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Brain most susceptible to cadmium induced oxidative stress in mice

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ABSTRACT

Accumulated evidence over the years indicate that cadmium (Cd) may be a possible etiological factor for neurodegenerative diseases. This may possibly be linked to excessive generation of free radicals that damages the organs in the body depending on their defence mechanism. Since Cd is a toxic agent that affect several cell types, the aim of this study was to shed light on the effect of Cd and its consequences on different organs of the mice body. To test the hypothesis of concentration dependent Reactive Oxygen Species (ROS) generation and DNA damage, observations were done in the serum of 4–5 weeks old male Swiss albino mice by treating with cadmium chloride ($CdCl_2$) in drinking water for 30 days. The expression of Bcl-2-associated X protein (Bax) an apoptotic marker protein was two times higher in brain compared to liver at an exposure level of 0.5 mg L^{-1} $CdCl_2$. Furthermore the correlation and linkage data analysis of antioxidant defence system revealed a rapid alteration in the brain, compared to any other organs considered in this study. We report that even at low dose of Cd, it impaired the brain due to lipid peroxidase sensitivity which favoured the Cd-induced oxidative injury in the brain.

Introduction

Cadmium (Cd), one of the biologically non-essential metals, is currently placed in the list of top 20 hazardous substances [1]. The role of Cd in diseases like Wilson's disease and Menkes syndrome is well known [2]. An increased production of Reactive Oxygen Species (ROS) which are potentially harmful for the cell components, is a common outcome of Cd exposure. Cd induces the generation of ROS by upregulating the expression of nicotinamide adenine dinucleotide phosphate oxidase 2 (NADPH oxidase 2) and its associated proteins [3]. It is evident that exposure to Cd may cause adverse health effects through formation of free radicals, that results in DNA damage, lipid peroxidation (LPO), and depletion of protein sulfhydryls [4]. Oxidative stress that results from the state of imbalance between the concentrations of ROS and the antioxidant defence mechanisms, may be connected to various pathological abnormalities [5–7] *e.g.* neurodegenerative diseases, diabetes, cancer [8]. Prolonged exposure of Cd, a toxic metal, targets the lung, liver, kidney, and testes following acute intoxication, and causing nephrotoxicity, immunotoxicity, osteotoxicity and tumours. Earlier report indicated that Cd stimulates free radical production, resulting in oxidative damages of lipids, proteins and DNA [9,10]. LPO and protein carbonylation are seen to be the two important parameters, increases with Cd intoxication [11,12]. Its damages were estimated through protein carbonylation and total sulfhydryls degradation [13,14]. The overexpression of Hyaluronan Binding Protein 1 (HABP1), a 34 kDa protein of the hyaladherin family generates ROS in normal murine fibroblasts resulting in induction of apoptosis [15]. Its interaction with various pathogenic proteins under condition of oxidative and pathological stress suggests its generic role in disease conditions [16,17].

In this paper, we report for the first time that Cd toxicity induces differential expression of HABP1 along with Bax, the apoptotic markers in different organs. We also observe that upon oral Cd treatment a significant dose dependent increase in oxidative stress in the brain compared to other organs, signifying the brain to be more vulnerable towards Cd toxicity.

Material and methods

Material

All reagents were obtained from Sigma Aldrich (St. Louis, MO, USA). The anti-HABP1 antibody was raised against purified recombinant human HABP1 in rabbit and characterized in our laboratory [18].

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Animals' exposure

Male Swiss Albino mice (4–6 week old and 25 ± 5 g body weight) were obtained from the animal facility of Jawaharlal Nehru University, New Delhi. Animals were categorized in five experimental groups and each group had 5 mice (5 × 5 = 25) of same breed progeny for treatment. To nullify the experimental variation of body weight the consumption of drinking water per day by average weight of a single group, was gauged and the estimated value of $CdCl_2$ concentration in 0.1, 0.5, 1.0, and 2.0 mg L^{-1} was dissolved in their drinking water and provided for 30 days treatment continuously with reference to earlier reports [19–23]. All the animals were housed in an air conditioned room, where the temperature was maintained at 25–27 °C with constant humidity (40–50%) and kept on 12 h/12 h light/dark cycle throughout the experiment. Animals were fed with standard food pellets (prepared by Brook Bond India Ltd., Backbay Reclamation, Mumbai, India), *ad libitum*. The spinal dislocation to sacrifice the mice for further experimental protocols for mice described in this study were approved previously by the Institutional Animal Ethical Committee (IAEC) and the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All subsequent animal experiments adhered to the 'Guidelines for Animal Experimentation' of the University.

Preparation of homogenate and cytosol fraction

After 30 days treatment mice were sacrificed by spinal dislocation and kidney, brain, liver, spleen and testis were perfused immediately with cold 0.9% saline and washed with chilled 0.15 mol L^{-1} Tris–potassium chloride (Tris–KCl) buffer (pH 7.4). Tissues were then blotted dry, weighed and homogenized in ice cold 0.15 mol L^{-1} Tris–KCl buffer (pH 7.4) for 10% homogenate. This homogenate (0.5 mL) was used for assaying glutathione level and remaining homogenate was centrifuged at 1047.19 radian s^{-1} for 20 min. Then supernatant was transferred into pre-cooled tubes and ultra-centrifuged at 10,500 × *g* for 60 min. The cytosolic fraction was collected and used for antioxidant enzyme assay, lactate dehydrogenase (LDH) and the pellet was dissolved in homogenizing buffer and used for assaying LPO.

Glutathione peroxidase (GSH-Px) assay

The activity of GSH-Px was measured in a freshly prepared cytosolic fraction. Glutathione reductase solution (2.4 U mL^{-1} in 0.1 mol L^{-1} potassium phosphate buffer, pH 7.0) was added to a 50 mmol L^{-1} potassium phosphate buffer (pH 7.0), 0.5 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 1 mmol L^{-1} sodium azide, 0.15 mmol L^{-1} Nicotinamide adenine dinucleotide phosphate (NADPH) and 0.15 mmol L^{-1} hydrogen peroxide (H_2O_2). After the addition of 1 mmol L^{-1} GSH (reduced glutathione), the NADPH-consumption rate was monitored at 340 nm as described by Flohe and Gunzler [24].

Superoxide dismutase (SOD) assay

The cytosolic fraction of tissue homogenate diluted 10 times with water, mixed with 5 μL Triton X-100, incubated for 30 min (25° C) and thereafter 3 mmol L^{-1} EDTA was added in assay mixture. The reaction was initiated by the addition of 100 μL of freshly prepared 2.6 mmol L^{-1} pyrogallol solution in 10 mmol L^{-1} hydrogen chloride (HCl) to attain a final concentration of pyrogallol of 0.13 mmol L^{-1} in the assay mixture. The assay mixture was transferred to a 1.5 mL cuvette and the rate of increase in the absorbance at 420 nm was recorded for 2 min from 1 min 30 s to 3 min 30 s in a spectrophotometer [25].

Catalase assay

Cytosolic fractions were first incubated with 0.3 mol L^{-1} ethanol and 2% (v/v) Triton X-100 in 0.01 mol L^{-1} sodium phosphate buffer at pH 7.0 for 10 min in a bath of ice cold water. Catalase activity was then measured at 0 °C in phosphate buffer at pH 7.0 in the presence of 1.5 mmol L^{-1} peroxide. The reaction was stopped at different times by adding the peroxidase reagent H_2O_2 and the disappearance of peroxide was then measured colorimetrically. Results were expressed in terms of the apparent first order reaction rate constant [26].

Lactate dehydrogenase (LDH) assay

Lactate dehydrogenase was assayed by measuring the rate of oxidation of NADH at 340 nm as described by Bergmeyer and Bernt [27]. One unit of enzyme activity was defined as that which causes the oxidation of one μmol of NADH per minute.

Lipid peroxidation (LPO) estimation

LPO in the microsomes was estimated spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method [28] and is expressed in terms of malondialdehyde (MDA) formed per mg of protein.

Reactive Oxygen Species assay

N,N-diethyl-para-phenylendiamine (DEPPD) was dissolved in 0.1 mol L^{-1} sodium acetate buffer (pH 4.8) to a final concentration of 100 μg mL^{-1} (R1 solution), and ferrous sulphate was dissolved in 0.1 mol L^{-1} sodium acetate buffer (pH 4.8) to a final concentration of 4.37 μmol L^{-1} (R2 solution). Five μL of either H_2O_2 standard solution (for generating a calibration curve) or serum was added to 140 μL of 0.1 mol L^{-1} sodium acetate buffer (pH 4.8) in 1 well of a 96-well microtiter plate, which reached a temperature of 37 °C after 5 min. One hundred μL of the mixed solution, which was prepared from R1 and R2 at a ratio of 1:25 before use, was added to each well as a starter. Then, after pre incubation at 37 °C for 1 min using a multi-mode microplate reader (Spectra Max M5), absorbance at 505 nm was measured for a fixed time (between 60 and 180 s) at intervals of 15 s. A calibration curve was automatically constructed from the slopes, which was calculated based on varying (delta) absorbance at 505 nm each time (min) corresponding to the concentration of hydrogen peroxide. ROS level in serum were calculated by the analyzer of Spectra Max M5 from the calibration curve, and expressed as equivalent to levels of H_2O_2 (1 unit = 1.0 mg H_2O_2 L^{-1}). The serum ROS levels determined by this assay system were compared with those measured by the conventional DROM test. In brief, 20 μL serum and 1.2 mL buffered solution (R2 reagent) were mixed in a cuvette, and 20 μL chromogen substrate (R1 reagent) was added to the cuvette. After mixing properly, the cuvette was centrifuged for 1 min and incubated for 5 min at 37 °C. Absorbance at 505 nm was monitored for 3 min [29]. In ROS measurement assay all data (10 experiments) were representative of five similar sets of exposure group having at least two mice from each group, in which the relative absorbance intensity was calculated by averaging the values as mean ± S.E.

Comet assay

Blood was collected (0.6–0.8 mL) from the orbital vessels of each animal post-treatment and the comet assay was performed as described by Singh et al. [30]. Slides were prepared in triplicate per blood cells sample. First the slide was dipped in methanol and burned over a blue flame to remove the machine oil and dust.