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Enzymatic Antioxidants in Erythrocytes Following Heavy Metal Exposure: Possible Role in Early Diagnosis of Poisoning

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Occupational and environmental exposure to heavy metals such as cadmium, mercury, nickel, and lead is known to cause health hazards due to their toxic action on the biological system. Metals have the potential to cause oxidative damage to various tissues, including erythrocytes (Sugiyama 1994) . Since erythrocytes are likely to be the primary target site for metal–induced damage, they may be useful as an early diagnostic tool. Erythrocytes are equipped with a variety of biochemical mechanisms operating against cellular damage. One such line of defense is provided by the enzymatic and non–enzymatic antioxidant system which helps to detoxify highly reactive species such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals that are being generated during oxidative stress (Gelman et al. 1978). Therefore, in the present study cadmium (Cd), chromium (Cr), nickel (Ni), mercury (Hg), lead (Pb), manganese (Mn), and zinc (Zn) were chosen to make a comparative evaluation of the metal–induced alterations in antioxidative enzymes of RBC's. Specifically, superoxide dismutase, catalase, and glutathione reductase activity in RBC was assessed following exposure to metals at 5, 15, and 30 ppm in drinking water for 30 days . We also determined if these RBC parameters are exclusively sensitive for any metal so that they can be used for early diagnosis and toxicity evaluation purposes.

MATERIALS AND METHODS

Nitroblue tetrazolium, oxidized glutathione, flavin adinine dinucleotide (FAD) and ß–NADPH were purchased from Sigma Chemical Company (St. Louis, MO, USA) and pyrogallol was obtained from J.T. Baker Chemical Company (Phillipsburg, NJ, USA). All other reagents used in the present study were of analytical grade.

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Male albino rats of Druckrey strain weighing in the range of 136-144 g were obtained from the central breeding facility of the Industrial Toxicology Research Centre, Lucknow, India. Twenty seven groups of animals having twelve rats per group were housed in stainless steel cages in an air-conditioned room maintained on a 12-hr light/dark illumination cycle. Rats were given a pelleted diet (Lipton Laboratory Feeds, India) ad libitum. Animals were exposed to 5, 15, and 30 ppm of a metal, viz Zn [$ZnCl_2Ž6H_2O_2$], Cr [$K_2Cr_2O_7$], Cd [$Cd(CH_3COO)_2$], Mn [$MnCl_2•6H_20$], Hg [$HgCl_2$], Ni [$NiCl_2$] or Pb [$Pb(CH_3COO)_2$], in their drinking water for 30 days. Control groups were given equivalent doses of NaCl or CH_3COONa. Body weight and water consumption were recorded on alternate days. Twelve rats from each group were selected randomly after 30 days of treatment and sacrificed by cervical dislocation. Heparinized blood collected through cardiac puncture from 2 rats was pooled to make six samples per group. Blood was centrifuged at 2,000g for 15 min at 4°C. RBC's were washed thrice with 5 volumes of chilled isotonic saline, lysed in 19 volumes of chilled distilled water and kept frozen. Samples were thawed, centrifuged at 5,000g for 10 min at 4°C and the hemolysates were used for enzyme assays. Hemoglobin was removed (McCord and Fridovich 1969) from a portion of hemolysate for the assay of superoxide dismutase [EC 1.15.1.1]. The enzyme was assayed according to the procedure of Shukla (1987), using pyrogallol as substrate and the activity was expressed as units/min/mL RBC. One unit is defined as the amount of enzyme required for a 50% inhibition in the pyrogallol auto-oxidation. Catalase [EC 1.11.1.6] was assayed spectrophotometrically and the activity was expressed as mmol of H_2O_2 utilized/min/mL RBC (Aebi 1983). Glutathione reductase [EC 1.6.4.2] was assayed using FAD as a cofactor (Beutler 1969). The activity was expressed as pmol NADPH oxidized/min/mL RBC. The significance of difference between control and a metal-treated group was evaluated by a Student's t-test at $p<0.05$.

RESULTS AND DISCUSSION

There was no significant change in body weight, water consumption (22±8 ml/day), RBC count, RBC protein level (Lowry et al. 1951) and visible behavior of metal-treated animals. The activities of selected RBC enzymes in NaCl and CH_3COONa controls were similar. The control values presented in Figures 1 to 3 are from NaCl treated controls.

Superoxide dismutase (SOD) activity was significantly inhibited (15%, $p<0.05$) at a 5 ppm Cd (Fig. 1). We have reported SOD inhibition in RBC and certain organs earlier also, following intraperitoneal administration

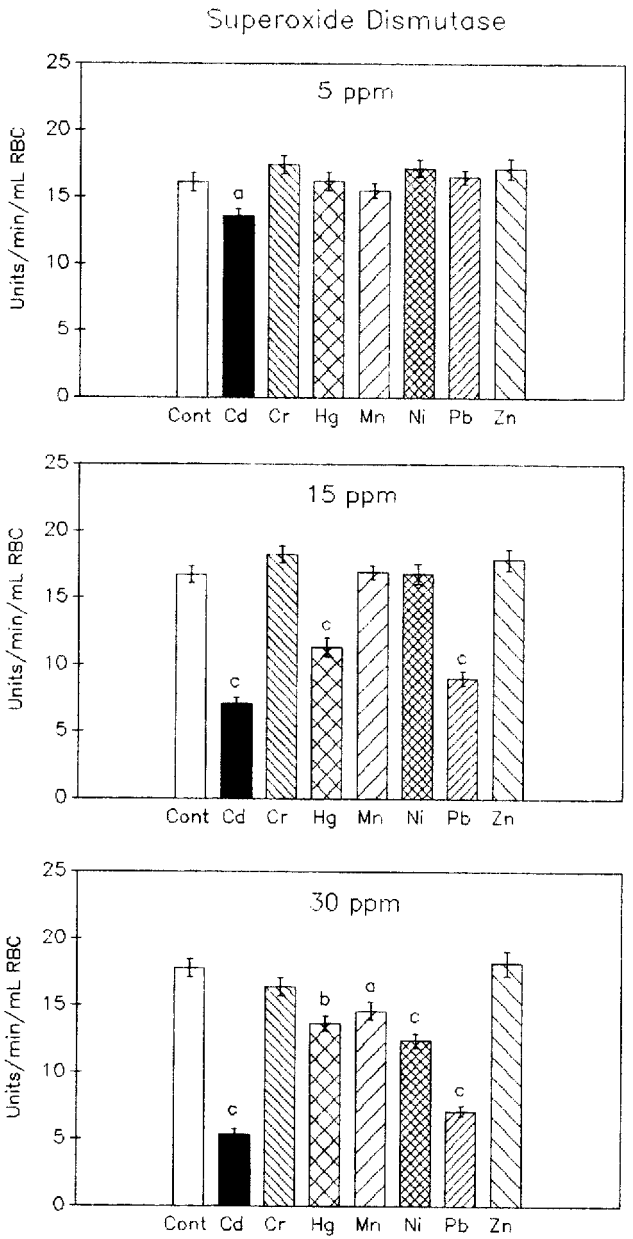

Figure 1. Superoxide dismutase activity following exposure to different concentrations of selected metals in drinking water. Control (Cont) values are derived from sodium chloride treated animals. Each bar represents the mean ±SE of 6 samples (derived from 12 rats) per group. The groups significantly different from controls are marked, [a]p<0.05; [b]p<0.0l, [c]p<0.001.

of Cd (0.4 mg/kg/day) for 30 days (Shukla et al. 1988). The inhibition of SOD at 5 ppm indicates a very high sensitivity of this enzyme towards Cd. Treatment with 15 ppm Cd, Pb, and Hg inhibited SOD activity markedly by 58% ($p<0.001$), 48% ($p<0.001$) and 31% ($p<O.001$), respectively. Exposure to 30 ppm metals in drinking water produced wide spread changes in SOD activity, which was decreased by Cd (70%,$p<0.001$), Pb (58%, $p<0.001$), Hg (22%,$p<0.01$), Ni (30%,$p<0.001$) and Mn (16%, $p<0.05$) compared to controls. High levels of Pb (Gelman et al. 1978) and Ni (Arsalane et al. 1992) have been reported to inhibit SOD activity in RBC and alveolar macrophages. SOD is a metalloenzyme, therefore, it is possible that the observed metal–induced SOD inhibition may be the result of an interaction of exposed metal with the metal(s) moiety of the enzyme. Cd has been reported to replace Zn in purified Cu,Zn–SOD and inhibit activity at very low concentrations (Bauer et al. 1980).

The effects of metals on the RBC catalase are shown in Fig. 2. At 5 ppm dose, catalase activity was only affected by Cd treatment, which produced an increase of 22% ($p<0.05$). The magnitude of increase was further enhanced to 40% ($p<0.01$.) in rats receiving 15 ppm of Cd. Furthermore, 15 ppm of Hg and Pb also increased the catalase activity by 85% ($p<0.001$) and 115% ($p<0.00l$), respectively. A stimulation of catalase activity by Pb has also been reported in the liver and brain of chicken embryos (Somashekaraiah et al. 1992). There is a report of increased catalase activity in house fly and cabbage looper moth following a low dose exposure to Hg (Zaman et al. 1994). At higher doses (30 ppm), Cd (62%,$p<0.001$), Pb (52%,$p<0.001$), Hg (34%,$p<0.01$), Ni (24%, $p<0.05$), and Cr (20%,$p<0.05$) produced an inhibitory effect on RBC catalase. It appears that the effects of certain metals on catalase are biphasic depending on the dose of exposure. We have reported earlier that a low dose of oral Cd (10 ppm) increased the activity of catalase in brain microvessels following 30 days of exposure; however, if the exposure continued for 90 days an inhibition was observed (Shukla et al. 1996). A relatively high Cd dose (0.4 mg/kg/ day, ip) to rats for 30 days has been reported to inhibit RBC catalase (Shukla et al. 1988).

Glutathione reductase (GR) was affected by Cd, Hg, and Pb; other metals did not produce significant changes (Fig. 3). Activity was decreased by 23% ($p<0.01$) and 25% ($p<0.0l$) in rats orally exposed to 5 ppm of Cd and Hg, respectively. A higher magnitude of inhibition was evident at 15 ppm Cd (31%,$p<0.01$), Pb (22%,$p<0.05$), and Hg 58% ($p<0.001$). The treatment with 30 ppm of Cd, Hg and Pb decreased the erythrocyte GR activity by 29% ($p<0.05$), 61% ($p<0.001$) and 48%

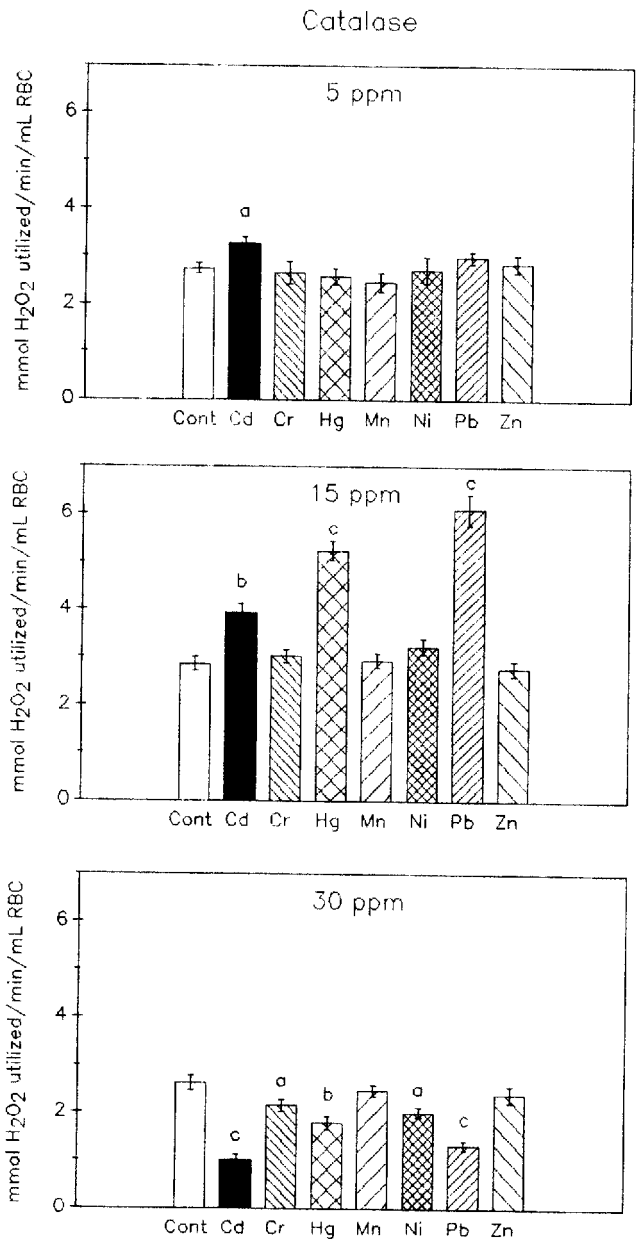

Figure 2. Catalase activity following exposure to different concentrations of selected metals in drinking water. Control (Cont) values are derived from sodium chloride treated animals. Each bar represents mean ±SE of 6 samples (derived from 12 rats) per group. The groups significantly different from controls are marked, [a]p<0.05; [b]p<0.01, [c]p<0.001.

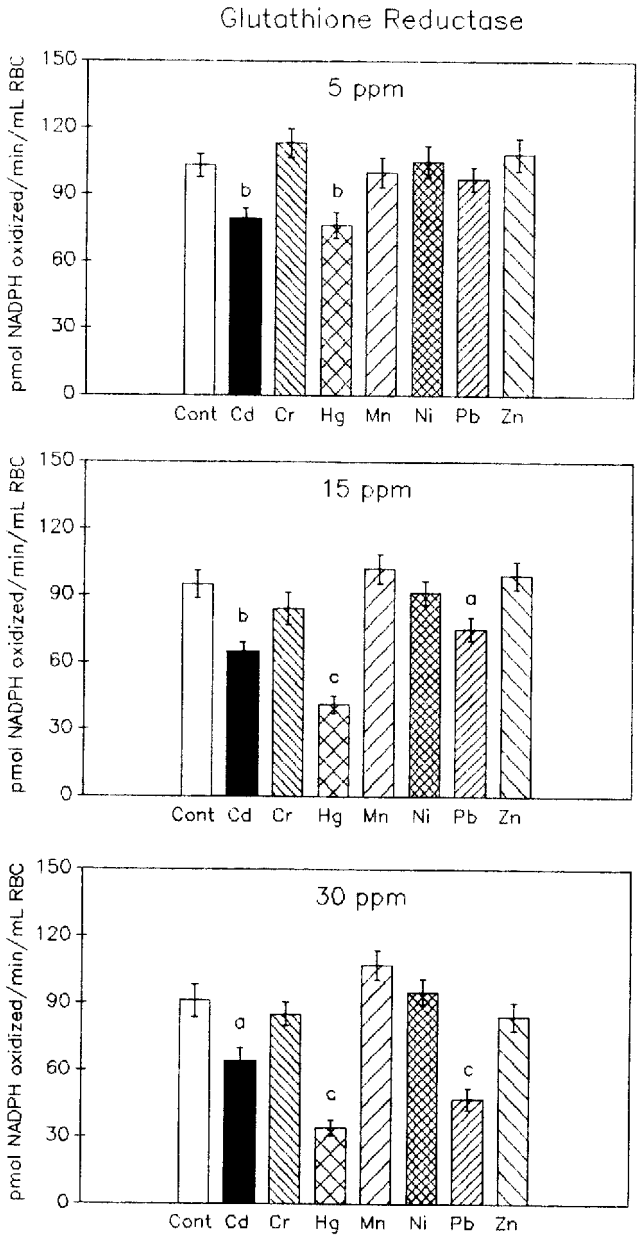

Figure 3. Glutathione reductase activity following exposure to different concentrations of selected metals in drinking water. Control (Cont) values are derived from sodium chloride treated animals. Each bar represents the mean ±SE of 6 samples (derived from 12 rats) per group. The groups significantly different from controls are marked, $^{a}p<0.05$; $^{b}p<0.01$, $^{c}p<0.001$.

(p<0.00l), respectively, as compared to the controls. It is possible that these heavy metals interact with sulfhydryl groups present at the active site of GR and produce inhibition in its activity. Our earlier studies have shown a very high sensitivity of GR towards Cd in vitro, inhibiting activity by 90% at a 10 µM concentration (Shukla et al. 1988a). The intraperitoneal administration of Cd (0.4 mg/kg/day) for 30 days was also found to inhibit this enzyme in RBC and certain organs in rats (Shukla et al. 1988, 1988a).

Although these RBC enzymes have been reported to change under certain pathological conditions, their use as early biomarkers of metal toxicity appear promising. Our results have shown that heavy metal exposure affects the RBC antioxidative enzymes and Cd, Hg, or Pb can produce changes at very low levels of exposure, indicating a high sensitivity of antioxidative enzymes towards these metals. Furthermore, Cd was found to be the most sensitive. The changes may not be transient in the case of Cd, as our earlier experiments have shown that the enzymatic alterations and Cd levels in RBC and certain soft tissues do not recover following withdrawal of the exposure for 15 days (Shukla et al. 1988, 1989). Our findings go well with the report of lower activities of SOD and catalase in erythrocytes from workers occupationally exposed to Pb (Sugawara et al. 1991). Recently, Perrin–Nadif et al. (1996) reported altered blood SOD and catalase in Hg–exposed workers and suggested these enzymes as biomarkers of oxidative stress. Although our investigation has shown dose–dependent changes in RBC antioxidant enzymes, further work correlating enzymatic changes with blood metal levels particularly in human subjects exposed to heavy metals is needed in order to validate the possible use of these parameters for an early diagnosis and toxicity evaluation.

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