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Unilateral spermatic cord torsion without ipsilateral spermatogenetic material: effects on testicular blood flow and fertility potential

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Abstract This experiment was planned to answer the question of how the elimination of ipsilateral spermatogenetic material, which is necessary for contralateral testicular damage caused by an autoimmune response, affects contralateral testicular blood flow and fertility potential in unilateral spermatic cord torsion (USCT). Thirty-four male and 68 female adult albino rats were divided into three groups. Group 1 rats underwent a control operation, group 2 rats underwent subepididymal orchiectomy to eliminate spermatogenetic material, and group 3 rats underwent USCT after subepididymal orchiectomy. Testicular blood flows of the rats were measured by ^{133}Xe clearance technique. Additionally, to determine fertility potential, each male rat was housed with two female rats. Numbers of impregnated and delivered rats were recorded. Both mean testicular blood flow and fecundity of group 3 were significantly lower than those of groups 1 and 2. When compared with groups 1 and 2, fertility and mean number of the impregnated rats of group 3 were lower but the differences were not significant. These findings suggest that absence of spermatogenetic material in USCT reduces contralateral blood flow and fertility potential. Therefore, contralateral testicular damage originating from blood flow alterations rather than autoimmune mechanism should be considered to explain fertility problems encountered following USCT.

Keywords Spermatic cord torsion · Contralateral damage · Testicular blood flow · Fertility

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Introduction

To date, many experimental and clinical studies have questioned the effects of unilateral spermatic cord torsion (USCT) on ipsilateral and contralateral testes [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]. Albeit some studies oppose bilateral effects of spermatic cord torsion [2, 8], its contralateral effects have been the focus of major attention because such a damage carries a high risk for infertility. Ipsilateral orchiectomy is recommended to prevent contralateral damage and infertility if a prolonged period has elapsed after USCT [11].

Recent studies performed by Tanyel et al. [3, 6] showed a reduction in contralateral testicular blood flow during USCT. In another theory for contralateral testicular injury, Williamson and Thomas [12] proposed that spermatogenetic cells enter the circulation after impairment of blood-testis barrier and cause autoimmunization through antisperm antibodies. In this autoimmune phenomenon, presence of a testis including spermatogenetic material is essential. On the other hand, in their histopathological evaluation, Karagüzel et al. [13] reported that the presence of ipsilateral testis is not necessary for the occurrence of contralateral testicular injury during USCT. However, the effects of USCT on contralateral testicular blood flow and fertility potential in the absence of spermatogenetic material are not clear. The present study has been planned to enlighten these unclear points on an experimental basis.

Materials and methods

The study was approved by the animal ethics committee of our university.

Thirty-four male and 68 female adult albino rats were used for the study. The animals were randomly allocated into three groups:

1. Group 1 (n = 13). Rats underwent a control operation and testicular blood flows were measured 24 h after the operation.
2. Group 2 (n = 10). Rats were subjected to two operations: I) a subepididymal orchiectomy and II) a control operation that was performed 4 weeks after subepididymal orchiectomy. Testicular blood flows were measured 24 h after the control operation.
3. Group 3 (n = 11). Each rat underwent two operations: I) subepididymal orchiectomy and II) USCT 4 weeks after subepididymal orchiectomy. Testicular blood flows were measured 24 h later following USCT.

Surgical procedures

All surgical procedures were performed under ether anesthesia employing a sterile technique and standard left side (ipsilateral) ilioinguinal incision. In our experiment, the rats were subjected to one or two of three kinds of operations:

1. *Control operations* were performed on groups 1 and 2 rats. In group 1, the operation included the exposure and fixation of the testis. The fixation was carried out to restrain the testis in its normal position by using a 4/0 atraumatic silk suture passing through tunica albuginea and the Dartos fascia of the scrotum. In group 2, the control operation included the exposure and fixation of epididymis in its normal position. Fixation suture passed through corpus epididymis was fixed to the Dartos fascia in these rats. In both groups, incisions were closed as single layer running sutures (5/0, atraumatic, silk).
2. *Subepididymal orchiectomy* was performed in the same manner on groups 2 and 3 rats. Briefly, after exposing testis and epididymis, epididymo-testicular attachments were cut by sharp dissection (Fig. 1). Then, spermatic vessels and caput epididymis were ligated closely to the testis and divided. Thus, epididymis was kept in place after this procedure and the incision was closed as defined in the control operation.

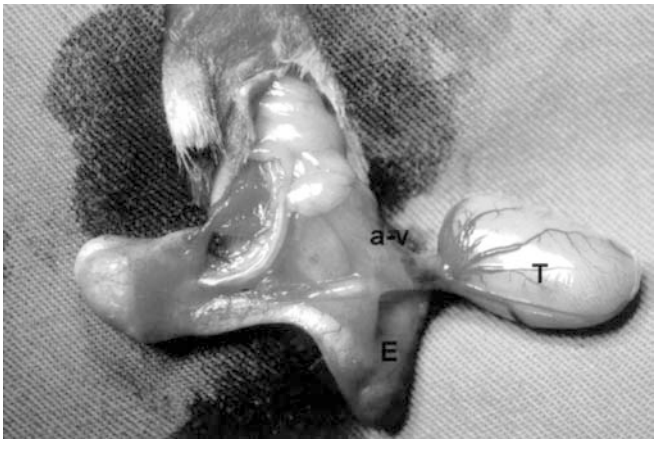

Fig. 1 Intra-operative photograph shows an isolated rat testis which has been divided from its epididymal attachments to perform subepididymal orchiectomy. Vascular pedicle has not yet been divided. *T* testis, *E* epididymis, *a-v* spermatic artery and vein

3. *Spermatic cord torsion* was performed on group 3 rats. The epididymis was found by entering the ilioinguinal region via the previous incision. The epididymis and proximal part of the spermatic cord were dissected from surrounding tissues. Then, the torsion was created by rotating both left epididymis and spermatic cord in a 720-deg clockwise direction. The twisted epididymis was fixed to the Dartos fascia with a 4/0 silk suture. Incision was closed similarly to those of other groups.

Measurement of testicular blood flow

In all rats, testicular blood flows were measured by ^{133}Xe clearance technique using a gamma camera (Toshiba GCA 602 A). Under ether anesthesia, the rats were placed on the gamma camera and contralateral testes were pulled into scrotum. Then, 100–200 mCi ^{133}Xe in a volume of 0.1 ml 0.9% NaCl solution was injected into the testis. Sequential 20-sec frames were collected for 20 min. The testicular washout curves were obtained by drawing regions of interest. The half-time of the exponential curve was calculated and testicular blood flow was estimated using the equation below (partition coefficient [λ] was generally around 0.85).

$$\text{Flow}(\text{ml}/100g/\text{min}) = 0.693 \times \lambda \times 100/\text{T}_{1/2} \quad (1)$$

Fertility

After first operations, all rats were kept in an animal facility for a 4-week period. The aim was to wait for spontaneous resorption of intraluminal spermatogenetic material in groups 2 and 3 animals but the same procedure was also applied to group 1 rats to maintain identical conditions between the groups. Then, all rats were individually housed with two female rats known to be fertile. If a male rat could not induce pregnancy in either of the two female rats during the mating period (maximum 2 weeks), the rat was considered infertile. If pregnancy had occurred during the mating period, the rats were observed until delivery. The number of female rats impregnated by each male rat and the number of rats delivered by each female rat were recorded. Fecundity was defined as total number of the rats sired by each male rat.

Statistical analysis

All measurements were expressed as mean ± SD. Data were statistically evaluated using SPSS computer software. For constant variables, multiple comparisons among groups were performed with one-way analysis of variance, which was followed by Scheffe post hoc test. Fertility values were compared with Kruskal Wallis test. A p value of less than 0.05 was considered to be statistically significant.

Results

Data pertaining to contralateral blood flows and fertility potential are summarized below.

Testicular blood flows

Mean testicular blood flows of groups 1, 2 and 3 were 9.1 ± 3.9 ml/100 g/min, 14.5 ± 2.1 ml/100 g/min and 4.9 ± 1.1 ml/100 g/min, respectively (Fig. 2). These values reflect significant differences among the groups. In such way, mean testicular blood flow of group 3 was

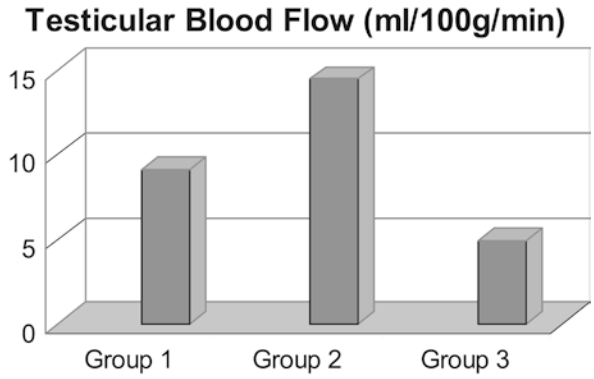

Fig. 2 This figure demonstrates our data related to mean contralateral testicular blood flow. There are significant differences among groups 1 and 2 ($p < 0.001$), groups 1 and 3 ($p < 0.01$), and groups 2 and 3 ($p < 0.001$)

Table 1 Three parameters related to fertility potentials of the rats. While there was no significant difference among groups in fertility and mean number of impregnated rats, the mean number of sired rats of group 3 was significantly lower than that of the other two groups

Groups	Fertility (Fertile/total)	Sired rats (Mean ± sd)	Impregnated rats (Mean ± sd)
Group I	13/13	13.4 ± 5.4	1.6 ± 0.5
Group II†	9/9	12.9 ± 4.3	1.5 ± 0.5
Group III	7/11	6.2 ± 5.7*	0.9 ± 0.8

*$p < 0.05$ vs. groups 2 and 3
†One rat died during mating period

significantly lower than those of group 1 ($p < 0.01$) and group 2 ($p < 0.001$). In addition, group 2 has a significantly higher testicular blood flow than group 1 ($p < 0.001$).

Fertility

All male rats in groups 1 and 2 were fertile while four male rats were infertile in group 3 (Table 1). However, the differences were not statistically significant ($p > 0.05$). Similarly, the number of impregnated female rats in group 3 was lower than in other groups but the difference between the groups was also not significant ($p > 0.05$).

Fecundity of group 3 was significantly lower than in group 1 ($p < 0.01$) and group 2 ($p < 0.05$), whereas there was no significant difference between groups 1 and 2 ($p > 0.05$).

Discussion

Current studies emphasize the role of germ cell apoptosis in testicular damage encountered following spermatic cord torsion, whatever its triggering mechanism [14, 15, 16]. Despite these sophisticated investigations, USCT-related bilateral testicular damage is still a controversial issue. However, it is obvious that there are many infertile men with a history of USCT [1, 17]. Thus, an event causing permanent contralateral testicular damage must be present. The exact mechanism of contralateral testicular damage after USCT has not yet been highlighted. There are several hypotheses on this subject, including an immunlogical phenomenon, release of acrosomal enzymes, blood flow alterations, underlying congenital testicular defect and subclinical attacks of contralateral testicular torsion [3, 5]. Although there is some evidence in favor of diminished blood flow, the hypothesis that includes an autoimmune response through impaired blood-testis barrier in the presence of spermatogenetic material is still popular [18, 19, 20]. Our study tested these two important hypotheses in a model based on the elimination of spermatogenetic material in ipsilateral side prior to USCT. This model was developed by Karagüzel et al. in 1994 [13] and was based on the information that spermatozoa could be maintained in a viable state only for several weeks within the epididymis [21]. The model has offered us to evaluate the two main effects of USCT in the absence of ipsilateral spermatogenetic material, namely testicular blood flow (a physiopathological parameter) and fertility potential (a clinical parameter).

Different techniques, including the use of doppler ultrasonography, electromagnetic flowmeters, hypoxic parameters and gamma camera, have been studied for the measurement of blood flow alterations during USCT [3, 8, 9, 22]. Because measuring the blood flow scintigraphically using ^{133}Xe clearance technique has several advantages over other techniques, we selected this technique [9]. Kızılcan et al. [23] measured testicular blood flow with systemic injection of ^{133}Xe during USCT and they found a significant decrease in contralateral blood flow during early period. In another study in which testicular microcirculation was examined using a videomicroscope and spermatic artery flow was measured with an ultrasonic flow probe, Kolettis et al. [24] found that USCT altered microcirculation and decreased total blood flow to the opposite testis. However, Lievano et al. [25] showed that contralateral testicular blood flow was unaffected following 8 h of USCT in a piglet model in which testicular blood flow was measured by using radiolabeled microspheres. Our findings showed that the rats which underwent 24 h of USCT 4 weeks after subepididymal orchiectomy had significantly lower blood flows than the rats that underwent control operation or subepididymal orchiectomy. This finding indirectly supports the idea that spermatogenetic material is not necessary for diminished contralateral blood flow. A further, also interesting finding was that excision of one-side testis without torsion caused a significant increase in contralateral testicular blood flow. Such a response may be due to a compensatory mechanism.

With respect to testicular function, clinical and experimental data suggest a deteriorated sperm pro-

duction in a substantial part of the patients affected from USCT. To determine the effects of testicular malfunction in animal studies, some biological parameters have been recommended. The best known is fertility; others are fecundity and the number of impregnated female rats by a male rat [5, 26]. There are other studies showing histopathological damage in contralateral testis after 24 h of torsion but none of the studies establishes a direct correlation between testicular blood flow and fertility potential [10, 13, 18]. In our study, fecundity of the rats subjected to USCT after subepidiymal orchiectomy was significantly lower than those of the rats undergoing control operation or subepididymal orchiectomy only. The low fecundity values of these rats showed parallelism to the decreased testicular blood flow. Furthermore, USCT without spermatogenetic material caused an additional decrease in fertility and mean number of impregnated rats. Although the decrease was not statistically significant, it was also a parallel finding to testicular blood flow alteration.

The deduction from the above-mentioned arguments is that fertility potential is at risk even in the absence of spermatogenetic material in USCT. For this reason, it seems that an autoimmune mechanism does not play a role in USCT-related fertility disorders. As a more specific finding which may explain mechanism of USCT-related fertility disorders, we showed that diminished contralateral testicular blood flow was associated with low fertility potential. However, further studies should be conducted to find the other potential factors because blood flow alteration has not caused complete infertility.

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