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The protection of selenium on cadmium-induced inhibition of spermatogenesis via activating testosterone synthesis in mice

Xiang-mei Ren [a,b], Gai-gai Wang [c], Dong-qing Xu [d], Kang Luo [a], Yu-xin Liu [a], Yi-hong Zhong [a], Yun-qing Cai [a,*]

[a] Department of Nutrition and Food Hygiene, School of Public Health, Nanjing Medical University, China
[b] Department of Nutrition, School of Public Health, Xuzhou Medical College, China
[c] State Key Laboratory of Reproductive Medicine, Nanjing Medical University, China
[d] The Pre-Clinical Medicine College, Nanjing University of Chinese Medicine, China

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ABSTRACT

Selenium (Se) is an essential trance element in testis. However, the potential protective effects of Se against cadmium (Cd)-induced reproductive toxicity remained to be elucidated. Male ICR mice were orally administered by gavage with Na_2SeO_3 (0.1, 0.2, 0.4 mg/kg BW) for 1 h prior to $CdCl_2$ (5 mg/kg BW) alone or in combination for 15, 25 or 35 days. Cd exposure caused a significant decrease in body weight, sperm concentration and motility as well as plasma testosterone level which was accompanied by decreased antioxidant enzymatic activity of SOD and GSH-Px and by increased lipid peroxidation (as malondialdehyde, MDA). Se pretreatment compensated deficits in the sperm parameters (concentration, motility and morphology) induced by Cd. Se (0.4 mg/kg BW) treatment significantly increased serum testosterone level that was reduced by Cd (on 15th, 25th and 35th day) ($P < 0.01$). Se treatment ameliorated Cd-induced reduction in testicular steroidogenic acute regulatory (StAR) and 17β-hydroxysteroid dehydrogenase (17β-HSD) activities. The present study suggest that the protective potential of Se against Cd-induced reprotoxicity might be due to up-regulation StAR and testosterone synthetic enzyme activity, which could be useful for increasing testosterone synthesis for achieving optimum protection in sperm quality and spermatogenesis.

1. Introduction

Cadmium (Cd) is a heavy metal and a ubiquitous environmental toxicant (IARC, 1993). The general population exposure to Cd predominantly results from smoking air pollution and consumption of Cd contaminated foods and water (Honda et al., 2010; Satarug et al., 2003), while occupational exposure to Cd usually takes place during mining or manufacturing of batteries and pigments that utilized Cd (Bernard, 2008; Satarug et al., 2010). Cd has an extremely long biological half-life of 20–40 years in humans (Klaassen et al., 2009) that essentially makes it an accumulative toxin mostly in the kidneys and the liver (Renugadevi and Prabu, 2009), however, its toxic effects extend to other target organs as the testes, prostate and bladder (Thompson and Bannigan, 2008; Valko et al., 2005).

Cd is acknowledged as a reproductive toxicant in humans (Cheng et al., 2011; Jurasovic et al., 2004). Increased evidence demonstrated that environmental exposure to Cd is associated with the poor semen quality and male infertility (Benoff et al., 2009). Recent studies indicated that even a low level of Cd accumulation in semen might contribute to male infertility by reducing sperm quality and sperm oxidative damage (Wu et al., 2008; Xu et al., 2003). Animal studies showed that Cd induces testicular lesions, with rodents being particularly affected (Siu et al., 2009). Cd intoxication has been reported to cause degeneration of spermatogenic and Leydig cells. In addition, several studies have demonstrated that treatment with Cd caused a significant decrease in sperm concentration, weight of testes and epididymis, and increase in dead and abnormal sperm (Monsefi et al., 2010; Oliveira et al., 2009). Androgens, primarily testosterone (T), are essential for adult mammalian spermatogenesis. Cd, as a well-known endocrine disrupting chemical, is not only a regulator of hypothalamus and pituitary hormone secretion, but also disrupts testicular testosterone production (Ji et al., 2010; Yeung et al., 2011). In vitro studies observed a decreased testosterone secretion in Cd-treated primary rat Leydig cells (Yang et al., 2003). Although Cd-mediated testicular toxicity was recognized decades ago, previous studies mainly focused on the histological and ultrastructural effects of Cd in the testis

* Corresponding author. Address: Department of Nutrition and Food Hygiene, School of Public Health, Nanjing Medical University, #140 Hanzhong Road, Nanjing 210029, China. Tel.: +86 25 86662941; fax: +86 25 86662930.
E-mail address: cai2941@163.com (Y.-q. Cai).

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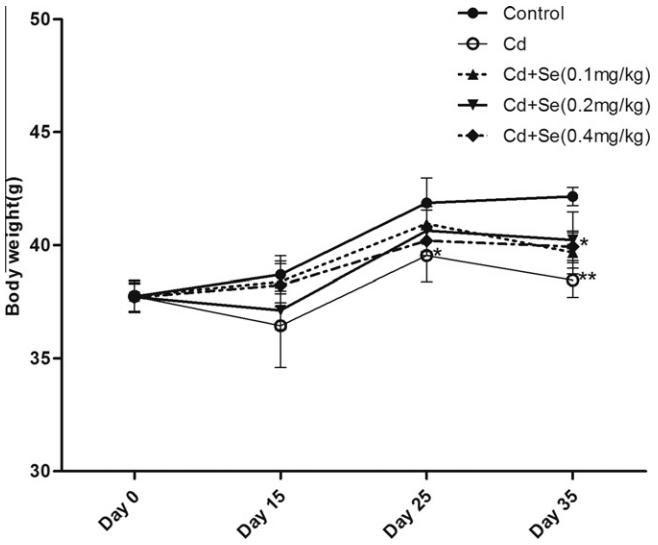

Fig. 1. Effects of Se on Cd-induced changes in body weight. Mice were exposed to Cd (5 mg/kg) or Cd + Se (0.1, 0.2, 0.4 mg/kg) for 15, 25 or 35 days. Data are expressed as means ± SD of 6 mice in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control groups, using one-way ANOVA.

(Ji et al., 2011). Studies have been equivocal about the effects of Cd administration on sperm quality, testicular spermatogenesis and steroidogenesis.

Oxidative stress has been considered a primary initiating mechanism during Cd-induced testicular damage (Manna et al., 2008; Siu et al., 2009). Several antioxidants were found to be effective in minimizing Cd-induced organ damages, including the testes with restoration of testicular spermatogenesis (Acharya et al., 2008; Ognjanovic et al., 2010). Selenium (Se), an important antioxidant nutrient, is essential for normal testicular development, spermatogenesis, spermatozoa motility and functions (Messaoudi et al., 2010). Some studies showed that Se supplementation in subfertile men with low Se status could improve sperm motility and increase the chance of successful conception (Ursini et al., 1999). Se has also been demonstrated to have the protective effects against the toxicity of metals in the male reproductive system of experimental animals (Said et al., 2010). In spite of some studies on the effects of Se on spermatogenesis on rodent testis, little is known about the effects of Se on testicular testosterone synthesis. In addition, to the best of our knowledge, no comprehensive study concerning the protective effect of Se on Cd-induced inhibition of spermatogensis and reduced steroidogenesis.

Our previous in vitro studies showed that Cd can induce apoptosis in LLC-PK1 cells via increasing cellular reactive oxygen species (ROS) production, and that Se has a protective effect against Cd cytotoxicity through the c-jun N-terminal kinase (JNK) phosphorylation activation and blocking ROS generation to restore the mitochondrial membrane potential collapse (Liu et al., 2007; Zhou et al., 2009). However, no comprehensive in vivo study concerning the protective effects of Se on Cd-induced impairment of testicular functions has been performed yet. Taking the above in account, the present study was carried out to evaluate the protective effects of Se on sperm quality, testicular spermatogenesis and steroidogenesis in mice exposed to Cd (as cadmium chloride). For this purpose, three concentrations of Se were tested and the effects on sperm parameters and serum testosterone levels were assessed at three experimental time points after treatment with Cd.

2. Materials and methods

2.1. Chemicals

Cadmium chloride ($CdCl_2$, purity ⩾ 99%) and Sodium selenite (Na_2SeO_3, purity ⩾ 98%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and experimental design

Male ICR mice were purchased from Shanghai SLAC laboratory animal Co., Ltd., Shanghai, China, at 6 weeks of age. All animals were housed in polycarbonate cages in an environmentally controlled room (temperature: 20–24 °C, relative humidity: 50% ± 10%, frequent ventilation and 12 h light cycle). The animals were acclimated to the laboratory for 1 week prior to the start of the experiments. They were fed with commercial standard pellet diet (Beijing Keao Ltd., China) and tap water *ad libitum* throughout the experimental period. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Nanjing Medical University.

For experimental treatments, $CdCl_2$ and Na_2SeO_3 were dissolved in ultrapure water. Total 90 animals were randomly divided into five groups of 18 animals in each group. GroupI normal mice orally received only the vehicle. GroupII: mice orally received Cd (as $CdCl_2$) at a dose of 5 mg/kg BW. GroupIII mice orally received Se (as Na_2SeO_3) at a dose of 0.1 mg/kg BW plus Cd 5 mg/kg BW. GroupIV: mice orally received Se 0.2 mg/kg BW plus Cd 5 mg/kg BW. Group V: mice orally received Se 0.4 mg/kg BW plus Cd 5 mg/kg BW. Mice were given daily gavages administration with Se for 1 h prior to the addition of Cd. Mice were euthanized by cervical dislocation on the 15th, 25th and 35th day after gavage ($n = 6$ per group and per period). A test dose of 5 mg/kg $CdCl_2$ was selected based on prior knowledge of testicular injury (Bu et al., 2011), and supplementation of selenium is based on the Chinese Dietary Reference Intakes (DRIs) and non-toxic in rodents (Nehru and Bansal, 1997).

2.3. Antioxidant enzymes activities and lipid peroxidation assessments

Serum SOD activity was assayed using pyrogallol as a substrate according to the method described by Marklund and Marklund (1974). One unit (U) of total SOD is defined as the amount of enzyme required to inhibit the rate of pyrogallol. GSH-Px activity was determined by the subsequent oxidation of NADPH at 412 nm with t-butylhydroperoxide as substrate (Gunzler et al., 1974). Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content according to the method described by Kaushal and Bansal with minor changes (Kaushal and Bansal, 2007).

2.4. Sperm functional parameters

2.4.1. Sperm morphology and sperm concentration

Sperm samples were collected as described by Elangovan et al. with minor changes (Elangovan et al., 2006). Cauda epididymis was removed and trimmed free of fat, and was placed in conical tubes containing HTF medium (Invitrogen, MD, USA) supplement with 10% fetal bovine serum. The HTF medium contained NaCl (90 mM), KCl (5 mM), $CaCl_2{\cdot}2H_2O$ (1.8 mM), $MgSO_4{\cdot}7H_2O$ (1 mM), $NaHCO_3$ (25 mM), glucose (6 mM), sodium lactate (10 mM), sodium pyruvate (0.27 mM), KH_2PO_4 (1.2 mM), Hepes (20 mM), penicillin (100 IU/ml) and streptomycin (100 IU/ml). Two or three deep cuts were made along the proximal and distal cauda

Table 1
Effects of Se on Cd-induced changes in serum SOD and GSH-Px activity as well as MDA concentration (35 days).

Experimental groups	Control	Cd (5 mg/kg)	Cd + Se (0.1 mg/kg)	Cd + Se (0.2 mg/kg)	Cd + Se (0.4 mg/kg)
SOD(u/ml)	167.03 ± 13.88$^{\#\#}$	136.49 ± 14.01**	152.32 ± 6.61$^{*\#}$	156.26 ± 5.61$^{\#}$	158.73 ± 14.70$^{\#\#}$
GSH-Px(u)	579.80 ± 46.16$^{\#}$	489.88 ± 64.30*	559.41 ± 56.36	544.41 ± 56.27	562.35 ± 40.91
MDA(nmol/ml)	5.79 ± 0.36$^{\#\#}$	7.21 ± 0.42**	5.95 ± 0.53$^{\#\#}$	6.05 ± 0.59$^{\#\#}$	5.72 ± 0.67$^{\#\#}$

Data represent the means ± SD of 6 animals per group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control groups; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with Cd-treated groups, using one-way ANOVA.