Toxicology Reports 2 (2015) 858–863

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Evaluation of zinc effect on cadmium action in lipid peroxidation and metallothionein levels in the brain

Marcos M. Braga[a,b,*], Tuiskon Dick[a], Diogo L. de Oliveira[a], Adriele Scopel-Guerra[a], Ben Hur M. Mussulini[a], Diogo O. Souza[a], João Batista T. da Rocha[b]

[a] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, CEP 90035-003 Porto Alegre, RS, Brazil
[b] Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil

ARTICLE INFO

Article history:
Received 26 March 2015
Received in revised form 29 May 2015
Accepted 31 May 2015
Available online 5 June 2015

Keywords:
Cadmium
Zinc
Metallothionein
δ-Aminolevulinate dehydratase
Food intake

ABSTRACT

Cadmium (Cd) is a known hepato- and nephrotoxic pollutant and zinc (Zn) metalloproteins are important targets of Cd. Hence, the administration of Zn may mitigate Cd toxic effects. However, the interaction of Cd and Zn has been little investigated in the brain. Previously, we reported a protective effect of Zn on mortality caused by Cd in rats. Here, we tested whether the protective effect of Zn could be related to changes in brain Zn-proteins, metallothionein (MT) and δ-aminolevulinate dehydratase (δ-ALA-D). Male adult rats were daily administered for 10 days with Zn (2 mg kg^{-1}), Cd (0.25 and 1 mg kg^{-1}) and 0.25 mg kg^{-1} of Cd plus Zn and 1 mg kg^{-1} of Cd plus Zn. The body weight loss, food intake deprivation, and mortality occurred in 1 mg kg^{-1} of Cd, but Zn co-administration did mitigate these effects. The brain Zn content was not modified by treatment with Cd, whereas cerebral Cd levels increased in animals exposed to Cd. The administration of 0.25 mg kg^{-1} of Cd (with or without Zn) induced lipid peroxidation and decreased MT concentration, but 2 mg kg^{-1} of Zn and 1 mg kg^{-1} of Cd did not change these parameters. Brain δ-ALA-D was not modified by Cd and/or Zn treatments. Since the co-administration of Zn did not attenuate the changes induced by Cd in the brain, our results suggest that the protective effect of Zn on impairments caused by Cd in animal status is weakly related to a cerebral interaction of these metals.

1. Introduction

The metal cadmium (Cd) is a widespread environmental contaminant [1] and a potential toxin that may harmfully affect human health. Cadmium has a long biological half-life [2] and testicular, hepatic and renal cells are particularly sensitive to exposure to Cd [3–6]. Accordingly, Cd intoxication has been associated with reactive species over production, mitochondrial injury, lipid peroxidation and cellular death in liver [7,8] and kidney [9].

Cadmium can have also neurotoxic effects. In several experimental models, it has been reported that Cd induces oxidative damage in brain mitochondria [10,11], impairs synaptic transmission [12,13], and reduces neuronal differentiation and axonogenesis [14]. Thus, occupational exposure to Cd have been linked to motor and memory deficiencies [15].

The molecular toxicity of Cd is mainly related to direct action in zinc (Zn)-dependent biological pathways [16]. Cadmium exposure is able to alter Zn transporters expression in zebrafish [17] and cause Zn accumulation in different tissues of rodents [16,18]. In addition, the Zn-dependent activity of the δ-aminolevulinate dehydratase (δ-ALA-D) is a potential target of Cd, which can replace Zn of the active site of the mammalian enzyme [19–21]. The expression of metallothionein (MT), an important protein in Zn homeostasis, has been also induced after Cd administration [22]. Overall, Cd effects may be mitigated by Zn, including Cd-induced oxidative stress [23]. The antagonist effect of Zn on Cd toxicity has led to proposition that Cd is an antimetabolite of Zn [16]. However, the interaction between Cd and Zn after systemic exposure has been little explored in the brain.

Zinc has important role in the brain and could be a highly sensitive target to action of Cd. There are a considerable number of proteins that bind to Zn in the central nervous system (CNS), for instance, δ-ALA-D [24] and MTs [25]. Moreover, a brain fraction of Zn, named chelatable Zn pool, is found free or loosely bound to biomolecules, and it is localized abundantly in synaptic vesicles [26]. This indicates that Zn participates in the regulation of synaptic

* Corresponding author at: Departamento de Bioquímica, ICBS Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2600-Anexo, CEP: 90035-003 Porto Alegre, RS, Brazil. Tel.: +55 51 33085555.
E-mail address: marcosmbraga@gmail.com (M.M. Braga).

http://dx.doi.org/10.1016/j.toxrep.2015.05.014

physiology. As corollary, we can suppose that the interaction between Cd and Zn could be more critical in the brain.

The aim of this work was to investigate whether the protective effect of Zn on systemic effects of Cd could be related to changes in brain Zn-proteins, similar with those observed in peripheral tissues of adult rats [27–29]. Thus, the animal status (*e.g.*, body weight gain and food intake) was evaluated during the exposure to the metals. Specifically, we measured the content of Zn and Cd in the brain of rats after *in vivo* exposure to metals. Furthermore, we evaluated the lipid peroxidation rate, the levels of MT, and the δ-ALA-D activity.

2. Materials and methods

2.1. Chemicals

δ-aminolevulinic acid (δ-ALA), DL-dithiothreitol (DTT), rabbit metallothionein-I, *o*-phenylendiamine, thiobarbituric acid (TBA) and malonaldehyde bis- (dimethyl acetal) were obtained from Sigma (St. Louis, MO, USA); mouse anti-metallothionein-I/II, and peroxidase-conjugated to goat anti-mouse IgG were purchased from Dako Corporation (Carpinteria, CA, USA).

2.2. Animals

Male Wistar rats (±80 days and ±240 g) were obtained from our animal facility. Animals were maintained under a controlled environment (three rats housed per cage, room temperature 22 °C, standard light/dark cycle of 12 h, and water and food provided *ad libitum*). Animal care was followed in accordance with the "National Institutes of Health Guide for Care and Use of Laboratory Animals", and all experiments were approved by Ethics Committee of Universidade Federal do Rio Grande do Sul.

2.3. Treatments and tissue preparation

The rats ($n = 9$ animals per group) were daily exposed to Cd and/or Zn during 10 days. The metals were injected *via* intraperitoneal (i.p.), as a model for parenteral exposure of Cd, *i.e.*, inhalation [30]. Based on previous studies [16,31,32], animals were administered with neurotoxic doses of Cd in the presence or absence of supplementary dose of Zn. Experimental groups consisted in (i) NaCl 0.9% (control group), (ii) 2 mg kg^{-1} of Zn, (iii) 0.25 mg kg^{-1} of Cd, (iv) 0.25 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, (v) 1 mg kg^{-1} of Cd; (vi) 1 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn. The metals were injected as acetate salts. Twenty-four hours after the last injection, the rats were anesthetized with sodium thiopental (40 mg/kg, 1 ml/kg, i.p.) and euthanized by decapitation. The whole brain was removed and separated in order to evaluate Zn and Cd levels and biochemical parameters. Importantly, the same brains were used to perform all tests. For biochemical analysis the brains were separately placed on ice and homogenized in saline (1:5) and centrifuged at 4000 g at 4 °C for 10 min. The remaining supernatant was used to determine the activity of the δ-ALA-D, MT content, and thiobarbituric acid-reactive substances (TBARS).

2.4. Body weight and food intake

The body weight gain of each animal was evaluated during exposure to the metals and the data were expressed as cumulative body weight gain per animal ($n = 9$ animals per group). The food intake per cage was also determined in each day of exposure to the metals, and the results were divided by number of animals in the cage. The food intake data were expressed as cumulative food intake (g) per animal ($n = 3$ independent experiments per group).

2.5. Zn and Cd determination

The content of Zn and Cd was determined by atomic absorption spectrometry following the US EPA 3052 protocol [33]. All analyses were carried out at the Centro de Ecologia of Universidade Federal do Rio Grande do Sul. The samples were digested in closed vials with 9 ml of 65% nitric acid and 3 ml of 30% hydrogen peroxide in boiling water. Zinc content in the samples (control, $n = 6$; 2 mg kg^{-1} of Zn, $n = 5$; 0.25 mg kg^{-1} of Cd, $n = 6$; 0.25 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 6$; 1 mg kg^{-1} of Cd, $n = 6$; 1 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 6$) was determined by a Perkin-Elmer 3300 flame atomic absorption spectrometer and Cd levels (control, $n = 5$; 2 mg kg^{-1} of Zn, $n = 3$; 0.25 mg kg^{-1} of Cd, $n = 6$; 0.25 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 5$; 1 mg kg^{-1} of Cd, $n = 5$; 1 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 4$) were estimated with a Perkin-Elmer SIMAA-6000 graphite furnace atomic absorption spectrometer. The minimum detection was 0.495 and 0.008 μg/g for Zn and Cd, respectively. All analyzes were determined with ultrapure reagents to avoid sample contamination.

2.6. Thiobarbituric acid-reactive substances

The lipid peroxidation was measured in the samples (control, $n = 6$; 2 mg kg^{-1} of Zn, $n = 5$; 0.25 mg kg^{-1} of Cd, $n = 6$; 0.25 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 8$; 1 mg kg^{-1} of Cd, $n = 6$; 1 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 6$) by relatively non-specific TBARS method according to Draper and Hadley [34]. Briefly, 80 μl of homogenized samples (2 μg/μl protein) were precipitated with 160 μl of TCA (15%) and centrifuged at 4000 g for 10 min. The supernatants (60 μl) and standard malondialdehyde (MDA) solutions were transferred to 96 well microplate and each well was filled with deionized water in order to obtain the equal volume. Next, 100 μl of TBA (0.67%) was added in each well and the microplate was sealed for heating in boiling water bath at 90 °C for 30 min. The TBARS levels were determined at 532 ηm and the values were expressed as μmol MDA equivalents/mg protein.

2.7. Metallothionein content

Metallothionein-I and -II (MT) were measured by enzyme-linked immunoassay (control, $n = 7$; 2 mg kg^{-1} of Zn, $n = 7$; 0.25 mg kg^{-1} of Cd, $n = 9$; 0.25 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 8$; 1 mg kg^{-1} of Cd, $n = 7$; 1 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 7$) [35]. The MT-III was not analyzed, because this isoform is not sensitive to Cd [36]. The experiment was conducted in 96-well microtiter plates at room temperature, and MT-I was used as standard. The plates were incubated with samples and standard concentrations of MT for 1 h. Then, the plates were washed three times with phosphate buffered saline plus 0.05% Tween 20 (washing solution). A blocking solution (washing solution plus 1% bovine serum albumin) was applied for 30 min, and the plates were washed three times. Then, mouse anti-metallothionein-I/II was incubated for 30 min and washed three times. Afterwards, peroxidase-conjugated to goat anti-mouse was incubated for 30 min and washed three times. Finally, *o*-phenylendiamine was incubated in the dark for 30 min for colorimetric reaction. The reaction was stopped with 3 M HCl and the plate was read at 490 ηm. The values were expressed as μg MT/mg protein.

2.8. δ-ALA-D activity

The enzyme activity (control, $n = 5$; 2 mg kg^{-1} of Zn, $n = 7$; 0.25 mg kg^{-1} of Cd, $n = 7$; 0.25 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 5$; 1 mg kg^{-1} of Cd, $n = 7$; 1 mg kg^{-1} of Cd plus 2 mg kg^{-1} of Zn, $n = 7$) was evaluated as previously described [37]. The formation rate of porphobilinogen (PBG) was obtained in medium containing