AUTOIMMUNE NATURE OF THE DISTURBANCE OF SPERMATOGENESIS IN RATS WITH EXPERIMENTAL VARICOCELE

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UDC 616.147.22-007.64-092.9-06:616.69-008.8-092:616-008.9-097.3

Measured disturbance of the venous outflow from the left testis in rats causes the development of destructive changes in the spermatogenic epithelium both in the testis on the side of the operation and also in the contralateral organ. Disturbances of spermatogenesis in rat testes (foci of desquamation of spermatogenic epithelium, disorganization and degeneration of the sex cells, emptying of the seminiferous tubules) are similar to those in men with varicocele, so that the results of such experiments can be regarded as an experimental model of varicocele. In the experimental rats the permeability and fine structure of the blood–testis barrier were disturbed in both testes, the pattern of the morphological changes was similar with that observed during the development of autoimmune orchitis, and lymphocytes sensitized to spermatozoal antigens were found in the lymphoid organs. Taken as a whole, the results suggest involvement of immunologic mechanisms in the development of the pathological changes in the testes in varicocele.

KEY WORDS: varicocele; spermatogenesis; autoimmune responses; blood–testis barrier.

Men with unilateral dilatation of the veins of the spermatic cord develop oligospermia and sterility [7, 10]. Rabokh et al. [3], Nekhvyadovich [2], Charny [6], and Lipshultz and Corriere [11] found different degrees of disturbance of spermatogenesis, up to diffuse aspermatogenesis, in the testes on the side of a varicocele. In later publications [12] pathological changes were described in the spermatogenic epithelium in the intact, contralateral testis in patients with varicocele.

Most workers [2, 3, 9] consider that the disturbance of spermatogenesis is caused by vascular disturbances which lead to hypoxia of the testis and elevation of its temperature, and also by the retrograde flow of blood from the renal vein into the internal testicular vein. However, appropriate investigations have yielded inconsistent results and the causes of the disturbance of spermatogenesis in varicocele still remain unexplained [8].

Meanwhile studies of orchitis of varied etiology (for details, see [4]) suggest the participation of immunologic mechanisms in the development of the bilateral disturbances of spermatogenesis in varicocele.

The object of the present investigation was to develop an experimental model of varicocele in rats and to study the role of immunologic factors in the disturbance of spermatogenesis following interference with the venous outflow.

EXPERIMENTAL METHOD

Experiments were carried out on 41 sexually mature male Wistar rats weighing 200-300 g. Under aseptic conditions and under ether anesthesia a ligature was applied to the pampiniform plexus of 31 rats near the anterior pole of the left testis, on a catheter 1 mm in diameter.

Laboratory of Human Embryonic Histogenesis, Institute of Human Morphology, Academy of Medical Sciences of the USSR. Department of Pediatric Surgery, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 1, pp. 44-48, January, 1979. Original article submitted May 16, 1978.

 0007-4888/79/8701-0052$07.50

TABLE 1. Effect of Measured Disturbance of Venous Outflow from Left Testis on Weight of Testes, Spermatogenesis, and Sensitization of Lymphocytes to Spermatozoal Antigens

Character of procedure	Left testis			Right testis			% of rosette-forming cells		
	weight, ‰ of body weight	% of seminiferous tubules with undisturbed spermatogenesis	diameter of seminiferous tubules, μ	weight, ‰ of body weight	% of seminiferous tubules with undisturbed spermatogenesis	diameter of seminiferous tubules, μ	in spleen	in thymus	in lymph node
Control (mock operation)	4,87 (4,5—5,5) n=10	92 (88—97) n=19	235,3 (200,9—266) n=10	4,80 (4,5—5,8) n=10	91 (87—93) n=10	234,7 (219—256) n=10	0 n=10	0 n=10	0 n=10
Application of ligature to pampiniform plexus:									
2—5 days	5,5 (4,9—5,9) n=7	73,4 (66—81) n=7	207 (201—219) n=7	5 (4,7—5,6) n=7	84,4 (75—97) n=7	227,6 (218,3—250,8) n=7	—	—	—
1 week	4,9 (4,4—5,9) n=3	69 (57—76) n=3	198,1 (190,4—202) n=3	5 (4,8—5,1) n=3	71 (62—84) n=3	216,4 (202,4—227) n=3	—	—	—
2 weeks	6,3 (5,6—7,0) n=2	88 (88—88) n=2	189 (135—243) n=2	6,4 (5,8—7) n=2	77 (64—90) n=2	219 (218—220) n=2	4 n=1	1 n=1	1 n=1
3 "	4,3 (2,2—6,5) n=6	30,5 (0—59) n=6	180 (156—220) n=6	5,4 (3,1—6,5) n=6	52 (40—58) n=6	208 (190—220) n=6	1,5 (1—2) n=3	2,7 (1,5—3) n=3	0,7 (0,5—1) n=3
4 "	3,25 (2,3—5,1) n=5	11,2 (0—24) n=5	150,7 (123,5—182) n=5	5,4 (4,5—5,9) n=5	49,6 (28—82) n=5	206,2 (198,9—222) n=5	1 (0,5—1,5) n=3	1,5 (1—2) n=3	0,5 (0,5—0,5) n=3
8 "	3,04 (2,4—5,7) n=3	36 (9—57) n=3	159,2 (143—183) n=3	5,37 (4,7—6,3) n=3	69 (43—94) n=3	194 (190—199) n=3	—	—	—
10 "	2,4 (1,8—3,4) n=5	14 (0—70) n=5	126 (110—142) n=5	4,5 (3,9—5,5) n=5	69 (61—85) n=5	176,3 (156—199) n=5	2,5 (2—3,5) n=3	1 (0,1—1,5) n=3	0 n=3

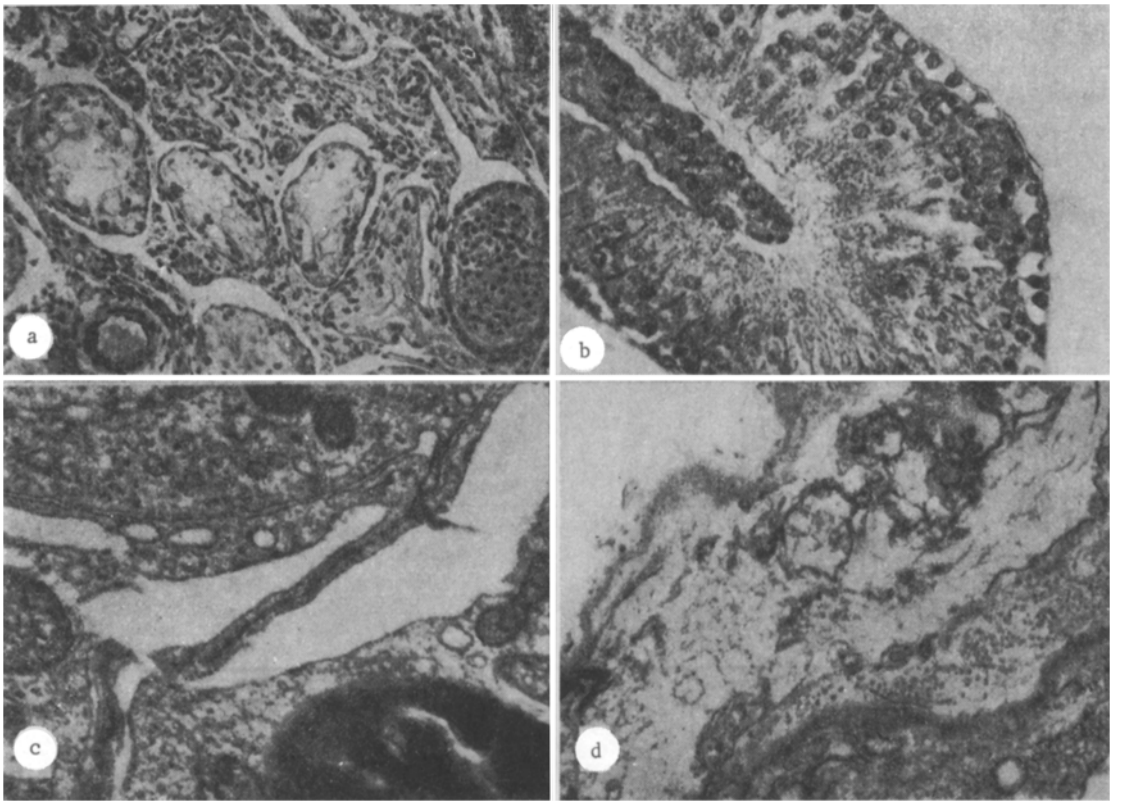

Fig. 1. Testes of rats at various times after measured disturbances of venous outflow from left testis: a) diffuse aspermatogenesis, infiltration of interstitial tissue by lymphocytes in left testis of rat 10 weeks after operation; b) desquamation of sex cells and vacuolation of cytoplasm of Sertoli cells in right testis of rat 2 weeks after operation; c) ultrastructure of contacts between Sertoli cells, parallel arrangement of plasmalemmas, dilatation of cisterns of endoplasmic reticulum in left testis of rat 3 weeks after operation; d) ultrastructure of tunica propria of convoluted tubules, myoid cells, and plasma membranes of acellular layers destroyed, collagen fibers haphazardly arranged and increased in number in left testis of rat eight weeks after operation. a, b) Hematoxylin-eosin. Magnification: a) 200×; b) 400×; c) 15,000×; d) 17,000×.

After the ligature had been applied the catheter was removed. A mock operation was performed on 10 control animals. The animals were killed 2-5 days and 1, 2, 3, 4, 8, and 10 weeks after the operation, two to seven rats at each time. The weight of the rats and of their testes was determined. For light microscopy the testes were fixed in Carnoy's fluid; paraffin sections 5 μ thick were stained with hematoxylin-eosin, methyl greenpyronine, and with fuchselin by Weigert's method and counterstained by Van Gieson's method. Sections of the seminiferous tubules for electron microscopy were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) and then postfixed with 1% OsO_4 in the same buffer, dehydrated, and embedded in a mixture of Epon and Araldite. Sections were cut on the LKB Ultratome, stained with uranyl acetate and lead citrate, and examined in the Tesla BS-516 electron microscope.

Permeability of the blood—testis barrier (BTB) for endogenous globulins and rivanol was investigated by Davydova's method [1]. Sensitization of lymphocytes from the spleen, thymus, and lymph nodes of the rats to antigens of allogeneic spermatozoa was determined by the rosette-formation test [5].

EXPERIMENTAL RESULTS

The weight of the left testes of the rats of the experimental group 2-5 days after the operation was increased compared with the right, and the tunica albuginea was stretched. In most convoluted tubules active spermatogenesis was found with edema of the interstitial tissue. Marked congestion of veins of different sizes and spasm of arterioles and arteries of small and medium caliber also were observed.

Foci of destructive changes in the spermatogenic epithelium in the testis on the side of operation were first found one week after the beginning of the experiment. Dying and desquamated sex cells and disorganization of the layers of the spermatogenic epithelium were found in the convoluted tubules. A decrease in the weight of the testes on the side of application of the ligature, accompanied by progression of the pathological changes in the spermatogenic epithelium and also by a reduction in the number of tubules with active spermatogenesis, were observed three weeks and more after the operation (Table 1). Destructive changes also were found at the points of contact between the Sertoli cells: a parallel arrangement of the plasmalemmas and widening of the cisterns of the endoplasmic reticulum (Fig. 1c). The diameter of the convoluted tubules was considerably reduced (Table 1). Inside most tubules only those Sertoli cells and spermatogonia which were close to the walls still remained. In some convoluted tubules all the contents had undergone coagulation necrosis. Venous congestion was replaced by emptying of the veins and obliteration of some of the arterioles and small arteries. The muscular wall of these arteries was atrophied, karyolysis was observed in the muscle cells, and the intima of the vessels filled their lumen with horseshoe-shaped evaginations. Similar changes in the vessels were found at later stages of the investigation and were evidence of the development of ischemia of the testis and reduction of the blood supply to the organ.

The number of convoluted tubules with active spermatogenesis in the left testis four weeks after the operation was reduced on average to 11.2%. Various disturbances of spermatogenesis (desquamation, disorganization, death of the sex cells leading to emptying of the tubules) were found in the remaining tubules.

In some animals 8 and 10 weeks after the operation diffuse aspermatogenesis developed in the testes with a disturbed venous outflow. The pattern of the morphological changes in this case was similar to that which develops during the induction of autoimmune orchitis [11]. In the blood vessels the leukocytes and lymphocytes were distributed around the periphery and migrated into the interstitial tissue. Cells of the spermatogenic epithelium either were absent or showed signs of degeneration (karyopycnosis, karyolysis, vacuolation of the cytoplasm (Fig. 1a). Myoid cells and plasma membranes of the acellular layers of the tunica propria of seminiferous tubules were destroyed (Fig. 1d).

In the contralateral testis dilatation and congestion of the arterioles and small arteries and edema of the interstitial tissue were found 2-5 days after the operation. Slight disturbances of spermatogenesis were observed for the first time one week after the beginning of the experiment. They were manifested as desquamation of the sex cells, death of individual cells, and the formation of empty spaces in various layers of the spermatogenic epithelium (Fig. 1b). Destructive changes also were observed in the tunica propria of the convoluted tubules: edema of the myoid cells, translucency of they cytoplasmic matrix, and folding of their basement membranes.

Similar changes were found in the right testes at all subsequent times of investigation, but 10 days after the operation active spermatogenesis was still preserved in 61-85% of the convoluted tubules. Two weeks and more after the operation, a juxtamural arrangement of the leukocytes and lymphocytes and their migration into the interstitial tissue were found in the blood vessels of these testes.

In the rats of the experimental group a persistent increase in permeability of the BTB to both indicators was first found two weeks after the operation in the left testis and three weeks after the operation in certain tubules of the right testis, and they persisted at subsequent times of investigation. Starting with the second week and at later times of investigation lymphocytes forming rosettes with allogeneic spermatozoa began to appear in varied numbers in the lymphoid organs (Table 1).

Consequently, after unilateral disturbance of the venous outflow from the testis destructive changes in the spermatogenic epithelium developed in both organs (although their intensity differed). In the testis on the side of the disturbed venous outflow a variety of forms of pathological changes was found in the spermatogenic epithelium: from desquamation and disorganization of the cells in some tubules to death of the whole contents in other and the development of diffuse aspermatogenesis. In the contralateral testis the disturbances of spermatogenesis were focal in character and consisted of desquamation and disorganization of the cells. These changes in both testes of the experimental rats correspond to the picture of pathological changes in the parenchyma of both testes in men with unilateral varicocele [6]. On the basis

of this fact the operation of measured disturbance of the venous outflow from the testis in rats can be regarded as an experimental model of varicocele.

Disturbance of the permeability and fine structure of the BTB in both testes of the experimental rats, morphological changes similar to thos during the development of autoimmune orchitis, and the presence of lymphocytes sensitized to spermatozoal antigens in the lymphoid organs all suggest that immunologic mechanisms are involved in the development of the pathological changes in the testes in varicocele.

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