Gülhane Tıp Dergisi 2007; 49: 67-71

ARAŞTIRMA/ORIGINAL ARTICLE

Histopathological changes and the effects of prednisolone treatment in contralateral testis in rats subjected to unilateral testicular torsion

Levent Sağnak (*), Hamit Ersoy (*), Berk Burgu (*), Rıfkı Hazıroğlu (**)

Summary

We aimed to determine the effects of unilateral testicular torsion on the contralateral testis and to evaluate different treatment approaches. Forty pubertal Sprague Downey rats were randomized into 8 groups (Five in each group). In the control group (Group 1), the sham operation was performed. In Group 2, the left testis underwent torsion and was returned to the scrotum. In the other groups, the left testis underwent torsion at 720º counterclockwise and was fixed to the scrotum wall. Then the surgical procedures like detorsion (Group 3, 24 hours later) and orchiectomy (Group 4, 24 hours later and Group 5, 48 hours later); intraperitoneal prednisolone injection in addition to orchiectomy (Group 6, 24 hours later and group 8, 48 hours later), and intraperitoneal prednisolone injection in addition to detorsion (Group 7, 24 hours later) were performed. All the contralateral testes were removed 4 weeks after the torsion and evaluated histopathologically. We observed degeneration at varying intensities in contralateral testes of all groups except for the control group. We found a significant difference in the diameters of seminiferous tubules of the contralateral testes in Groups 2, 3, 4, 5 and 8 compared with the control group. In Groups 6 and 7, no significant changes were observed in the diameters of seminiferous tubules of the contralateral testes, and hyperemia, edema and degeneration were less prominent. We demonstrated that the histopathological damage can be prevented by prednisolone even in the detorsioned group by suppressing the immunologic response.

Key words: Contralateral testis, histopathology, rat, testicular torsion

Özet

Unilateral testis torsiyonu yapılan ratlarda karşı testiste prednizolon tedavisinin etkileri ve oluşan histopatolojik değişiklikler

Bu çalışmada unilateral testis torsiyonunun kontralateral testise olan etkilerinin araştırılması ve değişik tedavi yaklaşımlarının değerlendirilmesi amaçlanmıştır. Bu amaçla pubertal (yaklaşık 2.5-3 aylık) 40 adet Sprague-Downey sıçan rastlantısal olarak 5'erli 8 gruba ayrıldı. Kontrol grubunda (Grup 1) testislere yalancı operasyon uygulandı. Grup 2'de sol testis torsiyone edilip bırakıldı. Diğer gruplarda sol testisler 720º saat yelkovanı tersine torsiyone edilip tespit edildi. Daha sonra bu testise detorsiyon (Grup 3'de 24 saat sonra), orşiyektomi (Grup 4'de 24 saat sonra ve Grup 5'de 48 saat sonra) gibi cerrahi müdahaleler ile bazı gruplarda orşiyektomi (Grup 6'da 24 saat sonra ve Grup 8'de 48 saat sonra) ve detorsiyona (Grup 7'de 24 saat sonra) ilaveten intraperitoneal kortizon uygulandı. Tüm kontralateral testisler torsiyondan 4 hafta sonra orşiyektomi ile alınarak histopatolojik değerlendirmeleri yapıldı. Histopatojik olarak kontrol grubu dışında tüm kontralateral testislerde değişen şiddetlerde dejenerasyon mevcuttu. Seminiferöz tübül çapları arasındaki fark, Grup 6 ve Grup 7 dışında önemli bulundu. Bu iki grupta kontralateral hiperemi, ödem ve dejenerasyon da belirgin olarak azdı. Bu bulguların eşliğinde, prednizolon ile histopatolojik hasarın detorsiyone grup da dahil olmak üzere immunolojik cevabın baskılanması yoluyla önlenebileceğini söyleyebiliriz.

Anahtar kelimeler: Kontralateral testis, histopatoloji, sıçan, testis torsiyonu

* **3rd Urology Clinic, Ankara Dışkapı Education and Research Hospital, Ankara**

** **Department of Pathology, Ankara University Faculty of Veterinary Medicine, Ankara**

Reprint request: Dr. A.Levent Sağnak, Angoraevleri, Altınbel Villaları, Hitit Bulvarı No: 38/A, Beysukent, Ankara

E-mail: leventsagnak@yahoo.com

Date submitted: November 15, 2006

Accepted: March 6, 2007

Introduction

The testicular torsion is an acute disease, which appears especially in male adolescents and observed in 1 of 158 males under 25 years old (1). Although the diagnosis of the testicular torsion is easy, the period that passes between the beginning of torsion and the treatment of the disease is very important (2,3) The effects of unilateral testicular torsion on the contralateral testis and fertility have been searched by several scientists but there is no consensus on the level of these effects, the treatment period and approaches (1,2,4).

Some authors suggest that differences in pathological findings

after the torsion in the contralateral testis are due to decreased blood flow and oxidative stress as a result of sympathetic activation (5-7), which is explained by immunological mechanisms (8).

In this study, we examined the effects of unilateral testicular torsion on the contralateral testis and evaluated different treatment approaches in an experimental rat model.

Material and Methods

For this study, 40 pubertal (2.5-3 months old) Sprague-Downey rats which are known to reach puberty 50±10 days after the birth were used. The experiment was performed on the rats by the permission of Ankara University Veterinary Faculty Ethic Commission. The rats were randomized into 8 groups (Five in each group) as follows: Group 1 (control): Sham operation was performed to the left testis. Group 2 (torsion): Left testis was subjected to 720° counterclockwise torsion and fixed to the scrotum subcutaneously with non-absorbable sutures. Then, the scrotum was closed with absorbable sutures. Group 3 (torsion/detorsion 24 hours later): The left testis was subjected to torsion with the same surgical procedures performed in Group 2. Twenty four hours later, left testis was returned to its normal position (detorsion) and the scrotum was closed with absorbable sutures. Group 4 (torsion/ orchiectomy 24 hours later): The left testis was subjected to torsion with the same surgical procedures performed in Group 2. Left orchiectomy was performed 24 hours after the torsion and the scrotum was closed. Group 5 (torsion/orchiectomy 48 hours later): The left testis was subjected to torsion with the same surgical procedures performed in Group 2. Left orchiectomy was performed 48 hours after the torsion and the scrotum was closed. Group 6 (torsion/orchiectomy 24 hours later/prednisolone): 2 mg/kg/day methyl prednisolone was administered intraperitoneally to the rats for 4 weeks after the same procedures performed in Group 4. Group 7 (torsion/detorsion 24 hours later/prednisolone): 2 mg/kg/day methyl prednisolone was administered intraperitoneally to the rats for 4 weeks after the same procedures performed in Group 3. Group 8 (torsion/orchiectomy 48 hours later/prednisolone): 2 mg/kg/day methyl prednisolone was administered intraperitoneally to the rats for 4 weeks after the same procedures performed in Group 5. Rats were anesthetized with intraperitoneal penthobarbital sodium (30 mg/kg body weight) and placed in the supine position. After local cleansing with povidone iodine, the left testes of the rats were explored following a scrotal incision. In the control group, after the scrotal incision and release of the left testis, the scrotum was closed with absor-bable sutures (Sham operation). After the surgical procedures, all rats were put into separate cages for 24 hours and then they were united with their groups.

The rats were housed and maintained in specific pathogen-free conditions. At the end of 4 weeks, orchiectomy was performed on the right testes of all rats. The rats were necropsied, and body weights and testicular weights were determined.

The removed right testes were fixed in Bouin's solution for 24 hours, sectioned, processed and embedded in paraffin. Sections (4- to 6-μm thick) of paraffin-embedded tissues were stained with Hematoxylin and Eosin (H&E) and evaluated under the light microscopy. For histopathological evaluation, we used a method similar to three-grade system of Mikuz (9). In this method, the histopathologic findings such as edema, hyperemia, degeneration and spermatogenesis are evaluated in most simple method as mild (+), moderate (++) and severe (+++) in all samples. According to this, in mild (+) grade, the testicular parenchyma shows edema of interstice, slight blood extravasation and desquamation of the germ cells. The moderate (++) grade shows, diffuse hemorrragic saturation of the interstice and partial necrosis of germ cells. The severe (+++) grade shows fully developed hemorrhagic infarction of the testis. The diameters of 10 seminiferous tubules were measured in each animal by the ocular micrometer under the light microscopy. The Kruskal Wallis Anova with the Mann Whitney U-test were then used for comparison.

Results

Histopathological changes appearing in contralateral testes in all groups are shown in Table I. The average testicular weights, body weights and the diameters of seminiferous tubules are summarized in Table II. We observed a decrease in the diameters of seminiferous tubules in all groups except for the control group (Figure 1).

In Group 1, seminiferous tubules in all contralateral testes had regular structure demonstrating normal spermatogenesis (Figure 1a).

Table I. Histopathological findings in contralateral testis

Groups	Hyperemia	Edema	Degeneration	Spermatogenesis
1	-/-	-/-	-/-	++/+++
2	-/+	-/+	++/+++	-/+
3	+/++	+/++	+/++	-/+
4	+/++	+/+++	+/+++	-/+
5	+/++	+/++	+/+++	-/+
6	-/+	-/+	+/+	-/+
7	-/+	-/+	+/++	-/++
8	-/++	-/+++	+/+++	-/++

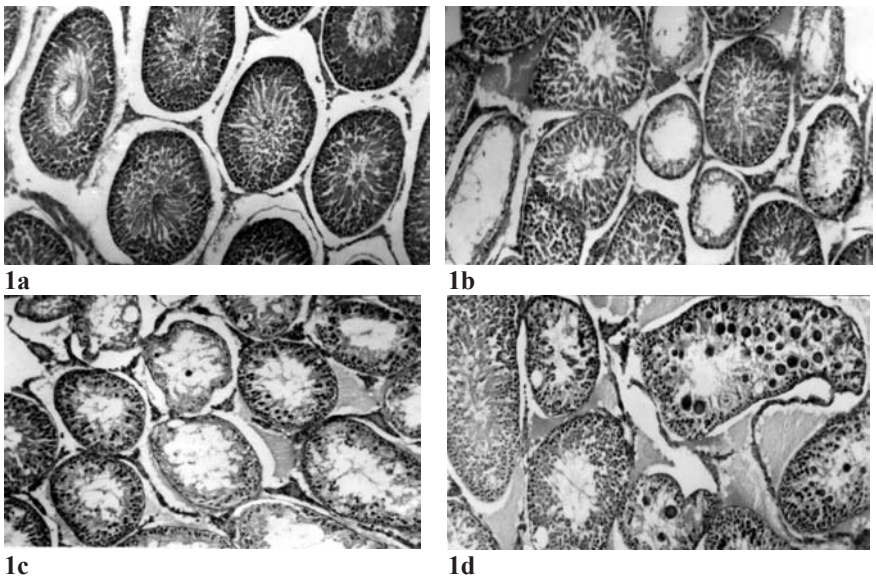
+: Mild, ++: Moderate, +++: Severe

Table II. The body weights, average contralateral testis weights, and the diameters of seminiferous tubules

Groups	Body weight (gr) (Mean±SD)	Testis weight (gr) (Mean±SD)	Seminiferous tubule diameter (micron) (Mean±SD)
1	229.1±18.92	2.58±0.115	197.0±6.67
2	220.0±16.78	2.49±0.117	*178.8±10.77
3	255.6±8.78	2.88±0.219	*177.4±6.52
4	275.6±16.0	2.99±0.111	*160.8±7.64
5	263.8±8.53	3.12±0.103	*160.2±2.82
6	237.4±7.83	2.62±0.084	192.2±4.97
7	268.2±19.0	2.89±0.235	193.4±9.10
8	268.8±14.5	2.42±0.216	*173.8±9.34

*: $p<0.05$ compared with the control group (Group 1)

In Group 2, mild hyperemia and interstitial edema were observed in all testes except for one animal. There were vacuolar and hydropic degenerations at varying degrees in germinal epithelium of the seminiferous tubules. In some tubules, the germinal epithelium was completely detached and the basal membrane was in wavy structure. Spermatogenesis was observed in two animals in 1-2 tubules (Figure 1b).



1a **1b**

1c **1d**

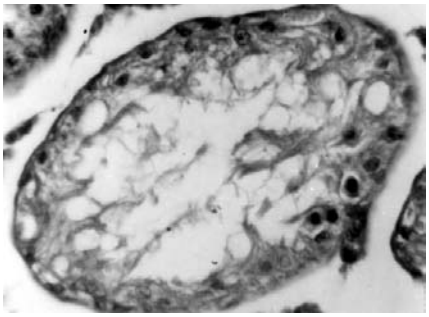
Figure 1a. Seminiferous tubules demonstrating normal spermatogenesis in the contralateral testis of a rat from the control group (Group 1). H&E x 90. **1b.** Disordered appearance of seminiferus tubules in the contralateral testis of a rat from the torsion group (Group 2). The germinal epithelium that covers some tubules are completely detached. H&E x 90. **1c.** Moderate to severe degenerative changes in the germinal epithelium and interstitial edema in the contralateral testis of a rat from the torsion/orchiectomy 24 hours later group (Group 4). H&E x 90. **1d.** Large nuclear cells in the degenerated germinal epithelium with interstital edema in the contralateral testis of a rat from the torsion/detorsion 24 hours later/prednisolone group (Group 5). H&E x 90.

In Group 3, mild hyperemia and interstitial edema were observed in the testes of four animals. In one animal hyperemia and edema were moderate. There were vacuolar and hydropic degenerations at varying degrees in germinal epithelium. Spermatogenesis was observed in all animals.

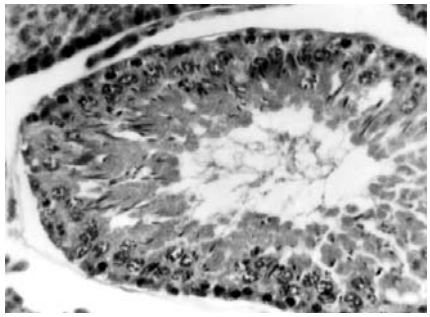
In Group 4, mild to moderate hyperemia and interstitial edema were observed in the testes of four animals. In one animal the edema was severe. The basal membrane was in wavy structure and the germinal epithelium of seminiferous tubules was completely detached. There were vacuolar and hydropic degenerations at varying degrees in germinal epithelium (Figure 1c). In one animal, the degenerative changes were severe. Spermatogenesis was observed in one animal in 1-2 tubules.

In Group 5, mild to moderate hyperemia and interstitial edema were observed. Degenerative changes at varying degrees were observed in the germinal epithelium (Figure 2a). In some portions, the tubular diameter was quite diminished and the basal membrane was in wavy appearance. Spermatogenesis was observed in two animals in 1-2 tubules.

In Group 6, mild hyperemia and interstitial edema were observed in two animals. In all seminiferus tubules, mild degenerative changes were observed (Figure 2b). No spermatogenesis was observed in two animals.



2a



2b

Figure 2a. Intensive degeneration in the germinal epithelium in the contralateral testis of a rat from the torsion/orchiectomy 48 hours later group (Group 6). H&E x 380. **2b.** Normal spermatogenesis together with moderate degenerative changes in the contralateral testis of a rat from the torsion/orchiectomy 24 hours later/prednisolone group (Group 7). H&E x 380.

In Group 7, mild hyperemia and interstitial edema were observed in three animals. Degenerative changes in the germinal epithelium were observed at varying degrees. In one animal, we observed large nuclear cells in the degenerated epithelial cells (Figure 1d). There was no spermatogenesis in one animal, and the others had spermatogenesis at varying degrees.

In Group 8, hyperemia and interstitial edema were observed in three animals at varying degrees. Degenerative changes were severe in two animals. In these animals, the germinal epithelium was completely detached and in some tubules numerous large nuclear cells were observed. There was no spermatogenesis in these two animals and the others had spermatogenesis at varying degrees.

The differences in the body weights and testicular weights between the groups were not significant ($p>0.05$). The difference in the diameters of seminiferous tubules between the groups was evaluated with Mann-Whitney U test. Significant decreases were found in Groups 2, 3, 4, 5, and 8 compared with the control group ($p<0.05$).

Discussion

Unilateral testicular torsion has been shown to affect the contralateral testis histopathologically in varying intensities depending on the treatment method (9,10). However, there is no consensus on when the effects of the torsion starts on the contralateral testis and how the treatment approach affects the outcome. A number of studies showed that 4-6 hours of torsion is adequate to affect the contralateral testis (11). In addition, the degree of rotation is also important. Sessions et al. reported varying degrees of torsion ranging from 180° to 1080° in patients who underwent surgical exploration for testicular torsion (12). It has been reported that medium level pathological changes start with 360 degree torsion, and full infarction is seen after 4 hours of 720 degree torsion (13).

Nagler et al. demonstrated that detorsion did not protect the contralateral testis from atrophy while orchiectomy could prevent the contralateral testis damage (7). Testicular torsion leads to an immunologic response that damages the contralateral testis. Orchiectomy results in removal of the antigenic stimulus, which in turn protects against the development of contralateral testicular damage (14). However, detorsion of the damaged testis might further increase the damage to the contralateral testis due to the remaining immune response. In the present study, we have demonstrated that unilateral testicular torsion caused a significant decrease in the diameters of seminiferous tubules of the contralateral testis with degenerative changes. Detorsion or orchiectomy performed 24 or 48 hours after the torsion did not protect the contralateral testis.

In experimental models of testicular torsion, it has been shown that antispermatozooal humoral immunity is activated following testicular torsion in rats (15). Immunocytochemical studies have demonstrated that IgM antibodies cross the blood-testis barrier of the contralateral testis 7 days after the infarction, which are then replaced by IgG antibodies after 28 days, affecting the spermatogenic and the supporting cells of the seminiferous tubules (16). In another study, anti-rat IgG antibodies against spermatozoa antigens were identified in the contralateral testes of animals subjected to unilateral testicular torsion for 12 and 24 hours, which were then subjected to detorsion (8). However, no antibody formation was detected in animals that underwent orchiectomy 24 hours following unilateral torsion. Immunosuppressive treatment with anti-lymphocyte globulin (ALG) has been shown to decrease the contralateral testicular damage induced by spermatic cord torsion, whereas ALG treatment plus splenectomy prevents the contralateral testicular damage completely (7). In addition, Gulmez et al. demonstrated that adjuvant

immunotherapy with prednisolone following detorsion protected the contralateral testis from ipsilateral testicular antigenic stimulation (14). In our study, we also noted that in groups that had undergone orchiectomy or detorsion (Groups 6 and 7) 24 hours following torsion, addition of prednisolone treatment protected the contralateral testes from immunologic damage. We clearly observed less hyperemia and interstitial edema in these two groups compared to the other groups. The histopathological findings correlated with minimum changes in the average seminiferous tubule diameters, which were close to the control group. Nevertheless, in Group 8, orchiectomy performed 48 hours following torsion plus prednisolone treatment did not prevent the contralateral testicular damage confirming that the duration of torsion is the most important factor that determines the degree of contralateral damage.

On the other hand, in some experimental animal studies, no damage to the contralateral testis was reported following unilateral testicular torsion with no changes in the semen parameters (1,15). In some publications, the immunological damage in the contralateral testis after the unilateral torsion were not also supported (1,17,18). Nagler et al. studied the effects of testicular torsion in prepubertal rats (19). They found no damage in the contralateral testis and concluded that prepubertal testicular torsion may not have the same implications as postpubertal torsion since the antisperm antibodies which cause immunologic damage to the contralateral testis do not appear before puberty. Cosentino et al. have also worked with prepubertal rats, and found that the decrease in spermatogenesis following torsion is more prominent in the contralateral testis of pubertal rats compared to the younger ones (20).

We conclude that the extent of histological damage to the contralateral testis is dependent on the duration of the torsion and the treatment approach. These changes are observed less intensively in animals that undergo orchiectomy, showing that the presence of torsioned testis might be responsible for the contralateral damage mediated by immunologic mechanisms. However, we have demonstrated that the histopathological damage can be prevented by prednisolone even in the detorsioned group by suppressing the immunologic response.

References

1. Anderson JB, Williamson RC. Fertility after torsion of the spermatic cord. Br J Urol 1990; 65: 225-230.
2. Madgar I, Lunenfeld B, Mashiach S, et al. R. Effect of testicular torsion on contralateral testis and fertility in mature rats. Arch Androl 1987; 19: 237-241.
3. Heindel RM, Pakyz RE, Cosentino MJ. Spermatic cord torsion. Contralateral testicular degeneration at various ages in the rat. J Androl 1990; 11: 506-513.
4. Madarikan BA. Testicular salvage following spermatic cord torsion. J Pediatr Surg 1987; 22: 231-234.
5. Tanyel FC, Buyukpamukcu N, Hicsonmez A. Contralateral testicular blood flow during unilateral testicular torsion. Br J Urol 1989; 63: 522-524.
6. Otcu S, Karnak İ, Kilinc A, et al. Orchidopexy or orchidectomy for preventing tissue hypoxia in contralateral testis following induction of ipsilateral abdominal testis associated with vas deferens obstruction. Urol Int 2003; 71: 91-95.
7. Nagler HM, White RD. The effect of testicular torsion on the contralateral testis. J Urol 1982; 128: 1343-1348.
8. Kosar A, Kupeli B, Alcigir G, et al. Immunologic aspect of testicular torsion: detection of antisperm antibodies in contralateral testicle. Eur Urol 1999; 36: 640-644.
9. Mikuz G. Testicular torsion: simple grading for histological evaluation of tissue damage. Appl Pathol 1985; 3: 134-139.
10. Bartsch G, Frank S, Marberger H, et al. Testicular torsion: late results with special regard to fertility and endocrine function. J Urol 1980; 124: 375-378.
11. Barkley C, York JP, Badalament RA, et al. Testicular torsion and its effects on contralateral testicle. Urology 1993; 41: 192-194.
12. Sessions AE, Rabinowitz R, Hulbert WC, et al. Testicular torsion: direction, degree, duration and disinformation. J Urol 2003; 169: 663-665.
13. Williamson RC, Thomas WE. Sympathetic orchidopathia. Ann R Coll Surg Engl 1984; 66: 264-266.
14. Gulmez I, Karacagil M, Sade M, et al. Effect of testicular torsion on the contralateral testis and prevention of this effect by prednisolone. Eur Urol 1987; 13: 340-343.
15. Ryan PC, Whelan CA, Gaffney EF, et al. The effect of unilateral experimental testicular torsion on spermatogenesis and fertility. Br J Urol 1988; 62: 359-366.
16. Lewis-Jones DI, Richards RC, Lynch RV, et al. Immunocytochemical localisation of the antibody which breaches the blood-testis barrier in sympathetic orchiopathia. Br J Urol 1987; 59: 452-457.
17. Cerasaro TS, Nachtsheim DA, Otero F, et al. The effect of testicular torsion on contralateral testis and the production of antisperm antibodies in rabbits. J Urol 1984; 132: 577-579.
18. Turner TT. Acute experimental testicular torsion. No effect on the contralateral testis. J Androl 1985; 6: 65-72.
19. Nagler HM, Deitch AD, deVere White R. Testicular torsion: temporal considerations. Fertil Steril 1984; 42: 257-262.
20. Cosentino MJ, Nishida M, Rabinowitz R, et al. Histological changes occurring in the contralateral testes of prepubertal rats subjected to various durations of unilateral spermatic cord torsion. J Urol 1985; 133: 906-911.