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Manganese pre-treatment attenuates cadmium induced hepatotoxicity in Swiss albino mice

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ABSTRACT

Cadmium (Cd) is a soft, malleable bluish-white metal with low melting point, a ubiquitous heavy metal and an environmental pollutant, found in soil, water and air. The presence of Cd in the components of the environment such as air, soil and groundwater is to a large part due to human activity, and the general population is exposed mainly by contaminated drinking water or food. Manganese (Mn) is a component in many enzymes, which play an important role in counteracting oxidative stress. *In vitro* experiments have revealed the ability of Mn to scavenge oxygen free radicals generated in differently mediated lipid peroxidation (LPO) conditions. The aim of the present study was to investigate the *in vivo* preventive effect of Mn^{2+} pre-treatment on acute Cd-intoxication with regard to oxidative stress biomarker and antioxidant defense system in liver of Swiss albino mice. On exposure to Cd a significant increase in LPO levels, decrease in thiol content and induction in glutathione metabolizing enzyme were observed. Mn pre-treatment attenuated the modulation caused in the above-mentioned parameters due to acute Cd exposure in mice. In conclusion, the results from this study demonstrate that the protective effect of Mn in Cd-induced systemic toxicity in mice. Further investigations are required on the relation between Mn accumulation and resistance to oxidative stress and on the factors influencing Mn/Cd transport in rodents are needed to elucidate the molecular basis of this protective effect.

Introduction

Cadmium (Cd) is a heavy metal which has been widely reported as an industrial and environmental pollutant. It has been implicated in various industrial uses such as electroplating, preparation of paints, dye stuffs and the mining industries [1]. Their damaging effects on physiological, biochemical, and behavioural dysfunctions have been documented in animals and humans by several investigators [2]. In humans, the primary route of exposure is through contaminated drinking water, food supplies, or tobacco. Cd is an extremely accumulative toxicant with very high retentive biological half-life [3]. Acute Cd exposure induced toxicities to the lung, testes, liver, and brain [4–6], while chronic exposure to Cd frequently leads to anaemia, osteoporosis, renal dysfunction, and bone fractures [7].

Liver is one of the most important target organs for digestion and detoxification and it is easily affected by Cd exposure. Cd seems to be instantly toxic to hepatic cells, but also indirectly acts on these cells through the activation of Kupffer cells [8]. There is an increasing recognition of evidence that the toxicity of Cd may be associated with the production of reactive oxygen species (ROS) [9]. It has been also demonstrated that Cd provokes free radical generation, resulting in oxidative derogation of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals [10]. The free radical scavengers strongly bind to Cd and this uptake leads to elevated ROS, a normal by-product of aerobic respiration and the major armamentarium behind accelerating oxidative stress [11]. Oxidative stress is a disturbance of the cellular redox balance in favour of the pro-oxidants, and can lead to disruption of cellular macromolecules such as degradation of lipid, proteins and DNA, and membrane fatty acid peroxidation [12]. Cd exerts its toxic effects through oxidative damage to cellular organelles induced by the ROS generation which comprise mainly of $^{\bullet}O_2$, H_2O_2 and $^{\bullet}OH$. Reactions of these ROS with cellular biomolecules have been shown to induce LPO, altered antioxidant system, DNA damage, altered gene expression and apoptosis [13]. In addition to that depletion of antioxidant defence glutathione (GSH) and other endogenous antioxidants may also contribute significantly to the development of Cd-induced toxic oxidative stress [14]. If these ROS mediated stress events are not balanced by repair processes, affected cells may be undergoing apoptosis or necrosis [15]. Thus it is believed

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that antioxidant should be one of the important components of an effective treatment of Cd toxicity. Several studies have been performed with different natural substances possessing antioxidant properties to investigate their possible protective effects in Cd-induced tissue damage [16].

Additionally several essential elements as potential agents have been studied to reduce severe side effects of xenobiotics including heavy metals. Manganese (Mn) has emerged as a crucial trace element in biological system. The correlation between Mn aggregation and resistance to oxidative stress has extended to the suggestion that, in addition to a role as a prosthetic group in antioxidant enzyme such as superoxide dismustase, Mn could exert its antioxidant potential *via* non-enzymatic redox reactions [17]. Pretreatment of trace elements has been preferred to protect protein synthesis and cell death in liver of rodents [18]. The antioxidant effect of pre-treatment completely prevented the adverse effect of Cd on LPO and the antioxidant defence system in mouse hepatic system intoxicated to Cd. Studies on rat hepatocytes incubated with Cd suggested that Mn ions may protect from Cd-induced LPO and the inhibition of antioxidant enzymes [19]. The present study was undertaken to evaluate the ameliorative effect of Mn pre-treatment on acute Cd-intoxication in a murine model with regard to oxidative stress biomarkers, enzymatic and non-enzymatic antioxidant defense system in the liver tissues of mice.

Materials and methods

Chemicals

Bovine serum albumin (BSA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), oxidized glutathione (GSSG), reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Butylated hydroxy toluene (BHT), ethylene diamine tetraacetic acid (EDTA), orthophosphoric acid (OPA), thiobarbituric acid (TBA) sulfosalicylic acid and trichloroacetic acid (TCA) were purchased from Merck Limited (Mumbai, India). Cadmium chloride ($CdCl_2$) and manganese chloride ($MnCl_2$) were obtained from SRL Labs (Mumbai, India).

Animals and treatment

Swiss albino male mice weighing 50–60 g were used for experiments and procured from the Central Animal House of Jamia Hamdard (Hamdard University), New Delhi, India. Mice were kept at temperature 23 ± 2 °C with relative humidity at 66 ± 10% and at a photoperiod of 12 h light/dark cycle. Food and water were supplied *ad libitum* prior to the start of the experiment. All the experiments were carried out according to the standard guidelines of Institutional Animal Ethics Committee (IAEC). The animals were assigned to four groups of 6–7 animals:

I. Control group
II. Mn group
III. Cd + Mn group
IV. Cd group

The mice in II group received Mn treatment only (a single *i.p.* dose of Mn at a dose of 20 mg/kg body weight) and the control group received saline only. The animals from Cd + Mn group received a single dose of Mn at a dose of 20 mg/kg body weight, administered *i.p.* 24 h prior to *s.c.* Cd intoxication at a dose of 7 mg/kg body weight. The mice in IV group were injected *s.c.* Cd at a single dose of 7 mg/kg of body weight.

Sample preparation

After 24 h treatment with Cd the mice were sacrificed by cervical dislocation without anaesthesia. The liver was rapidly excised on a petri-dish placed on ice, the blood and external vessels were carefully removed, weighed and separately homogenized in 10 volumes (1:10, w/v) of 0.1 M sodium phosphate buffer, pH 7.4 with a Potter-Elvehjen homogenizer. The homogenate of liver tissue was centrifuged at 1800 × *g* for 20 min at 4 °C to discard nuclei and cell debris. The resulting pellet was the primary mitochondrial pellet and the supernatant was post mitochondrial supernatant (PMS).

Oxidative stress marker

Determination of lipid peroxidation

LPO was measured using the procedure of Uchiyama and Mihara [20] as modified by Tabassum et al. [21]. Briefly, 0.25 mL of 10% homogenate prepared from liver tissue was mixed with 10 mM BHT, OPA (1%) and TBA (0.67%) were added and mixture was incubated at 90 °C for 45 min. The absorbance was measured at 535 nm. The rate of LPO was expressed as μmoles TBARS formed/h/g tissue based on the molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Non-enzymatic antioxidant assays

Reduced glutathione (GSH) content

GSH was assessed by the method of Jollow et al. [22]. The reaction is based on the fact that the thiol group of GSH reacts with the –SH reagent, (DTNB) to form thionitro-benzoic acid. The PMS (10%) was mixed with 4% sulphosalicylic acid. It was then incubated at 4 °C for a minimum time period of 1 h and then centrifuged at 4 °C at 1200 × *g* for 15 min. The reaction mixture contained 0.1 M phosphate buffer (pH 7.4), DTNB and 0.4 mL PMS prepared from 10% homogenate of mice liver tissue. The yellow colour developed was read immediately at 412 nm on spectrophotometer. The reduced glutathione concentration was calculated as μmoles GSH/g tissue using a molar extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$.

Non protein thiol (NP-SH) level

NP-SH was determined in the liver tissue samples by using the method of Sedlak and Lindsay [23] as adopted by Govil et al. [24]. For the determination of NP-SH 0.5 mL of the supernatant was precipitated with 0.5 mL of 40% TCA and then centrifuged at 3000 × *g* for 15 min. Then the PMS was used for the measurement by adding 0.4 M Tris buffer (pH 8.9), and 10 mM DTNB. The molar extinction coefficient of $13{,}100\ M^{-1}\ cm^{-1}$ at 412 nm was used for the determination of thiol content. The value was expressed μmoles NPSH/g tissue.

Glutathione reductase (GR) activity

GR activity was assayed by the method of Mohandas et al. [25]. The assay system consisted 0.1 M phosphate buffer (pH 7.4), 0.5 mM EDTA, 1 mM GSSG, 0.1 mM NADPH and 0.3 mL PMS of mice liver in a total volume of 2.0 mL. The enzyme activity was quantitated at 25 °C by measuring the disappearance of NADPH at 340 nm, and was calculated as nmoles NADPH oxidized/min/mg protein using a molar extinction coefficient of $6.223 \times 10^3\ M^{-1}\ cm^{-1}$.

Protein determination

The protein content was determined in PMS of liver tissue by the method of [26] using BSA as a standard.