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Mannitol has a protective effect on testicular torsion: An experimental rat model

Omer Kurt [a], Cenk Murat Yazici [a], Mustafa Erboga [b], Cuneyt Turan [c], Yeliz Bozdemir [b], Alpaslan Akbas [d], Polat Turker [a], Cevat Aktas [b], Murat Aydin [e], Ebru Yesildag [f]

[a]Namik Kemal University, School of Medicine, Department of Urology, Tekirdag, Turkey

[b]Namik Kemal University, School of Medicine, Department of Histology and Embryology, Tekirdag, Turkey

[c]Namik Kemal University, School of Medicine, Department of Anesthesiology and Reanimation, Tekirdag, Turkey

[d]18 Mart University, School of Medicine, Department of Urology, Canakkale, Turkey

[e]Namik Kemal University, School of Medicine, Department of Biochemistry, Tekirdag, Turkey

[f]Namik Kemal University, School of Medicine, Department Pediatric Urology, Tekirdag, Turkey

Correspondence to: O. Kurt, Namik Kemal University School of Medicine, Department of Urology, Tekirdag, Turkey

drkurtomer@gmail.com (O. Kurt)

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Summary

Objective
Testicular torsion is an emergency condition that causes testicular injury. Any treatment opportunity reducing the destructive effect of testicular torsion is important for the future life of patients. In this experimental study we investigated the protective effect of mannitol on ischemia–reperfusion (I/R) injury in a rat testes torsion model.

Method
In total, 32 male Sprague Dawley rats were included. Four experimental groups included eight rats each. Group A was a sham group in which the right testis was brought out through a scrotal incision and then replaced in the scrotum without torsion. In Group B, the right testis was torsioned, by rotating 720° clockwise and fixed to the scrotum with no treatment. In Group C, the same testicular torsion process was performed with saline infusion just after testicular torsion. In group D, mannitol infusion was used just after testicular torsion. Testicles were detorsioned after 3 h and left inside for more than 2 h before orchiectomy. Histopathological, immunohistochemical, and biochemical analyses were performed.

Results
Testicular architecture was disturbed significantly in the torsion groups without mannitol infusion. However, testicular tissue structure was significantly better in the mannitol-treated group, demonstrating a protective effect. Similar findings were also shown for the proliferating cell nuclear antigen (PCNA) index and antioxidant activity; both were higher in the mannitol group than in the no-treatment and saline groups ($p < 0.01$). The apoptotic index was also significantly lower in the mannitol-treated group compared with the no treatment and saline groups ($p < 0.01$).

Conclusions
The seminiferous tubule structure in testicular torsion without mannitol treatment was significantly disturbed, whereas the structural disruption was considerably less in the mannitol group. Mannitol treatment also decreased reactive oxygen radical levels significantly and was able to decrease apoptosis. These results were consistent with other organ model studies that evaluated the protective effects of mannitol treatment in I/R injury. Mannitol infusion had a protective effect against I/R injury in testicular torsion in rats. This experimental study may guide clinicians to evaluate the effectiveness of mannitol in human testicular torsion.

Table Histopathological evaluation of study groups.

	Apoptotic index	PCNA index	MTBS	MSTD
Group A	3.88 ± 0.83[b]	36.47 ± 1.36[b]	9.23 ± 0.17[b]	273.25 ± 5.14[b]
Group B	25.88 ± 2.91[a,b]	22.22 ± 2.34[a,b]	4.57 ± 0.22[a,b]	209 ± 6.11[a,b]
Group C	25.52 ± 2.87[a,b]	22.51 ± 2.99[a,b]	4.76 ± 0.14[a,b]	210.63 ± 5.09[a,b]
Group D	15.25 ± 1.48[a]	29.28 ± 1.66[a]	6.72 ± 0.25[a]	229.63 ± 6.18[a]

MSTD = mean seminiferous tubular diameter; MTBS = mean testicular biopsy score; PCNA = proliferating cell nuclear antigen; I/R = ischemia-reperfusion.
Group A was a sham operation group, Group B (I/R) had 3 h ischemia and 2 h reperfusion, Group C (I/R + Saline) had 3 h ischemia and 2 h reperfusion and saline bolus treatment, Group D (I/R + Mannitol) had 3 h ischemia and 2 h reperfusion and mannitol bolus treatment.
[a] $p < 0.01$ compared with group A.
[b] $p < 0.01$ compared with group D.

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Introduction

Testicular torsion is an emergency condition with an incidence of 1/4000 in males under 25 years of age [1]. It causes testicular injury, leading to potential infertility and subfertility; thus, immediate diagnosis and intervention are important [2]. Although the main pathological mechanisms of testicular injury in torsion are only partially understood, overproduction of reactive oxygen species (ROS) has been implicated as one of the main factors in cellular and tissue damage [3]. Several antioxidant molecules, such as alpha-lipoic acid, quercetine, and melatonin, were found to be effective against ischemia–reperfusion (I/R) injury, but these molecules have not been used widely in clinical practice because of their toxic side effects [4–6].

Mannitol has traditionally been administered before partial nephrectomy to reduce ischemic renal damage as an intravascular volume expander with free-radical scavenging properties, as well as being an osmotic diuretic [7]. It reduces oxidant-derived injury in kidneys, heart, and lungs [8–10]. In this experimental study, we hypothesized that mannitol may have antioxidant protective effects against I/R injury in testicular torsion and sought to investigate this protective effect with histopathological and biochemical analyses in rat testes.

Materials and methods

With the approval of the local animal care and use committee, in total, 32, 6-month-old (mature) male Sprague Dawley rats, weighing 240–280 g, were used in the study. We randomly divided them into four experimental groups, each with eight rats (Table 1). All animals were housed in a temperature- and light-controlled room, with *ad libitum* access to water and rat chow. All animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals*.

Experimental design

Surgical procedures were performed under ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia and sterile conditions. A scrotal midline incision was made and torsion was induced by rotating the right testis 720° clockwise and maintained by fixing the testis. The same surgical procedure was performed in the sham group except there was no testicular torsion. Saline (NaCl, 0.09%, 10 mL/kg/min) was administered during the procedure to all groups for hydration. After a 3-h torsion period, the suture was removed with a detorsioning procedure. The testis was replaced in the scrotum for an additional 2-h period. At the end of study, the rats were decapitated, and a right orchiectomy was performed for biochemical and histopathological examinations (Table 1).

Testicular specimens were individually immersed in Bouin's fixative, dehydrated in alcohols, and embedded in paraffin wax. Sections of 5 μm were obtained, deparaffinized, and stained with hematoxylin and eosin (H&E) for evaluation by a histologist in a random order under blinded conditions with standard light microscopy. Three slides, prepared from the upper, lower, and mid-portions of the testes were examined. Mean seminiferous tubule diameter (MSTD) was measured, in micrometers. Spermatogenesis was assessed histopathologically using Johnsen's mean testicular biopsy score (MTBS) [3,4]. A score of 0–10 was given to each tubule according to epithelial maturation. Preparations were evaluated with a bright-field microscope (Olympus CX41, Japan) and photographed. We determined testicular tissue antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). Testicular tissue levels of lipid peroxidation products, malondialdehyde (MDA), and xanthine oxidase (XO) activity, were also determined.

Immunohistochemical reactions were performed according to the avidin-biotin complex technique described by Hsu *et al*. [11]. Sections were incubated with a specific monoclonal antibody to proliferating cell nuclear antigen (PCNA; Cat. # MS-106-B, Thermo LabVision, USA). To quantify the incidence of PCNA, 10 seminiferous tubules were counted in each slide. Both stained and non-stained germ cells were counted, and the ratio of stained cells to the total number of germ cells, the "PCNA index," was calculated for each seminiferous tubule.

Table 1 Experimental groups.

Sham group (Group A): A sham procedure was performed to determine biochemical and histopathological basal values. The right testis was brought out through the incision and then returned to the scrotum without torsion. A 4/0 silk suture was used to fix the testis in the scrotum. After a 2-h period, the right testis was removed for evaluation.
Ischemia-reperfusion (I-R)/untreated group (Group B): After 3 h of unilateral testicular torsion, detorsion was performed and the testis was replaced in the scrotum and fixed. The rats in this group received only saline solution (NaCl at 0.02%, 10 mL/kg/min) during the procedure, and did not receive any treatment after the detorsion process. After 2 h of detorsion, the right testis was removed for evaluation.
Ischemia-reperfusion (I-R)/saline bolus treated group (Group C): The same surgical procedure (torsion and detorsion) was performed as in Group B. The rats were given a bolus of saline solution (1 mg/kg; i.v.) immediately after detorsion. The bolus injection was given intravenously in a 2-min period, and the testis was removed 2 h after detorsion.
Ischemia-reperfusion (I-R)/mannitol bolus treated group (Group D): The same surgical procedure (torsion and detorsion) was performed as in other groups. The rats were treated with a bolus of mannitol (1 mg/kg; i.v.) immediately after detorsion. The bolus injection was given intravenously in a 2-min period that started immediately after reperfusion, and the testis was removed 2 h after detorsion.