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Short communication

Sub-chronic administration of diphenyl diselenide potentiates cadmium-induced testicular damage in mice

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Abstract

Sub-chronic cadmium (Cd) exposure causes testicular damage in mice. The mode of action may involve oxidative stress and especially lipid peroxidation. The present study has monitored the pathogenesis of testicular damage during sub-chronic Cd exposure and has evaluated the potential protective effect of antioxidant therapy with diphenyl diselenide $(PhSe)_2$. Male mice were dosed with 2.5 mg/kg $CdCl_2$ (2.5 mg/kg) with or without $(PhSe)_2$ (5 μmol/kg) at 30 min post-exposure using a model of five weekly subcutaneous injections. Histological evaluation of the testis was performed across a 4 week test period. Animals exposed to $CdCl_2$ and $CdCl_2$ plus $(PhSe)_2$ displayed a reduction in body weight gain and testicular weight. Progressive damage and histolopathological changes in the testis were not remediated with, but rather were potentiated by, $(PhSe)_2$ therapy. We conclude that $(PhSe)_2$ enhances testicular injury in an animal model for sub-chronic Cd exposure mice.

Keywords: Antioxidant; Cadmium; Selenium; Testicular toxicity; Mice

1. Introduction

Cadmium (Cd) is an important heavy metal widely used in batteries, metal plating, pigments, plastics, and alloys. In addition to occupational exposures, environmental Cd exposure in humans may occur through cigarette smoking and dietary consumption [1,2]. It has long been known from studies in rodents that testicular tissue is a sensitive target to Cd toxicity [3]. In experimental models, Cd exposure can affect testis weight and induce pathogenesis leading to reduced sperm counts and impaired sperm motility to adversely affect male fertility [4–6]. Lipid peroxidation has been considered a primary initiating mechanism during Cd injury [6–8]. Therefore, antioxidant therapy could be important for treatment of Cd poisoning.

Various natural and synthetic substances possessing antioxidant properties should be investigated as to the possible protective effects on Cd-induced tissue damage. Among them are selenium (Se) compounds. Se is as an essential trace element with important roles in normal growth, development, and metabolism [9–13]. Se compounds have antioxidant properties and may counter the effects of cellular injury caused by free radicals and oxidative stress [14,15]. Recent studies have shown the diaryl diselenides to act as potent antioxidants in mice [16,17], and a variety of organo-selenium compounds are being considered for their pharmacological properties [17–20].

Recently, we demonstrated the efficacy of diphenyl diselenide $(PhSe)_2$ towards reversal of acute testicular injury in male mice exposed to Cd [6,21]. The efficacy was similar to the chelating compounds succimer [6] and 2,3-dimercapto-1-propanesulfonic acid [21]. In the present study, we have monitored the potential efficacy of $(PhSe)_2$ therapy toward testicular injury associated with sub-chronic Cd exposure.

2. Materials and methods

2.1. Chemicals

$CdCl_2$ was purchased from Sigma (St. Louis, MO, USA). $(PhSe)_2$ was synthesized as described previously [22]. Analysis of the ^1H NMR and ^{13}C NMR

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Table 1
Effect of sub-chronic exposure with cadmium and $(PhSe)_2$ on body weight gain

Group	1st week	2nd week	3rd week	4th week
I. Control	1.86 ± 0.54	0.68 ± 0.58	1.66 ± 0.41	1.34 ± 0.42
II. $(PhSe)_2$	0.32 ± 0.58*	0.08 ± 0.51	1.34 ± 0.61	1.60 ± 0.54
III. Cd	−0.88 ± 0.54*	−0.80 ± 0.50*	−0.26 ± 1.05*	−0.26 ± 0.91*
IV. Cd + $(PhSe)_2$	−1.25 ± 1.69*	−0.22 ± 0.70*	−0.13 ± 0.51*	−0.23 ± 0.38*

Data are mean ± S.D. from six to eight animals per group for each week.
* Denoted $p < 0.001$ as compared to the control group (one-way ANOVA/Duncan).

spectra showed analytical and spectroscopic data in full agreement with its assigned structure. Chemical purity (99.9%) was confirmed by GC/HPLC. All other reagents were of analytical grade and obtained from standard commercial suppliers.

2.2. Animals and exposures

Male adult Swiss albino mice (25–35 g) from an in-house breeding colony were used in these studies. The animals were housed in separate room and kept on a 12 h light:12 h dark cycle, at room temperature, with access to food and water ad libitum. The animal protocols were approved by the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil. A total of 112 animals were used for the research described here.

For experimental treatments, $CdCl_2$ was dissolved in saline (0.25 mg/ml) and $(PhSe)_2$ was dissolved in dimethylsulfoxide (DMSO). The animals were divided into four treatment groups: Group I received saline + DMSO; Group II received saline + $(PhSe)_2$; Group III received $CdCl_2$ + DMSO; and Group IV received $CdCl_2$ + $(PhSe)_2$. Mice were injected subcutaneously with the respective test agent or vehicle five times weekly for a test period of up to 4 weeks. The DMSO or $(PhSe)_2$ treatment was administered 30 min after the saline or $CdCl_2$ exposure. A test dose of 2.5 mg/kg $CdCl_2$ was selected based on prior knowledge of testicular injury [23], and 5 μmol/kg $(PhSe)_2$ was previously shown to be effective and non-toxic in rodents [20].

2.3. Evaluations and assessments

To assess testicular injury, several animals ($n = 6$–8) per treatment group were anesthetized with ether and sacrificed by decapitation immediately following the 1st, 2nd, 3rd, or 4th weeks of treatment. Body weight gain was continuously monitored during the study period and absolute testicular weight was determined at autopsy to assess the relative testis weight/body weight ratio. Data are expressed as mean ± S.D. and statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test when appropriate. Values of $p < 0.05$ were considered statistically significant. For histological evaluation, one testis from each animal at autopsy was fixed in Bouin's solution for 7 days. The Bouin's-fixed testis was embedded in paraffin and sectioned at 5 μm for routine hematoxylin and eosin staining and light microscopy.

3. Results

3.1. Body weight gain and ratio of testis weight/body weight

Mice exposed to $CdCl_2$ (Group III) or $CdCl_2$ + $(PhSe)_2$ (Group IV) showed a significant reduction in the body weight gain across the 4-week test period (Table 1). Treatment with $(PhSe)_2$ alone (Group II) caused a decrease in the body weight gain in the animals after the 1st week only (Table 1). The relative testis weight, assessed by the ratio of testis weight/body weight, was reduced by Cd exposure (Groups III and IV) at all weeks of the study, irrespective of Se (Table 2). Therefore, the results of this study do not show evidence for intervention of Cd-induced testicular injury by $(PhSe)_2$.

3.2. Histopathological findings

Histological observation of the control (Group I) testis showed normal features of spermatogenesis with spermatogenic cells (Fig. 1). After 1 week in mice treated with Cd (Group III), we observed coagulative necrosis of seminiferous tubule cells, as well as cytoplasmic acidophilia, nuclear pyknosis, multifocal vascular necrosis, and fibrin in the interstitium (Fig. 2). We assessed the type of vascular necrosis observed here as fibrinoid necrosis, an immune-mediated vascular damage marked by deposition of fibrin-like proteinaceous material along the arterial walls. The testis of Group III mice treated with Cd for 2-weeks showed severe necrosis and degeneration of seminiferous tubules, with partial loss of the spermatogenic cells and an associated relative increase of the interstitial tissue (Fig. 3). Few cells remained in the seminiferous tubules, in which the most evident were Sertoli cells. Calcium-like deposition in granular or plaque-like forms was observed in some seminiferous tubules

Table 2
Effect of sub-chronic exposure with cadmium and $(PhSe)_2$ on the ratio of testis weight/body weight

Group	1st week	2nd week	3rd week	4th week
I. Control	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001
II. $(PhSe)_2$	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.006 ± 0.001
III. Cd	0.004 ± 0.001*	0.003 ± 0.0002*	0.002 ± 0.001*	0.002 ± 0.0002*
IV. Cd + $(PhSe)_2$	0.004 ± 0.001*	0.004 ± 0.001*	0.003 ± 0.001*	0.002 ± 0.0004*

Data are mean ± S.D. from six to eight animals in each group for each week.
* Denoted $p < 0.001$ as compared to the control group (one-way ANOVA/Duncan).