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Protective effect of soybeans as protein source in the diet against cadmium-aorta redox and morphological alteration

Matías F.F. Pérez Díaz, Mariano Acosta, Fabián H. Mohamed, Mariana L. Ferramola, Liliana B. Oliveros, María S. Gimenez *

Department of Biochemistry and Biological Sciences, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Argentina
IMIBIO-San Luis CONICET, Argentina

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ABSTRACT

We investigated the effects of cadmium exposition on thoracic aorta redox status and morphology, and the putative protective effect of soybeans in the diet.
Male Wistar rats were separated into 6 groups: 3 fed with a diet containing casein and 3 containing soybeans, as protein source. Within each protein group, one was given tap water (control) and the other two tap water containing 15 and 100 ppm of Cd^{2+}, respectively, for two months.
In rats fed with casein diet, 15 ppm of Cd induced an increase of thiobarbituric acid-reactive substances (TBARS), and of the catalase (CAT) and glutathione peroxidase (GPx) activities, which were even higher with 100 ppm of Cd^{2+}, in aorta.
Also, 100 ppm Cd^{2+} exposure increased superoxide dismutase (CuZnSOD) activity; CAT, GPX, SOD, Nrf2 and metallothioneine II mRNA expressions and CAT, GPx and NOX-2 protein levels, compared with control. Aorta endothelial and cytoplasmic alterations were observed.
However, with the soybeans diet, 15 and 100 ppm of Cd^{2+} did not modify TBARS levels; CAT, GPX and Nrf2 mRNA expressions; CAT, GPx and NOX-2 protein; and the aorta morphology, compared with control.
The soybean diet attenuates the redox changes and protects against morphological alterations induced, in a dose-dependent way, by Cd in aorta.

Introduction

Cadmium (Cd) is among the most toxic pollutants, being widely distributed in the environment. Each year the EPA (Environment Protection Agency) lists a number of inorganic substances of high environmental impact. This list is led by metals such as lead (CASRN 7439-92-1), arsenic (CASRN 7440-43-9) and cadmium (CASRN 7440-43-9). High level exposure to Cd is usually the result of environmental contamination from human activities, such as mining, smelting, fossil fuel combustion and industrial use (Nordberg, 1972). In addition, it has been well established that Cd is one of the major contaminants in tobacco smoke (Li et al., 2000).

Gastrointestinal ingestion of Cd, through food and drinking water, is a major route of intake in non-smoking and non-occupationally exposed populations (ATSDR, 1999). It has been demonstrated, in humans and animals, that soluble Cd^{2+} salts accumulate and injure several tissues, including kidney (Renugadevi and Prabu, 2008), liver (Larregle et al., 2008), brain, lung (Luchese et al., 2007), adenohypophysis (Calderoni et al., 2010), prostate (Alvarez et al., 2006), and heart (Manna et al., 2008; Soares et al., 2007), depending on exposure time and dose. Some studies have attempted to correlate environmental Cd exposure with lung (Nawrot et al., 2006), kidney and prostate cancer (Järup, 2003; Satoh et al., 2002; Waalkes, 2003). Furthermore, the vascular endothelium has been suggested as a critical target of Cd toxicity, leading to many cardiovascular complications such as hypertension, atherosclerosis and cardiomyopathy (Prozialeck et al., 2006). Considerable evidence suggests that the hypertensive effect of Cd exposure results from complex actions on both, the vascular endothelium and vascular smooth muscle cells (VSMCs) (Prozialeck et al., 2008). Cd-fed ApoE −/− mice has been shown to exhibit a substantial increase of aortic plaque surface area (Knoflach et al., 2011). Blood and urinary Cd has been associated with peripheral arterial disease in a representative sample of the U.S. population (Selvin and Erlinger, 2004; Navas-Acien et al., 2005). Cadmium exposure has also been associated with future peripheral artery disease, supporting the concept that Cd exposure in the population has proatherogenic effects (Fagerberg et al., 2012).

It is known that Cd increases oxidative stress, affecting antioxidant enzyme activities (Ognjanović et al., 2008; Sinha et al., 2008). Cadmium could replace iron and copper in a number of cytoplasmic and membrane proteins, like ferritin, which in turn releases and increases the concentration of unbound iron or copper ions. These free ions participate in

* Corresponding author at: Department of Biochemistry and Biological Sciences, Chacabuco 917, National University of San Luis 5700, San Luis, Argentina. Fax: +54 266 4431301.
E-mail address: marisofigime44@gmail.com (M.S. Gimenez).

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the Fenton reaction, generating reactive oxygen species (ROS) (Flora et al., 2008). The production of ROS and reactive nitrogen species (RNS), appears to be a relevant mechanism for Cd toxic effects in many tissues and organs, including cardiovascular system (Beyersmann and Hartwig, 2008; Stoths and Bagchi, 1995; Waisberg et al., 2003), which can be diminished by the presence of oxygen radical scavengers. The increased ROS production induced by Cd, can trigger lipid peroxidation, DNA damage and oxidative modifications of proteins, which can eventually lead to cellular dysfunction and necrotic cell death (Thévenod, 2009; Valko et al., 2007).

NADPH oxidase (NOX), is a multisubunit enzyme that catalyzes the reduction of molecular oxygen to form superoxide ($O_2^{\bullet -}$). Gp91phox, also known as NOX2, is the NADPH oxidase prototype and emerges as a major source of $O_2^{\bullet -}$ in vascular cells and myocytes (Griendling et al., 2000; Kim et al., 2005). The first line of defenses towards $O_2^{\bullet -}$ and H_2O_2 mediated injury, are antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Helmut, 1997). These enzymes, among others, are regulated by NF-E2-related nuclear factors (Nrf1 and Nrf2), which bind to the antioxidant response element (ARE) and regulate ARE-mediated gene expression and induction (Leonard et al., 2006; Thimmulappa et al., 2002).

Increased oxidative stress and endothelial dysfunction have been suggested to be risk factors for hypertension and atherosclerosis (Förstermann, 2008). Several autopsy studies have found association between tissue lead or Cd levels and atherosclerotic lesions (Aalbers and Houtman, 1985; Voors et al., 1982). Also, it has been proposed that Cd may partially mediate the effect of smoking on peripheral arterial disease in the general US population (Navas-Acien et al., 2004). However, the biochemical and molecular bases underlying Cd intoxication in the mammalian arteries have not been fully elucidated.

Moreover, soy proteins are becoming increasingly important in the human diet. Among the beneficial health effects, they have been described to: lower cholesterol and LDL cholesterol (Borodin et al., 2009), prevent heart disease, reduce weight in obesity (von Post-Skagegard et al., 2006; Zemel et al., 2010) and protect against breast and prostate cancer (Friedman and Brandon, 2001). Evidence suggests that a diet rich in soy protein and your isoflavone, has a scavenging activity and antioxidant properties, and can inhibit lipoprotein oxidation and reduce the incidence of coronary heart disease (Anderson et al., 1995; Clarkson, 2002; Lissin and Cooke, 2000; Tikkanen and Adlercreutz, 2000).

Drinking water containing 15 ppm of Cd^{2+} for 2 months, has been previously used in our laboratory (Calderoni et al., 2010; Larregle et al., 2008) to intoxicate rats and attain serum Cd^{2+} concentrations up to the World Health Organization (WHO) toxic limit. Furthermore, it has been shown that higher Cd doses (50 ppm or 200 ppm in drinking water for 3 months), modified the vascular reactivity of an isolated and perfused rat mesenteric bed (Skoczynska and Martynowicz, 2005). Thus, the purpose of this study was to chronically expose rats, via drinking water, to two different Cd doses (15 and 100 ppm of Cd^{2+}) for two months, to evaluate in the aorta: (1) the prooxidant effects of Cd on the activity and expression of antioxidant enzymes, (2) the relation between possible aorta structural alterations and Cd serum levels and, (3) to investigate the protective role of soybeans, as the diet protein source, against the possible Cd toxic effects.

Materials and methods

Diet and experimental design. Adult male Wistar rats, weighing 180–200 g at the onset of treatment, were used. They were bred in our animal facilities (National University of San Luis, San Luis, Argentina). The experimental protocols were approved by the Committee for Care and Use of Laboratory Animals of the National University of San Luis, and were in accordance with the Rat Care and Treatment Recommended Guidelines (U.S. Public Health Service, 1985). Animals were handled under standard laboratory conditions of 12 h light/12 h dark cycles in a temperature and humidity controlled room.

Rats were given free access to food and water throughout the entire experimental period. Rats were randomly divided into 6 groups of 6 animals each. Regarding the protein source, 3 groups were fed with a diet containing casein and the other 3 with a diet containing soybeans as the dietary protein.

Within the groups fed with each protein, one was given tap water (control) and the other two tap water containing 15 and 100 ppm of Cd^{2+}, (as $CdCl_2$), respectively. Rats were euthanized after 2 months of treatment.

Since ingestion is the most important route of Cd human exposure, drinking water was chosen as the exposure carrier. The Cd concentrations and exposure time were consistent with previous studies (Calderoni et al., 2010; Larregle et al., 2008; Skoczynska and Martynowicz, 2005; Thijssen et al., 2007). Furthermore, exposure to 100 ppm of Cd^{2+} was reported as environmentally realistic (Thijssen et al., 2007).

Casein and soybean diets were prepared according to AIN-93M-CAS and AIN-3M-SOY (Reeves, 1996) for laboratory rodents, respectively. No Cd was detected in the control rat drinking water. After treatment, rats were fasted overnight and euthanized by decapitation at 09:00 h. Immediately after, trunk blood samples were collected for serum separation.

The thoracic aorta was quickly excised, washed several times with ice-cold isotonic saline solution and cleaned to remove the surrounding tissue. Afterwards, aorta samples were placed in liquid nitrogen for storage. Analyses were carried out within 1–2 weeks of obtaining the samples. Additionally, enzyme determinations were performed in fresh tissues.

Serum cadmium determination. Cd in serum and aorta was determined by electrothermal atomic absorption spectrometry, using a Perkin Elmer Analyst 200 Gf, equipped with a graphite tube with a L'vov platform, with DL 0.001 μg/l and QL 0.01 μg/l (detection and quantification limits of 1 ppt and 10 ppt, respectively). A matrix modifier was used (ammonium phosphate–ammonium nitrate). The calibration curve was made with an aqueous Cd standard, to which a tensoactive agent and matrix modifier were added, in a range of 0.5 to 5 μg/l; MLD: 0.035 μg/l (Imbus, 1963). Validation was carried out on a synthetic sample (cow liver homogenate), with the addition of a standard Cd solution, traceable to standard reference material from NIST, following method 200.0 revision 1.2 4/91 protocol. Cd recovery was about 98–99%. Samples DL and QL were 0.01 and 0.1 μg/l, respectively.

Thiobarbituric acid reactive substance (TBARS) determination. Aorta lipid peroxidation levels (assessed as thiobarbituric acid reactive substances, TBARS), were measured spectrophotometrically, according to Draper and Hadley (1990). Briefly, aorta tissue was homogenized in 120 mM KCl and 30 mM phosphate buffer, pH 7.4. Proteins were precipitated with 20% tricholoroacetic acid (TCA, Sigma-Aldrich Co.) and a supernatant containing malondialdehyde (MDA), the end product of the lipid peroxidation, was incubated with a 0.7% thiobarbituric acid solution (TBA, Sigma-Aldrich Co.) to measure the TBARS content. An acid hydrolysis product of 1,1,3,3-tetramethoxy propane (TMP) was used as standard. Aorta TBARS were expressed as nmol of MDA/mg protein.

Antioxidant enzyme activities. In order to process the thoracic aorta for determination of the antioxidant enzyme activity (20 mg of wet weight), it was homogenized in 30 mM PBS buffer, with 120 mM KCl, pH 7.4, containing 1× protease inhibitors (Pepstatin A and PMSF) and 50× Triton, followed by centrifugation at 3000 rpm, for 30 min at 4 ºC. The pellet was discarded and the supernatant was used as homogenate (Gonzales-Flecha et al., 1991). The enzyme determinations were performed immediately after. CAT activity was determined by measuring the decrease in absorption, at 240 nm, of H_2O_2 decomposition (Chance et al., 1979). The results were expressed as units per milligram of protein (U/mg protein). One CAT unit is defined as the amount of enzyme required to decompose

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