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Experimental Testicular Torsion: Effect on Endocrine and Exocrine Function and Contralateral Testicular Histology

G. Janetschek[1], F. Schreckenberg[1], G. Mikuz[2] and M. Marberger[1]

1 Department of Urology, University of Mainz Medical School, Mainz, Federal Republic of Germany
2 Department of Pathology, University of Innsbruck Medical School, Innsbruck, Austria

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Summary. In order to investigate whether unilateral testicular torsion exerts a negative influence on the previously undisturbed contralateral side, exocrine and endocrine testicular function were evaluated before and two months after torsion. A rat model with 6 hours', 12 hours' or permanent extravaginal 540° torsion of the right testis was used; a sham operated group of animals served as controls. Ejaculates were collected by electrostimulation; LH, FSH and testosterone serum levels were determined by radioimmunoassays. Eight weeks after torsion sperm output had decreased by half in the experimental groups, and LH levels increased significantly, whereas the other hormone levels, as well as the controls, remained unchanged. Morphometry of the contralateral testis revealed no alterations except a significant increase of the Leydig cells and interstitial cells in some subgroups. All observed changes correlate with the functional loss of one testis; definite evidence for contralateral damage was not observed.

Key words: Testicular torsion – Contralateral damage – Morphometry – Endocrinology – Spermiograms

Introduction

Unilateral testicular torsion evokes subfertility in a high percentage of patients. Assuming that one healthy testis can maintain normal fertility, the contralateral testis must either be primarily defective or secondarily damaged [1, 11]. Clinical studies focussed attention on this problem, but have failed to solve the issue so far. A multitude of animal experiments provided controversial information, due in part to poorly comparable models [16, 18, 23]. As the formation of normal spermatozoa depends on an undisturbed testicular anatomy, the first step of this study was to investigate whether the amount of germinal epithelium and other testicular components was altered by contralateral torsion. Stereological methods were introduced to obtain quantitative morphological information allowing direct comparison with data on the sperm output. As the exocrine and endocrine testicular function depend on each other, simultaneous hormone determinations are a prerequisite for a thorough understanding of functional interrelationships.

Materials and Methods

Animals

110 male sexually mature Wistar albino rats – varying in weight from 300–350 g – were used for the present investigation. The animals were housed in a temperature and light controlled room and maintained on pellet food and water ad libitum.

Experimental Design

The animals were divided into 3 groups of 30 each: 1) unilateral right testicular torsion for 6 h, 2) right torsion for 12 h, 3) permanent right torsion. In a fourth group 4) of 20 animals, a sham operation was performed as control.

Spermiograms and plasma hormone levels of each individual animal were determined before and 9 weeks after torsion, so that each animal served as its own control. Morphometry of the contralateral testis and conventional microscopic examination of the twisted testis were performed at the end of the study. At this time, because some of the animals had died, group 1 was reduced to 25, group 2 to 20, group 3 to 25 and the control group to 15 rats.

Induction of Torsion

All operations were carried under sterile conditions and using ether for anaesthesia. Testicular torsion was performed by scrotal incision. As described before, an extravaginal torsion of 540° was found to produce maximal and reproducible hemorrhagic infarction, which mimics the clinical situation best [13]. The testis was then replaced into the scrotum and fixed in place with a suture on each side. Torsion was maintained for 6 or 12 h as previously indicated and then released, or left permanently.

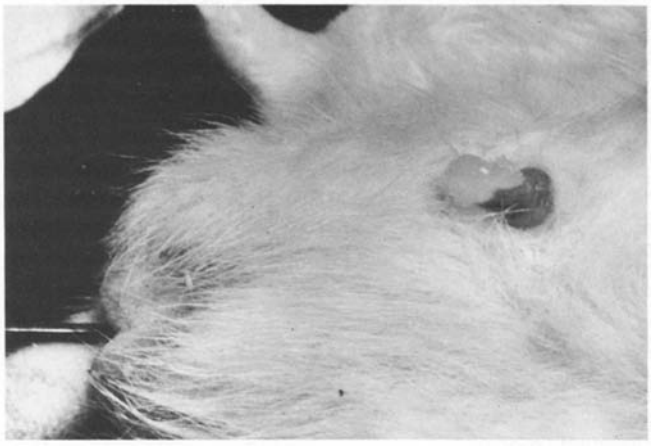

Fig. 1. Ejaculates were collected by electrical stimulation using a single bipolar electrode inserted into the rectum

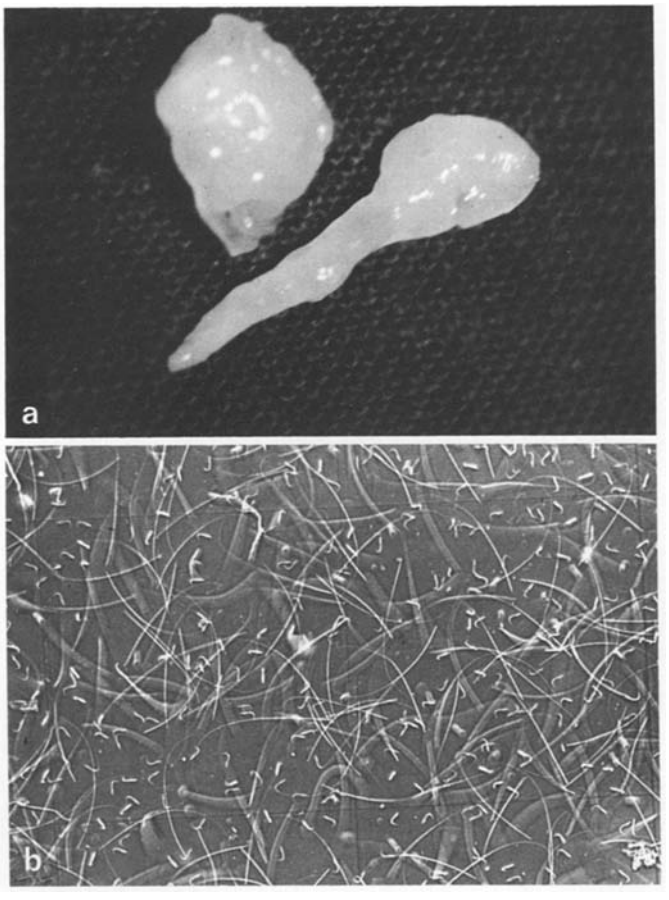

Fig. 2. a The sperm are entrapped in a solid coagulum. **b** Micrograph of Neubauer haemocytometer counting chamber containing liquefied ejaculate with spermatozoa

Spermiograms

Semen was collected from each rat by electroejaculation. The animals were anaesthesized lightly with ether to facilitate handling. Electrical stimulation was provided by a 50-cycle alternating current transformer, which was constructed by ourselves. It could be varied uninterruptedly from 0 to 20 V; amperage was between approximately 5 and 20 mA. The stimulus was applied by a single bipolar electrode [2]. The probe was 0.4 cm in diameter, one electrode forming the tip and the second being 2 cm away from it. The probe was introduced through the anus until the ring electrode was just insisde the rectum. Good electrical contact was provided by adding a small amount of sodium chloride to the lubricant, thereby increasing the conductivity. The electrical stimulus was maintained over a period of 20 s, which was followed by an interval of one minute. The voltage was gradually increased with each succeeding stimulus, starting with 1 V until a peak of 15–20 V was reached. Stimuli at this level were continued until semen was obtained (Fig. 1).

Since during electroejaculation the coagulation gland and the seminal vesicles are also stimulated, the ejaculated spermatozoa are entrapped in a solid plug, which prevents accurate counting (Fig. 1 and 2a) [2, 21]. This problem can be overcome by removal of these organs. Since we did not want to introduce another operation which could possibly interfere with the experimental torsion, the ejaculate was liquefied with a solution of 3% alpha-chymotrypsin in sterile water, thus making spermatozoa countable [21]. Penicillin was added to prevent bacterial contamination during incubation at 37 °C for 24 h. During this process heads and tails of the spermatozoa may separate, so that sperm motility cannot be evaluated. Sperm counts were performed using a Neubauer hemocytometer counting chamber. Photographs of the light microscopic samples were projected onto a screen, thereby facilitating counting (Fig. 2b). The volume of the ejaculate was measured by pipetting it after liquidization. From this, total sperm output was calculated. To reduce individual variations in sperm counts, each spermiogram constituted the mean of two or three determinations at 5 day intervals.

Hormone Measurements

The plasma levels of testosterone, luteinizing hormone (LH) and folliclestimulating hormone (FSH) were determined. 2 ml of blood were taken by direct puncture of the external jugular vein between 10 and 12 o'clock. The blood was allowed to clot for 2 h at room temperature. Serum was collected by centrifugation at 800 g for 10 min at 2 °C and stored at −20 °C until required for the hormone assays. All assays were carried out in duplicate. Serum testosterone was measured by double-antibody radioimmunoassay using commercial kits obtained from Radioassay Systems Laboratories, Inc., California, USA. Serum FSH and LH were measured by double-antibody radioimmunoassay [6] using kits supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD) (rat pituitary hormone distribution program, Dr. A. F. Parlow). The results are expressed in terms of their respective NIAMDD-RAT-RP-1 reference preparations. The radioiodination was carried out after the method of Greenwood et al. [9]. Separation of ^{125}I-labelled hormones from free ^{125}I was achieved by Sephadex G-75 column chromatography. The specific activity of ^{125}I-LH was 168 mCi/mg and of ^{125}I-FSH 201 mCi/mg.

Histologic Investigation

9 weeks after torsion and sham operation, respectively, both testes were removed and fixed in Bouin's solution for 2 days. They were then stored in 80% alcohol until they were embedded in paraffin. 5 μm thick sections were prepared and stained with haematoxylin and eosin.

The degree of necrosis of the ipsilateral testis was estimated by conventional light microscopy.

Stereological techniques were employed to obtain quantitative morphological information on the contralateral testis, thus allowing

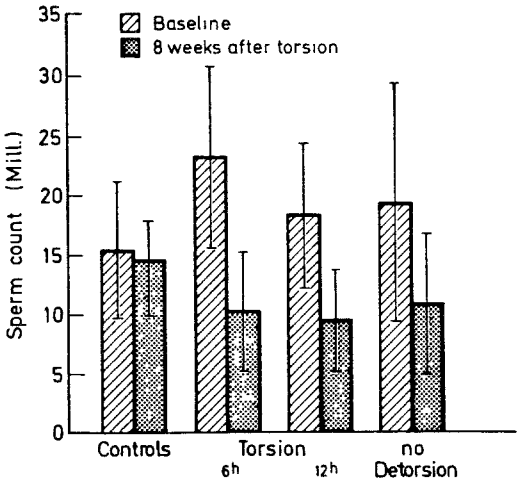

Fig. 3. Sperm counts before and two months after a unilateral testicular torsion of indicated duration ($\bar{x} \pm SD$)

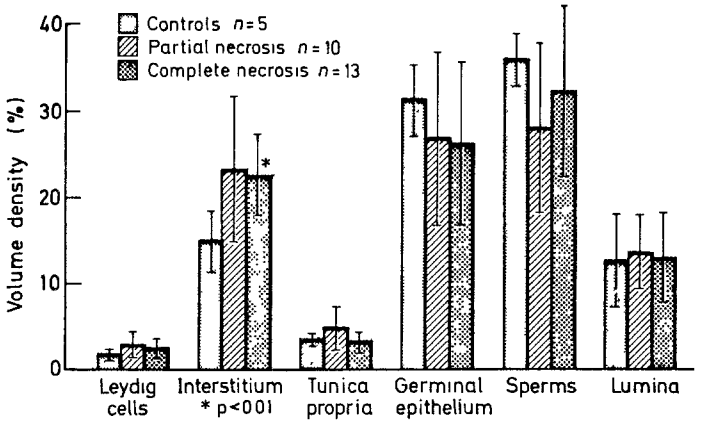

Fig. 4. Morphometry of contralateral testis: The volume fractions of the different testicular components were compared to the controls according to whether a partial or complete necrosis could be observed in the twisted testis ($\bar{x} \pm SD$)

statistically defined comparisons. In practice, the volume of tissue components can be determined by superimposing a systematic set of test points over the cross section and then counting the number of points lying over the different tissue components [7]. 10 visual fields in each of 3 histologic sections per rat testis were evaluated by light microscopy (x200). Sampling was done on a strictly random basis. A well established mathematical correlation exists between the number of points counted within a distinct area of a given tissue and the corresponding relative volume of this component. The volume density of the Leydig cells, the interstitial tissue, the tunica propria, the germinal epithelium, the spermatozoa and the tubular lumina was calculated. This was performed in 9 animals of group 1, 7 of group 2, 7 of group 3 and 5 in the control group.

Statistics

The hormone values and the spermiograms were statistically compared using the paired Student's t-test.

The data of the morphological parameters and sperm counts were analyzed for differences among the groups using analysis of variance and the Student's-test. All data showed normal distribution.

Results

Spermiograms

Average total sperm output was 15.4 ± 5.7 millions in the control group, where a slight, but statistically insignificant decrease to 14.5 ± 3.6 millions could be observed after 8 weeks. In the animal groups with 6 h, 12 h or permanent torsion, there was a significant decrease ($P < 0.001$) of the sperm counts to about half of the inital values (Fig. 3). Within these three groups no significant difference could be observed, neither before torsion nor at the end of the experiment. Exactly the same is true when the ipsilateral testicular histology – partial or complete necrosis of the germinal epithelium – is taken as discriminating factor instead of the length of time of torsion.

Hormone Measurements

The mean plasma levels of testosterone, LH and FSH at the beginning and at the end of the study are indicated in Table 1. The only significant changes observed were an increase of LH in group 1 ($P < 0.001$) and 3 ($P < 0.005$). All other hormone levels remained essentially unchanged.

Morphometric Results

The relationship between contralateral testicular histology and the duration of torsion is shown in Table 2. When compared to the control group, the volume density of the Leydig cells was significantly higher and the fraction of spermatozoa lower after 6 h torsion. An increase of the volume density of interstitial tissue was also noted after 12 h or permanent torsion. Any difference was demonstrable using analysis of variance.

Contralateral testicular histology was also analyzed and compared with the controls depending on whether partial or complete necrosis could be observed in the twisted testis (Fig. 4). With the exception of a significant increase of interstitial tissue after complete necrosis, absolutely no morphological changes were demonstrable in the contralateral testis. Using analysis of variance, the increase of interstitial tissue was not significant either.

Histology of Twisted Testes

Conventional light microscopy demonstrated that the germinal epithelium of the twisted testis was either severely damaged or completely destroyed with no exceptions. Some correlation between duration of torsion and the degree of damage could be observed. Some testes had disappeared completely.

Table 1. Plasma hormone levels of testosterone, LH and FSH (mean ± standard deviation)

Group	Procedure	Before torsion			2 months after torsion		
		Testosterone ng/ml	LH ng/ml	FSH ng/ml	Testosterone ng/ml	LH ng/ml	FSH ng/ml
1	6 h torsion	2.6 ± 1.8	32.9 ± 9.0	452 ± 157	1.9 ± 1.3	40.2 ± 8.4[a]	521 ± 95
2	12 h torsion	3.3 ± 1.8	30.8 ± 8.6	426 ± 79	3.1 ± 2.3	35.1 ± 8.6	469 ± 114
3	permanent torsion	3.1 ± 2.3	34.1 ± 7.8	437 ± 140	2.1 ± 1.6	42.9 ± 9.4[a]	500 ± 162
4	sham (control)	2.0 ± 1.4	37.7 ± 5.9	472 ± 86	2.0 ± 1.3	35.2 ± 8.0	437 ± 61

[a] significant difference between hormone level before and two months after torsion in indicated group: $P < 0.05$

Table 2. Morphometry of contralateral testis: volume densities of the different testicular components (mean values ± standard deviation)

Group	Procedure	Leydig cells %	Interstitial tissue %	Tunica propria %	Germinal epithelium %	Spermatozoa %	Tubular lumina %
1	6 h torsion	3.2 ± 1.3[a]	20.5 ± 7.2	4.4 ± 2.1	30.4 ± 6.8	26.5 ± 6.6[a]	14.9 ± 3.6
2	12 h torsion	2.3 ± 1.2	23.8 ± 3.4[a]	3.2 ± 1.5	26.8 ± 6.9	30.8 ± 5.7	13.2 ± 4.4
3	permanent torsion	2.1 ± 1.4	25.2 ± 7.5[a]	3.3 ± 0.7	21.9 ± 12.7	35.7 ± 14.8	11.7 ± 6.1
4	sham (control)	1.6 ± 0.6	14.9 ± 3.6	3.4 ± 0.9	31.4 ± 4.1	35.9 ± 3.1	12.8 ± 5.4

[a] significant difference between the controls and indicated group: $P < 0.05$

Discussion

The disorders of spermatogenesis frequently found in unilateral testicular torsion can either be explained by preexisting testicular pathological conditions, such as damage to the germinal epithelium by recurrent subtorsion with short ischemic periods, or by immunological processes affecting the contralateral testis. A review of the recent literature provides enough arguments for each of these theories [1, 11].

Jules Freund et al. [8] first demonstrated germ cell-specific immune induced aspermatogenesis, and his experiments were confirmed by others in various animals [3] and even in man [20]. This well documented autoimmune orchitis appears very appealing as a theroretical basis for the mechanism of contralateral testicular damage after torsion. Aspermatogenesis, as an induced autoallergic response, is usually elicited by intradermal injection of antigen and adjuvant together. Testicular torsion cannot provide sensitization comparable to that. Differences in the presentation of the antigen to the immune system may explain why such an effect was observed in some studies [16, 23], and was not demonstrable in others [18, 29]. There is also strong evidence that the aspermatogenic response is reversible to some extent [14, 15]. Some of the studies demonstrating severe damage of the contralateral germinal epithelium were performed within two weeks after torsion before recovery could occur [16, 23]. But there are also some contradictory long-term results [19, 24]. This raises the question of a common explanation for the conflicting data found.

Testicular torsion usually results in hemorrhagic infarction. With an increasing degree of torsion the testicular artery is finally also obstructed, thereby producing complete ischemia. The biological effect thereof may not be the same as that of hemorrhagic infarction, and the theory has been presented that some degree of circulation of the manipulated testis seems to be necessary to trigger an immune response [4]. Although this has in part been refuted [16], experimental torsion should mimic the clinical situation by achieving maximum hemorrhagic infarction and not ischemia. It does not seem reasonable to rely on different time periods of torsion in the experimental design if the hemodynamic changes are not exactly defined. The hemodynamic effects of experimental testicular torsion were therefore investigated in a first study [13]. In the rat extravaginal torsion of 360° and 540° only reliably produces hemodynamic alterations comparable to the clinical situation of testicular torsion.

Quantitative histological analysis by stereological techniques demonstrated that 2 months after torsion the amount of contralateral germinal epithelium was not reduced in any of the groups with different duration of torsion. As it could be speculated that the maintenance of an autoimmune orchitis might depend on the presence of some damaged germinal tissue to induce and maintain antibody formation, histological analysis of the twisted testis was performed to determine whether any germinal tissue was left in the twisted testis or not. The following quantitative histological analysis of the contralateral testis was then performed with respect to the presence or absence of damaged germinal epithelium in the twisted testis. Also with this

approach alterations of the germinal epithelium could not be observed.

Sperm output decreases to half of the initial values two months after torsion. This corresponds exactly to the functional loss of one half of a paired organ as was observed by others after semi-castration [22, 25], since compensatory testicular hypertrophy will not occur in rats 45 days of age or older [17]. Neither the duration of torsion nor the degree of damage to the twisted testis had any demonstrable effect on spermatogenesis. This corresponds to the fact that the sperm number within the lumina of seminiferous tubules as determined with morphometry also remained unchanged. The only exception to this was noted in the 6 h torsion group, but the observed decrease of spermatozoa within the lumina was not correlated in the sperm output. In this group FSH values were normal as in all other groups, which indicates a functionally intact germinal epithelium [12].

Besides unchanged FSH levels an isolated increase of LH was noted, which was not paralleled by a decrease of plasma testosterone. The same findings have been described after semi-castration and could be explained by the simultaneous finding of a twofold increase of the intraparenchymal testosterone level of the remaining testis [5]. This compensatory mechanism may be responsible for the increase of Leydig cells after torsion, although this was not a constant finding.

The increase of interstitial tissue is difficult to understand and may indicate some non-specific reaction.

The simultaneous finding of a normal histology of the contralateral testis, normal plasma FSH values and undisturbed spermatogenesis 2 months after torsion indicates that in the rat damage to one testis does not necessarily result in a permanent lesion of the other side. As experimental studies undoubtedly have proved that such damage occurs shortly after torsion, it appears permissible to conclude that the noxious effect exerted by the injured testis is only transient in nature and will be followed by recovery, which is complete two months later. Further studies are required to investigate the time course of the presumed damage and to determine the regenerative capacity of the germinal epithelium. Also one should not overlook that there is strong evidence that the target of an immunologic attack is not only the contralateral germinal epithelium, but also the mature spermatozoa on its way out through the male genital tract [10].

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Dr. G. Janetschek
Klinik für Urologie
Anichstrasse 35
A-6020 Innsbruck
Austria