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Assessment of Immunological Mechanism in Infertility of the Rat After Experimental Testicular Torsion

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Summary. A necrotic torted testis left in the scrotum affects the contralateral testis, resulting in depressed fertility. It was suggested that testicular torsion provoked a systemic response of an immunologic nature. In order to prove this theory, 56 rats were operated. Ten had a sham operation, in 17 a testis was torted and excised after 24 h, and in 29 a testis was torted and excised after 24 h, and in 29 a testis was torted but left in situ till the sacrifice. Antisperm-antibodies were not found in any phase of the experiment in the sham group. Using indirect immunofluorescence, antisperm-antibodies were found in the sera of 8/17 rats of the orchiectomy group and in 24/29 of the detorted group. In the direct immunofluorescence study, all the antibodies were localized in the tail of the spermatozoid. The antisperm-antibodies dissappeared after 3 to 6 months.

Key words: Testicular torsion, Immunologicol infertility, Autoimmune orchitis, Spermatogenesis, Antisperm-antibodies, Immunofluorescence.

Introduction

The agressive approach of early surgical exploration in testicular torsion has yielded the highest rates of testicular salvage in terms of preservation of the testes [4]. The incidence of abnormal semen analysis is apparently dependent on the duration of the torsion and the treatment rendered [2]. Patients in whom torsion exists for longer than 24 h before undergoing detorsion were uniformly found to have persistent abnormal semen analysis. In contrast, patients with torsion of similar duration undergoing orchiectomy demonstrated post-operative semen analyses that were normal. This latter observation provokes the question of whether a contralateral testis abnormality can be induced by the presence of testicular torsion [20].

Unilateral experimental testicular damage, performed in a variety of ways, can result in changes in the contralateral testicle which produce impaired fertility [19], defective sperm production [5, 13] and focal changes of allergic orchitis [7, 8, 25]. Autoimmune orchitis has been demonstrated following thermal or traumatic testicular damage [17]. In a previous study [19] it was proved that leaving a torted testis in situ provoked a severe reduction in fertility and some lesser reduction in sperm motility in the contralateral testis. It was then suggested that the damage was caused by an immunological response. The present study was undertaken to prove this immunological influence.

Material and Methods

Adult male Charles River strain rats were operated on. In ten rats, a sham operation was performed: through an abdominal incision, the left testicle was luxated and then replaced in the scrotom. In fifty rats, the abdomen was opened, the left testicle was luxated, its artery and vein were ligated as reported in our previous experiment [19]. 24 h later, the wound was reopened. In some of the rats, the damaged testis was resected and in others the ligature was released and the "torted" testicle was left in situ.

A week preoperatively, 2 ml of blood were withdrawn from the rat's orbit and the serum was separated and frozen. Blood was withdrawn again 1 month and 2 months postoperatively. Some of the rats of each group were sacrificed after 3 months. Blood was withdrawn from their heart and the right testicle was removed for examination. In the remaining rats, blood was again withdrawn from the orbits, and they were sacrificed after six months.

The epididymis was separated and cut into minute pieces and put in 5 ml of M-199 warmed to 34 °C (M-199 Bio-Lab Product, with 20 mM Hepes Buffer and with Hank's Salts). The suspension was then filtered and washed three times with 10 ml phosphate buffered saline (PBS) by centrifugation for 10 min at 1,400 x G at 4 °C as described by Kolk et al. [14]. The washed spermatozoa were resuspended in PBS and adjusted to a concentration of 2×10^6 per ml. A drop of 20 µl sperm suspension was placed in each of the ten squares of a microscope slide for IF. (Immunofluorescence – Institute Pasteur Production, code number 50,564). This was dried by a warm air current, the temperature not exceeding 37 °C. The slides were then fixed in methanol for 30 min (Absolute methanol Bio-Lab Laboratories Ltd. production). After drying, the slides were used for immediate immunofluorescence study or stored at a

temperature below −20 °C for further control. A Leitz Orthoplan microscope with epi-illumination was used and as well as a filter combination for fluorescein isothiocyanate fluorescence.

For direct immunofluorescence study, the slide was incubated with fluorescein conjugated Rabbit-anti-rat immunoglobulins antiserum diluted 1:10 (produced by DAKO Immunoglobulin A/S, code number F234). After 30 min the slide was washed with PBS 3 times and dried as before, mounted in glycerol PBS 1:1, covered with 20 x 50 mm coverslip and examined for antigens.

For indirect immunofluorescence study, sperm of a normal adult rat was prepared as previously described. A drop of diluted serum 1:10 collected from the experimental rats was layered on each spot of the slide and incubated for 30 min, and at the end of the procedure was mounted in glycerol PBS 1:1 and examined.

Results

Fifty-six rats were alive at the end of the experiment: 10 of the sham operation group, 17 of the orchiectomy group and 29 of the torted testis group.

Antisperm-antibodies were not found in any phase of the experiment in the sham operation group, neither by direct immunofluorescence study nor by the indirect one.

In four rats, antisperm-antibodies were found in the serum already preoperatively. These antibodies persisted till the end of the second month in the orchiectomy group and till the end of the third month in the torted group and then disappeared.

Antisperm-antibodies were found in the sera of 5 of the orchiectomy group (5 out of 17 rats) at the end of the first month, and in 14 of the detorted group left in situ (14 out of 29 rats). These antibodies persisted till the end of the second month in 1 of the orchiectomy group and in 6 of the detorted group. The antisperm-antibodies were still there at the end of three months in 3 of detorted group, and even at 6 months in one of them.

At the end of the second postoperative month, antisperm antibodies appeared de novo in 3/17 of the orchiectomy group but persisted till the end of three months only in one of them. In the detorted group, there appeared antisperm-antibodies in 6/29 rats which persisted till they were sacrificed. Antisperm-antibodies were not found in any of the orchiectomy group at the end of 6 months.

Summing up (Table 1) antisperm antibodies were found in the sera of 8/17 rats of the orchiectomy group and in 24/29 of the detorted group. They persisted longer in the detorted group, in 4 of them even at 6 months.

Direct immunofluoroscopy study of the spermatozoids of the sacrificed rats showed the presence of antisperm antibodies in 1/17 of the orchiectomy group and 6/29 of the detorted group. All the other rats remained negative to direct fluoroscopy. Those who were positive in the direct immunofluoroscopy study were also positive in the indirect study of the sera.

Localization of the antibodies in the spermatozoids was as follows:

In the orchiectomy group, the antibodies were located in the tail of the spermatozoids of 5 rats; in 3, both in the tail and in the head. In none of them antibodies were found merely in the head.

In the detorted group, antibodies were found in the tail in 14 rats, both in the head and in the tail in 8 rats, and in 2 there were antibodies only in the head.

In the direct immunofluorescence study, all the antibodies were localized in the tail only.

Discussion

The necessity to excise a non-viable torted testicle is commonly accepted nowadays [2, 5, 11, 19, 20, 22, 26, 27]. There exists clinical evidence that a necrotic torted testis left in the scrotum will affect the contralateral testis [2, 15, 18, 20]. Bartsch et al. [2] found sperm analysis to be normal when orchiectomy was performed in 3 patients, while in another 4 patients, where the damaged testis was left in situ, sperm analyses were pathological. There is ample experimental proof to corroborate this finding [3, 5, 9, 13, 19, 20, 27].

Etiology, on the other hand, is not clear. Horica and Hadziselimovic claim that it is not possible to determine definitely whether the disorders of spermatogenesis frequently found in unilateral testicular torsion are the result of congenital dysplasia or damage to the germinal epithelium caused by recurrent subtorsion [12]. It was also suggested that testicular torsion provoked a systemic response, possibly of an immunologic nature that was damaging the contralateral testis by depressing fertility and causing histologic changes [2, 15, 18, 20, 21, 23]. Detorsion after 24 h did

Table 1

Group	Antisperm Antibodies in the Serum				Direct Immunofluorescence	
	Pre-operative		Pos-operative			
	Positive	Negative	Positive	Negative	Positive	Negative
Sham Operation (10)	0	10	0	10	0	10
Orchiectomy (17)	1	16	8	9	1	16
Detorted (29)	3	26	24	5	6	23

not protect the contralateral testicle from tubular atrophy, whereas orchiectomy afforded significant protection [20]. Therefore, if the testis is ischaemic and non-viable, it should be removed [5, 11, 19, 20, 22, 26, 27].

The actual site of induction of immunological response remains unknown, as well as the time of onset and the duration of this influence. Damage induced in the contralateral testis is likely to be permanent, and structural changes in the opposite testicle may occur as early as 16 days after the torsion [5]. Wallace et al. [26] found impaired spermatogenesis in the contralateral testes of rats at 4 and 8 weeks after injury. Harrison et al. [11] proved that just a manipulation of the testis and its blood supply was sufficient to cause spermatogenic damage and initiate an immunological response.

In our present experimental study, we showed that there were no antisperm antibodies in the control group at any stage, whether by direct or indirect immunofluorescence study. On the other hand, antibodies appeared after torsion. The antibodies had a tendency to disappear much more quickly in the orchiectomy group rather than in the detorted group. It is interesting to note that antisperm antibodies were found even in those rats who had their torted testis resected after 24 h. These antibodies tended to disappear after 3 months in the orichetomy group, but persisted even 6 months in the detorted group.

It is presumed that antisperm antibodies result from direct contact of spermatozoa and immune competent cells, but the actual site of induction remains a mystery [9]. T-cells were necessary for the induction of experimental allergic orchitis, but antibodies may also be necessary for the full expression of this disease [3]. The histologic changes in the contralateral testis can be suppressed with removal of the antigenic stimulus (orchiectomy) as well as by immunosuppression of the host animal with antilymphocyte globulin and splenectomy [20].

The antisperm antibodies in our experiment were concentrated especially on the tail of the spermatozoid. We have no explanation for that. We cannot specify their class, since we used polyvalent antisera.

Extrapolation of animal models to humans should be done with great caution. In a large series of infertile patients, none had a history of testicular torsion [1, 22].

We have shown in our experiment that antisperm antibodies appear after testicular torsion. The great majority of these antibodies disappeared after a certain lapse of time. The same phenomenon probably exists in man. Yet we feel that non-viable torted testes should be excised in spite of the disappearance of antibodies with time, because till then the damage to the contralateral testis might become irreversible.

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