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ROLE OF VITAMIN C AS AN ANTIOXIDANT IN CADMIUM CHLORIDE INDUCED TESTICULAR DAMAGE

Rekha D Kini[1*], Tripathi Y[2], CV Raghuveer[3], SheilA R Pai[4], Ramaswamy C[5], Priya Kamath[6]

[1]Dept. of Physiology, Kasturba Medical College, Mangalore, Manipal University, Karnataka
[2]Dept .of Physiology, Santhosh Medical college, New Delhi
[3]Dept.of Pathology, Srinivas Medical College, Mangalore, Karnataka
[4]Dept .of Physiology, Kasturba Medical college, Mangalore, Manipal University, Karnataka
[5]Dept. of Physiology, Saveetha Medical college, Saveetha Nagar, Thandalam, Chennai.
[6]Dept. of OBG, AJ Institute of Medical sciences, Mangalore

ABSTRACT: Cadmium is one of the most toxic heavy metals and potential for human exposure has generally increased with increase in industrial usage of this element. The purpose of the present study was to determine anti-oxidative role of vitamin C against cadmium chloride induced oxidative stress on rat testis. Adult male rats(n=6/group) were divided into five groups ,one control (Gr.I- 0.9% Saline treated) & two untreated experimental & two pretreated experimental groups. The untreated groups were injected with single dose of 0.5 & 1 mg /kg bw cadmium chloride (Gr.II &Gr.III) intraperitoneally. Vitamin C (30mg/kg bw,ip) was orally administered for 30 days prior to the exposure to 0.5 and 1mg/kg bw(Gr,IIa &Gr.IIIA) of cadmium chloride. In all the groups, rats were sacrificed 15 days after the final cadmium chloride or saline administration and the changes in the testicular weight and testicular level of Melonaldehyde , glutathione & superoxide dismutase were studied. Exposure to cadmium chloride lead to significant decrease in the testicular weight& level of GSH & SOD and increase in the level of testicular MDA compared to normal control. Pretreatment with vitamin C (30mg/kg bw) significantly prevented the increase in MDA level of the testis & ameliorated the fall in GSH & SOD as well as testicular weight compared to 0.5mg/kg bw cadmium chloride group. But pretreatment with vitamin C did not show any beneficial effect with 1mg/kg bw cadmium treated group. The study reports the antioxidative role of vitamin C in ameliorating lower doses of cadmium chloride induced testicular damage.
Key Words: Cadmium chloride, oxidative stress, vitamin C, rat testis

INTRODUCTION

The roles that heavy metals play in the etiology of reproductive pathology have been debated for several decades. Exposure to heavy metals has become an increasingly recognized source of illness worldwide[1] . Most, if not all, metals are toxic, even those known to be essential. Heavy metals are among one of the most widespread potential chemical contaminants in the environment[2]. Effect of metal or multimetal exposure on the testis is of great concern as occupational exposure to certain metals results in impaired reproductive function[3]. Heavy metals like zinc, manganese and selenium are essential for normal testicular function whereas lead, cadmium, mercury and cobalt are toxic[4].

Cadmium compounds have been shown to exert toxic and carcinogenic effects in humans and experimental animals[5]. The International Agency for Research on Cancer (IARC) has classified Cadmium as a "Category I" human carcinogen. Cadmium is known to affect various organs like kidney, liver, bone and testis in human beings and experimental animals[6]. Testes are exquisitely sensitive to cadmium toxicity[7] .Many studies suggested that generation of reactive oxygen species (ROS) and its interference with cellular antioxidant system is one of the major mechanisms by which toxic effect of cadmium is mediated[8].
Oxidative stress is a harmful condition that occurs when there is an excess production of oxygen free radicals. Vitamin C acts as a potent water soluble antioxidant by scavenging reactive oxygen species and reactive nitrogen species[9] .Vitamin C is an excellent source of electron and thus donates electron to free radicals such as hydroxyl radical and superoxide radical and quenches their reactivity[10]. Vitamin C offers effective protection against lipid peroxidation[11]. In addition to scavenging action vitamin C can regenerate other small molecule antioxidants such as α- tocopherol, glutathione, urate from their respective radical species[9]. Even though several studies indicated that ascorbic acid pre-treatment had a protective effect on cadmium induced toxicity in different tissues, the mechanisms by which it reduces toxicity remains to be defined. Therefore the present study was designed to evaluate the effect of vitamin C against cadmium chloride induced testicular toxicity

MATERIALS AND METHODS

The present study was conducted following approval from Institutional Bioethical Committee and strict internationally accepted guidelines, for the usage of animals in experimental study were followed. Inbred adult male albino rats of wistar strain weighing 200-250g were used in the present study. Animals were housed in polypropylene cages (4-5 rats per cage) under standard laboratory conditions and fed ad libitum with commercial rodent chow (Hindusthan lever limited) and water**.** Cadmium chloride (CdCl2) (Loba Chemie, India) was dissolved in normal saline. Vitamin C was dissolved in normal saline and administered orally

Experimental protocol & drugs

Animals were divided into five groups of six rats in each group. In the normal control group (Gr. I) rats were administered with the normal saline intraperitoneally. In untreated experimental control groups (cadmium treated group) rats were administered with single dose of 0.5mg/kg bw (Gr.II) & 1 mg/kg bw(Gr.III) cadmium chloride intraperitoneally. In pretreated groups rats were pretreated with vitamin C (30mg/kg bw) for 30 days orally and then injected with 0.5mg/kg bw (Gr.IIa) & 1mg /kg bw (Gr.IIIa) cadmium chloride intraperitoneally. In all the groups, rats were sacrificed under anesthesia 15 days after the final cadmium administration. Following the completion of the experimental protocol animals in each group were anaesthetized by injecting sodium pentabaritone (40mg/kg bw) intraperitoneally under aseptic conditions. Laparotomy was performed and the reproductive organs were exposed. Both the testes were removed and cleaned of fat tissue and blood and weighed.. Pieces of the testis were transferred into a glass homogeniser containing 10ml of cold phosphate buffer saline solution of pH 7.4.

PREPARATION OF TISSUE HOMOGENATE

The minced testicular tissue (1g) was transferred to a homogenizer containing cold 10ml of 10mM cold potassium phosphate buffer (pH 7.4). The tissue was homogenized using a manual homogenizer. The unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 10 minutes by using Remi C 24 refrigerated centrifuge(- 4^0C) . The obtained supernatant was used for the following biochemical estimations. Testicular level of MDA, GSH & SOD were measured in all the groups.

ESTIMATION OF TESTICULAR LIPID PEROXIDATION

Lipid peroxidation was estimated according to the method of Kartha & Krisnamurthy [12].

ESTIMATION OF TISSUE GLUTATHIONE

Glutathione content in the tissue homogenate [10% w/v in 10mM potassium phosphate buffer (7.4pH)] was estimated by the method of Beutler et al[13].

Superoxide Dismutase Assay

Superoxide Dismutase (SOD) was estimated by original method of Beauchamp and Fridovich[14].

STATISTICAL ANALYSIS

Values were expressed in mean ± SEM. One way (ANOVA) with post hoc comparison was used for statistical comparison. P <0.05 was taken as significant.

RESULTS

Exposure to 0.5mg/kb bw of cadmium chloride did not show significant decrease in the testicular weight, but resulted in the significant increase (P< 0.001) in the tissue level of lipid peroxidation (Table 1). Cadmium administration (0.5mg/kgbw) also showed a significant decrease(p<0.001) in the level of Glutathione and SOD(Table 1). Pretreatment with 30mg/kg bw vitamin C did not show any significant change in the testicular weight, but showed a significant increase (P<0.001) in the tissue level of SOD and GSH and decrease in level of lipid peroxdidation in the rats administered with 0.5mg/kg bw cadmium chloride(Table1). Exposure to 1mg/kb bw of cadmium chloride showed the significant decrease in the testicular weight, level of SOD &GSH as well as significant increase(P<0.001)in the tissue level of lipid peroxidation (Table2). But, vitamin C pretreatment in the rats administered with1mg /kg bw cadmium chloride did not show any increase in the testicular weight as well as level of GSH & SOD compared to untreated group. The level of lipid peroxidation was also high in this group compared to untreated experimental control (Table2).

Table 1: Effect of vitamin C pre-treatment with 0.5mg/kg bw on rat testis

Group	Testicular weight (g/100g bw)	MDA (nmol/g wet tissue)	SOD (units/g protein)	GSH (nmol/mg protein)
Gr.I	0.619±0.02	5.113±0.277	12.451±0.655	5.951±0.379
Gr.II	0.611±0.02^{NS}	26.687±1.229***	7.496±0.376***	3.570±0.08***
Gr.IIa	0.605±0.02^{NS}	7.410±3.144***	11.085±0.981***	5.658±0.249***

The values are expressed as mean ± SEM. In each group eight animals were used. NS= Not Significant, Gr. II vesus Gr.I & Gr.IIa versus Gr.II, ***P<0.001, Gr. II versus Gr.I and Gr. IIa versus Gr. II.

Table 2: Effect of vitamin C pretreatment with 1mg/kg bw cadmium chloride on rat testis

Group	Testicular weight (g/100g bw)	MDA (nmol/g wet tissue)	SOD (units/g protein)	GSH (nmol/mg protein)
Gr.I	0.619±0.02	5.113±0.277	12.451±0.655	5.951±0.379
Gr.III	0.312±0.01***	26.687±1.229***	5.078±0.191***	3.414±0.176***
Gr.IIIa	0.335±0.02^{NS}	50.453±2.193^{NS}	5.805±0.480^{NS}	3.522±0.151^{NS}

The values are expressed as mean ± SEM. In each group eight animals were used. ***P<0.001, Gr. III versus Gr.I. NS= not significant, Gr. III a versus Gr.III.

DISCUSSION

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant which is present in the soil, water, air, food and in cigarette smoke. Cadmium causes poisoning in various tissues of humans and animals[15,16] .In the present study, exposure to cadmium decreased the testicular weight and increased the LP level in the testis which was accompanied by increased formation of ROS. As a consequence, enhanced lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis as well as marked disturbances of antioxidant defense system occurred[15,17]. Pretreatment with Vitamin C was very effective in the prevention of oxidative damage induced by lower doses of cadmium which resulted in significantly lower LP concentration and increase in the testicular weight (Fig. 1). In animals exposed to cadmium chloride, the activities of SOD as well as concentration of GSH were significantly decreased .The depletion of GSH & SOD due to cadmium exposure in the present study may be due to oxidative stress induced by cadmium and compromising the cellular defense mechanism against such stress.

Vitamin C (Ascorbate) is a potent water soluble antioxidant found in human plasma. It scavenges reactive oxygen species and nitrogen species including hydroxyl radical, peroxyl radical, superoxide anion, nitrogen dioxide as well as non-radical species such as hypochlorous acid, ozone and singlet oxygen. It has been shown that marginal vitamin C deficiency results in intracellular oxidative damage in the guinea pigs[18] . Our previous studies have showed that treatment with cadmium induces decrease in vitamin C concentration in testicular tissue of rats.

In the present study, pretreatment of rats with vitamin C (30mg/kg bw) prior to 0.5mg/kg bw cadmium chloride exposure showed a significant increase in the testicular level of SOD and GSH and decrease in MDA level of the testis. This indicates the protective role of vitamin C on cadmium induced oxidative stress.

In the present study pre-treatment of animals with vitamin C prior to 1mg /kg bw cadmium chloride administration did not show significant increase in the tissue levels of glutathione and superoxide dismutase compared to untreated experimental control group. Similarly, vitamin C pre-treatment prior to 1mg /kg bw cadmium chloride administration did not show any significant decrease in the tissue lipid peroxidation compared to untreated experimental control groups. This suggested that ascorbic acid failed to protect the testes from damaging effect of higher doses of cadmium chloride. In the present study failure of ascorbic acid in protecting the testes from toxic effects of higher doses of cadmium chloride may be ascribed to dosage of vitamin C used and/or may be the dosage of cadmium chloride administered.

It can be concluded from the present study, cadmium induced oxidative damage in the testis leads to increase in the level of lipid peroxidation and alteration in the level of GSH and SOD. Our results show that vitamin C expressed protective role against toxic influence of lower doses of cadmium.

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