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Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium

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Abstract

Cadmium (Cd) is a potential pollutant of the environment. It manifests cyto-toxic effects in different organs in animals. In the present study, intraperitoneal injection of $CdCl_2$ (1 mg/kg body weight) increased lipid peroxidation in Swiss mice testes indicating oxidative stress during 5th to 8th week of post-treatment .The enzymatic activity of superoxide dismutase (SOD), catalase (CT) and peroxidase (PD) were significantly decreased over the post-treatment phase in Cd-treated mice testes compared to vehicle controls. Further, ascorbic acid content also declined significantly in Cd-exposed mice testes. Following Cd treatment, a marked increase in sperm abnormality percentage and significant decrease in sperm count was observed. The purpose of the present study was to evaluate the effect of vitamins C and E supplementation on Cd-treated mice testes. Therefore, Cd-treated mice groups were injected with vitamins C and E, separately, to assess the effect of the vitamins in combating Cd-induced cytotoxicity and other manifestations. Supplementation of vitamin C (10 mg/kg body weight) and vitamin E (100 mg/kg body weight) to Cd-induced mice groups declined lipid peroxidation, increased sperm count profile, depressed the percentage of sperm abnormality, increased the activity of antioxidant enzymes mentioned above and also increased the concentration of ascorbic acid to a measurable extent. The role of vitamins in reducing oxidative stress-related effects on spermatogenesis in Cd-treated Swiss mice testes have been reported.

Keywords: Swiss mice; Testes; $CdCl_2$; Vitamin C; Vitamin E; Lipid peroxidation; Sperm count; Sperm abnormality; SOD; CT; PD

1. Introduction

Cadmium (Cd) is a ubiquitous environmental pollutant [1]. In addition to occupational exposure, the two main sources of non-occupational exposure to Cd are cigarette smoking and diet [2]. Cd exposure has been associated with adverse toxic effects in various target organs including the testes [3,4]. A number of cases from infertile or sterile subjects have been correlated with Cd pollution [5]. Tobacco smoke containing approximately 1–2 μg of Cd [6] reportedly causes reproductive impairments in smokers [7].

The molecular mechanism of Cd toxicity is incompletely understood. Studies showed that Cd may not generate reactive oxygen species (ROS) in tissues through the Harber–Weiss reaction [8] but can increase lipid peroxidation through generation of noxious radicals such as superoxide anion radicals, hydroxyl radicals, nitric oxide and hydrogen peroxide [9]. These radicals are transitory due to their high chemical reactivity and thus can stimulate lipid peroxidation and deleterious modification of complex lipoprotein assemblies in biomembranes and cellular dysfunction [9,10]. In the testis these changes may underlie the etiology of defective sperm formation, decreased sperm counts and male infertility [11].

Male germ cells are more susceptible to oxidative stress than somatic cells for several reasons. One is their close proximity to the free radical-generating, phagocytic Sertoli cell [12]. Another is the inclusion of higher polyunsaturated fatty acids in their membranes. To protect them from oxidative injury, testicular cells are well-equipped with enzymatic and low-molecular weight antioxidants to maintain redox homeostasis [13]. This defense includes superoxide dismutase (SOD), catalase (CT) and glutathione peroxidase (GPX) activities, glutathione and antioxidant vitamins that can scavenge ROS or prevent ROS formation. On the other hand, excessive ROS may over-ride antioxidant defenses or exceed scavenging ability of the antioxidant defense system leading to oxidative stress and permanent

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tissue injury. These systems provide surrogate measures of local oxidative stress.

Oxidative stress plays a critical role in the etiology of defective sperm formation, function, sperm count profile and male infertility [14]. In contrast, alterations in the antioxidant defense system and the possible role of the antioxidants [15] in the prevention of male infertility is controversial. Whereas several studies indicate the effect of Cd-induced oxidative stress on the testes only few monitored sperm counts and sperm abnormalities as endpoint parameters. Thus, the present study was conducted to examine the possible adverse effects of Cd (as cadmium chloride) on testicular parameters. The results corroborate the usefulness of extraneous supplementation with vitamins C and E in protection from Cd-induced oxidative stress.

2. Material and methods

2.1. Chemicals

Cadmium chloride ($CdCl_2$) manufactured by qualigens fine chemicals, Glaxo India Ltd., Mumbai was used as the test agent. Taking the quantitative exposure and tissue retaining capacity of various Cd-compounds by humans into consideration, a dose of 1 mg/kg body weight (BW) was selected for study. L-Ascobic acid (vitamin C) manufactured by Loba chemicals, India was used to test its efficacy as an antioxidant. Tocopheryl acetate (vitamin E) was purchased from E-Merck, India. The dose of vitamin C (10 mg/kg BW) and vitamin E (100 mg/kg BW) were chosen in this study basing on our previous study which showed that this dose was effective against the toxicity of metals [16–18].

2.2. Animals and administration schedule

Swiss albino mice at 10-week-old of age were obtained from M/S Gosh enterprises, Kolkota, India. Mice were acclimated to laboratory conditions (temperature $23 \pm 2^{\circ}$) and fed a balanced diet and water *ad libitum*. In total, 96 mice ranging from 20 to 25 g body weights were selected and randomly divided into four groups each containing 24 animals. The first group was injected with distilled water; the second group was injected with $CdCl_2$. The third group was injected with $CdCl_2$ + vitamin E and the fourth group was injected with $CdCl_2$ + vitamin C. All treatments were through a single intraperitoneal dose and the volume of each injection was adjusted to deliver 0.33 ml/30 g BW. Mice were euthanized by cervical dislocation at the 5th, 6th, 7th and 8th weeks after injection ($n = 6$ per time).

2.3. Biochemical parameters

The testes were dissected from accessory tissues under ice-cold normal saline. A portion was weighed and processed for the determination of lipid peroxidation potential (LPP) by a modified thiobarbituric acid (TBA) test [19]. The amount of TBA reactive species (TBARS) produced was expressed in terms of μmol/g wet weight. Ascorbic acid was extracted after homogenizing and centrifuging the tissue with 5% cold trichloroacetic acid (TCA). The TCA-supernatant was used to estimate ascorbic acid content [20], as modified by [21].

For enzyme assays, the testes were gently homogenized in phosphate buffer, pH 7.4, at 4 °C using a glass potter-type homogenizer at 500–800 rpm. The homogenate was filtered through a muslin cloth and then centrifuged at $1000 \times g$ for 30 min at 4 °C to obtain a crude homogenate. The resultant supernatant was immediately used for measuring enzyme activities of SOD, PD and catalase (CT). Cu/Zn–SOD activity was measured spectrophotometrically [22]. One unit of SOD activity is defined as the amount of extract causing 50% inhibition of the nitroblue tetrazolium reduction rate. Substrate devoid of enzyme served as the negative control. Data are expressed as units (U) of enzyme/mg of tissue protein. PD activity was determined following a standard method [23]. The concentration of purpurogalin formed was determined spectrophotometrically (Systronics, 106) at 430 nm. Specific activity was expressed as units of enzyme/mg of tissue protein. CT activity was estimated spectrophotometrically at 570 nm [24]. Specific activity was expressed as units of enzyme/mg of tissue protein. Protein content was determined by the Lowry method [25].

2.4. Sperm parameters

For assessing sperm head abnormalities, the vas deferens was dissected and kept in phosphate buffer saline (pH 7.4). Sperm were squeezed from the vas and centrifuged for 1 min at 1000 rpm. Small drops of sperm suspension were smeared gently on a glass slide and dried overnight. The slides were stained with 10% Giemsa diluted in fresh Soreson's buffer (pH 6.8). Morphologically abnormal sperm were recorded as described [26]. The same suspension was used for sperm counting using a haemocytometer.

2.5. Statistical analysis

All data were subjected to a two-way analysis of variance (ANOVA) to determine the level of significance between control and treated mice at different exposure period. Student's *t*-test was utilized for comparison of data between control and experimental groups. A difference was considered significant at the $p < 0.05$ level. Data are reported here as mean ± standard error of measurement (S.E.M.).

3. Results

Cd-exposed mice showed a significant increase of LPP in the testis compared to vehicle control animals across the post-treatment period; however, in vitamin (vit) supplemented groups the LPP declined significantly with relative to Cd-exposured groups, albeit not to the control level (Fig. 1). Ascorbic acid content of the Cd-treated tissues significantly declined compared to control over the post-treatment phase, whereas vit supplementation significantly protected testicular ascorbic acid content (Fig. 2). Testicular SOD also declined in Cd-exposed mice versus vehicle controls and subsequently increased following supplementation with vit C and vit E (Fig. 3). Specific activities of CT (Fig. 4) and PD (Fig. 5) also declined significantly in Cd-exposed mice but increased after vitamin supplementation.

We observed a significant increase in sperm abnormalities in Cd-treated mice versus the controls and this change was minimized after vit supplementation (Fig. 6). Similarly, a significant decrease in sperm count profile was recorded in Cd-treated groups (Fig. 7). Vitamin supplementation reversed the effect on sperm count.

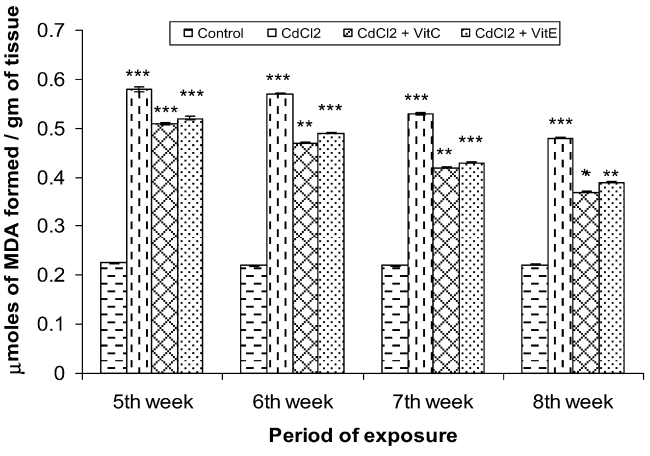

Fig. 1. Showing the effect of vitamin C and vitamin E on LPP in $CdCl_2$ (1 mg/kg b.w., i.p.) treated Swiss mice testes.