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Involvement of selenoprotein P and GPx4 gene expression in cadmium-induced testicular pathophysiology in rat

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ABSTRACT

To investigate the effect of co-exposure to cadmium (Cd) and selenium (Se) on selenoprotein P (SelP) and phospholipid hydroperoxide glutathione peroxidase (GPx4) gene expression in testis and to evaluate their possible involvement in Cd-induced testicular pathophysiology, male rats received either tap water, Cd or Cd + Se in their drinking water for 5 weeks. Cd exposure caused a down-regulation of SelP and GPx4 gene expression and a significant decrease in plasma and testicular concentrations of Se. These changes were accompanied by decreased plasma testosterone level, sperm count and motility, GSH content, protein-bound sulfhydryl concentration (PSH), enzymatic activities of catalase (CAT) and glutathione peroxidase (GSH-Px) as well as by increased glutathione-S-transferase (GST) activity, lipid peroxidation (as malondialdehyde, MDA) and proteins carbonyls (PC). The decrease of testicular SelP and GPx4 gene expression under Cd influence was significantly restored in Cd + Se group. Co-treatment with Cd and Se also totally reversed the Cd-induced depletion of Se, decrease in plasma testosterone level and partially restored Cd-induced oxidative stress and decrease in sperm count and motility. Taken together, these data suggest that down-regulation of SelP and GPx4 gene expression induces plasma and testicular Se depletion leading, at least in part, to Cd-induced testicular pathophysiology.

1. Introduction

Cadmium (Cd) is regarded as one of the most toxic heavy metal elements. Acute and chronic Cd toxicity is associated with severe damage in various organs, particularly the testes, in both humans and animals [1,2]. Cd exposure can lead to testosterone suppression [3,4] and reduced sperm motility [5]. Although the testicular damage induced by Cd was recognized decades ago [6], the precise mechanisms underlying its toxicity to the testes remained unclear.

Selenium (Se), an essential trace element, is a potent antioxidant and is required for maintenance of male reproduction [7,8]. In experimental mammals, moderate to severe Se deficiency reduces testicular mass and morphology and causes flagella defects in sperms [9]. The glutathione peroxidase (GSH-Px) enzyme activity, levels of serum follicle-stimulating hormone, luteinizing hormone, testosterone and male fertility were also greatly reduced in testes of Se-deficient animals [7,9].

The function of Se is believed to be mediated through selenoproteins that contain Se as a selenocysteine residue. To date, several selenoproteins are known, such as selenoprotein P (SelP) and phospholipid hydroperoxide glutathione peroxidase (GPx4). SelP is involved in the distribution of Se to tissues [10] and maintaining Se homeostasis in the body [11]. Deletion of the SelP gene was associated with a decrease in Se concentrations in tissues [12]. The mRNA that encodes SelP was selectively expressed by Leydig cell, suggesting that SelP participated in testosterone production [13]. The high expression of GPx4 in the testis underlines the relevance of this gene to spermatogenesis. Deficiency of GPx4 is considered one of the causes of male infertility [14–16]. In hypophysectomized rats and in testosterone-deprived rats, testicular GPx4 activity and mRNA content were significantly decreased and partially restored by human chorionic gonadotropin or testosterone administration, respectively [9,17].

It is well known that many of the toxic effects of Cd result from interactions with essential elements such as Se. Yiin et al. [18] have reported that Cd exposure decreased the testicular Se concentration through undefined mechanisms. On the other hand, treatment with Se during Cd exposure has been demonstrated to have protective effects on Cd-induced toxicity in various organs including testes [18–23]; however the exact mechanism behind this protective effect remains largely unexplored. Although molecular studies have indicated that aberrant gene expression can be an important factor in Cd-induced toxicity, no information about Cd-induced changes in selenoproteins gene expression, such as SelP and GPx4

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genes, in the testes are available. Therefore, it was considered of interest to investigate the effect of orally co-exposure to Cd and Se on SelP and GPx4 gene expression in testis and to evaluate their possible involvement in Cd-induced testicular pathophysiology as well as in the protective effect of Se against Cd toxicity.

2. Materials and methods

2.1. Animals and experimental design

Twenty four 10-week-old male Wistar rats and weighing 222 ± 23 g were divided into three experimental groups of eight animals each: the control group was not treated with Cd and the remaining two experimental groups received 200 μg Cd/L (as $CdCl_2$) or 200 μg Cd/L + 0.1 μg Se/L (as Na_2SeO_3), in their drinking water for 5 weeks. Exposure duration, Cd and Se doses as well as the manner of administration were chosen on the basis of our previous reports in which we have demonstrated the effectiveness of Se in alleviating Cd-induced organ pathologies in rats treated with the same experimental protocol used in the current work [24–26]. The animals were housed, according to the EEC 609/86 Directives regulating the welfare of experimental animals, in individual stainless steel cages at 23 ± 1 °C and exposed to 12-h light–dark cycle. They had access to a standard rodent laboratory diet (SICO, Sfax, Tunisia) and drinking water *ad libitum*. Concentrations of Cd and Se in the diet are, respectively, 3 and 5 (10^{-5} μg/kg dry weight). To evaluate the daily intakes of Cd and Se in each of the experimental groups and express them as ppm/kg b.w., the 24-h consumption of drinking water and body weight were monitored during the whole experiment. Drinking water consumption and daily Cd intake were investigated according to the method described by Brzoska and Moniuszko-Jakoniuk [27].

On the last day of experimentation, final body weight of each animal was recorded and rats were killed under anesthesia. Blood was taken by cardiac puncture and was immediately collected into heparinized tubes. Plasma samples, for testosterone, Cd and Se analysis, were obtained by centrifugation at 5000 rpm for 5 min at 4 °C. The testes were excised, cleared of adhering connective tissues, and weighed for relative testis weight (RTW) determination. The RTW was expressed in grams per 100 g body weight. The left testis was flash frozen in liquid nitrogen and stored at −80 °C until oxidative stress markers determination as well as for Cd and Se estimation. The right testis was kept in RNA later solution (Sigma–Aldrich, St. Louis, USA) at −80 °C until molecular analysis.

2.2. Analytical procedures

2.2.1. Measurement of cadmium and selenium concentrations

Testicular slices destined for Cd and Se determination were oven-dried (60 °C) to constant weight. Dried tissues and plasma samples were digested with concentrated nitric acid (Merck, 65%) at 120 °C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 5 mL with ultra-pure water. All samples were analyzed to determine Se and Cd concentrations by Graphite-Furnace atomic absorption spectrophotometry. These measures were implemented using a ZEEnit 700-Analytik-Jena (Germany) equipped with deuterium and Zeeman background correction, respectively, as recommended by the manufacturer. Samples were analyzed in triplicate and the variation coefficient was usually less than 10%. Concentrations of the trace elements were expressed as micrograms per gram dry weight in testis and as micrograms per liter in plasma.

2.2.2. Epididymal sperm concentration and motility

Epididymal sperm count was evaluated by the method of Linder et al. [28]. Accordingly, epididymal spermatozoa were obtained by mincing the epididymis with anatomical scissors in 5 mL of physiological saline and incubated at 32 °C for 2 min. An aliquot of this solution was placed into a Malessez cells and motile sperm were counted by using microscope at 400× magnification. Non-motile sperm numbers were first determined, followed by counting of total sperm. Sperm motility was expressed as a percent of motile sperm of the total sperm counted.

2.2.3. Testosterone determination

Plasma testosterone levels were measured by electrochemiluminescence by automat (Elecsys, Rochediagnostics) according to the method of Wheeler [29]. Briefly, the Elecsys testosterone assay is based on a competitive test principle using a monoclonal antibody specifically directed against testosterone. Endogenous testosterone released from the sample by ANS (8-anilino-1-naphtalene sulfonic acid) and norgestrel competes with the added testosterone derivative labeled with a ruthenium complex for the binding sites on the biotinylated antibody.

2.2.4. Measurement of antioxidant enzymes activities

Testicular slices were homogenized in cold sodium phosphate buffer (pH 7.4) containing 1 mM EDTA. The homogenates were then centrifuged at 4000 rpm for 15 min at 4 °C. The supernatants were separated and used for enzyme assays and protein determination.

CAT activity was determined using the method described by Beers and Sizers [30] by measuring hydrogen peroxide decomposition at 240 nm, while GSH-Px activity was assayed by the subsequent oxidation of NADPH at 240 nm with t-butyl-hydroperoxide as substrate [31]. Testicular glutathione-S-transferase (GST) activity was assayed through the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Habig et al. [32]. The protein content in supernatant was estimated by the Bradford method using bovine serum albumin as standard [33].

2.2.5. Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content according to the method of Buege and Aust [34].

2.2.6. Determination of total glutathione, protein-bound sulfhydryls and protein carbonyl contents in testis

Protein-bound sulfhydryl concentration (PSH) was determined by the method of Sedlack and Lindsay [35] by subtracting the non-protein sulfhydryl (NPSH) content from the total sulfhydryl content (TSH). TSH in the testis homogenate was measured after the reaction with dithionitrobisbenzoic acid (DTNB) using the method of Ellman [36]. NPSH content of the testis was assayed by treating the sample with 10% trichloroacetic acid (TCA) and then centrifuged. The clear supernatant was treated with DTNB. The total GSH content of the testis was estimated as the NPSH content according to the method of Ellman [36].

As a hallmark of protein oxidation, total protein carbonyl content (PC) was measured by the quantification of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) as described by Levine et al. [37]. Briefly, proteins were precipitated by the addition of 20% TCA and redissolved in DNPH and the absorbance was read at 370 nm.

2.2.7. Quantitative real-time polymerase chain reaction

2.2.7.1. RNA isolation and cDNA synthesis. Total RNA was extracted from about 10 mg frozen testis tissues using the Trizol reagent