Subsequent alterations in the contractile property of the vas deferens according to duration of spermatic cord torsion

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Objective: To determine whether twisting of the ipsilateral vas deferens results in alteration of its contractility.

Design: Experimental study.

Setting: University animal lab.

Animal(s): 24 male Wistar rats.

Intervention(s): All the rats in the experimental groups underwent spermatic cord torsion. Durations of torsion were 45 minutes, 3 hours, and 24 hours in groups 2, 3, and 4, respectively. In groups 2 and 3, subgroups b were created to evaluate late effects using in vitro pharmacological techniques.

Main Outcome Measure(s): The contractility of the vas deferens was evaluated in groups 1, 2a, 3a, and 4 right after and in groups 2b and 3b 48 hours after the initial operation.

Result(s): Group 4 and subgroups 2b and 3a had significantly diminished responses compared with the control group, whereas in subgroups 2a and 3b, the responses to noradrenaline and to single-pulse field stimulation were not significantly different.

Conclusion(s): The impairment of contractility with the twisting of the vas deferens might be another factor responsible for subfertility, particularly that related to sperm transport. The unfavorable late change in short duration of torsion may be the result of either ischemia and reperfusion injury or sympathetic overactivation in the acute period of torsion. (Fertil Steril 2011;–:

Key Words: Spermatic cord, torsion, vas deferens, contractility, fertility

Spermatic cord torsion represents one of the most serious emergencies encountered in surgical practice in terms of both urgent management and long-term potential serious sequelae. Although series in spermatic cord torsion using an aggressive approach with rapid scrotal exploration have achieved testis survival rates higher than 90% (1), diminished fertility rates resulting from spermatic cord torsion, especially in experimental studies, are still noted (2–4). The diminished fertility in unilateral testicular torsion might be attributed to the proven secondary effects of torsion of ipsilateral testis to the contralateral side. Most of these insults are observed to be time dependent and require long periods in order to exert biochemical and autoimmune effects (5–7). Additionally, some studies have suggested that a reflexive vasospasm preceded by sympathetic activation is another important factor resulting in contralateral testicular damage in unilateral spermatic cord torsion (8–10). However, the local effects of twisting of the spermatic cord to the vas deferens regarding its contribution to subfertility are still open to investigation.

The vas deferens, which is one of the organs that get twisted during testicular torsion, mainly functions as a conduit for the transport of sperm from the epididymis to the urethra and plays an important role in the production of seminal emissions, which occurs in response to rhythmic contractions of the male secondary sex organs (11, 12). These contractions of the vas deferens are directly induced by adrenergic mediators and are modulated by a variety of local endogenous factors.

To our knowledge, the effects of pure local damage to vas deferens motility in case of torsion that also might play an important role in infertility previously have not been studied with in vitro pharmacological techniques. In this experimental study, we aimed to determine the effects of ipsilateral vas deferens torsion to the contractility function of this organ in different durations. We also investigated the possible persistency of this damage by evaluating the detorsioned vas deferens 48 hours after the intervention.

MATERIALS AND METHODS

Twenty-four sexually mature male Wistar rats weighing 200–240 g (which corresponds to an age of 9–12 weeks) were used in this study. They were individually housed in metal cages in a quiet, temperature- and humidity-controlled room (22 ± 3°C and 60% ± 5%, respectively) in which a 12:12 hour light-dark cycle was maintained. The experiment was performed under relevant institutional rules after institutional review board approval.

Four groups were created to examine the ipsilateral effects of twisting on vas deferens contractility by using in vitro pharmacological techniques. All the rats in this study, except the ones in the control group (group 1), underwent clockwise 720° spermatic cord torsion.

In group 1 (n = 4), a sham procedure was carried out. In groups 2 and 3, the spermatic cords were initially subjected to torsion and subsequently divided into two subgroups a and b. In subgroup 2a, the vas deferens was examined at the end of the torsion time, and in subgroup 2b, the testis was relocated in the scrotum...
after detorsion and reoperated to retrieve the vas deferens 48 hours after the initial operation. Further, in group 2 (n = 8; subgroup 2a, n = 4; subgroup 2b, n = 4), the duration of torsion was 45 minutes, and in group 3 (n = 8; subgroup 3a, n = 4, subgroup 3b, n = 4), the duration of torsion was 3 hours.

Subgroups 2b and 3b were particularly designed to find out whether the alterations of contractility after the torsion of the vas deferens were transient or not. Finally, group 4 (n = 4) was designed to determine the effects of torsion that lasts for 24 hours.

For the in vitro assay, the vas deferens was quickly removed from the rat and dissected free from blood vessels and adjacent tissues (Fig. 1). The vas deferens was flushed free of sperm with a syringe. The epididymal portions of the vas deferens were dissected and placed in preheated (37 °C) Krebs-Henseleit physiological solution of the following composition (mM): NaCl = 118, KCl = 5.6, CaCl2 = 2.5, MgSO4 = 1.2, KH2PO4 = 0.9, NaHCO3 = 25, and glucose = 11. The lower end was fixed and the upper end was attached to an isometric force transducer (Grass FT03) under an initial tension of 1 g. Tissues were equilibrated for at least 45 minutes. The tension was recorded on a Grass polygraph (model 7A).

In the first setting of the experiment, cumulative concentration–response curves to noradrenaline for the epididymal portion of control and torsion groups (from 10⁻⁸ to 10⁻⁴ M) were obtained by dosing at 1 log unit interval, with the use of (—)-noradrenaline hydrochloride (Sigma). Increasing concentrations were added to the organ bath after the response to the previous one had reached peak value. The second step was electrical field stimulation provided by a Grass S88 stimulator via gold-coated silver electrodes positioned on each side, parallel to the axis of the ring. Single square wave pulses (60 V, 0.5 ms, 5–30 Hz) were used.

Results are expressed as mean ± SEM. Contractions are reported as percentage of the control response. Statistical differences between the control and torsion groups were assessed by two-way analysis of variance (Bonferroni test). P values < .05 were regarded as statistically significant.

**RESULTS**

The concentration-dependent increase in tension with increasing concentrations of noradrenaline and the contractile response to single-pulse field stimulation were recorded for all groups.

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**TABLE 1**

<table>
<thead>
<tr>
<th>% Maximum response</th>
<th>Control</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tbody>
<tr>
<td>Noradrenaline (M)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10⁻⁸</td>
<td>5.8 ± 2.5</td>
<td>9.8 ± 2.0</td>
<td>16.5 ± 3.4</td>
<td>3.6 ± 2.4*</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>31.2 ± 5.3</td>
<td>26.8 ± 5.1</td>
<td>58.3 ± 5.5*</td>
<td>6.9 ± 3.3*</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>77.4 ± 6.2</td>
<td>72.7 ± 11.5</td>
<td>66 ± 1.6*</td>
<td>16.7 ± 7.1*</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>100</td>
<td>96.0 ± 9.7</td>
<td>23.3 ± 7.6*</td>
<td>69.2 ± 10.9</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Electrical field stimulation (Hz)</th>
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<tr>
<td>5</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>15</td>
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<td>20</td>
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<td>25</td>
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Note: Values presented as mean ± SD. Group 1 (n = 4), control. Groups 2 (45 minutes) and 3 (3 hours) are designed to mimic the clinical orchiectomy population. (In subgroup a, the vas deferens was examined at the end of the torsion time. In subgroup b, we relocated the testis in the scrotum after detorsion and reoperated to retrieve the vas deferens 48 hours after the initial operation.) Group 4 (n = 4) was designed to have a torsion duration of 24 hours.

* Statistically important set.

Contractions in each study group are studied as a percentage of control response. These findings are listed in Table 1.

Group 4 and subgroups 2b and 3a had significantly diminished responses to noradrenaline and to single-pulse field stimulation compared with the control group. In subgroups 2a and 3b, the responses to noradrenaline and to single-pulse field stimulation were not significantly different (Fig. 2).

Analysis of the orchiopexy groups revealed two distinct results. In group 2 (duration of torsion 45 minutes), a worsening response after detorsion in the 48-hour period was observed whereas in group 3 (duration of torsion 3 hours), a healing response was detected on comparison of the subgroups with the control group (Fig. 2). These results revealed that the initial effect of twisting as contractility damage after the 3-hour torsion period was not apparent any more at the postinterventional 48th hour. Group 4 representing the orchiectomy group in practice had significantly diminished responses, as expected (Fig. 3).

**DISCUSSION**

Various conditions that affect the morphology of the vas deferens may also influence its function and, ultimately, male fertility. Experimental studies evaluating the influence of local trauma on the vas deferens with manipulations or extensive mobilizations demonstrated significant damage, which may contribute to infertility in animal models (6, 7).

The isolated vas deferens has proven to be a useful specimen for a variety of pharmacological and physiological experiments and it is also well known that the rat prepubertal vas deferens serves as a good model for investigation of the human vas deferens (4, 13).

The spermatic cord torsion mainly occurs at the epididymal part of the vas deferens. Because the segments from the epididymal part are more responsive to noradrenaline, we preferred to use noradrenaline for evaluating the effects of spermatic cord torsion on the ipsilateral vas deferens (14). The results allowed us to conclude that contractility after torsion can be studied with in vitro techniques in the rat vas deferens, because the exogenously applied noradrenaline and electrical field stimulation resulted in different responses with different torsion durations.

Although local effects have been evaluated histopathologically in experimental models, we did not find any study concerning the evaluation of contractility of the vas deferens in spermatic cord torsion with in vitro pharmacological techniques. In 1989, Gautam et al. (15) showed that degeneration of muscular layers of the vas deferens...
showed marked inhibition of its contractility. The present study also has shown that twisting of the vas deferens is a cause of inhibition of the contractile responses to electrical stimulation and noradrenaline in the rat ipsilateral vas deferens, which depends on the duration of torsion.

The most dramatic inhibition of contractile responses was found in the 24-hour torsion group, whereas no significant difference was noted in the acute phase of the 45-minute duration group. Further, contractile responses were also decreased significantly in the acute phase of the 3-hour torsion group, which demonstrates that we cannot decide easily whether a testis is viable. Thus, the study results led us to conclude that delayed presentation of torsion continued to be an inherent obstacle not only for testicular salvage but also for vas deferens contractility.

We also sought to investigate if the vas deferens was permanently affected by the twisting under experimental torsion. We found out that the damage to the vas deferens was transient in terms of contractile responses in the 3-hour group when reexamined 48 hours after detorsion. In the 45-minute torsion group, a significant decrease was observed in contractile responses after detorsion. These different responses in relation to relapse time in the 3-hour and the 45-minute torsion groups led us to consider two possible mechanisms: the ischemia and reperfusion injury after detorsion and sympathetic overactivation in the acute period of torsion.

It is important to appreciate that ischemia is not an isolated event and when it is temporary or reversible, ischemia is followed by reperfusion. It has been shown that short-term ischemia and injury by reperfusion are more detrimental than the effects of ischemia alone (13, 16). The more significant decrease of contractility after detorsion in the 45-minute group compared with the 3-hour group may support the same hypothesis: more severe injury with reperfusion after short-term ischemia.

Because the rat isolated vas deferens is characterized by a predominantly sympathetic innervation through the hypogastric plexus, a homogenous population of $\alpha_1$-adrenergic receptors mediates the contractile response of this tissue to noradrenaline (14, 17). This tissue is therefore a useful model to compare the changes in receptor density or properties with changes in functional responsiveness to adrenergic receptor stimulation. A lower tissue sensitivity to exogenous noradrenaline, which would occur with abundant endogenous adrenergic neurotransmitter, points to sympathetic overactivation (18). Our study also supports the hypothesis that an activated sympathetic reflex produced by the ipsilateral testis under stress in acute periods has resulted in decreased response to noradrenaline.

The current study showed that in addition to all the theories regarding subfertility after unilateral torsion, the impairment of contractility with the twisting of the vas deferens may be another factor responsible for subfertility in some of these cases, apart from the testicular effects. It is of utmost importance to say that if a patient has any congenital or acquired pathology of the contralateral side, this possibility must be kept in mind, as some of the subfertility cases, particularly those related to transport and emission of sperm, may be affected by alterations in the contractility of the vas deferens smooth muscle. However, whether these studies in the rat can be extrapolated to the circular smooth muscle of human vas deferens remains to be determined.

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REFERENCES


