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Effectiveness of lycopene on experimental testicular torsion

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ABSTRACT

Aim: We aimed to demonstrate the long term effectiveness of lycopene, a precursor of vitamin A, on the testes for ischemia-reperfusion injury.
Materials and methods: Seventy male Wistar albino rats were used for this experiment. The rats were divided into seven groups. Group 1 served as the control group; group 2 was sham-operated; group 3 received 20 mg/kg/day lycopene (intraperitoneally); in group 4, the right testes of rats were kept torted for 2 hours and then were detorted and the animals lived for three days; in group 5, the right testes of rats were kept torted for 2 hours and then were detorted and the animals lived for ten days; in group 6, the right testes of the rats were kept torted for 2 hours and then detorted and the animals received 20 mg/kg/day lycopene (intraperitoneally) for three days; in group 7, the right testes of the rats were kept torted for 2 hours and then were detorted and the animals received 20 mg/kg/day lycopene (intraperitoneally) for ten days. Lycopene was used intraperitoneally. Some of the testes tissues were used for biochemical analyses and the other tissues were used for histological procedures. The Johnsen's score was used for seminiferous tubule deterioration. The TUNEL method was utilized to show apoptosis of testicular tissue. Testosterone levels were measured from blood samples and SOD, MDA, TNF-α, IL-1β and IL-6 measurements were recorded from tissue samples. The results were analyzed statistically.
Results: In groups 1, 2 and 3 there was normal testicular structure. Rats in groups 4 and 5 had damaged testicular tissues. In groups 6 and 7, in which we used lycopene, the testes were not better than those in groups 4 and 5. The MSTD and JTBS values were better in group 6, but not in group 7 among the torsion groups. As a result, MDA, SOD, TNF-α and IL-1β were increased and serum testosterone and IL-6 levels were decreased in groups 4 and 5 compared to group 1. There was no improvement in the groups treated with lycopene for therapeutic purposes.
Conclusion: It was shown that lycopene, as an antioxidant agent, is not effective for testicular torsion in the long term. This study can be considered as a preliminary study showing the need for further researches using different antioxidant agents to determine their long term effects in ischemia-reperfusion injuries in an appropriate experimental design.

Testicular torsion, which was first described in 1840 by Delasiavue, is the most important emergency in childhood [1]. The testes twist around the longitudinal axis of the testicular cord. Then, the blood supply of the testes is interrupted and if this situation goes on for a long time, necrosis will not be a surprising result. Minutes become more important for the diagnosis and management of testicular torsion [1]. Many substances have been shown to be useful for decreasing ischemic effects in the postoperative period in previous studies [2].

Lycopene is a red carotenoid found in large amounts in tomatoes. There has been more attention paid in studies about lycopene as a precursor of vitamin A, instead of β-carotene, in the last 20 years [3]. It has been proposed as a useful molecule for several cancers, coronary artery disease, bone health, male fertility, hypertension and neurodegenerative diseases [3,4].

We aimed to study the effectiveness of lycopene, as an antioxidant, in the long term after torsion.

1. Materials and methods

1.1. Animals

Sexually mature male Wistar rats, which were obtained from the Hakan Çetinsaya Experimental and Clinic Research Center, at Erciyes University, Kayseri, Turkey, were used for this study. They were housed in plastic cages which were placed in a well-ventilated rat house, allowed ad libitum access to rat chow and water and subjected to a

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natural photoperiod of 12-hour light-darkness cycle. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Ethical approval for the study was obtained from Erciyes University Animal Researches Local Ethics Committee and the ethical regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments (Public Health Service (PHS), 1996). The rats were randomly assigned to seven groups of ten rats per group. Group 1 served as the control group; group 2 was sham-operated; group 3 received 20 mg/kg/day lycopene (intraperitoneally); in group 4, the right testes of the rats were kept torted for 2 hours and then were detorted and the animals lived for three days; in group 5, the right testes of the rats were kept torted for 2 hours and then were detorted and the animals lived for ten days; in group 6, the right testes of the rats were kept torted for 2 hours and then were detorted and the animals received 20 mg/kg/day lycopene (intraperitoneally) for three days; in group 7, the right testes of the rats were kept torted for 2 hours and then were detorted and the animals received 20 mg/kg/day lycopene (intraperitoneally) for ten days. Lycopene was dissolved in corn oil.

1.2. Surgical procedure

All surgical procedures were performed under xylazine/ketamine (10/90 mg/kg, i.p.) anesthesia using sterile conditions. The scrotum was incised by a midline incision. The tunica vaginalis was opened, and the right testis was delivered to the surgical field. The right testis was rotated 720° in a clockwise direction and maintained in this torsion position by fixing the testicle to the scrotum with a 4-0 silk suture [5]. The ischemia period was 2 hours and orchiectomy was performed on the third day in groups 4 and 6 and on the tenth day in groups 5 and 7 after detorsion. Lycopene (L9879, Sigma Aldrich, St. Louis, MO, USA) was freshly prepared and administered once daily, 30 minutes after detorsion and presented at the same time of the day (10:00 am).

At the end of the experimental period, the animals were killed by decapitation under intraperitoneal ketamine (90 mg/kg) + xylazine (10 mg/kg) anesthesia. After decapitation, testes tissues were quickly removed. Some of the testes tissues were used for biochemical analyses and the other tissues were used for histological procedures.

1.3. Histopathologic evaluation

All of the testicular tissues were examined and evaluated in random order under blindfold conditions with standard light microscopy by the same histologists. Mean seminiferous tubule diameter (MSTD) was measured in micrometers (Analysis LS Research Program). More than 20 seminiferous tubular sections per testis were each given a Johnsen's score (JTBS) from 1 to 10, as described previously [6]. In this system of classification, all tubular sections in each section of the testicular biopsy are evaluated systematically and each is given a score from 1 to 10. Complete spermatogenesis with many spermatozoa is evaluated as score 10.

1.4. Apoptosis (TUNEL)

The TUNEL method was utilized to show apoptosis of testicular tissue. An in situ Cell Death Detection Kit, the Fluorescein Kit (Roche), was used. For the process, first of all 5–6 μm thick testis tissues were obtained and after being deparaffinized and rehydrated, they were washed with PBS. After washing for antigen retrieval, the tissues were placed in a 0.01 M sodium citrate buffer in a microwave oven at 350 W for five minutes. Then, they were left to cool for 20 minutes at room temperature. Having been washed with PBS three times for 5 minutes, the tissues were incubated with a TUNEL reaction mixture in a damp and dark place at 37 °C for 60 minutes. After washing with PBS three times for five minutes, the tissues were contrast colored with 4,6-diamidine-2′-phenylindol. After covering the tissues with a solution containing glycerol, they were all examined with a Olympus BX–51 fluorescent microscope at 450–500 nm wavelength. In order to estimate the apoptotic index, the TUNEL-positive cells in seminiferous tubules in 20 randomly chosen fields were counted. The apoptotic index was calculated as the percentage of TUNEL positive cells [7].

1.5. ELISA

Blood samples were collected in empty tubes to obtain sera and were centrifuged at 3000 rpm for ten minutes. The resulting sera were used for the determination of testosterone levels (201-11-5126, SunRed) using ELISA kits. Testis tissues collected for ELISA analyses were stored at -80 °C until analyses were performed, at which time they were removed to room temperature and left to warm for 15 minutes. They were then homogenized on ice according to the procedures of the ELISA kits. After homogenization, the testis tissues were centrifuged at +4 °C for 30 minutes at 12,000 rpm; the supernatants were then aliquoted and prepared for ELISA procedures. Then SOD (201-11-0169, SunRed), MDA (201-11-0157, SunRed), TNF-α (201-11-0765, SunRed), IL1-β (201-11-0120 SunRed), and IL-6 (Invitrogen KRC0061) ELISA kits were used and their amounts in testis tissue homogenates were determined.

1.6. Statistical analysis

One-way analysis of variance (ANOVA) and the post hoc Tukey test were used to determine the differences between groups. Results are presented as mean ± S.E.M. Values were considered statistically significant if $p < 0.05$. The SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used for the statistical analysis.

2. Results

2.1. Histopathological findings

Group 1 (Fig. 1A), group 2 (Fig. 1B) and group 3 (Fig. 1C) testes showed the presence of normal testicular architecture and regular seminiferous tubular morphology with normal spermatogenesis and the presence of primary and secondary spermatocytes, spermatids, and spermatozoa. Desquamation of epithelial cells into the lumen, disorder of seminiferous tubule germinal epithelium (Fig. 1F), multinucleated giant cells (Fig. 1D) and necrosis of some seminiferous tubules (Fig. 1E) were determined in group 4 and group 5. Similar findings were also seen in groups 6 (Fig. 1G and H) and 7 testes tissue subjects (Fig. 1I). We observed that lycopene does not improve this damage.

The MSTD and JTBS values for the testes in each group are shown in Table 1. It was observed that the MSTD and JTBS values of testes in of the groups 4 and 5 were statistically significantly low when compared with the control rats. However, a curative effect was determined with the administration of lycopene in the group 6 but not group 7. The MSTD and JTBS values in groups 2 and 3 were similar to those in the control group.

2.2. Apoptosis (TUNEL) findings

TUNEL staining was performed to determine apoptotic cells in testicular tissue (Fig. 2). The apoptotic index results are given in Table 1. Apoptotic cells were observed in the spermatogenic cells in the seminiferous tubules. The number of apoptotic cells in testicular tissues was increased in groups 4 and 5 in comparison to group 1. We observed that lycopene improved the number of apoptotic cells in group 6 compared to group 4. The number of apoptotic cells in groups 2 and 3 was similar to that in the control group.