

Lycopene prevents adriamycin-induced testicular toxicity in rats

Ahmet Ateşşahin, Ph.D.,^a Gaffari Türk, Ph.D.,^b İzzet Karahan, Ph.D.,^a Seval Yılmaz, Ph.D.,^c Ali Osman Çeribaşı, Ph.D.,^d and Özgür Bulmuş, Ph.D.^e

^aDepartment of Pharmacology and Toxicology, ^bDepartment of Reproduction and Artificial Insemination, ^cDepartment of Biochemistry, and ^dDepartment of Pathology, Faculty of Veterinary Medicine, and ^eDepartment of Physiology, Faculty of Medicine, University of Firat, Elazığ, Turkey

Objective: To investigate a possible protective role of lycopene on adriamycin (ADR)-induced spermiotoxicity using quantitative, biochemical and histopathological approaches.

Design: Experimental study.

Setting: Firat University Medical School, Experimental Research Centre, Elazığ, Turkey.

Animals: Twenty four Sprague Dawley rats (8-weeks old)

Intervention(s): Adriamycin (10 mg kg⁻¹) was intraperitoneally injected and lycopene (4 mg kg⁻¹) was administered by gavage in corn oil.

Main outcome measure(s): Reproductive organ weights were evaluated along with epididymal sperm concentration, motility and morphology. Testicular histological findings, oxidative status and plasma testosterone levels were also determined.

Result(s): Lycopene ameliorated ADR-induced reductions in both testes and epididymis weights. ADR decreased sperm motility, increased total abnormal sperm rates, but epididymal sperm concentration was not changed compared to control. A marked normalization was achieved in sperm motility and morphology in pretreatment with lycopene. Although testosterone level was decreased in ADR group compared to control, no changes were observed in pretreatment group. An increase in malondialdehyde and a decrease reduced glutathione concentrations were detected in alone ADR group compared to control. Pretreatment with lycopene restored significantly malondialdehyde and reduced glutathione concentrations. ADR caused severe degenerative changes in germinal cells, atrophy in the diameter size of seminiferous tubules and germinal cell thickness. However, ADR-induced histopathological alterations were effectively reverted by pretreatment with lycopene.

Conclusion(s): This study clearly indicates that ADR treatment markedly impaired testicular function and that pretreatment with lycopene might prevent this toxicity. (Fertil Steril® 2006;85(Suppl 1):1216–22. ©2006 by American Society for Reproductive Medicine.)

Key Words: Lycopene, adriamycin, testicular function, toxicity

Adriamycin (ADR) (also named doxorubicin) is an anthracycline antibiotic that has been used for more than 30 years for the treatment of a wide variety of cancers. It causes DNA double strand breaks by intercalating into the DNA and by stabilizing topoisomerase II–DNA complex formation. However, the clinical use of ADR in long-term treatment is limited due to its serious side effects, particularly the development of a dose-dependent form of cardiomyopathy (1, 2). In addition, ADR has toxic effects on liver and testis tissue. Occurrence of sterility in testicular cancer and Hodgkin's disease after treatment with anticancer drugs is an important concern (2–4).

Biochemical mechanism of ADR toxicity is currently unknown. However, inhibition of DNA synthesis and the formation of oxygen radicals following lipid peroxidation in tissues have implicated the toxicity of ADR (5, 6). In order to protect spermatogenesis from xenobiotics, many antioxi-

dants, such as vitamin E, vitamin C, carotenoids, selenium, melatonin, lipoic acid, etc., have been attempted (1, 3, 5, 7).

Lycopene, an aliphatic hydrocarbon, is one of the 600 known naturally occurring carotenoids. Recently, lycopene, a naturally occurring carotenoid in tomatoes, has attracted considerable attention as a potential chemopreventive agent. In addition, lycopene has received particular attention in recent years as a result of studies indicating that it is a highly efficient antioxidant and has a singlet-oxygen and free radical scavenging capacity (8–10).

The aims of this study were to investigate the effects of ADR on sperm characteristics, plasma testosterone levels, and histopathologic and biochemical changes related to oxidative stress in testis tissue and to highlight the protective effect of lycopene on these parameters.

MATERIALS AND METHODS

Chemicals

Adriamycin (Adriblastina 10 mg, doxorubicin hydrochloride) was purchased from Carlo Erba (Istanbul, Turkey) and

Received June 7, 2005; revised and accepted November 23, 2005.
Reprint requests: Ahmet Ateşşahin, Ph.D., Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim, Firat Üniversitesi, Dali 23119, Elazığ, Turkey (FAX: +90-424-238 81 73; E-mail: aatessahin@hotmail.com).

lycopene 10% FS (Redivivo, code 7803) from DSM Nutritional Products (Istanbul, Turkey). All other chemicals were obtained from Sigma (St. Louis, MO).

Animals and Treatments

In this study, 24 healthy adult male Sprague-Dawley rats (eight weeks old weighing 190–240 g) were used. The animals were obtained from the Firat University Medical School, Experimental Research Center, Elazig, Turkey, and were kept under standard laboratory conditions (12 h light:12 h dark and $24^{\circ}\text{C} \pm 3^{\circ}\text{C}$). The rats were fed standard commercial laboratory chow (pellet form, Elazig Food Company, Elazig, Turkey). Food and water were provided *ad libitum*.

The rats were divided into four groups, each group containing six rats. Adriamycin was injected intraperitoneally at the dose of 10 mg kg^{-1} , which is well documented to induce testicular toxicity in rats (5, 6). Lycopene were suspended in corn oil and administered to the animals by gavage at the dose of 4 mg kg^{-1} . The dose of lycopene used in this study was selected on the basis of the previous studies (8–10). Group 1 (control) received a single-dose intraperitoneal injection of 1 mL isotonic saline following treatment with 0.5 mL corn oil for 10 days. Group 2 received a single-dose intraperitoneal injection of 1 mL isotonic saline following 10 days of lycopene treatment. Group 3 received a single dose of ADR following 10 days of treatment with 0.5 mL corn oil. Group 4 received a single dose of ADR following 10 days of lycopene treatment.

Sample Collection

The rats in all groups were decapitated on the third day after ADR injection, which induced testicular toxicity within this time. Blood samples were collected into tubes containing sodium oxalate (2%) and centrifuged at 3,000 rpm for 10 min. Measurement of testis weight, length, thickness, epididymal, seminal vesicle, and prostatic weight were evaluated along with epididymal sperm concentration, sperm motility, and sperm morphology. One of the testes was fixed in 10% formalin for histopathologic examinations. Plasma and other testis samples were stored at -20°C until biochemical analyses.

Epididymal Sperm Concentration, Motility, and Abnormal Sperm Rate

Spermatozoa in the right epididymis were counted by a modified method of Yokoi et al. (11). Briefly, the epididymis was minced with anatomic scissors in 5 mL physiologic saline, placed in a rocker for 10 min, and then allowed to incubate at room temperature for 2 min. After incubation, the supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%), and 25 mg eosin per 100 mL of distilled water. Total sperm number was determined by using a hemocytometer. Approximately

10 μL of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 min. The cells settled during this time were counted with the help of light microscope at $200\times$ magnification.

The progressive motility was evaluated by an earlier method described by Sönmez et al. (12). The fluid obtained from the left cauda epididymis with a pipette was diluted to 0.5 mL with Tris buffer solution. A slide was placed on light microscope with heater table, an aliquot of this solution was placed on the slide, and percentage motility was evaluated visually at a magnification of $400\times$. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were kept at 35°C .

The described method by Ateşşahin et al. (10) was used for determination of the percentage of morphologically abnormal spermatozoa. According to the method, slides were prepared with India ink. A total of 300 sperm cells were counted on each slide under light microscope at $400\times$ magnification.

Biochemical Assays

The testis tissue was homogenized in Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. Concentrations of malondialdehyde (MDA), as proceeding from lipid peroxidation, were measured in the homogenate. Then homogenates were centrifuged at $18,000g$ ($+4^{\circ}\text{C}$) for 30 min to determine reduced glutathione (GSH) levels. The MDA concentrations were assayed according to a modified method of Ohkawa et al. (13), based on the reaction with thiobarbituric acid, and were expressed as nmol g^{-1} tissue.

Tissue GSH concentrations were measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman (14) and were expressed as $\mu\text{mol g}^{-1}$ tissue. Protein concentrations were measured according to Lowry et al. (15). Plasma testosterone levels (catalogue no. L2KTT; Immulite Diagnostic Products Corporation, Los Angeles, CA) were determined by chemiluminescence immunoassay and expressed as ng mL^{-1} (16).

Histopathologic Examinations

Fixed testis tissue samples in 10% formalin were embedded in paraffin sectioned at $5\text{ }\mu\text{m}$ and were stained with hematoxylin and eosin (H&E). Light microscopy was used for the evaluations. The diameter and germinative cell layer thickness of the seminiferous tubule (ST) from five different areas of each testicle were measured using an ocular micrometer in a light microscope, and the average size and thickness of ST were calculated.

Statistical Analyses

All values were presented as mean $\pm$ SEM. One-way analyses of variance (ANOVA) and post hoc Duncan test were

TABLE 1**Testes, epididymis, and accessory gland weights and testis dimensions in all groups.**

	Control	ADR	Lycopene	Lycopene + ADR
Testis weight (mg)				
Right	1240.0 ± 30.8 ^A	1090.0 ± 27.9 ^B	1236 ± 28.8 ^A	1220.0 ± 31.1 ^A
Left	1223.3 ± 31.9 ^a	1033.3 ± 70.4 ^b	1230.3 ± 30.2 ^a	1236.0 ± 17.7 ^a
Testis length (cm)				
Right	1.83 ± 0.02 ^A	1.68 ± 0.03 ^C	1.91 ± 0.09 ^A	1.78 ± 0.02 ^{A,B}
Left	1.83 ± 0.02 ^a	1.73 ± 0.04 ^a	1.82 ± 0.02 ^a	1.84 ± 0.02 ^a
Testis thickness (cm)				
Right	0.93 ± 0.06 ^a	0.97 ± 0.05 ^a	0.91 ± 0.07 ^a	0.93 ± 0.02 ^a
Left	0.91 ± 0.04 ^a	0.89 ± 0.06 ^a	0.90 ± 0.05 ^a	0.92 ± 0.02 ^a
Epididymis weight (mg)				
Right	363.3 ± 10.2 ^A	281.7 ± 9.1 ^B	360.7 ± 12.5 ^A	392.0 ± 15.9 ^A
Left	366.7 ± 12.0 ^A	288.3 ± 18.5 ^B	370.7 ± 10.7 ^A	380.0 ± 22.3 ^A
Seminal vesicles (mg)	595.0 ± 64.6 ^A	308.0 ± 48.9 ^B	607.0 ± 43.8 ^A	330.0 ± 54.0 ^B
Prostate (mg)	305.0 ± 13.4 ^a	246.0 ± 25.4 ^a	298.0 ± 10.4 ^a	248.0 ± 41.6 ^a

^{A,B} Different letters within same line showed significant ($P < .01$) differences among the groups.^{a,b,c} Different letters within same line showed significant ($P < .05$) differences among the groups.

Ateşşahin. Lycopene prevents adriamycin-induced spermiotoxicity. Fertil Steril 2006.

performed to determine the differences among all groups in the whole parameters using the SPSS/PC computer program (version 12.0; SPSS, Chicago, IL).

RESULTS

Organ Weights and Dimensions

The values of testis weights and dimensions, epididymis, and accessory gland weights in all groups of rats are given in Table 1. Although a significant decrease was found in both right ($P < .01$) and left ($P < .05$) testis weights in the ADR group compared to the control group, no difference was observed in the pretreatment group as compared to the control group. There was a significant difference ($P < .01$) between the control and ADR alone groups with respect to the

lengths of the right testes, but for the left testes no differences were determined among any of the groups.

When the weight of the epididymis were analyzed it was determined that all the groups receiving ADR alone had significantly lighter epididymis, both right and left, as compared to the control group ($P < .01$). The weight of seminal vesicles were significantly lower in the ADR alone and pretreatment groups than in the control group ($P < .01$). No differences were found in the weight of the prostate among any groups.

Sperm Characteristics

Epididymal sperm concentration, sperm motility, and abnormal sperm rates are presented in Table 2. No significant

TABLE 2**Epididymal sperm concentrations, sperm motility, and abnormal sperm rates in all groups.**

	Control	ADR	Lycopene	Lycopene + ADR
Epididymal sperm concentration (million g ⁻¹)	301.83 ± 14.02 ^a	288.66 ± 43.91 ^a	310.25 ± 21.34 ^a	294.00 ± 26.95 ^a
Sperm motility (%)	69.16 ± 1.63 ^A	32.22 ± 2.74 ^B	71.09 ± 0.97 ^A	60.33 ± 2.78 ^A
Abnormal sperm rate (%)				
Head	1.17 ± 0.36 ^a	3.84 ± 2.35 ^b	1.21 ± 0.40 ^a	2.00 ± 0.40 ^a
Tail	3.58 ± 0.37 ^a	4.67 ± 1.07 ^a	3.06 ± 0.23 ^a	2.90 ± 0.57 ^a
Total	4.75 ± 0.36 ^a	8.51 ± 3.41 ^b	4.27 ± 0.36 ^a	4.90 ± 0.62 ^a

^{A,B} Different letters within same line showed significant ($P < .01$) differences among the groups.^{a,b} Different letters within same line showed significant ($P < .05$) differences among the groups.

Ateşşahin. Lycopene prevents adriamycin-induced spermiotoxicity. Fertil Steril 2006.

TABLE 3

Testis tissue MDA concentrations, reduced GSH levels, plasma testosterone levels, and diameter size and germinative cell thickness of ST in all groups.

	Control	ADR	Lycopene	Lycopene + ADR
MDA (nmol ml ⁻¹)	0.80 ± 0.09 ^A	1.94 ± 0.26 ^B	0.69 ± 0.12 ^A	0.64 ± 0.08 ^A
GSH (μmol mg protein ⁻¹)	0.16 ± 0.01 ^A	0.10 ± 0.04 ^B	0.15 ± 0.02 ^A	0.13 ± 0.02 ^{A,B}
Testosterone (ng mL ⁻¹)	2.99 ± 1.33 ^a	0.05 ± 0.01 ^b	1.99 ± 0.32 ^a	0.06 ± 0.01 ^b
Diameter size of ST (μm)	210.50 ± 2.93 ^A	146.33 ± 5.37 ^B	216.87 ± 2.19 ^A	206.66 ± 3.49 ^A
Germinative cell thickness (μm)	53.83 ± 1.28 ^A	32.83 ± 1.48 ^B	54.13 ± 1.08 ^A	53.50 ± 0.78 ^A

^{A,B} Different letters within same line showed significant ($P < .01$) differences among the groups.

^{a,b} Different letters within same line showed significant ($P < .05$) differences among the groups.

Ateşşahin. Lycopene prevents adriamycin-induced spermiotoxicity. Fertil Steril 2006.

differences in sperm concentrations were observed among any groups. Lycopene was able to prevent the ADR-induced reduction in sperm motility of the group receiving ADR alone, which had significantly lower ($P < .01$) sperm motility than the control group; this ADR-induced reduction in sperm motility was absent in the pretreatment with lycopene groups.

Adriamycin treatment did not have any significant effect on the tail abnormalities of the sperm in any group, but the ADR alone group had significantly higher head abnormalities compared to the control group ($P < .05$). When total sperm abnormalities were analyzed, it was found that the ADR alone group had a significantly higher level of abnormalities. Pretreatment with lycopene had a preventive effect on ADR-induced total and head sperm abnormalities.

Lipid Peroxidation in Testis Tissue and Plasma Testosterone Levels

The levels of testis tissue MDA, GSH, and plasma testosterone levels are presented in Table 3. Although the ADR alone group had significantly higher MDA levels compared to control group, the pretreatment with lycopene groups had lower levels of MDA than the ADR group ($P < .01$). Although there were significant decreases ($P < .01$) in the GSH levels of the ADR group compared to the control group, GSH levels in pretreatment with lycopene groups were higher than in the group receiving ADR alone. A significant decrease ($P < .05$) in levels of testosterone was observed in both ADR alone and pretreatment with lycopene groups.

Effect of ADR and Lycopene Treatment on Testis Histology

In the microscopic examination, severe degenerative changes were detected in germinative cells in the ADR alone group compared to the control group (Fig. 1). The diameter size of ST and germinative cell thickness ($P < .001$) in the ADR group was rather smaller than the control group (Table 3). Several primary spermatocyte and degenerative giant cells

were observed in ST lumen. In addition, thickening in the basal layer of ST was seen. However, all ADR-induced alterations in histopathologic findings were effectively reverted by pretreatment with lycopene (Fig. 2).

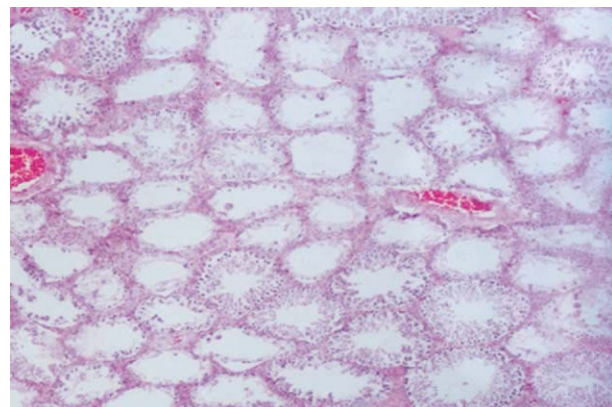
DISCUSSION

Treatment with cytotoxic chemotherapy is associated with significant gonadal damage in the male reproductive organs. Spermatogenic cells are targeted by cytotoxic agents because of their high mitotic activity. Damages in spermatogonia result in prolonged sterility or oligozoospermia (4, 17). The chance of recovery of spermatogenesis following cytotoxic insult, and also the extent and speed of recovery, are related to the agent used and the dose received (18, 19).

Adriamycin therapy results in damage of different tissues, such as heart, kidney, liver, and testes. Recently, it has

FIGURE 1

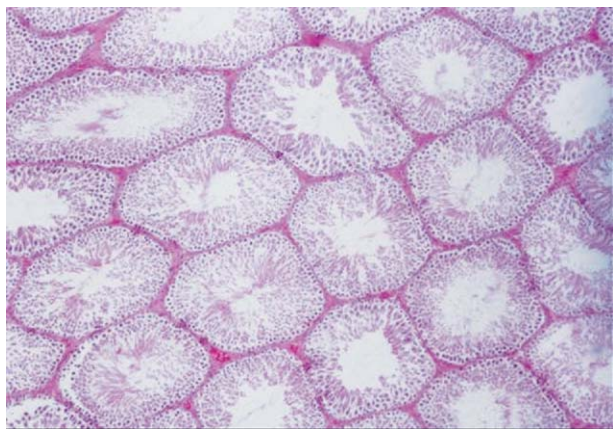
Light microscopy of testis tissue from rat treated with adriamycin alone. Seminiferous tubules show severe degenerative changes (H&E, 20×).



Ateşşahin. Lycopene prevents adriamycin-induced spermiotoxicity. Fertil Steril 2006.

FIGURE 2

Light microscopy of testis tissue from rat pretreated with lycopene before adriamycin. Seminiferous tubules show nearly normal seminiferous tubules (H&E, 20×).



Ateşşahin. Lycopene prevents adriamycin-induced spermiotoxicity. *Fertil Steril* 2006.

attracted more attention owing to impairment in testicular function following chemotherapy. The toxicity of ADR has been investigated in detail and well documented. Adriamycin-induced testicular toxicity in animal models has been reported by many investigators (1, 4, 5, 20). In the present study, the degree of ADR-induced damage in the reproductive organs of rats was assessed with the determination of testicular, epididymal, and accessory gland weights and testis dimensions, sperm concentration, sperm motility, abnormal sperm rates, histopathology, plasma testosterone, and testicular tissue MDA and GSH levels.

Kato et al. (6) reported that chronic exposure to ADR can decrease reproductive organ weights, but according to Endo et al. (4) ADR had no significant effect. However, in this study, testis and epididymis weights of the group treated with ADR alone were significantly lower than the control group. It is thought that severe parenchymal atrophy in the ST of rats after ADR administration causes this reduction. Interestingly, lycopene pretreatment in ADR-administered rats showed normal testis weights.

The results of the present study indicated that ADR administration at the dose of 10 mg kg^{-1} resulted in a significantly decrease in sperm motility and plasma testosterone levels and increase in total sperm abnormality rates in the rats. But epididymal sperm concentrations were not changed in the ADR alone group compared to control group. Several investigators (1, 2, 5) have suggested that ADR treatment may adversely affect the quality and quantity of sperm, which is responsible for male fertility. It is widely accepted that oxidative stress and the production of free radicals are involved in ADR action, in terms of both antitumor effects

and other organ toxicity. Increased oxidative stress damages the sperm membranes, proteins, and DNA associated with male fertility (3, 7, 21).

In this study, ADR did not affect the epididymal sperm concentration compared to the control group. In histopathologic examination of testis tissue, ADR induced the damage in the spermatogenesis due to degeneration in ST. As the period of administration of ADR is limited and the rats were killed on the third day after administration in the present trial, epididymal sperm concentration was not significantly changed in spite of the damaged ST in testes.

Sperm motility significantly reduced in ADR alone group compared to the control group. The loss of sperm function is due to the peroxidation of unsaturated fatty acids in sperm plasma membrane, as a consequence of which the latter loses its fluidity and the cells lose their function (6, 10).

It has been reported (1, 2, 22, 23) that ADR leads to direct oxidative injury to DNA and generates lipid peroxidation. Two different pathways of free radical formation by ADR have been described. The first implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the ADR to the corresponding ADR semiquinone. In the presence of oxygen, redox cycling of ADR-derived quinone-semiquinone yields superoxide radicals. In the second pathway, ADR free radicals come from a nonenzymatic mechanism that involves reactions with iron. For example, Fe^{+3} reacts with ADR in a redox reaction, after which the iron atom accepts an electron and an Fe^{+2} -ADR free radical complex is produced. This iron-ADR complex can reduce oxygen to hydrogen peroxide and other active oxygen species. It has been reported that testis lipid peroxidation determined by thiobarbituric acid-reactive substance production was increased in several days after a single dose of ADR (1, 5). In the present study, a significant increase was observed in testis MDA concentrations in three days following ADR administration, and these findings are compatible with previous work.

Reduced glutathione plays a critical role in several important biologic processes, including the maintenance of essential sulphhydryl groups on membrane proteins, drug detoxification reactions involving glutathione-S-transferase, and the breakdown of intracellular peroxides or free radicals. It is important in regulation of the cellular redox state, and a decline in its cellular level has been considered to be indicative of oxidative stress. Several investigators reported that administration of chemotherapeutics such as ADR, cyclophosphamide, cisplatin, etc. reduces the GSH levels. Glutathione reductase mediates reduction of oxidized glutathione (GSSG) to GSH. This reduction reaction requires reduced nicotinamide-adenine-dinucleotied phosphate (NADPH). Available NADPH is lower in ADR-treated rats. In conclusion, the level of GSH may be lower because of imbalance in the GSSG/GSH redox couple (1, 5, 24). The observations of those investigators support our finding that there was a

decline in GSH levels. Administration of lycopene helps to overcome the oxidative stress by increasing the GSH levels.

Excessive production of semen reactive oxygen species (ROS), which causes abnormality in spermatozoa, could be an indicator for infertility. High concentrations of hydrogen peroxide induce lipid peroxidation and result in cell death. The consequences of such oxidative stress are a loss in motility and fertilizing ability of sperm and the induction of DNA damage in sperm nucleus. The loss of sperm function is due to peroxidation of unsaturated fatty acids in the sperm plasma membrane, as a consequence of which the latter loses its fluidity and the cells lose their function (4, 6, 21).

The germinal epithelium is far more sensitive to the effects of cytotoxic drugs than the Leydig cells. There are some studies finding a reduction in testosterone concentrations after treatment with gonadotoxic agents, and there is some evidence to suggest that the Leydig cell impairment following chemotherapy may be clinically important (4, 17). In the present study, Leydig cell impairment following ADR treatment in rats was shown, and pretreatment with lycopene changed the impairment of Leydig cells. But plasma testosterone levels, induced by ADR, could not be recovered by administration of lycopene. The reason for this conflict situation is not known and further studies are required.

To protect spermatogenesis from toxicant exposure, many clinical and experimental trials of antioxidant agents have been attempted. Carotenoids, as potential antioxidants, are well known as highly efficient scavengers of singlet oxygen ($^1\text{O}_2$) and other excited species. During $^1\text{O}_2$ quenching, energy is transferred from $^1\text{O}_2$ to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, such as OH, NO_2 or peroxyxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins, and DNA (25–27). Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against hydrogen peroxide and nitrogen dioxide radical components. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo (8–10).

In the present study, pretreatment with lycopene significantly inhibited the increase in testis MDA and the depletion of testis GSH induced by ADR. The possible explanation for the protective effects of lycopene against the increase in ADR-induced lipid peroxidation is its ability to react with the oxygen metabolites. Gupta and Kumar (27) documented that oral lycopene therapy in men with idiopathic infertility provided an improvement in male infertility, especially in sperm characteristics.

In our study, significant decrease in sperm motility and increase in abnormal sperm rates was observed in rats treated with ADR alone, but normalization of these parameters was observed in the group pretreated with lycopene. A rational mechanism for potential anticarcinogenic and antimutagenic

effects of β -carotene and other carotenoids is their ability to scavenge free radicals that cause oxidative DNA damage. These findings are in agreement with the data of the present study. The protective effects of pretreatment with lycopene against ADR-induced abnormal sperm rates may be attributed to the antioxidant properties of lycopene. These observations might also indicate that lycopene has protective and therapeutic effects on ADR induced oxidative stress.

In conclusion, this study clearly indicated that ADR treatment markedly impaired testicular function and that pretreatment with lycopene might prevent this toxicity in rats. Our results also indicate that lycopene may have a potential for clinical applications such as impairment of testicular function by cytotoxic chemotherapeutics.

REFERENCES

1. Quiles JL, Huertas JR, Battino M, Mataix J, Ramires-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. *Toxicology* 2002;180:79–95.
2. Suominen JS, Linderborg J, Nikula H, Hakovirta H, Parvinen M, Toppari J. The effects of mono-2-ethylhexyl phthalate, adriamycin and N-ethyl-N-nitrosourea on stage-specific apoptosis and DNA synthesis in the mouse spermatogenesis. *Toxicol Lett* 2003;143:163–73.
3. Kalender Y, Yel M, Kalender S. Doxorubicin hepatotoxicity and hepatic free radicals in rats. The effects of vitamin E and catechin. *Toxicology* 2005;209:39–45.
4. Endo F, Manabe F, Takeshima H, Akaza H. Protecting spermatogonia from apoptosis induced by doxorubicine using the luteinizing hormone-releasing hormone analog leuporelin. *Int J Urol* 2003;10:72–7.
5. Prahalathan C, Selvakumar E, Varalakshmi P. Lipoic acid ameliorates adriamycin-induced testicular mitochondriopathy. *Reprod Toxicol* 2005;20:111–6.
6. Kato M, Makino S, Kimura H, Ota T, Furuhashi T, Nagamura Y. Sperm motion analysis in rats treated with adriamycin and its applicability to male reproductive toxicity studies. *J Toxicol Sci* 2001;26:51–9.
7. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front Biosci* 1996;1:78–86.
8. Jonker D, Kuper CF, Frail N, Estrella A, Rodrigues Otero C. Ninety-day oral toxicity study of lycopene from *Blakeslea trispora* in rats. *Regul Toxicol Pharmacol* 2003;37:396–406.
9. Cohen LA. A review of animal model studies of tomato carotenoids, lycopene, and cancer chemoprevention. *Exp Biol Med* 2002;227:864–8.
10. Ateşşahin A, Karahan İ, Türk G, Gür S, Yılmaz S, Çeribaşı AO. Protective role of lycopene on cisplatin induced changes in sperm characteristics, testicular damage and oxidative stress in rats. *Reprod Toxicol* 2006;21:42–7.
11. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. *Biol Trace Elem Res* 2003;93:141–53.
12. Sönmez M, Türk G, Yüce A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Theriogenology* 2005;63:2063–72.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351–58.
14. Ellman G. Tissue sulphhydryl groups. *Arch Biochem Biophys* 1959;82:70–7.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *J Biol Chem* 1951;193:265–75.
16. Mourao CA, Schmidt SL, Manhaes AC. Testosterone and free thyroxine blood in congenitally acallosal male BALB/cCF mice. *Neuroendocrinol Lett* 2003;24:459–62.
17. Howell SJ, Shalet SM. Testicular function following chemotherapy. *Hum Reprod Update* 2001;7:363–9.

18. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer Inst Monogr* 2005;34:12–7.
19. Ishikawa T, Kamidono S, Fujisawa M. Fertility after high-dose chemotherapy for testicular cancer. *Urology* 2004;63:137–40.
20. Prahalathan C, Selvakumar E, Varalakshmi P. Remedial effect of DL- α -lipoic acid against adriamycin induced testicular lipid peroxidation. *Mol Cell Biochem* 2004;267:209–14.
21. Sanocka D, Kurpisz M. Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol* 2004;2:1–7.
22. Jahnukainen K, Hou M, Parvinen M, Eksborg S, Söder O. Stage-specific inhibition of deoxyribonucleic acid synthesis and induction of apoptosis by anthracyclines in cultured rat spermatogenic cells. *Biol Reprod* 2000;63:482–7.
23. De Beer EL, Bottone AE, Voest EE. Doxorubicine and mechanical performance cardiac trabeculae after acute and chronic treatment: a review. *Eur J Pharmacol* 2001;415:1–11.
24. Eklow L, Moldeus P, Orrenius S. Oxidation of glutathione during hydroperoxide metabolism. A study using isolated hepatocytes and the glutathione reduction inhibitor 1,3-bis (2-chloroethyl)-1-nitrosourea. *Eur J Biochem* 1984;138:459–63.
25. Stahl W, Sies H. Antioxidant activity of carotenoids. *Mol Aspects Med* 2003;24:345–51.
26. Wertz K, Siler U, Goralczyk R. Lycopene: modes of action to promote prostate health. *Arch Biochem Biophys* 2004;430:127–34.
27. Gupta NP, Kumar R. Lycopene therapy in idiopathic male infertility—a preliminary report. *Int Urol Nephrol* 2002;34:369–72.