Varicocele in the rat: a new experimental model

Effect on histology, ultrastructure and temperature of the testis and the epididymis

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Summary. With no consistent animal prototype for the study of varicocele, we set out to create a model in the rat by complete ligation of the main branch of left spermatic vein (MBSV) or by partial ligation of the left renal vein. Three months later, the histology, ultrastructure and temperature of the testis and epididymis were studied. Microscopically, spermatogenic arrest was the most frequent anomaly seen. The most frequently noted ultrastructural change of the testis was distension of smooth endoplasmic reticula in Sertoli cells. The microvilli of columnar epithelia in epididymis were sparse and showed local defects. Lesions and increased temperatures in the testis and epididymis induced by the ligation of the left MBSV were similar to those seen in partial ligation of the left renal veins, with no significant differences between left and right. Significant differences were found, however, on comparison with the controls.

Key words: Varicocele - Rat, Histology - Ultrastructure - Temperature - Infertility

It is well known that varicocele is implicated in male infertility, but the exact mechanism by which the varicocele gives rise to the infertility is still an enigma [8]. Since the mid-1970s, experimental study of this subject has intensified, and many experimental models have been created in the dog, monkey, and rat [3]. However, the studies in these models have been inconsistent. Thus, it is necessary to continue to search for an ideal model for the study of varicocele.

Anatomical studies on the testicular vein system of the rat have shown that the pampiniform plexus drains up the internal inguinal ring into two efferent veins, a thick and a thin one [10, 13]. The thick efferent vein has been named the main branch of the spermatic vein (MBSV), and it leads into the common iliac vein or into the caudal end of the inferior vena cava. The thin one is named the testicular vein. On the left, 95% of the testicular veins went into the renal vein, and 5% into the inferior vena cava, while on the right, 10% of the testicular veins went into the renal vein, and 90% into the inferior vena cava. Thus, on the basis of the results of these anatomical studies, we used ligation of the left MBSV to create the varicocele, and compared it with the status following partial ligation of the left renal vein with reference to the testicular histology and ultrastructure and to the temperature of both the testis and the epididymis of the rat.

Materials and methods

Seventy-eight adult male Spraque-Dawley rats weighing 250-350 g were subdivided randomly into three groups as classified below.

Group I

The animals were anesthetized with sodium pentobarbital 40 mg/kg i.p. Through a left paramedian incision, the left renal vein was partially ligated to give an external diameter of 0.85 mm. A consistent stenosis was achieved by using a 3-0 silk suture which was tied around both the renal vein and a metal probe. The probe was carefully removed and the vein was allowed to expand against the loop of the suture. The suture was positioned at the junction of the renal vein and the inferior vena cava. The incision was closed, and the animals were returned to the vivarium.

Group II

In this group of animals the same procedure as above was carried out, the only difference being that the left MBSV was completely ligated under the operating microscope without partial ligation of the left renal vein.

Group III

This group served as controls, each rat undergoing a “sham” operation.

Three months later, the animals in groups I, II, and III (10 rats each group) were anesthetized again. Biopsies were obtained from
the center of each testis and from the tail of each epididymis. These specimens were immediately fixed by immersion in 4% gluteraldehyde in 0.2 M phosphate buffer, then post-fixed in 1% osmium tetroxide. Next, they were dehydrated, first in ethanol and then in acetone, and finally embedded in Epon 812-Araldite. Thin sections were taken and double-stained with uranyl acetate and lead citrate, then viewed with H-600 transmission electron microscopy (Tokyo, Japan). The remains of the testis and epididymides were completely removed, fixed in Bouin's liquid and embedded in paraffin. Two sections were taken from each testis and each epididymis for viewing with light microscopy. One specimen was stained with hematoxylin and eosin, while the other was stained with periodic acid-Schiff. In each section of the right and left testes, 50 tubules were randomly selected, for examination and a record kept as to whether the tubules were normal or abnormal in configuration. The square roots of the percentages of abnormal tubules were then subjected to arc-sine transformation prior to statistical evaluation by *t*, *t'* and *q* tests.

The animals in groups I1, II and III (8 rats per group) were positioned in the supine position under anesthesia. The temperatures of the testes and epididymides (both right and left) were measured by puncturing the centers of the testes and the tails of the epididymides with a 23 gauge microprobe (CDT-I, Chengdu, China). The temperature in the laboratory was 23 ± 0.5°C and the rectal temperatures of the rats were controlled at 37 ± 0.1°C. Statistical analyses involved analysis of variance and Dunnett's test.

Results

Histology and ultrastructure

In groups I1 and II, all the rats with left testicular vein dilatation had abnormal histology bilaterally except two of the rats in group I1 (one showed no alteration, the other showed only a slight lesion of the left testis, both without testicular vein dilatation). Slight abnormalities were also noted in the tubules near the tunica albuginea in group III.

Lesions of the testes observed by light microscopy showed that both impaired and normal tubules intermingled and impaired tubules presented "patchy" alteration among normal tubules. The predominant lesion was that of spermatogenic arrest at the spermatid and preliminary spermatocytic phases and the next frequent lesion was that of premature sloughing of spermatogenic cells (spermatids and spermatocytes) into the lumina of the tubules. Some rats showed minimal atrophy of the lumen and a decreased number of cells in the lumen. Most rats had significant atrophy of the lumen, disorganization of the germinal epithelia, and sloughing of spermatocytes and/or spermatids into the lumen. In the more severe lesions no cells were noted in the lumina. The Leydig cells showed vacuolar degeneration (Figs. 1, 2). The tubular wall was normal in every case. No significant histological alterations in the epididymides were observed by light microscopy.

The semi-quantitative data of abnormal tubules are shown in Table 1. There were no significant differences between left and right testes in any group, and no significant difference was found between groups I1 and II1 (Ps > 0.05). It is obvious that there are many more abnormal tubules of the testes in group I1 and group II1 than in group III1 (Ps < 0.01).

Transmission electron microscopy showed that the most frequent change was distension or vacuolization of the smooth endoplasmic reticula in Sertoli cells, even when there was no spermatogenetic disorder (Fig. 3). The extension of vacuolization could cause breaks in the plasma membrane, resulting in the release of immature germ cells, whereas the junctions between Sertoli cells remained intact. The nuclei vacuoles of early spermatids often occurred, and there were particularly large vacuoles

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Left</th>
<th>Right</th>
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</thead>
<tbody>
<tr>
<td>I1</td>
<td>10</td>
<td>52.24 ± 13.25*</td>
<td>53.35 ± 12.24*</td>
</tr>
<tr>
<td>II1</td>
<td>10</td>
<td>56.01 ± 13.86*</td>
<td>51.93 ± 9.02*</td>
</tr>
<tr>
<td>III1</td>
<td>10</td>
<td>27.71 ± 3.45</td>
<td>27.23 ± 4.06</td>
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* *P* < 0.01 vs III1.