

·Original Article·

The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats

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Abstract

Aim: To investigate the effect of formaldehyde (FA) on testes and the protective effect of vitamin E (VE) against oxidative damage by FA in the testes of adult rats. **Methods:** Thirty rats were randomly divided into three groups: (1) control; (2) FA treatment group (FAt); and (3) FAt + VE group. FAt and FAt + VE groups were exposed to FA by inhalation at a concentration of 10 mg/m³ for 2 weeks. In addition, FAt + VE group were orally administered VE during the 2-week FA treatment. After the treatment, the histopathological and biochemical changes in testes, as well as the quantity and quality of sperm, were observed. **Results:** The testicular weight, the quantity and quality of sperm, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione (GSH) were significantly decreased whereas the level of malondialdehyde (MDA) was significantly increased in testes of rats in FAt group compared with those in the control group. VE treatment restored these parameters in FAt + VE group. In addition, microscopy with hematoxylin-eosin (HE) staining showed that seminiferous tubules atrophied, seminiferous epithelial cells disintegrated and shed in rats in FAt group and VE treatment significantly improved the testicular structure in FAt + VE group. **Conclusion:** FA destroys the testicular structure and function in adult rats by inducing oxidative stress, and this damage could be partially reversed by VE. (*Asian J Androl* 2006 Sep; 8: 584–588)

Keywords: formaldehyde; vitamin E; testis; rats; oxidative stress; reproductive toxicity

1 Introduction

Formaldehyde (FA) is widely used in hospitals, laboratories and industrial settings. In addition, FA is often found in domestic environment [1] (e.g. paint, plywood, fabrics, cosmetics, heating and cooking emissions, etc). Humans are exposed to FA from both direct environ-

mental sources and the metabolism of xenobiotics [2]. The current Chinese occupational exposure limit (OEL) for FA is 0.5 mg/m³ [3], however, studies have shown that the levels of FA in occupational environment were far higher than this limit [3, 4].

FA can react with different macromolecules such as proteins and nucleic acid, or with low molecular weight substances such as amino acids [5, 6]. Furthermore, FA is a highly water soluble compound which can rapidly diffuse into many tissues [2].

These properties of FA might bring about human health problems. Various studies have focused on the harmful effects of FA on the respiratory system and hematological system [7, 8]. To date, however, reports regarding the

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effects of FA on male reproduction are still scarce and insufficient.

It has been shown that FA can increase the production of reactive oxygen species (ROS) in many tissues [2, 9]. ROS including singlet oxygen, hydrogen peroxide, superoxide anions and hydroxyl radicals are important mediators of cellular injury and play an important role in oxidative damage.

Vitamin E (VE), a well known antioxidant, plays an important role in scavenging free oxygen radicals and stabilizing the cell membranes [2]. A number of studies have shown that VE pretreatment significantly protects testes against oxidative damage [10, 11].

Therefore, the present study was designed to investigate the adverse effects of FA on the testes, and whether the effects are caused by oxidative stress and whether VE can reverse the effects.

2 Materials and methods

2.1 Animals and treatment

Thirty healthy adult male Sprague–Dawley rats weighing 208–216 g were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). They were acclimatized at a 12 h light:12 h dark cycle and fed a standard diet and tap water *ad libitum* for 1 week before the experiments commenced. The experiments were carried out in accordance with the Animal Experimentation Committee Regulation.

The rats were divided at random into three groups, each comprising 10 rats; (1) control group: rats were orally administered physiological saline (Hantang, Xi'an, China) and were not exposed to FA for 2 weeks (one seminiferous epithelial cycle in rats), (2) FA treatment group (FAT): rats were orally administered physiological saline and exposed to FA (Huagong, Xi'an, China) by inhalation at a concentration of 10 mg/m³ (12 h per day) for 2 weeks, and (3) VE treated group (FAT+VE): rats were orally administered with vitamin E (Shuangjing, Qingdao, China) at the dose of 30mg/kg body weight per day and exposed to FA by inhalation at a concentration of 10 mg/m³ (12 h per day) for 2 weeks.

The concentration of FA in the chambers was monitored by formtector (4160-2, Interscan, Chatsworth, CA, USA).

2.2 Testicular histopathology

At the end of the exposure, the rats were killed using

an overdose (50 mg/kg body weight i.p) of pentobarbital sodium (Sigma, Chicago, USA), and the testes were immediately removed and weighed. The left testis of each rat was used for histopathological examination and the right for biochemical assay. The left testis was fixed in fresh Bouin's solution for 24 h and then dehydrated and embedded in paraffin, finally 4 µm sections were cut and stained with hematoxylin-eosin (HE). The tissue sections were observed under a light microscope (Olympus, Tokyo, Japan) for the testicular histopathology according to Bustos-Obregon *et al.* [12].

2.3 Epididymal sperm analysis

One epididymis was minced with fine scissors in 4 mL physiological saline at 37°C and then filtered through one piece of gauze. One drop of the filtrate (sperm suspension) was placed on a slide for light microscope observation of sperm motility at a magnification of × 100, a total of 200 sperm per sample were evaluated. The spermatozoa density was estimated using Neubauer hemocytometer (Xinya, Shanghai, China) according to the methods described by Tang *et al.* [13] and expressed as 10⁷/g epididymal weight. An aliquot of the sperm suspension was smeared on a clean glass, stained with Comassie G250 (Sigma, Chicago, USA) and a total of 500 sperm per sample were evaluated, under a light microscope (Olympus, Tokyo, Japan) at a magnification of × 400, for the percentage of abnormal sperm.

2.4 Biochemical assays

The right testis of each rat was immediately decapsulated, cleaned and washed in precooled physiological saline several times and homogenized in 10 volumes of precooled phosphate buffered saline (pH 7.4), the homogenate was centrifugated at 3 000 × g for 15 min and the supernatant was used for biochemical assays. Protein concentration was estimated by the method of Lowry *et al.* [14]. The activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and the content of glutathione (GSH) and malondialdehyde (MDA) in testes were detected using commercial Assay Kits (Jiancheng, Nanjing, China).

2.5 Statistical analysis

All statistical analyses were carried out using SPSS statistical software version 11.5 (SPSS, Chicago, USA). All data were expressed as mean ± SD. Distribution analysis was conducted using the Shapiro-Wilk test. Data on oxidative stress parameters and testicular weight were

analyzed using one-way ANOVA. However, data on sperm parameters among the three groups were analyzed by Kruskal-Wallis test, and dual comparisons between groups were evaluated using the Mann-Whitney *U*-test. $P < 0.05$ was considered as significant.

3 Results

3.1 The testicular weight

The testicular weight was significantly decreased in rats of FAT group compared with that in the control ($P < 0.05$). Treatment with VE significantly prevented the decline of testicular weight ($P < 0.05$, Figure 1).

3.2 Testicular histopathology

Compared with the control group (Figure 2A), there were significant histopathological changes in testis of rats in FAT group. The main pathological changes included the atrophication of seminiferous tubules (Figure 2B), decrease in spermatogenic cells, and seminiferous epithelial cells disintegrated and shed into the lumina (Figure 2C). The interstitial tissue was edematous with vascular dilatation and hyperemia (Figure 2B, 2C). The lumina were azoospermic (Figure 2B, 2C). In FAT + VE group, the pathologic changes in seminiferous tubules had partly recovered, but the number of sperm in the lumina was still less than that in the control (Figure 2D).

3.3 Epididymal sperm

Compared with the control group, the sperm count and the percentage of motile sperm were significantly decreased, whereas the percentage of abnormal sperm was significantly increased in rats of FAT group ($P < 0.05$,

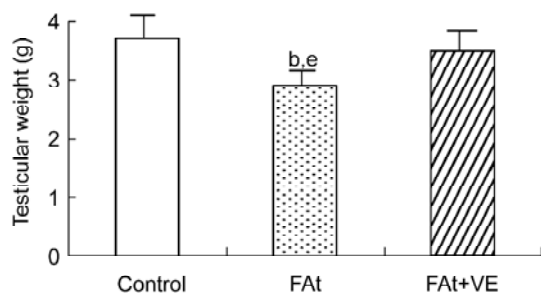


Figure 1. Effects of formaldehyde (FA) and vitamin E (VE) on testicular weight in rats (mean \pm SD, $n = 10$). ^{b,e} $P < 0.05$, compared with the control and FAT + VE groups. FAT, FA treatment group.

Table 1). Treatment with VE significantly prevented the decline of sperm quantity and quality in FAT + VE group ($P < 0.05$, Table 1).

3.4 Biochemical analysis

The activities of SOD, GSH-Px and GSH in rats of FAT group were significantly lower than those in the control group ($P < 0.05$). Treatment with VE significantly elevated the activities of SOD, GSH-Px and GSH ($P < 0.05$, Table 2).

Furthermore, MDA levels in the testicular tissue were found to be significantly higher in the FAT group than those in the control group. Treatment with VE prevented elevation of MDA levels significantly in FAT + VE group ($P < 0.05$, Table 2).

4 Discussion

FA is a common environmental contaminant. Although preventive measures aimed at reducing FA levels have been implemented, exposure to FA remains one of the most prominent occupational and environmental health problems [1–4].

The present study showed that the testicular weight

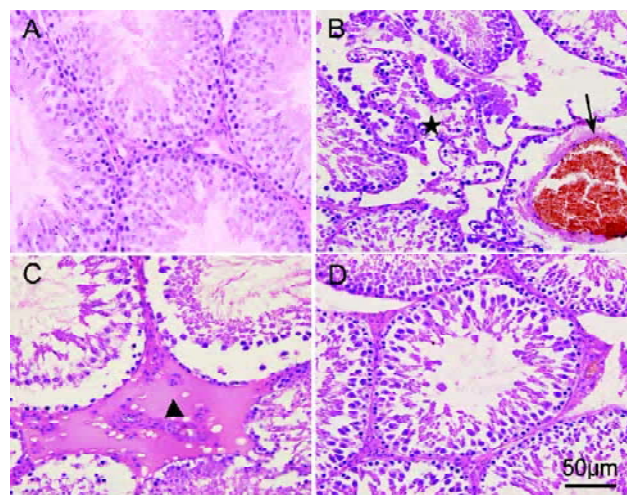


Figure 2. Light micrographs of testicular sections stained with hematoxylin-eosin (HE) ($\times 400$). (A): Testis in a rat of control group. (B): Testis in a rat of formaldehyde treatment (FAT) group showing seminiferous tubular atrophy (star) and interstitial vascular dilatation and hyperemia (arrow). (C): Testis in a rat of FAT group showing seminiferous epithelial cells disintegrated and shed into the lumina and interstitial edema (triangle). (D): Testis in a rat of FAT + vitamin E (VE) group. Scale bar = 50 μ m.

was significantly decreased in rats exposed to FA, which supports the results of other authors [13, 15]. In addition, FA caused regressive histological changes in the seminiferous tubules resulting in the suppression of spermatogenesis. Seminiferous tubules atrophy and spermatogenic cells decreasing were morphologic indicators of spermatogenesis failure [16]. Seminiferous epithelial cells sloughing were usually the result of the damage of Sertoli cells and interruption of the intercellular bridge [17].

Furthermore, the decrease in sperm count and increase in abnormal sperm were consistent with an earlier report on mice [13]. The changes in sperm indicate the genotoxicity of FA.

From the above results, we could conclude that FA has a harmful effect on male reproduction.

Pro-oxidant and antioxidant balance is vital for normal biological functioning of the cells and tissues [18]. The antioxidant system comprises enzymatic antioxidants such as SOD, GSH-Px and non-enzymatic antioxidants such as GSH. SOD and GSH-Px are major enzymes that scavenge harmful ROS in male reproductive organs [18]. GSH repairs oxidized and damaged molecules and plays a role in regulating a variety of cellular functions. Oxidative stress occurs when the oxidative homeostasis is damaged [19]. Excessive ROS are generated and then cause lipid peroxidation. MDA is one of the most important products of lipid peroxidation, which interferes with protein biosynthesis by forming adducts with DNA, RNA and protein [19]. It is known that human testes and spermatozoa are extremely sensitive to ROS-induced damage. Excessive ROS increases germ cells apoptosis

and inhibits the activity of spermatozoa [18]. Sheweita *et al.* [20] reported that almost 40% of infertile males show abnormally increased ROS levels.

The present study showed an obvious decrease of testicular antioxidant system, whereas there was a prominent increase of the testicular lipid peroxidation product MDA in the testis of FA exposure rats. Similar phenomena are often observed after exposure to chemicals and gamma radiation that cause testicular damage [10, 11]. This suggests that oxidative stress is an important mechanism of testicular damage.

The present study found that VE treatment significantly protected the testicular structure and sperm quantity and quality. This protective effect of VE could be the result of direct free radical scavenger properties [2]. VE could also react with membrane phospholipid bilayers to break the chain reaction initiated by ROS [11]. The improvement of the activities of antioxidant systems might be one of the results of the free radical scavenging effect of VE [2]. The detailed mechanisms are worthy of further investigation.

In conclusion, FA destroys the testicular structure and function in adult rats by inducing oxidative stress, which could be partially reversed by VE.

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Table 1. Effects of formaldehyde (FA) and vitamin E (VE) on sperm count, the percentage of motile sperm and abnormal sperm in rats (mean \pm SD, $n = 10$). ^{b,e} $P < 0.05$, compared with the control and FAt + VE groups. FAt: formaldehyde treatment group.

Group	Sperm count ($10^7/g$ epididymal weight)	Percentage of motile sperm	Percentage of abnormal sperm
Control	4.91 \pm 0.38	93.81 \pm 3.99	4.82 \pm 1.72
FAt	3.02 \pm 0.68 ^{b,e}	76.38 \pm 2.05 ^{b,e}	17.33 \pm 4.31 ^{b,e}
FAt + VE	4.13 \pm 0.43	82.42 \pm 2.97	10.56 \pm 2.73

Table 2. Effect of formaldehyde (FA) and vitamin (VE) on lipid peroxidation and antioxidative defense in testes of rats (mean \pm SD, $n = 10$). ^{b,e} $P < 0.05$, compared with the control and FAt + VE groups. FAt: formaldehyde treatment group; GSH: glutathione; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; SOD: superoxide dismutase.

Group	SOD (NU/mg protein)	GSH-Px (U/mg protein)	GSH (mg/mg protein)	MDA (nmol/mg protein)
Control	38.96 \pm 2.71	13.72 \pm 3.49	58.97 \pm 7.63	0.52 \pm 0.12
FAt	27.25 \pm 3.57 ^{b,e}	10.03 \pm 2.26 ^{b,e}	36.65 \pm 8.17 ^{b,e}	0.75 \pm 0.16 ^{b,e}
FAt + VE	35.34 \pm 3.05	13.36 \pm 2.73	51.81 \pm 7.76	0.61 \pm 0.13

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