International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue: 4 2014 Pages: 89-92

(p-ISSN: 2348-5213; e-ISSN: 2348-5221)

RESEARCH ARTICLE

SCAVENGING ROLE OF BETA CAROTENE IN OXIDATIVE STRESS INDUCED BY CADMIUM CHLORIDE ON RAT TESTIS

REKHA D KINI, NAYANATARA AK, ANUPAMA N, BHAGHYALAKHMI K, SHEILA R PAI, AND SNEHA B SHETTY

Department of Physiology, Kasturba Medical College, Manipal University Mangalore, India
*Corresponding author e-mail: rekhadk2004@yahoo.com

Abstract

Cadmium is a transitional metal that exists in different oxidational or transitional states in the environment. It is a well-known caricinogen and a potent testicular toxicant. Cadmium is known to affect various organs like kidney, liver, bone and testis in human beings and experimental animals. The present study was aimed to study the anti-oxidative role of beta carotene against cadmium chloride induced oxidative stress on rat testis. Adult male rats(n=8/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 body weight cadmium chloride (Gr.II &Gr.III) intraperitoneally. Beta carotene (10mg/kg body weight) was orally administered for 30 days prior to the exposure to 0.5and 1mg/kg body weight (Gr,IIa&Gr.IIIA) of cadmium chloride. In all the groups, rats were sacrificed15 days after the final cadmium chloride or saline administration and the changes in the testicular weight and testicular level of Malonaldehyde , 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. Beta carotene pretreatment significantly prevented the increase in MDA level of the testis & ameliorated the fall in GSH & SOD as well as testicular weight when compared to 0.5mg/kg body weight cadmium chloride group. But pretreatment with beta carotene did not show any beneficial effect with 1mg/kg body weight cadmium treated group. Thus the results of the present study showed that the antioxidative role of beta carotene in ameliorating lower doses of cadmium.

Keywords: Cadmium, Glutathione, Beta Carotene, lipid peroxidation

Introduction

Pollution and industrial practices result in concentration of heavy metals in the environment (Novelli et al.,1998).Cadmium is a transitional metal that exists in different oxidational or transitional states in the environment (Donald et al,1996). It is a well-known caricinogen and a potent testicular toxicant. Cadmium is known to affect various organs like kidney, liver, bone and testis in human beings and experimental animals (Misra et al., 1998).Cadmium toxicity is dependent on dose, duration and route of exposure (Jarup et al., 1998). The liver injury is also of acute toxicity dominated by apoptosis and necrosis, two modes of cell death (Habbeebu et al.,1998). Among the various tissues testis are more susceptible for cadmium toxicity (Anders Bergh,1990). Exposure to cadmium metal is known to induce the formation of reactive oxygen species (ROS) like superoxide radical, hydroxyl ion and hydrogen peroxide (Christopher O Ikediobi, 2004). An antioxidant is a substance that inhibits the oxidation of other molecules; it protects the body from free radicals .Vitamins C, E and selenium, Beta-carotene are known to be protective anti-oxidants (Stohs et al., 2001; Das et al., 2007). They cause the inhibition of peroxidation, mopping up of free oxygen radicals and disorganization and

breakage of peroxidation chain reactions (Murray et al., 2000) by an inhibition of glutathione peroxide. Beta-carotene, like all carotenoids, is an antioxidant. Beta-Carotene is a purported anticancer agent, which is believed by some to have antioxidant action of a radical-trapping type. However, there are not many studies on the type of correlation between biochemical indices and the cadmium chloride toxicity on testis and their function. Information regarding antagonistic effects of beta carotene on cadmium induced testicular toxicity is rare. Hence, it is therefore in this study, we seek to establish the detailed correlation between biochemical stimulation of testis using cadmium chloride and their function on one hand, and the effect of anti-oxidant beta caroteneon the other hand.

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) (LobaChemie, India) was dissolved in normal saline. Beta carotene is dissolved in coconut oil and administered orally (10mg/kg bw).

Experimental protocol and drugs

Animals were divided into five groups of eight 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 beta caroteen (10mg/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 (**-** 40C) . 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 and Krisnamurthy (1978).

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., (1963).

Superoxide Dismutase Assay

Superoxide Dismutase (SOD) was estimated by original method of Beauchamp and Fridovich (Beauchamp et al.,1971).

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/kg bw of cadmium chloride showed a significant decrease in the testicular weight (P<0.05), but resulted in the significant increase (P< 0.001) in the tissue level of lipid peroxidation. Cadmium administration (0.5mg/kgbw) also showed a significant decrease (p<0.001) in the level of Glutathione and SOD.

Table 1. Effect of beta carotene pre-treatment with 0.5mg/kgbw Cadmium chloride on rat testis

GROUP	TESTICULAR WEIGHT(g/100g bw)	MDA (nmol/g wet tissue)	SOD (units/g protein)	GSH (nmol/mg protein)
GROUP I	0.617±0.02	4.124±0.276	11.551±0.656	5.251±0.379
GROUP II	0.596±0.03*	25.672±1.319***	6.246±0.356***	3.321±0.08***
GROUP IIa	0.614±0.02*	16.210±3.244***	10.125±0.881***	5.658±0.249***

The values are expressed as mean ± SEM. In each group eight animals were used.*P<0.05 Gr.II versus Gr.I&Gr.IIa versus Gr.II . ***P<0.001, Gr. II versus Gr.I and Gr. IIaversusGr. II

Table 2. Effect of beta carotene pre-treatment 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
GROUP I	0.619±0.02	4.123±0.277	12.431±0.645	5.891±0.368
GROUP III	0.314±0.01***	58.022±1.229***	5.102±0.191***	3.114±0.174***
GROUP IIIa	0.334±0.01NS	46.734±0.344NS	5.692±0.1NS	3.378±0.151NS

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

Pretreatment with 10mg/kgbw beta carotene showed a significant increase in testicular weight (P<0.05) as well as 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.

Exposure to 1mg/kgbw 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 . But, beta carotene pretreatment in the rats administeredwith1mg /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 pretreated group compared to untreated experimental control (Table 2).

Discussion

One of the areas of concern in the field of research today is the effect of heavy metal exposure on testes (biology of reproduction). Cadmium is a potent environmental pollutant and known to induce oxidative stress by generating oxygen free radical (RonjoySen Gupta et al., 2004). Lipid peroxidation is involved in cadmium-related toxicity. In the present study, Our results showed that, administration of cadmium chloride (0.5mg and 1 mg /kg BW) decreased the weight of testis as well testicular SOD and GSH. But there was significant increase in the lipid peroxidation after exposure to cadmium chloride. This indicated testicular damage induced by cadmium chloride. Thus the results of the present study was in accordance with previous study that cadmium depletes glutathione and protein bound sulphadryl groups in the testes, resulting in enhanced production of reactive oxygen species such as superoxide ion, hydroxyl radicals and hydrogen peroxide (Faix et al., 2005)

An antioxidant is a substance that inhibits the oxidation of other molecules; it protects the body from free radicals. Beta-carotene is an effective antioxidants and free radical scavengers.β-carotene is a strongly-colored red-orange pigment abundant in plants and fruits. It is the precursor of vitamin A. In the present study, pre-treatment with beta carotene orally for 30days prior to the 0.5 mg/kg bw cadmium administration showed a significant increase in the testicular weight, SOD and GSH level of the testis over the untreated group and significant decrease in the lipid peroxidation level. Thus the present showed that beta carotene reversed the cadmium chloride induced testicular damage. But pretreatment with beta carotene prior 1mg/kg bw cadmium chloride was not beneficial

in ameliorating cadmium induced testicular damage. In the present study failure of beta carotene protecting the testes from toxic effects of higher doses of cadmium chloride may be ascribed to dosage of beta carotene 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 testisleads to increase in the level of lipid peroxidation and alteration in the level of GSH and SOD. Our results show that beta carotene expressed protective role against toxic influence of lower doses of cadmium chloride

References

Anders RJ Bergh. The acute effects of cadmium in the testis do not require the presence of Leydig cells. Toxicology.1990: 63; 183-186.

Beauchamp C, Fridovich I. Superoxide dismutase, improved assay and an assay applicable to acrylamide gel. Ann Biochem.1971: 44; 276-287.

Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. J Lab Clin Invest.1963: 61; 883-887.

Christopher O Ikediobi, Veera L Badisa, Lambert T Ayuk- Takem, Lekan M Latinwo and John West. Response of antioxidant enzymes and redox metabolites to cadmium –induced oxidative stress in CRL-1439 normal rat liver cells. International Journal of Molecular Medicine. 2004:14; 87-92.

Das, E. N. and King, G. L.The role of protein kinase C activation and the vascular complications of diabetes.*Pharmacol Res.2007:*55; 498–510.

Donald, B.C.A., Edward, A. and Ashwood, A.D. Tiezts- Fundermental Clinical*Chemistry.*1996:416; 145.

Faix S, Faixova, Boldizarova K, Javorsky P. The effect of long term intake of heavy metal intake on lipid peroxidation of gastrointestinal tissue in sheep.Vet .Med. Czech. 2005: 50; 401-405.

Habbeebu, S.S. Liu J. and Klaassen, C.D. Cadmium-induced apoptosis in mouse Liver.Toxicol.Appl Pharmacol.(1998): 149(2);203-209.

Jarup L. Berglund, M. Elinder, C.G. Nordberg, G.andVahter, M. Health effects of cadmium exposure -a review of the literature and a risk estimate.Scand J Work Environ Health.1998: 24(suppl 1);1–51.

Kartha R, Krishnamurthy S. Factors affecting invitro lipid peroxidation in rat brain homogenate. Ind J Physiol Pharmacol.1978: 22; 44-52.

Misra RR, Smith GT, Waalkes MP.Evaluation of the direct genotoxic potential of cadmium in four different rodent cell lines. Toxicology.1998: 126; 103-14.

Murray, K.M. Granner, D.K. Mayer, P.A. and Rodwell, V.W. Harpers Biochemistry,25th Edition.2000: 8: 83-85.

Novelli EL, Vieira EP, Rodrigues NL, Ribas BO. Risk assessment of cadmium toxicity on hepatic and renal tissues of rats. Environmental Res.1998:79; 102-5.

RonojoySen Gupta, EnakshiSen Gupta, Bijaya Kumar Dhakal, Ashoke Ranjan Thakur, Joohong Ahnn. Vitamin C and Vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Mol. cells. 2004:17; 132-139.

Stohs, S. J. Bagchi, D., Hassoun, E. and Bagchi, M. Oxidative mechanisms in the toxicityof chromium and cadmium ions. J. Environ.Pathol. Toxicol. Oncol.2001: 20; 77-88.

.

