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Vitamin C and Vitamin E Protect the Rat Testes from Cadmium-induced Reactive Oxygen Species

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Cadmium is an environmental and industrial pollutant that affects the male reproductive system of humans and animals. However, the mechanism of its adverse effect on Leydig cell steroidogenesis remains unknown. The present study points to the possible involvement of oxidative stress in the suppression of steroidogenesis. Cadmium administration caused an increase in reactive oxygen species (ROS) by elevating testicular malondialdehyde (MDA) and decreasing the activities of testicular antioxidant enzymes such as glutathione peroxidase and superoxide dismutase. The mRNA of Steroid Acute Regulatory (StAR) protein was substantially reduced. The activities of testicular Δ^5-3β and 17-β-hydroxysteroid dehydrogenases (HSD) as well as serum testosterone level were also lowered, suggesting that cadmium-induced ROS inhibit testicular steroidogenesis. Supplementation with vitamin C (VC) and or vitamin E (VE) reduced testicular ROS and restored normal testicular function in Cd-exposed rats. We conclude that VC and VE prevent oxidative stress and play vital roles in co-regulating StAR gene expression and steroid production in cadmium-exposed rats.

Keywords: Cadmium; ROS; Testes; Vitamin C; Vitamin E.

Introduction

Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH·) are generated *in vivo* from the incomplete reaction of oxygen during aerobic metabolism or in stimulated host phagocytes, as well as from exposure to environmental agents such as radiation and redox cycling agents (Babior, 1992; Matés *et al.*, 1999).

Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defense mechanisms, causing damage to macromolecules such as DNA, proteins and lipids (Bartsch and Nair, 2000; Halliwell and Gutteridge, 1989). At low concentration, ROS have been implicated in the regulation of several physiological processes such as proliferation (Shibanuma *et al.*, 1990), differentiation (Allen and Balin, 1989), apoptosis (Hockenbery *et al.*, 1993) and senescence (deHaan *et al.*, 1996). At high concentrations, ROS are extremely toxic to cells, causing extensive DNA damage, lipid peroxidation, and protein degradation (Sun, 1990).

Preventing lipid peroxidation is essential in all the aerobic organisms, as it is a chain reaction and if unchecked can cause extensive DNA damage (Matés, 2000; Packer, 1995). To counteract the damaging effect of ROS, aerobic cells are provided with extensive antioxidant defense mechanisms. These consist mainly of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase, etc.), antioxidant proteins [e.g., thioredoxin and metallothionein (MT)], and small molecular antioxidants (e.g., glutathione, *N*-acetyl-$_L$-cysteine, vitamin E and vitamin C) (Baker *et al.*, 1997; Kaynoki *et al.*, 1996; Kondo *et al.*, 1997; Manna *et al.*, 1998).

Small molecule antioxidants, such as vitamin E (VE) (α-tocopherol) and vitamin C (VC) (ascorbic acid or ascorbate) interact with oxidizing radicals (Burton and Ingold, 1986; Jones *et al.*, 1995). VE terminates the chain reaction of lipid peroxidation in membranes and lipoproteins (Dieber-Rotheneder *et al.*, 1991), and VC scavenges aqueous ROS by rapid electron transfer and thus inhibits lipid peroxidation (Halliwell *et al.*, 1987), as well as re-

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Abbreviations: ROS, reactive oxygen species; StAR, Steroid Acute Regulatory.

ducing the level of oxidized VE (tocopheroxyl radicals). Ascorbate can regenerate tocopherol from the tocopheroxyl radical, which is formed when tocopherol reacts with a lipid peroxyl radical (Packer *et al.*, 1979). Antioxidant compounds are known to be tightly linked in interlocking cycles of regeneration and recycling in which vitamin E appears to play a major role (Kagan *et al.*, 1992; Packer and Kagan, 1992).

Several lines of evidence indicate that ROS are involved in cadmium-induced testicular damage (Oteiza *et al.*, 1999). Acute, as well as chronic, cadmium exposure is associated with elevated lipid peroxidation in lung, brain, kidney, liver, erythrocytes and testes (Bagchi *et al.*, 1997; Klimczak *et al.*, 1984; Koizumi and Li, 1992; Manca *et al.*, 1991; Sugawara and Sugawara, 1984) and the testes are the most susceptible of these organs (Kar and Das, 1960). Cadmium has a profound effect on sex organ weight, the primary indicator of a possible alteration in androgen status (Biswas *et al*., 2001). The significant reduction in testis and accessory sex organ weights observed in our previous study suggests that androgen secretion was sub-optimal or that testicular endocrine function had been compromised. Cadmium can directly inhibit primary Leydig cell testosterone levels (Laskey and Phelps, 1991) but the mechanism of this effect is not known.

We undertook the present study to investigate whether treatment with vitamin C (VC) or vitamin E (VE) protects rat testis by inhibiting the production of ROS in response to cadmium (Cd). We found that Cd caused an increase in ROS either by elevating testicular malondialdehyde (MDA) or by decreasing the level of endogenous testicular antioxidants. It also resulted in significant reduction of Steroid Acute Regulatory (StAR) mRNA. A marked reduction in the activities of testicular steroidogenic enzymes was observed along with a lowering of serum testosterone, suggesting that cadmium has a direct inhibitory effect on testicular steroidogenesis. Supplementation with VC or VE reduced ROS production and restored normal testicular function. Our findings indicate that vitamin C and Vitamin E prevent oxidative stress and play vital roles in co-regulating steroid production and StAR gene expression in cadmium-poisoned rats.

Materials and Methods

Chemicals Cadmium chloride, dehydroisoandrosterone (5-androsten-3β-ol-17-one), D, L-α-tocopherol (VE), ascorbic acid (VC), ethylenediaminetetraacetic (EDTA) disodium salt, potassium phosphate dibasic, tetrasodium pyrophosphate, β-nicotinamide adenine dinucleotide, testosterone (4-androsten-17β-ol-3one; 17β-hydroxy-4-androsten-3-one;trans-testosterone), and TRI reagent were purchased from Sigma (USA). The ^{125}I RIA kit was purchased from ICN, Biochemical Inc., Diagnostic Division (USA).

Animals Eight-week-old laboratory-bred male Sprague-Dawley rats, weighing 200–250 g were allowed to acclimatize for one week before the experiments were begun. Animals were housed in plastic cage under controlled temperature (22°C) and photoperiod (12 h L: 12 h of D), with free access to water and rat chow. All treatments and procedures were conducted in accord with the highest standards of humane animal care as outlined in Guiding Principles in the Use of Animals in Toxicology.

Study design Each experimental group consisted of eight animals. Eighty animals were divided into ten groups to study the effect of vitamin C and / or vitamin E in the cadmium treated groups. The groups received: (1) a (-vit) basal diet without vitamin supplements; (2) 500 mg VC/ l drinking water; (3) 150 mg VE/kg chow; (4) 300 mg VE/kg chow; (5) 300 mg VC/l drinking water + 150 mg VE/kg chow. The animals in each group were divided into Cd-exposed and control subgroups: the latter received a single dose of physiological saline 0.1 ml/100 g BW, subcutaneously (sc), the former a single dose of cadmium chloride 0.20 mg / 100 g BW. Vitamin C and / or vitamin E were administered for three consecutive days. To facilitate absorption of lipid-soluble VE, the chow was supplemented with 2% soybean oil. The aqueous-soluble VC (500 mg/L) was added to the drinking water. All animals were sacrificed 48 h after the first treatment.

Blood collection The animals were killed by lethal injection of sodium pentobarbital (100 mg/kg BW) intra-peritoneally (ip) to facilitate the testicular perfusion technique (Sinha Hikim and Swerdloff, 1993). Blood samples were collected from the inferior vena cava of each animal immediately after death with a heparin-coated syringe, and centrifuged to separate out the serum, which was kept at –20°C until testosterone was assayed. The testes were removed, dissected free of surrounding connective tissue, and stored at –70°C until processed for Northern blot analysis, testicular steroidogenic enzymes, lipid peroxidation level, superoxide dismutase and glutathione peroxidase assays.

RNA isolation and Northern blot analysis Total RNA was isolated from 100–500 mg of frozen tissue by the acid phenol guanidium method (Chomczynski and Sacchi, 1987). One ml TRIZOL reagent was used to homogenize 100 mg of tissue. 400 µl of chloroform was added to the homogenate and the mixture vortexed vigorously for 30 s then allowed to stand for 5 min on ice. The suspension was centrifuged at 14,000 rpm for 15 min at 4°C and the upper aqueous phase transferred to a fresh tube; an equal volume of isopropanol was added, mixed thoroughly by vortexing, followed by centrifugation at 14,000 rpm for 15 min at 4°C. The RNA pellet was washed with 70% ethanol and after centrifuging again for 15 min at 14,000 rpm, ethanol was removed and the RNA was air-dried. Approximately 20–30 µg of total RNA was loaded in each lane and separated on a 2.2 M formaldehyde denaturing agarose gel as described previously

(Cho *et al.*, 2002). Then the RNA was transferred to a nylon membrane followed by UV-cross linking. The blot was preincubated in prehybridization solution (50% formaldehyde, 6× SCC, 0.1% SDS, 5× Denhardt's solution, 10 μg/ml salmon sperm DNA) containing [^{32}P]-labeled mouse StAR cDNA for 20 h at 42°C. It was then washed three times at RT (5 min) in 0.1× SCC, 0.1% SDS and three times at RT (10 min each) in 0.1× SSC/ 0.1% SDS, and exposed to X-ray film.

Determination of 3β-HSD and 17β-HSD activities To assay testicular Δ^5-3β-HSD and 17β-HSD activities (Jarabak, 1962; Talalay, 1962) testicular and adrenal tissues frozen at –70°C were homogenized with homogenizing fluid containing 20% spectroscopic grade glycerol, 5 mM potassium phosphate, and 1 mM EDTA at a tissue concentration of 25 mg/ml, and centrifuged at 10,000 × *g* for 30 min in an ultracentrifuge at 4°C. One ml of the supernatant was mixed with 100 μmol sodium pyrophosphate buffer (pH 8.9), 0.9 ml double distilled water, and 30 μg DHEA in a total of 3 ml. Δ^5-3β-HSD activity was measured after addition of 0.5 μmol NAD^+ in a UV spectrophotometer at 340 nm, against a blank without NAD^+. One unit of enzyme activity gives a change in absorbance of 0.001 U/min at 340 nm.

To assay 17β-HSD activity, one ml of the same supernatant was mixed with 440 μmol sodium pyrophosphate buffer (pH 10.2), 25 mg crystalline bovine albumin (BSA), and 0.3 μmol testosterone in a total of 3 ml. Enzyme activity was measured, after the addition of 1.1 μmol NADP, in a spectrophotometer at 340 nm against a blank without NADP. One unit of enzyme activity was equivalent to a change in absorbance of 0.001 U/min at 340 nm.

Testosterone assay The radioimmunoassay (RIA) for serum T was carried out with a testosterone ^{125}I RIA Kit (ICN, Biochemical Inc., Diagnostic Division, USA) according to the manufacturer's protocol, as reported previously (Biswas, 2001). Radioactivity was determined by gamma scintillation counting. All samples were run in duplicate in a single assay to avoid inter-assay variation. The inter-assay coefficient of variation of T was 6.5%.

Determination of lipid peroxidation Lipid peroxidation in testes was measured by the thio-barbituric acid (TBA) method (Wilbur *et al.*, 1949) that determines aldehyde formed by degradation of hydroperoxide, including ma-londialdehyde (MDA). Briefly, 0.5 ml HCL (0.8 M) containing trichloroacetic acid (TCA) (12.5%) was added to the incubation mixture followed by TBA (1.23%). Samples were boiled for 15 min, cooled and centrifuged at 1,500 × *g* at 4°C for 15 min, and the pink color developed by the reaction was measured at 530 nm. All assays were performed in triplicate. Values are expressed in nmol of malondialdehyde /100 mg of wet tissue using an extinction coefficient of 1.56×10^5 cm^2/mmol (Sinhuber *et al.*, 1958).

Assays of antioxidant enzymes Superoxide dismutase (SOD) activity was determined by the inhibition of the rate of superoxide-dependent cytochrome c reduction (Flohé and Otting, 1984). Glutathione peroxidase activity was determined by following the oxidation of NADPH at 340 nm (Flohé and Günzler, 1984). Testes were homogenized and antioxidant enzyme activities evaluated in 1,000 × g supernatants. Protein was determined as described by Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical analysis All values are means ± SD. Group differences were analyzed by Student's *t*-test and were regarded as significant at $P < 0.05$.

Results

Previously, Liu *et al.* (2001) and Cheng *et al.* (2003) have reported that lead and manganese inhibit steroidogenesis in rat Leydig cell cultures by disrupting StAR protein expression. To determine whether Cd has a similar effect we performed a Northern blot analysis of StAR gene expression. When animals were exposed to Cd (0.20 mg / 100 g BW), testis StAR mRNA was significantly reduced (Fig. 1A, lanes 1 and 6). However when vitamin C and / or vitamin E were added, and the animals exposed to Cd, the StAR mRNA level was maintained (Fig. 1B, lanes 2–5).

Since both Δ^5-3β-HSD and 17-β-HSD are considered key steroidogenic enzymes (Norman and Litwack, 1997), we assayed both testicular Δ^5-3β-HSD and 17-β-HSD activities to examine the effect of ascorbic acid on testicular steroidogenesis in cadmium-exposed rats. In the group not supplemented with vitamins (-Vit), testicular Δ^5-3β and 17-β-HSD activities were reduced by 17 and 22% respectively in Cd-exposed rats ($P < 0.05$) (Figs. 2A and 2B), and VC and VE were equally effective in maintaining normal testicular Δ^5-3β-HSD and 17-β-HSD activities in cadmium-exposed rats.

Cadmium is known to decrease luteinizing hormone (LH) receptor mRNA levels as well as cyclic adenosine monophosphate (cAMP) levels in testicular tissue (Gunnarsson *et al.*, 2003). To determine the effect of ascorbic acid on testosterone biosynthesis in cadmium-exposed rats, radioimmuno-assay of serum testosterone was carried out. In the (-Vit) group, serum testosterone level were significantly reduced by Cd ($P < 0.05$) (Fig. 3), and again vitamin C and vitamin E were equally effective in maintaining normal testosterone levels. Thus vitamin C and vitamin E protect normal Leydig cell function in the testes of cadmium-exposed animals.

The cellular damage induced by ROS was estimated by monitoring the lipid peroxidation level, a well-known indicator of cellular damage from oxidative stress (Slater, 1984). As a result of lipid peroxidation, a variety of aldehydes can be produced, including hexanal, malonaldialdehyde (MDA), and 5-hydroxynonenal, and MDA is

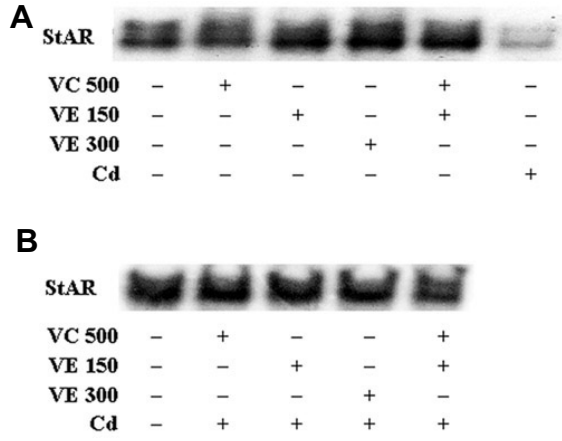

Fig. 1. Effects of vitamin C and vitamin E supplementation on StAR mRNA level in the testis of cadmium-treated rats. Total RNA was isolated and analyzed by Northern blotting. **A.** Effects of VC and / VE or cadmium on StAR mRNA levels. Lane 1, vitamin-unsupplemented (-Cd); lane 2, VC 500 (-Cd); lane 3, VE 150 (-Cd); lane 4, VE 300 (-Cd); lane 4, VC (500) + VE 150 (-Cd); lane 5, + Cd. **B.** Effect of VC and VE on StAR mRNA levels in cadmium treated rats. Lane 1, vitamin-unsupplemented (-Cd); lane 2, VC 500 + Cd; lane 3, VE 150 + Cd; lane 4, VE 300 + Cd; lane 5, VC 500 + VE 150 + Cd. The negative control for both cases is shown in lane 6 (A).

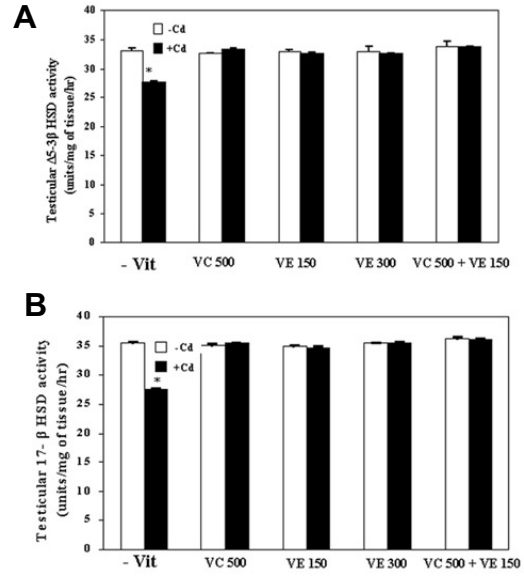

Fig. 2. Testicular steroidogenic enzyme activities after supplementation with vitamin C or vitamin E in cadmium-exposed rats. **A.** Effect of vitamin C or vitamin E supplementation on testicular Δ^5-3β-HSD activity, after a single s.c. injection of either 0.20 mg / 100 g BW cadmium (+Cd), or normal physiological saline (-Cd). * $P < 0.05$ compared with the corresponding rats not exposed to cadmium (-Cd) and rats receiving vitamin supplements and exposed to cadmium (+Cd). **B.** Effects of vitamin C or vitamin E supplementation on testicular 17β-HSD activity, after a single s.c. injection of either 0.20 mg / 100 g BW cadmium (+Cd), or normal physiological saline (-Cd). * $P < 0.05$ compared with the corresponding rats not exposed to cadmium (-Cd) and rats receiving vitamin supplements and exposed to cadmium (+Cd).

widely used as an indicator of oxidative stress (Favier, 1995). In our experiments, we evaluated lipid peroxidation levels in the testes of rats exposed to cadmium by measuring testicular MDA. In the (-Vit) group, testicular MDA increased by 67% in the Cd-exposed rats (Fig. 4) and vitamin C or vitamin E were equally effective in preventing this increase.

To evaluate the activities of antioxidant systems in testes affected by Cd exposure, we assayed testicular superoxide dismutase and glutathione peroxidase. Superoxide dismutase destroys the free radical superoxide by converting it to peroxide that can in turn be destroyed by glutathione peroxidase reactions (Mates, 2000). Hence any reduction in these enzymatic antioxidant systems will increase ROS. In the (-Vit) group, superoxide dismutase and glutathione peroxidase activities were reduced by 63 and 73% respectively, in the Cd-exposed rats ($P < 0.05$) (Figs. 5A and 5B) whereas in the vitamin supplemented groups, these activities were unchanged.

Discussion

Our observation that exposure to a single dose of Cd (0.20 mg / 100 g BW) results in decreased StAR mRNA level is consistent with the previous suggestion that Cd can affect DNA and RNA synthesis (Niyogi *et al.*, 1981; Sirover and Loeb, 1976). We have also demonstrated that Cd exposure decreases the activities of testicular Δ^5-3β and 17-β HSD, consistent with the low production of testosterone by Leydig cells. Cd being a divalent heavy metal has a strong affinity for the thiol groups of proteins and enzymes and can cause conformational changes that interfere with their function (Hassoum and Stohs, 1996). Since testicular Δ^5-3β- HSD and 17β-HSD activities were also affected, testosterone biosynthesis would be further reduced. In cadmium-exposed animals the expression of the StAR gene was reduced, the activities of the two steroidogenic enzymes were decreased by 17–22%, and serum testosterone level fell by 67%. These phenomena indicate that StAR is indeed critical for steroidogenesis.

We were interested in investigating whether Cd-induced suppression of steroidogenesis is due to oxidative damage to the testis. Cd compounds are known to induce various forms of oxidative damages, such as increased lipid peroxidation (Manca *et al.*, 1991), a reduction in glutathione peroxidase (Garcia-Fernandez, 2002), and DNA strand breaks (Snyder, 1988). However, Cd is not capable of accepting or donating electrons under physio-

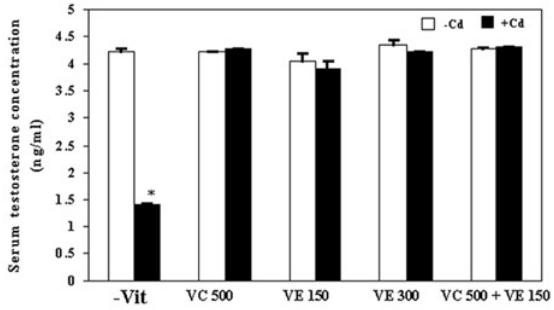

Fig. 3. Effects of vitamin C and vitamin E on serum testosterone level after s.c. injection of a single dose of 0.20 mg cadmium chloride / 100 g BW (+Cd) or normal physiological saline (-Cd). * $P < 0.05$ compared with the corresponding rats not exposed to cadmium (-Cd) and rats receiving vitamin supplements and exposed to cadmium (+Cd).

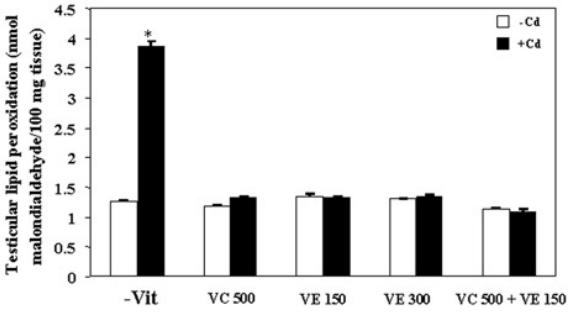

Fig. 4. Effects of single s.c. injection of single dose of 0.20 mg cadmium chloride / 100 g BW (+Cd) or normal physiological saline (-Cd) on lipid peroxidation product, malondialdehyde (nmol/ 100 mg wet tissue) among vitamin C and / or vitamin E supplement groups. * $P < 0.05$ compared with the compared with the corresponding rats not exposed to cadmium (-Cd) and rats receiving vitamin supplements and exposed to cadmium (+Cd).

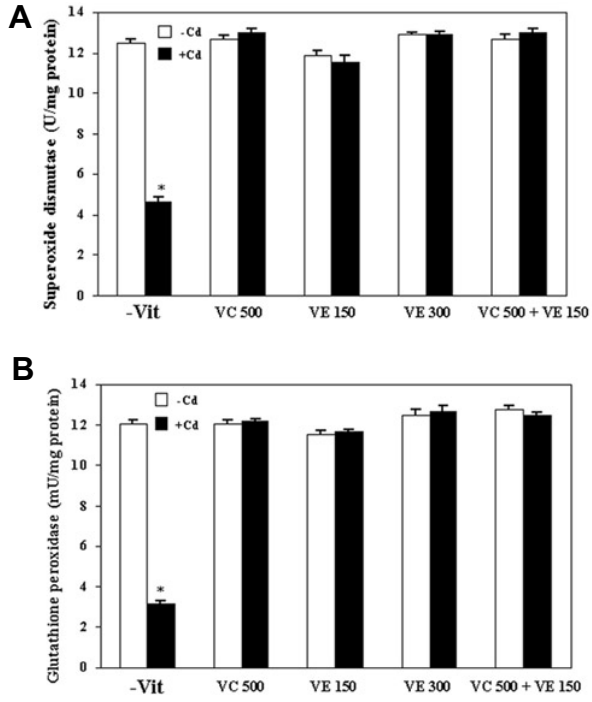

Fig. 5. Activities of testicular antioxidant enzymes in cadmium-exposed rats supplemented with vitamin C and / or vitamin E. **A.** Effects of vitamin C and vitamin E supplementation on testicular superoxide dismutase activity, after a single s.c. injection of either 0.20 mg / 100 g BW of cadmium (+Cd), or normal physiological saline (-Cd). * $P < 0.05$ compared with the corresponding rats not exposed to cadmium (-Cd) and rats receiving vitamin supplements and exposed to cadmium (+Cd). **B.** Effects of vitamin C and / or vitamin E supplementation on testicular glutathione peroxidase activity, after rats received a single s.c. injection of either 0.20 mg / 100 g BW of cadmium (+Cd), or normal physiological saline (-Cd). * $P < 0.05$ compared with the corresponding rats not exposed to cadmium (-Cd) and rats receiving vitamin supplements and exposed to cadmium (+Cd).

logical conditions (Ochi *et al*., 1987), and the mechanism by which it induces oxidative stress remains to be clarified. When the parameters of oxidative damage were evaluated, we observed that cadmium treatment led to higher levels of lipid peroxidation in the testis; testicular MDA increased, and the activities of the testicular antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) fell. Since Cd interacts with cell membranes, lipid peroxidation may be a direct consequence of membrane damage (Casalino *et al*., 1997) or of weakened antioxidant defense (Jamall and Sprowls, 1987). The reduction of SOD activity is known to be due replacement of the zinc (Zn) and/ or manganese (Mn) of the SOD molecule by Cd (Jacobson and Turner, 1980). Zn is an essential component of the oxidant defense system, functioning at many levels (Sato and Bremner, 1993). The reduction in activity of glutathione peroxidase might be due to depletion of selenium (Se) by cadmium (Zumkley, 1988). The changes in oxidant defense systems associated with cadmium exposure could increase the steady-state level of oxidants in the testis. Moreover, ROS are produced continuously in cells as a by-product of mitochondrial and microsomal electron transport and other metabolic processes (Hales, 2002). Mitochondrial respiration consumes 85–90% of the oxygen used by cells, and represents the greatest potential source of ROS. Steroidogenic cytochrome P450 enzymes also produce ROS as a by-product of their catalytic reaction mechanism (Hornsby, 1987; Quinn and Payne, 1984a; 1984b; 1985). Hydrogen peroxide, a potent oxidant, is known to inhibit steroidogenesis in Leydig cells (Dimer *et al*., 2003). Toxic lipid hydroperoxides and hydrogen peroxide produced during free radical-initiated oxidative stress are detoxified by selenium-dependent GSH-PX using glutathione (Mates,

2000). Hence an imbalance between O_2^-. and H_2O_2 might occur in the testes of Cd treated rats; the decrease in SOD and GSH-PX activities might indirectly lead to an increase in oxidative stress, causing ROS-induced damage to macromolecules such as DNA, proteins and key enzymes involved in testicular steroidogenesis and spermatogenesis. This idea is consistent with previous evidence that Cd depletes glutathione and protein-bound sulfhydryl groups, resulting in enhanced production of reactive oxygen species such as superoxide ion, hydroxyl radicals, and hydrogen peroxide (Oteiza *et al.*, 1999). These reactive oxygen species promote lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA and membrane damage, altered gene expression, and apoptosis (Stohs *et al.*, 2000).

The prevention of lipid peroxidation is essential for all aerobic organisms, and so the organism is well equipped with antioxidants that directly or indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals (Halliwell, 1995). Ascorbic acid (VC) is an important water-soluble antioxidant that reduces sulfhydryls, scavenges free radicals, and protects against endogenous oxidative DNA damage (Fraga *et al.*, 1991). Ascorbic acid, after being converted to dehydroascorbic acid by free radical reactions, is regenerated via the glutathione enzyme complex (Halliwell *et al.*, 1987). Ascorbic acid can prevent increased lipid peroxidation levels resulting from cadmium toxicity (Gupta and Kar, 1998), and our results indicate that it reduces lipid peroxidation in the testis. This is also consistent with the report that vitamin C and vitamin E can ameliorate ROS-related testicular toxicity in lead-exposed animals (Hsu *et al.*, 1998). Vitamin C supplementation in the present study helped to reduce MDA levels and at the same time increased the activity of testicular antioxidants, superoxide dismutase and glutathione peroxide in Cd-exposed rats. These actions of vitamin C help protect testicular steroidogenesis. Vitamin E (VE) is an important antioxidant, residing mainly in cell membranes. It is thought to interrupt the chain reactions involved in lipid peroxidation, and to scavenge ROS generated during the univalent reduction of molecular oxygen (Palamanda and Kehrer, 1993). Buettner (1993) found that VC and VE jointly protected lipid structures against peroxidation. Although VE is located in membranes and VC exists in the aqueous phase, VC was able to recycle oxidized VE (Frei, 1991). Recent findings show that damage to sperm by highly spermatotoxic ROS such as hydrogen peroxide and hydroxyl radicals can be prevented by the addition of VE and VC (Sharma and Agarwal, 1996). In the present study also, vitamin C and vitamin E reduced cellular toxicity caused by cadmium-induced ROS and protected the testicular antioxidant system. However, we did not observe a synergistic effect of VC and VE.

In summary, our results indicate that cadmium inhibits steroidogenesis by reducing cholesterol transfer mediated by the StAR protein. This effect is caused by excess generation of ROS in the testes resulting from depletion of antioxidant enzymes like SOD and GSH-PX. Both vitamin C and vitamin E can protect testicular function from ROS-induced oxidative stress.

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