ORIGINAL ARTICLES

Cadmium-mediated Induction of Cellular Defence Mechanism: a Novel Example for the Development of Adaptive Response against a Toxicant

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Abstract: Early response to the exposure of cadmium includes the enhancement in lipid peroxidation with the concomitant impairment in antioxidative defence mechanism. This investigation deals with the delayed response of cadmium induced stimulation of endogenous defence response against its oxidative damage. The administration of cadmium led to an increase in the hepatic enzymatic and nonenzymatic defence armory in a dose dependent manner 72 hrs post its administration. This includes respectively an increase in the activities of superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase and in the levels of glutathione, metallothionein and zinc. Cadmium administration also stimulated serum ceruloplasmin activity in a dose dependent manner. These changes are accompanied by the concomitant decrease in the peroxidative damage to lipids. Our results suggest the development of a delayed adaptive/defence response as a result of exposure to cadmium.

Key words: Antioxidative defence—Oxidative damage—Superoxide dismutase—Glutathione peroxidase—Glutathione—Glucose-6-phosphate dehydrogenase—Metallothionein—Ceruloplasmin—Zinc

INTRODUCTION

The exposure to cadmium is well known to induce a variety of toxicity symptoms in both experimental animals and exposed population¹⁻³. Recent investigations have drawn considerable attention on the mechanism of cadmium toxicity⁴⁻¹⁰. It has been demonstrated that the early response (up to 16 hrs) to the exposure of cadmium includes the enhancement in lipid peroxidation^{6,9} with the concomitant impairment in the antioxidative defence mechanism including

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the levels of glutathione which serves as a first line of defence against cadmium toxicity^{4, 5, 7, 10-14, 15}). The delayed response, however, includes induction of metallothionein which is synthesized in response to the presence of cadmium and functions as a metal storage protein. Metallothionein has also been shown to act as an efficient interceptor of hydroxyl radical ($\text{OH}^\bullet$) and superoxide anion ($\text{O}_2^{\bullet-}$), which may serve as a second line of defence against oxidative damage to lipid rich membrane^{4, 10, 16-18}). Further support to this hypothesis is evident from the studies where administration of a small dose of cadmium protects animals against the lethal response to a high dose of cadmium¹⁹). These results prompted us to investigate the delayed response of cadmium on the antioxidative defence armory in an animal model system. In the present study, the results of the delayed effect of cadmium on hepatic levels of defence system which include glutathione, metallothionein, zinc, superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and serum ceruloplasmin and their correlation with the peroxidation of membrane lipids is reported.

MATERIALS AND METHODS

Female albino rats (Industrial Toxicology Research Centre Colony) weighing 150 ± 15 g were housed in an airconditioned room and had free access to pellet diet (Hindustan Lever Ltd., Bombay, India) and water. The animals were divided into five groups consisting of six rats each. The first four groups received, respectively, a single intraperitoneal (ip) injection of cadmium(II) chloride solution at the doses of 0.25, 0.50, 1.0 and 3.0 mg/kg body wt. The fifth group received only normal saline and served as control. Animals of all the groups were sacrificed by decapitation 72 hrs after treatment. Blood was collected for separation of serum. Liver was immediately removed, cleaned free of blood and extraneous material and processed for either biochemical assay or metal estimation.

The hepatic levels of glutathione and activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase in post mitochondrial supernatant and superoxide dismutase in 3000xg supernatant were assayed according to known methodologies, the details of which are described earlier^{5, 11, 12, 20, 21}). Lipid peroxidation in whole homogenate was assayed by the modified procedure of Uchiyama and Mihara²²) as reported by Sunderman Jr. *et al.*²³) Serum ceruloplasmin was assayed by the procedure described by Ashby *et al.*,²⁴) while hepatic metallothionein was assayed by cadmium-heme-method¹⁷). For metal estimation a portion of liver samples was subjected to acid digestion and analysed for zinc on a Perkin Elmer 5000 Atomic absorption spectrophotometer.

RESULTS

Glutathione and glutathione peroxidase system provide first line of defence against oxidative damage to cells^{14,15}. The importance of glutathione in cadmium toxicity is evident from the studies where depletion in its level augments cadmium toxicity^{7,14}. Administration of cadmium has earlier been shown to deplete the organ levels of glutathione at initial hours (12 hrs) which is maintained upto 36 hrs and then exhibits an increasing trend¹⁰. The results of the present study show a dose dependent increase in its level 72 hrs after cadmium administration

Fig. 1: Dose dependent effect of cadmium on the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase and on the level of glutathione in rat liver.

Each point represents mean $\pm$ SE of six animals.

* represents significance ($p < 0.05$) when compared to saline treated control.

Fig. 2: Dose dependent effect of cadmium on the hepatic activity of superoxide dismutase. Each point represents mean $\pm$ SE of six animals.

Fig. 3: Dose dependent effect of cadmium on the activity of serum ceruloplasmin and on the levels of hepatic metallothionein and zinc. Each point represents mean $\pm$ SE of six animals.

* represents significance ($p < 0.05$) when compared to saline treated control.

Fig. 4: Regression lines representing correlations between the inhibition of lipid peroxidation and the enhancement in levels of metallothionein, zinc and ceruloplasmin activity and between the induction of metallothionein and zinc levels.

(Fig. 1). Similar to the increase in glutathione, an increase in the activity of glutathione peroxidase was also observed 72 hrs post cadmium administration. However, the magnitude of increase was not significant (Fig. 1). A significant decrease in the activity of glutathione reductase has also been reported during the early phase of cadmium administration^{5,11}. Our results exhibit no significant change in its activity at any of the dose levels studied (Fig. 1). Previous studies have also reported a significant decrease in the activities of glucose-6-phosphate dehydrogenase during the early phase of cadmium exposure^{5,8,11}. The results of our experiment exhibit significant stimulation in the activity of this enzyme after the administration of cadmium (Fig. 1).

Cadmium also exhibit a dose related increase in the activity of superoxide dismutase with the maximum increase of 22% observed at 3 mg cadmium dose

Fig. 5: Dose dependent effect of cadmium on lipid peroxidation. Each point represents mean $\pm$ SE of six animals.

* represents significance ($p < 0.05$) when compared to saline treated control.

level (Fig. 2). Our data corroborates with earlier findings where increased superoxide dismutase activity was observed in lung 72 hrs after intratracheal cadmium administration¹²⁾. The effect of cadmium on the activity of serum ceruloplasmin is not known during early hours of cadmium exposure. However, 24 hrs after its exposure, a two fold increase in activity is reported²⁵⁾. Our results on the dose dependent effect of cadmium confirm early observation and show a three fold increase in its activity at the highest administered dose (Fig. 3).

Consistent with the previous observations, the results of the present study show a dose dependent increase in the hepatic levels of zinc and metallothionein (Fig. 3).

Expectedly a significant high positive correlation was observed between hepatic zinc and metallothionein concentration (Fig. 4). Lipid peroxidation is a sensitive marker of oxidative damage to lipid rich membrane⁹⁾. Our results at 72 hrs exhibit a decreasing trend in hepatic lipid peroxidation with increasing dose of cadmium (Fig. 5). The mathematical correlations between lipid peroxidation and hepatic metallothionein, zinc or serum ceruloplasmin established in the form of linear regression equations reveal a significantly high correlation between the decreasing trend in lipid peroxidation with the increase in metallothionein, zinc

and ceruloplasmin (Fig. 4).

DISCUSSION

Nature has provided a strong biological defence mechanism against the deleterious effect of reactive oxygen species which are the by products of normal metabolism of the organism. Both enzymatic molecules such as catalase, superoxide dismutase, glutathione peroxidase and ceruloplasmin and nonenzymatic molecules such as glutathione, metallothionein and ascorbic acid present in the cell have enormous capabilities to mitigate their otherwise potential harmful effects²⁸⁾. The chemicals which impair antioxidant defence armory mediate their toxic response through the unscavenged oxidants. Cadmium is one such chemical, exposure to which leads to a biphasic response in this regard. Early effects of cadmium include the inhibition of various antioxidant enzymes such as superoxide-dismutase, glutathione peroxidase and depletion in the levels of glutathione^{5, 8, 10, 26)}. Of these, the alterations in the thiol content seems to be important for the onset of cadmium toxicity²⁶⁾. Therefore the early toxic manifestation as a result of exposure to cadmium appears to be triggered by the increased oxidative stress. One of the consequences of increased oxidative stress may be related to cadmium mediated enhancement in lipid peroxidation, although it does not appear to be the sole cause of cadmium cytotoxicity⁵⁻⁸⁾. It has also been recently shown that cadmium evokes alterations in the level of inositol phosphates leading to the change in calcium fluxes²⁷⁾. These events may play a coordinated role in the manifestation of its toxicity. The increase in the antioxidant status observed in the present investigation, as evidenced by the increased activities of superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase and ceruloplasmin in the levels of glutathione, metallothionein and zinc after cadmium administration, suggests the development of adaptive/delayed response which may cope with further insult of cadmium.

Our results provide a useful toxicological information regarding the stimulation of cadmium induced endogenous defence response to protect the system against oxidative damage. It is of interest to mention that cadmium mediated peroxidative damage to membrane is more pronounced in male species than in female^{6, 29)}. Recent studies also provide evidence that iron induced peroxidative damage, leading to increase risk of cancer, is quite low in female than in male^{30, 31)}. The molecular mechanisms by which the system triggers the sex related difference in the antioxidant production after cadmium intoxication needs thorough investigation in view of some recent findings^{14, 27, 29-31)}.

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