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·*Original Article*·

Modulatory effects of diallyl sulfide against testosterone-induced oxidative stress in Swiss albino mice

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Abstract

Aim: To investigate the protective effect of diallyl sulfide (DAS), a constituent of garlic, against testosterone-induced oxidative stress in male Swiss albino mice. **Methods:** The animals were given low (250 mg/animal) and high dose (500 mg/animal) of DAS in corn oil for 7 days along with testosterone (5 mg/kg body weight, i.p.). At the end of the study period, the prostate and the liver were dissected to determine various antioxidant enzyme levels (catalase, superoxide dismutase, glutathione reductase, glutathione-s-transferase) and lipid peroxidation. **Results:** In testosterone treated mice, depleted antioxidant enzyme level was accompanied with enhancement in lipid peroxidation in prostate and liver. DAS significantly restored the testosterone-induced antioxidant enzymes and lipid peroxidation in the both organs. These changes appear to be mediated by the antioxidant-enhancing effects of DAS. **Conclusion:** The results of the present study suggest that DAS is effective in exerting antioxidant effects by inhibiting testosterone-induced oxidative stress and might be helpful in preventing prostate cancer. *(Asian J Androl 2006 Nov; 8: 719–723)*

Keywords: oxidative stress; antioxidants; lipid peroxidation; diallyl sulfide; testosterone

1 Introduction

Prooxidants are generated in our body during the normal metabolic processes and the exposure to adverse pathophysiological conditions. Exposure to prooxidants results in oxidative stress that shifts the balance in favor of prooxidants [1]. Reactive oxygen species (ROS) are generated during oxidative stress, including hydroxyl radical superoxide, peroxyl radical, hydrogen peroxide and singlet oxygen. These ROS are known to play a major role in either the initiation or progression of carcinogenesis [2]. Physiological concentration of androgen is associated with a shift in the prooxidant: oxidant balance of the prostate towards more oxidative stresses [3]. Occasionally, these changes result in oxidative stress mediated stimuli to specific changes of gene expression, thereby resulting in dysregulated cell growth and, therefore, tumor development in tissue, particularly in the prostate [4]. The liver, featuring key steroid enzymes, represents a major site for biotransformation, conjugation and catabolism of gonadal steroids. Increased circulating levels of androgens might also affect liver cancer risk as observed in hepatitis C virus infected women [5].

Consumption of fruit and vegetables containing large amounts of antioxidative nutraceuticals has been associated with the balance of the free radicals/antioxidants

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status, which helps to minimize the oxidative stress in the body and to reduce the risk of cancers [6]. Garlic has been recognized since ancient times not only as a flavoring agent for food but also for its medicinal properties, including bactericidal, antineoplastic, hypolipidaemic and hypocholesterolaemic effects [7]. Epidemiological studies provide evidence that an increased intake of garlic is associated with a decreased risk of cancer [8]. When garlic is cut, chopped or crushed, the clove's membrane is disrupted and S-allylcysteine sulfoxide is transformed enzymatically into allicin by allinase. The main components of the volatile oil are sulfur compounds, especially allicin, diallyl sulfide (DAS), diallyl disulfide, diallyl trisulfide and ajoene. DAS has been shown to inhibit several chemically induced forms of cancer, such as benzo(a)pyrene-induced forestomach tumors and pulmonary adenomas [9], diethylstilbestrol-induced breast cancer in rats [10] and cyclophosphamide-induced chromosomal aberrations in Swiss albino mice [11].

All these findings suggest a link between polyphenols and prevention of carcinogenesis. Among all the sulfides present in garlic, studies on DAS are comparatively few; therefore, further investigations are required to elucidate the mechanisms involved in its chemopreventive effects. The present study attempts to evaluate the antioxidative potential of DAS against testosterone-induced oxidative stress in Swiss albino mice.

2 Materials and methods

2.1 Chemicals

Testosterone, DAS, phenazine methosulfate, 1-chloro-2, 4-dinitrobenzene (CDNB), 2-thiobarbituric acid, 1,1, 3,3-tetramethoxy propane (TMP), nitro blue tetrazolium, nicotinamide adenine dinucleotide phosphate reduced (NADPH), nicotinamide adenine dinucleotide reduced, reduced glutathione (GSH), and oxidized glutathione were obtained from Sigma Chemical Company (St. Louis, MO, USA). The rest of the chemicals used in the present study were of analytical grade and were procured locally.

2.2 Animals and treatment

Male Swiss albino mice of 8 weeks of age (20–24 g body weight) were taken from the animal colony of Industrial Toxicology Research Centre and acclimatized for 1 week. They were randomly divided into five groups, each consisting of 6 animals. Animals were kept under standard conditions (25 ± 2°C, relative humidity 57% ± 2% and a 12 h:12 h Light:Dark cycle) and were fed with a synthetic pellet diet (Ashirwad, Chandigharh, India). Mice in group I were kept untreated and given normal drinking water, whereas animals in group III and IV were given low doses (250 μg/mouse) and high doses (500 μg/mouse) of DAS (dissolved in ethanol and diluted in corn oil), respectively. Testosterone (5 mg/kg body weight dissolved in ethanol and diluted in normal saline) was given i.p. to the animals of group II, III and IV. Animals of group V served as vehicle controls and were given DAS (500 μg/mouse). The feeding regimen was followed for 7 days. Animals from all the groups were examined every day for gross morphological changes and fluid consumption. On day 8, all the animals were killed by cervical dislocation and prostate and liver from each animal were excised and washed immediately with ice cold saline. The tissues were homogenized in ice-cold phosphate buffer (pH 7.4) containing 0.15 mol/L KCl and used as enzyme source.

2.3 Biochemical estimation

The activity of catalase (CAT) was analyzed according to the method of Sinha *et al.* [12] using H_2O_2 as substrate. The enzyme activity was measured following the disappearance of H_2O_2 at 570 nm using an spectrophotometer and was expressed as μmoles of H_2O_2 consumed/min/mg protein. Superoxide dismutase (SOD) was analyzed as per the protocol of Kakkar *et al.* [13]. A single unit of enzyme activity is defined as the quantity of SOD required for 50% inhibition of reaction. Glutathione reductase (GR) activity was determined using the procedure of Carlberg and Mannervic [14]. The activity was expressed as nmoles NADPH consumed/min/mg protein. Glutathione S-transferase (GST) was analyzed using the method of Habig *et al.* [15]. The activity was expressed as nmoles CDNB-GSH conjugate/min/mg protein. Lipid peroxidation was analyzed using the method of Ohkawa *et al.* [16]. The peroxides were expressed as nmoles of thiobarbutric acid reactive substance/mg of tissue protein using TMP as standard. The protein content of the tissue was determined using the method of Lowry *et al.* [17], using bovine serum albumin as standard at 660 nm.

2.4 Statistical analysis

Significance difference of variance in antioxidant level data between positive control group II and untreated group I as well as that between group II and experimen-

tal groups (groups III–IV) was analyzed using paired *t*-test, $P < 0.05$ was considered to be significant.

3 Results

No significant differences were detected in gross morphological changes between the control and treated groups. As expected, there was no significant difference observed in the levels of antioxidant enzymes and lipid peroxidation in group I (untreated) when compared to group V (DAS alone), indicating nontoxic effects of DAS.

In the prostate, testosterone (*vs.* no treatment) significantly reduced the levels of antioxidant enzymes (CAT, SOD, GR and GST) by 32.3–45.8%; whereas in testosterone-treated animals, low and high DAS recovered these enzymes by 15.5–25.1% and 32.2–59.4%, respectively (Table 1). Similar findings were observed in liver specimens (Table 2). Testosterone (*vs.* no treatment) significantly reduced the levels of antioxidant enzymes by 26.5–31.8%; whereas in testosterone treated animals, low and high DAS recovered these enzymes by 13.3–26.2% and 30.2–35.2%, respectively.

Enhancement in lipid peroxidation was found to be 93.2% and 35.0% in prostate and liver by testosterone administration (Table 3). Lipid peroxidation significantly diminished by 20.3% and 52.7% with low and high DAS

Table 1. Modulatory effects of diallyl sulfide (DAS) in testosterone-induced oxidative stress in mouse prostate. Values are expressed as mean ± SE of 6 animals of three independent experiments. †Significant increase over testosterone-treated group ($P < 0.05$). ‡Significant decrease over untreated control group ($P < 0.05$). Testosterone: 5 mg/kg body weight; ↑, increased percentage; ↓, decreased percentage.

Treatment	Catalase (μmoles/min /mg protein)	Superoxide dismutase (U/min/mg protein)	Glutathione reductase (nmoles/min/mg protein)	Glutathione S-transferase (nmoles/min/mg protein)
Untreated	173.5 ± 16.3	3.7 ± 0.2	59.4 ± 4.2	55.9 ± 3.4
Testosterone	96.5 ± 10.3‡	2.5 ± 0.2‡	32.1 ± 3.2‡	32.6 ± 2.6‡
(%)	44.4 ↓	32.3 ↓	45.8 ↓	41.7 ↓
Testosterone + DAS (250 μg/animal)	120.6 ± 14.8†	2.9 ± 0.1†	40.2 ± 3.8†	38.5 ± 2.2†
(%)	25.0 ↑	15.5 ↑	25.1 ↑	18.1 ↑
Testosterone + DAS (500 μg/animal)	153.8 ± 14.7†	3.3 ± 0.2†	52.6 ± 5.1†	48.7 ± 4.8†
(%)	59.4 ↑	32.1 ↑	63.7 ↑	49.4 ↑
DAS (500 μg/animal)	170.7 ± 18.8	3.8 ± 0.3	61.9 ± 5.8	59.8 ± 5.4

Table 2. Modulatory effects of diallyl sulfide (DAS) in testosterone-induced oxidative stress in mouse liver. Values are expressed as mean ± SE of 6 animals of three independent experiments. †Significant increase over testosterone treated group ($P < 0.05$). ‡Significant decrease over untreated control group ($P < 0.05$). Testosterone: 5 mg/kg body weight; ↑, increased percentage; ↓, decreased percentage.

Treatment	Catalase (μmoles/min/mg protein)	Superoxide dismutase (U/min/mg protein)	Glutathione reductase (nmoles/min/mg protein)	Glutathione S-transferase (nmoles/min/mg protein)
Untreated	285.2 ± 27.5	2.2 ± 0.2	32.5 ± 2.3	82.2 ± 6.6
Testosterone	206.8 ± 22.2‡	1.5 ± 0.1‡	22.6 ± 2.2‡	60.4 ± 3.5‡
(%)	27.5 ↓	31.8 ↓	30.7 ↓	26.5 ↓
Testosterone + DAS (250 μg/animal)	247.5 ± 23.5†	1.8 ± 0.1†	25.5 ± 1.5†	76.2 ± 4.9†
(%)	19.7 ↑	20.0 ↑	13.3 ↑	26.2 ↑
Testosterone + DAS (500 μg/animal)	279.7 ± 28.3†	2.0 ± 0.2†	29.4 ± 2.4†	81.1 ± 8.0†
(%)	35.2 ↑	33.3 ↑	30.2 ↑	34.2 ↑
DAS (500 μg/animal)	290.0 ± 28.8	2.3 ± 0.2	33.7 ± 2.6	86.9 ± 7.8

Table 3. Status of lipid peroxidation (nmoles thiobarbutric acid reactive substance/mg protein) induced by diallyl sulfide (DAS) against testosterone in prostate and liver of Swiss albino mice. Values are expressed as mean ± SE of 6 animals of three independent experiments. †Significant decrease over testosterone treated group ($P < 0.05$). ‡Significant increase over untreated control group ($P < 0.05$). Testosterone: 5 mg/kg body weight; ↑, increased percentage; ↓, decreased percentage.

Treatment	Prostate	Liver
Untreated	1.3 ± 0.1	2.20 ± 0.2
Testosterone	2.5 ± 0.3‡	3.0 0.4‡
(%)	93.2 ↑	35.0 ↑
Testosterone + Low DAS (250 μg/animal)	2.1 ± 0.2†	2.6 ± 0.2†
(%)	20.3 ↓	13.3 ↓
Testosterone + DAS (500 μg/animal)	1.7 ± 0.2†	2.4 ± 0.2†
(%)	52.7 ↓	23.2 ↓
DAS (500 μg/animal)	1.3 ± 0.1	2.1 ± 0.1

treatment in the prostate, respectively. Lipid peroxidation was also lowered in the liver by 13. 3% and 23.2% in the low and high DAS pretreated groups, respectively.

4 Discussion

Androgen are essential for normal prostate physiology and play a key role in either the initiation or progression of prostate cancer by inducing oxidative stress [4]. They are toxic to the liver in increased concentrations in the circulatory system [5]. ROS associated oxidative damage is well documented in prostate cancer [3, 4] and down modulation of oxidant enzymes observed in human prostate carcinoma cell lines; namely, DU 145 and LNCaP [3].

The liver, rich in reduced GSH, supplies the GSH to various extrahepatic tissues through a distinct GSH transport system. GSH maintains the integrity of the liver when the organ is challenged with a wide variety of xenobiotics, ROS and toxic compounds. GSH in conjuction with GR and GST detoxify ROS, thereby enhancing resistance against oxidative stress [18]. The depletion of antioxidant enzymes like GR and GST in prostate and liver, resulting from their increased use to scavenge ROS, might shift the redox status towards oxidative stress. Oxidative stress arising as a result of ROS overproduction coupled with deficiency of host antioxidant defense mechanisms observed in the present study might be an important factor contributing to the development of prostate and liver cancer.

Oxidative stress arising as a result of overproduction of radical non-radical ROS is inactivated by CAT, SOD, GR and GST [19]. Treatment with DAS in the present study effectively reduced the frequency of occurrence of testosterone-induced lipid peroxidation and enhanced the level of antioxidant enzymes CAT, SOD, GR and GST in prostate and liver in a dose dependent manner. These results indicate protective effects of DAS against testosterone-induced oxidative stress. By increasing the GPx and SOD activity which removes peroxides and superoxides, DAS might prevent the accumulation of ROS by trapping them. These modulations in the antioxidant enzyme system, which upregulate the host detoxification process, might be associated with reduced risk of prostate and liver cancer. Lipid peroxides cause damage to cellular macromolecules by generation of ROS [20], which is considered to enhance carcinogenesis.

The antioxidant property of DAS might result from the contribution of sulfur components at different steps of the process. Sparnins *et al.* [9] suggested that the protective effect of DAS might be caused by increased activity of GST, which catalyzes conjugation of electrophillic compounds with GSH. The intracellular content of GSH increases within the cells following treatment with DAS. This phenomenon is beneficial to the detoxification and antioxidation capabilities of hepatocytes [8], because they protect cells from the toxic effects of ROS.

The World Health Organization recommends 2–5 g of fresh garlic, 0.4–1.2 g of dried powder, 2–5 mg of oil, 300–1 000 mg of extract, or other formulations that are equal to 2–5 mg of allicin daily in adults. In the present study, we used 250 μg and 500 μg of DAS per mouse. Concentration of DAS varies from 30–100 mg/g of garlic, depending upon the geographical conditions. Therefore, the dose used in the present study seems to be achievable in humans.

These findings might open up new possibility for cancer prevention. Although mechanisms of antioxidant activities of DAS are not clear enough, various factors seem to contribute to it. The present study demonstrates that DAS inhibits testosterone mediated oxidative injury in prostate and liver. The data also imply that antioxidant enzymes can be used as a target for studies on prevention of different types of cancer, including prostate and liver cancer, and that DAS merits further investigations for developing strategies against carcinogenesis.

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