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Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: A study with relation to time

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Cadmium induced lipid peroxidation (LPO) and the activity of antioxidant enzymes after the administration of a single dose of $CdCl_2$ (0.4 mg kg^{-1} body wt, ip) was studied in rat erythrocytes. Cd intoxication increased erythrocyte LPO along with a decrease in superoxide dismutase (SOD) up to three days of Cd treatment. The decrease in erythrocyte catalase (CAT) activity was marked within 9 h of Cd intoxication. After three days of Cd treatment, LPO decreased towards normal, along with an increase in erythrocyte SOC and CAT activity. Blood glutathione (GSH) decreased significantly within 24 h of Cd treatment, followed by an increase towards normal. Erythrocyte glutathione S-transferase (GST) activity increased up to 10 days of Cd intoxication, probably in an attempt to reduce Cd toxicity. Serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and serum bilirubin increased up to 10 days of Cd intoxication. Blood urea increased significantly up to three days, followed by a decrease towards normal. The results show that Cd induced LPO was associated with a decrease in antioxidant enzymes and GSH in erythrocytes; as these antioxidants increase in erythrocytes with recovery from Cd intoxication, the Cd induced LPO reversed towards normal. The increase in the SGPT, SALP and serum bilirubin correlated with LPO. The results suggest that Cd intoxication induces oxidative stress and alters the antioxidant system, resulting in oxidative damage to rat erythrocytes.

Keywords: catalase, glutathione, glutathione peroxidase, lipid peroxidation, superoxide dismutase

Introduction

Cadmium is a known toxicant to humans and living organisms (Webb 1979). The deleterious effects of Cd have been shown to be due to oxidative damage by enhancing the peroxidation of membrane lipids in different tissues and in erythrocytes (Sarkar *et al.* 1995, Bansal & Bhatnagar 1996). It has been suggested that heavy metal ions such as Hg^{2+}, Pb^{2+} and Cd^{2+} have a prooxidant catalytic activity and can initiate membrane peroxidation by generating free radicals and thereby interfering with the antioxidant system in tissues and erythrocytes (Sunderman 1986). Cd induced LPO in erythrocyte membranes may cause hemolysis (Bansal & Bhatnagar 1996); however, the mechanism responsible for Cd induced anemia is not precisely known. The treatment of erythrocytes with thiol reactive agent causes shortened *in vivo* survival and splenic sequestration as well as decreased deformability (Chiu *et al.* 1989). Ageing erythrocytes are less deformable and lose their discoidal shape and intracellular potassium (Kunimoto *et al.* 1985). It is probable that Cd induced changes in membrane peroxidation and antioxidant enzymes may cause age related changes in rat erythrocytes *in vivo*. The antioxidant enzymes

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such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) dismutate the free radicals and reduce H_2O_2 toxicity by its decomposition or by reduction of peroxides by glutathione (GSH) via GSH-Px. The antioxidants such as vitamin E, ascorbic acid and GSH protect the erythrocyte membrane from oxidative damage (Sarkar *et al.* 1997a,b). Cd may alter the antioxidant system in erythrocytes and render the erythrocytes more vulnerable to oxidative damage. Therefore, in the present study, we investigated the effect of Cd on LPO and the antioxidant system in erythrocytes in order to show that Cd at a nonhemolytic concentration induces oxidative stress, which may be a mechanism responsible for Cd toxicity in erythrocytes.

Materials and methods

Male Wistar rats weighing 150–180 g were housed in polypropylene cages. The animals were maintained ethically under standard conditions of the animal house and were allowed free access to drinking water and basal diet. $CdCl_2$ was dissolved in distilled water and injected (0.2 ml, ip) at a single dose of 0.4 mg kg^{-1} body wt) to overnight fasted rats. The control animals received a placebo injection with an equal volume of normal saline. The animals were sacrificed by decapitation at 3, 6, 9, 12 and 24 h, and 3, 7 and 10 days of Cd administration. The selection of time periods was such as to monitor the changes in erythrocyte LPO and antioxidant enzymes after Cd administration. Blood was collected by heart puncture in vials containing 2% sodium citrate. The erythrocytes were washed three times with 0.1 M phosphate buffered saline (PBS) (1:9), pH 7.4. The blood was centrifuged and the packed cell volume (PCV) was adjusted to 5% with PBS, pH 7.4.

Lipid peroxidation in erythrocytes

LPO was measured in erythrocytes as malondialdehyde (MDA) formed by thiobarbituric acid (TBA) reaction (Stocks & Dormandy 1971). Two ml of 5% PCV were exposed to 10 mM H_2O_2 and 2 mM sodium azide in 4 ml phosphate buffer (1 M), pH 7.4. The mixture was incubated at 37°C for 1 h, after which 2 ml TCA (28%) were added. A tissue blank was prepared for each sample without exposing the erythrocytes to H_2O_2. The cell suspension was centrifuged at 1000 *g* for 5 min. Four ml of supernatant were transferred to a boiling tube to which 1 ml of TBA (1%) was added. The contents were boiled for 15 min, cooled immediately, and the absorbance at 532 nm was measured in a spectrophotometer. LPO was expressed as nmoles of MDA formed mg^{-1} protein using a molar extinction coefficient for MDA of 1.56×10^5.

Glutathione content in erythrocytes

Glutathione content in erythrocytes was determined by the method of Beutlar *et al.* (1963) using Ellman's reagent.

Antioxidant enzymes in erythrocytes

The activities of SOD, CAT and GST were determined in erythrocyte lysate prepared by the method of McCord & Fridovich (1969). SOD activity was measured by the autooxidation of pyrogallol (Marklund & Marklund 1974). CAT activity was determined by the method of Aebi (1983) by the decomposition of H_2O_2. GST activity was determined on the basis of conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) (Habig *et al.* 1974). Hemoglobin was estimated according to the method of Dacie & Lewis (1984). Serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (SALP) were estimated as described by Wootton (1964), and blood urea and serum bilirubin were determined as described by Varley (1969). Protein was determined according to the method of Lowry *et al.* (1951). The statistical analysis was performed by Student's *t*-test, with values less than 5% considered as significant.

Results

The treatment with Cd increased LPO up to three days and thereafter followed a decrease (Figure 1). The LPO decreased to control levels after 10 days of a single dose of Cd administration.

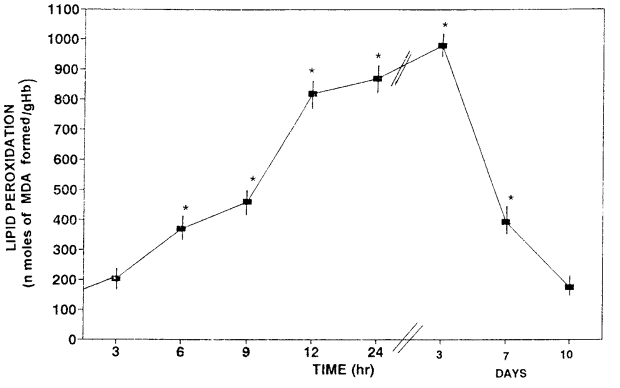

Figure 1. Effect of Cd on LPO in rat erythrocytes. The animals were treated with a single dose of $CdCl_2$ (0.4 mg kg^{-1} body wt, ip) and LPO in erythrocytes was measured in treated and control animals at 0, 3, 6, 9, 12 and 24 h, and 3, 7 and 10 days of Cd treatment. LPO in erythrocytes in control rats at 0 h was 185.5 ± 18.6 nmoles of MDA formed g^{-1} Hb. The different values over a period of 10 days in saline treated control rats were not significantly different as compared with 0 h. Values are mean ± SD of six and four animals in test and control groups, respectively. $^*P < 0.05$.

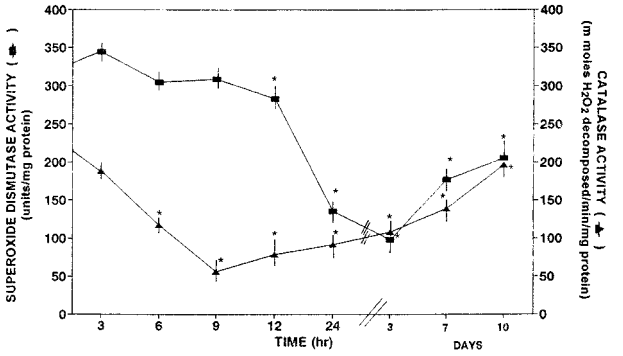

Figure 2. Effect of Cd on SOD and CAT activities in rat erythrocytes. SOD and CAT activities in erythrocytes were determined in test and control animals at 0, 3, 6, 9, 12 and 24 h, and 3, and 10 days of Cd treatment. SOD and CAT activities in erythrocytes in control animals were 351 ± 62 units mg^{-1} protein and 258 ± 51 nmoles of H_2O_2 decomposed min^{-1} mg^{-1} protein, respectively. The erythrocyte SOD and CAT activity at different time periods showed no significant difference in control animals as compared with 0 h. Values are mean ± SD of six and four animals in test and control groups, respectively. $^*P < 0.05$.

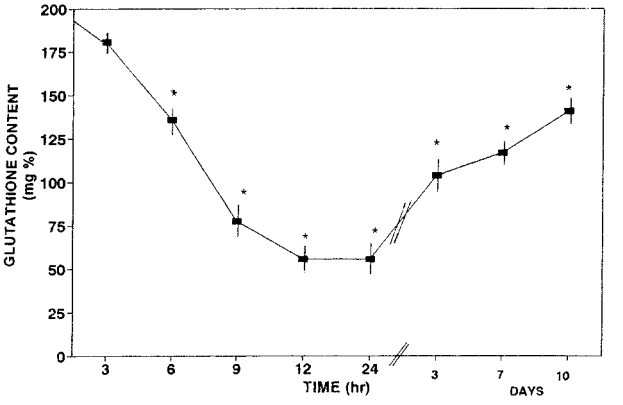

Figure 3. Effect of Cd on blood GSH. Blood GSH was determined in test and control animals at 0, 3, 6, 9, 12 and 24 h, and 3, 7 and 10 days of Cd treatment. Blood GSH in control rats at 0 h was 185 ± 42 mg% and the different values over a period of 10 days were not significantly different as compared with 0 h. Values are mean ± SD of six and four animals in test and control groups, respectively. $P < 0.05$.

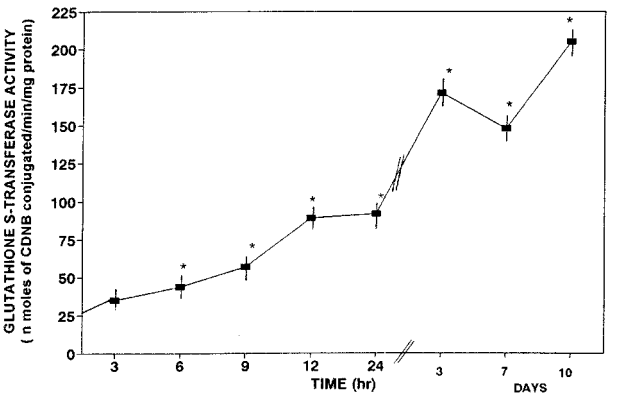

Figure 4. Effect of Cd on GST activity in rat erythrocytes. GST activity in erythrocytes was determined in test and control animals at 0, 3, 6, 9, 12 and 24 h, and 3, 7 and 10 days of Cd treatment. GST activity in erythrocytes in control animals was 25.5 ± 2.3 nmoles of CDNB conjugated min^{-1} mg^{-1} protein. The erythrocyte GST activity at different time periods showed no significant difference in control animals as compared with 0 h. Values are mean ± SD of six and four animals in test and control groups, respectively. $^*P < 0.05$.

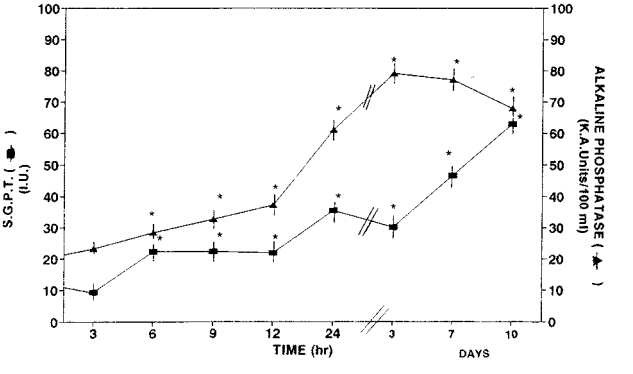

Figure 5. Effect of Cd on SGPT and SALP. SGPT and SALP activities were determined in test and control animals at 0, 3, 6, 9, 12 and 24 hr, and 3, 7 and 10 days of Cd treatment. SGPT and SALP activities in control rats were 10.1 ± 2.5 IU 1^{-1} and 20.5 ± 3.5 KA units 100 ml^{-1}, respectively. The SGPT and SALP activity at different time periods showed no significant difference in control animals as compared with 0 h. Values are mean ± SD of six and four animals in test and control groups, respectively. $^*P < 0.05$.

The effect of Cd on the activities of SOD and CAT is shown in Figure 2. The activity of SOD and CAT in erythrocytes decreased up to three days of Cd intoxication, followed by an increase up to 10 days. However, the decrease in CAT activity was more marked within 9 h of Cd administration, followed by an increase up to 10 days.

The GSH content in erythrocytes decreased within 24 h of Cd administration, followed by an increase up to 10 days (Figure 3).

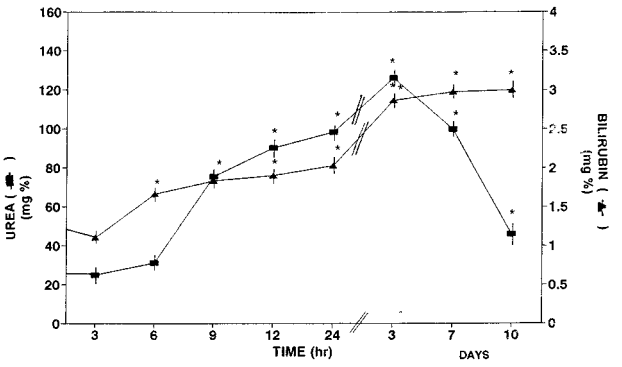

Figure 6. Effect of Cd on blood urea and serum bilirubin. Blood urea and serum bilirubin were determined in test and control animals at 0, 3, 6, 9, 12 and 24 h, and 3, 7 and 10 days of Cd treatment. Blood urea and serum bilirubin in control rats were 25.2 ± 3.2 mg% and 1.2 ± 0.2 mg%, respectively. Blood urea and serum bilirubin at different time periods showed no significant difference in control animals as compared with 0 h. Values are mean ± SD and four animals in test and control groups, respectively. $^*P < 0.05$.

The activity of GST in erythrocytes increased due to Cd administration and was high up to 10 days of intoxication (Figure 4).

The SGPT and SALP activities increased continuously up to 10 days of Cd administration (Figure 5).

Serum bilirubin increased up to 10 days of Cd treatment, while blood urea increased up to three days of Cd treatment, followed by a decrease towards normal by 10 days (Figure 6).

Discussion

The effect of Cd on LPO and the antioxidant system in erythrocytes was studied. Cd may induce oxidative stress by enhancing LPO and by altering the antioxidant system in erythrocytes. Cd intoxication showed increased erythrocyte LPO associated with lowered antioxidant enzymes; as the activity of SOD and CAT increased, the LPO decreased. The decrease in SOD and CAT activities in erythrocytes may be due to the inactivation of these enzymes, as superoxide anions have been shown to reduce the activity of these enzymes (Hodgson & Fridovich 1975, Kono & Fridovich 1982). LPO may, therefore, be exacerbated by the failure in antioxidant enzymes, which may be brought about by Cd intoxication. The recovery of erythrocyte LPO also correlated with the increase in SOD and CAT activity, of which the values reached were comparable with control values after 10 days of intoxication. The depletion of erythrocyte GSH along with the decreased in SOD and CAT activity may affect the ability of erythrocytes to scavenge superoxide anions and hydroxyl radicals. The decrease GSH content in erythrocytes by Cd intoxication may be due to depletion of GSH, Cd–SH binding, or an effect on glutathione reductase activity. The formation of Cd–GSH complexes occurs at an early stage of Cd accumulation in liver and in rat trachea (Iguchi & Ikeda 1991). The increase in GSH content in blood after 24 h of Cd intoxication indicates that repletion of GSH may also lower LPO and increase SOD and CAT activities in erythrocytes.

GST detoxifies a variety of electrophilic components to less toxic forms by conjugation with –SH groups such as GSH. GST has been shown to have an additional ability to reduce lipid peroxides such as Se-independent glutathione peroxidase (Masukawa & Nishimura 1974). The elevated activity of GST was probably an attempt by erythrocytes to counteract the increased peroxides in these erythrocytes.

The increase in SGPT, SALP, serum bilirubin and blood urea was in response to Cd intoxication and indicates liver function impairment due to increased Cd induced oxidative stress. The decrease in blood urea levels after three days of Cd intoxication correlates with the decreased LPO in erythrocytes after this period and indicates partial recovery from Cd induced oxidative stress.

In conclusion, the erythrocytes undergo peroxidation of membrane lipids and a decrease in SOD, CAT and GSH content due to Cd intoxication. The increase in erythrocyte GST activity was in response to Cd induced oxidative stress. The changes in enzymes such as SGPT and SALP, and serum bilirubin and blood urea were in correlation with increased LPO and decreased antioxidant enzymes. The alteration in antioxidant enzymes with increased LPO may possibly contribute to the susceptibility of erythrocytes to Cd induced oxidative injury.

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