip injection of Ni (11.8 mg/kg/10 ml in water) followed by single sc administration of $CdCl_2$ or $AgNO_3$ (1.5mg/kg/10 ml as Cd or Ag) or saline. The fourth group received only normal saline and served as control.

Twelve mice of all treated groups from all the three experiments were observed for survival upto d 14, while six were sacrificed at 24 hrs after Ni or saline treatment for biochemical assays and Ni estimation. Blood was obtained by cardiac puncture and serum was separated and used for CP analysis (Schosinsky et al. 1974). Liver, kidneys, heart, lung and spleen were removed immediately, washed free of extraneous material with cold normal saline and subjected to acid digestion for Ni-estimation (Misra et al. 1988) on a Perkin-Elmer

5000 Atomic Absorption Spectrophotometer (Norwalk, Conn. USA). A portion of liver was also homogenised (10% w/v) in cold KCl (154 mM) and subjected to fractionation to obtain cytosol (Witschi et al. 1968). Lipid peroxidation was measured in whole liver homogenate (10% w/v) in terms of TBA-reacting species as described by Sunderman Jr. et al. (1985) and expressed as n moles malondialdehyde (MDA) equivalent/g wet tissue. MT concentration was determined by Cd-haem method (Onosaka and Cherian 1982). The analysis of significance of difference between the groups was performed by means of student's `t' test (Fisher 1954).

## RESULTS AND DISCUSSION

The activity of CP in serum, hepatic MT content and lipid peroxidation after Cd, Ag or Ni treatment is shown in Table 1. Administration of two doses of Cd (0.75 mg/kg) produced significant enhancement in the activity of serum CP and hepatic MT content compared to single administration of Cd (1.5 mg/kg). Ag pretreatment in contrast significantly reduced the activity of serum CP whereas it caused marked induction in MT content. Cd or Ag treatment however, did not produce any alterations in hepatic lipid peroxidation. Effect of Ni alone on these parameters was also studied to evaluate the possible protective role of Cd or Ag pretreatments. Ni administration significantly enhanced hepatic lipid peroxidation, lowered the activity of serum CP, and did not result in any alterations in hepatic MT content.

When Ni was administered to either Cd or Ag pretreated mice, there was a significant inhibition in the Ni-induced hepatic lipid peroxidation. The protective effect of these metals on the lipid peroxidation could not be observed in our experiment when they were administered after Ni treatment. Our results further reveal that in Cd or Ag pretreated mice Ni treatment did not produce any significant change in CP activity. Such an effect was not observed when these two metals were administered after Ni treatment hence data are not represented.

The results of *in vitro* experiment demonstrate that addition of cytosol or serum (obtained from Cd pretreated mice) to the whole liver homogenate of Ni-treated mice significantly inhibited lipid peroxidation to the extent of 48% and 23% respectively (Fig.1). Addition of cytosol from Ag treated mice also inhibited (34%) hepatic lipid peroxidation.

The results presented in Fig. 2 exhibit alterations in the tissue Ni content when it was administered to Cd

Table 1. Effect of Cd or Ag pre-exposure in mice on Ni-mediated alterations in serum CP activity, hepatic MT content and hepatic lipid peroxidation (LP)

| Treatments | Serum CP (U/L) | | Hepatic MT (n moles/g wet tissue) | | Hepatic LP (n moles MDA/g wet tissue) | |
|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
| Sal. | 25.1±1.2 | 25.1±1.2 | 0.5±0.1 | 0.5±0.1 | 95±3 | 95±3 |
| Sal-Cd | 47.7±1.9* | 36.9±1.6* | 15.8±2.0* | 11.2±1.2* | 90±6 | 110±5 |
| Sal-Ag | 13.1±1.1* | 18.6±0.9* | 10.6±1.4* | 7.8±0.9* | 105±5 | 128±6 |
| Sal-Ni | 17.7±1.1* | 17.7±1.1* | 0.7±0.1 | 0.7±0.1 | 495±15* | 495±15* |
| Cd-Ni | 44.3±2.1* | 33.1±1.4* | 11.8±1.8* | 8.8±1.1* | 206±12* | 285±11* |
| Ag-Ni | 12.2±1.3* | 16.9±1.0* | 7.7±1.0* | 4.5±0.7* | 301±13* | 369±15* |

Experiments (Exp.) are described in detail under "Materials and Methods". Each value represents mean ± SE of six mice
* $p < 0.05$ when compared to sal.

pretreated mice. The alterations in tissue Ni content were more pronounced in the group which were pre-exposed with two doses of Cd. The effect of Cd on the alterations in tissue Ni content could not be observed when it was administered after Ni treatment. Ag pretreatment did not produce notable alterations in tissue Ni content hence data are not shown.

Cd pretreatment also resulted in greater protection against Ni-induced mortality in mice compared to Ag (Table 2). The protective effect of these metals could not be observed when they were given after Ni treatment The significant protection by Cd or Ag pretreatments in Ni-induced toxic responses viz. lipid peroxidation and mortality in mice and decrease in tissue Ni content observed only in Cd pretreated mice reflect the possible interplay of the induced level of MT and CP or both. The absence of protective effects of Cd or Ag upon their administration after Ni treatment further confirm the involvement of Cd or Ag induced antioxidants.

Our results further suggest that MT may be playing a major role in the protection against Ni-induced lipid peroxidation since this inhibition was proportional to the level of MT. The significant inhibition in hepatic lipid peroxidation by Ag pretreatment, inspite of the

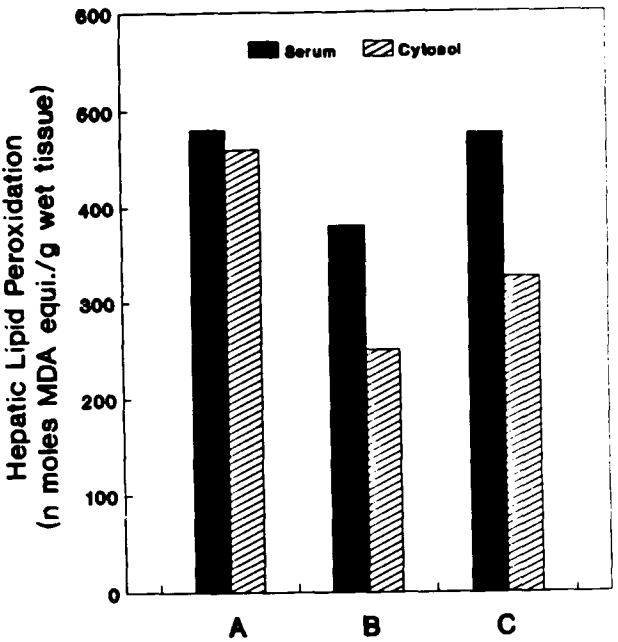


Figure 1. The effect of in vitro addition of serum or hepatic cytosol from (A) saline (B) Cd or (C) Ag treated mice to the liver homogenate of Ni treated mice on the alterations in Ni-induced lipid peroxidation

Serum (1.0ml)or hepatic cytosol (1.0 ml of 10% homogenate, w/v) obtained from saline (control), Cd or Ag treated mice (from Expt.1) was mixed with the whole liver homogenate (1.0ml, 10% w/v) of only Ni treated mice. Lipid peroxidation was measured as described under Materials and Methods. Broken line represents normal control values. Solid line represents value of Ni treatment alone. The results are the means of three experiments in which the values deferred by less than ± 1%.

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reduced level of CP further support the role of MT in inhibiting peroxidative damage. This contention is in conformation with our in vitro observations.

In our previous communication it was shown that hydroxyl radicals are the proximal lipid oxidants during Ni toxicity (Athar et al. 1987). Metallothioneins have earlier been demonstrated as an efficient interceptor of hydroxyl radical and superoxide anion. These data thus suggest that the decrease in lipid peroxidation is largely due to the ability of MT to scavenge ROS generation by Ni treatment. Although CP has been shown to scavenge ROS but its efficiency is manifold less than superoxide dismutase. The reduced efficacy of CP in inhibiting lipid peroxidation could

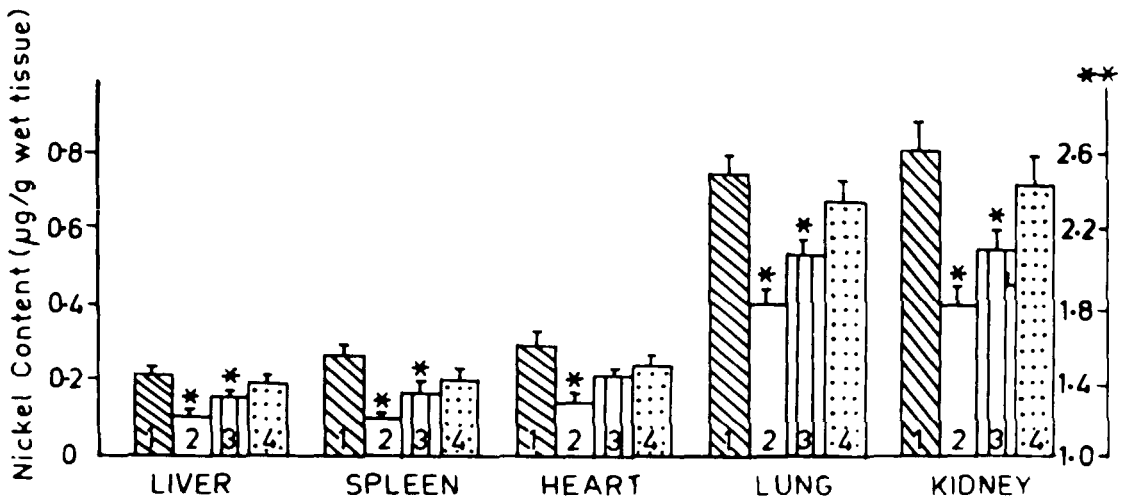


**Figure 2. Effect of Cd treatments on the alterations in tissue Ni level from Ni-exposed mice.**

Ni (11.8 mg/kg) was administered in mice 48 h after (1) saline (2) two dose of Cd (2x0.75 mg/kg) or (3) single dose of Cd (1.5 mg/kg) treatment and (4) Cd (1.5 mg/kg) administered immediately after Ni treatment. Details as under Materials and Methods.

* $p < 0.05$ when compared to Ni-Sal group

** Scale on the right axis represents Ni content in kidney only

Table 2. Effect of Cd or Ag exposure on the protection against Ni-mediated mortality in mice

| Treatment | Percent cumulative survival upto day 14 | | |
|---|---|---|---|
| | Experiment 1 | Experiment 2 | Experiment 3 |
| Saline | 100 | 100 | 100 |
| Sal-Ni | 60 | 60 | 60 |
| Cd-Ni | 92 | 83 | 67 |
| Ag-Ni | 80 | 76 | 56 |

*Details of experiments are described under "Materials and Methods".*

be due to its ineffectiveness in scavenging hydroxyl and other reactive oxygen radicals (Samokyszyn et al. 1989).

The alterations in tissue Ni contents observed only in Cd pretreated mice could be one of the manifestations of Cd- induced enhancement in the activity of CP rather than MT. This is further evident from the results of Ag pretreated mice where the tissue Ni content remained unaltered inspite of induced level of MT. This could be ascribed due to the lack of affinity of Ni to bind MT (Behari et al. 1984). The proportional decrease in tissue Ni contents with corresponding increase in the endogenous level of CP further demonstrates the role of CP in Ni homeostasis thereby suggesting the affinity of Ni to bind CP (McKee and Frieden 1971). The data of Cd pretreatment also reveal a correlation between lipid peroxidation and tissue Ni content. However, the absence of such correlation in Ag-pretreated group may be due to the lack of affinity of Ni towards MT.

The significant protection by Cd pretreatment(s) in reversing Ni-mediated toxic response could thus be attributed to the combined effects of both CP and MT. Considering oxidative stress as an adequate cause of damage in Ni toxicity (Athar et al.1987, Dutta et al. 1992, Kasprzak et al. 1992), our results thus suggest the need for the evaluation of nutritional sources that may serve as inducers of both CP and or MT in the amelioration of Ni toxicity.

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