Blood Flow in the Testes and Epididymides of Male Rats with Experimental Blockade of Testicular Veins

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Blood flow in reproductive organs was studied during experimental cessation of blood outflow in the testicular vein. This pathological state was accompanied by disturbances of the complex pathogenesis. The major component was autoregulatory focal ischemia, which resulted from the venoarterial response to venous hypertension.

Key Words: *testis; epididymis; macrocirculation; microcirculation; venoarterial reaction*

In recent years, peripheral circulatory disturbances are considered as a major pathogenetic stage of various somatic diseases. It also concerns andrology. However, little is known about diseases due to circulatory disorders in the gonads (varicocele, vein ligation and excision for varicocele, hydrocele, *etc*.) [2,3]. Varicocele results in the appearance of heterogeneous clinical signs for disturbances in scrotal blood flow. It is impossible to evaluate changes in blood flow in scrotal organs during subfertility. Our work was designed to perform an experimental study of this problem.

MATERIALS AND METHODS

Anatomical features of blood supply to the testes in 20 albino rats were evaluated to develop an adequate model for local circulatory disturbances in reproductive organs. Corrosive preparations of vessels were obtained after filling of the blood system with latex.

The specific feature of venous outflow from rat testes was termination of the right and left testicular veins (TV) in the inferior vena cava or common iliac veins (n=19). Experimental occlusion of venous outflow from the testes was produced by TV ligation at the site of drainage into the inferior vena cava or common iliac veins. The arterial vessels were intact. Venous outflow from the testis was partially preserved in the vein of the seminal duct (SD) and external TV.

Surgery was performed on ether-anesthetized rats under aseptic conditions. The anterior abdominal wall was opened by the midline in the lower third of the abdomen. The left internal TV was completely ligated at the site of drainage into the inferior vena cava or common iliac vein to produce unilateral block in venous outflow from the testes. Ligation was immediately followed by swelling of veins in the vascular cone and superior pole of the testis. The surface of the testis became strained and cyanotic. Surgical wound of the abdominal wall was sutured layer-by-layer. Sutures were removed after 3-4 days.

The animals were divided into 3 groups. Unilateral block of venous outflow from the testis was induced in group 1 rats (n=75). Sham operation in group 2 rats (n=85) suggested similar manipulations without ligation. Group 3 consisted of 49 intact animals. Group 1 and 2 rats were euthanized under ether anesthesia on days 1, 7, 14, 30, 60, 90, and 120 after surgery.

The state of the vascular bed was evaluated by light and electron microscopy of fixed samples

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from testicular tissue. At each stage of the study, the testes and epididymides were isolated from 5 animals immediately after euthanasia. They were fixed in Bouin's fluid, dehydrated in ascending alcohols, and embedded into paraffin. Deparaffinized sections (5 μ) were stained by a modified Papanicolaou method and with hematoxylin and eosin.

Experimental and clinical samples were studies using a morphometric method. We evaluated the number of arteries, veins, and capillaries in the field of view, the distance between capillaries, and the diameter and lumen of reductive and volume vessels. Experimental vital study of blood flow in the testes and epididymides was performed under a luminescence microscope equipped with filters FS-1 and ZhS-18, contact objectives (×10, ×20, ×53) [1], and television camera connected to a phototelevision device.

RESULTS

Ligation of the internal testicular vein was followed by dilation of veins in the pampiniform plexus, increase in sinuosity of veins, and cyanosis of the gonadal membrane. Venous dilation in the testicular membrane was observed after 1 day. Cyanosis of the surface was more pronounced and spread to the head of the epididymis in this period. The degree and area of macroscopic signs for venous stasis progressively increased over the first 2 weeks of the study. Veins of the epididymis and SD became sinuous and dilated. The sharpness of contours in the straight and subcapsular testicular arteries decreased. The color of these arteries changed from rich red to pale rose. Dilation and swelling of arteries in the small pelvis were revealed at later terms (day 30). These changes were found even in vessels of the paired organ. The size of the pampiniform plexus in several animals returned to normal during this period. It was related to the formation of alternative pathways for venous blood outflow. Our results indicate that the early stage of venous stasis is characterized by the formation of collateral pathways for blood outflow through veins of the epididymis and SD and cremasteric vein (CV) to venous collectors in the pelvis. Reconstruction of the vascular bed and the formation of collateral venous pathways are required for the adaptation of blood flow to post-ligation conditions. Changes in macroarchitectonics of the venous bed with high plasticity are probably related to an increase in venous pressure (phlebohypertension). Intrasystemic and intersystemic anastomoses between vessels of the pampiniform plexus, epididymal veins, SD vein, and CV have an important role in venous drainage of the gonad during phlebohypertension resulting from varicocele. The period for the formation of anastomoses in our experiment was comparable to that observed during occlusion of veins having similar diameter and localized in the broad ligament of the uterus.

Microscopy showed that an increase in the volume of the extraorgan venous bed and the formation of venovenous anastomoses are accompanied by changes in the microcirculatory bed of the testes and epididymides. Contact television microscopy showed that the blood flow rate in capillaries and veins of the microcirculatory bed significantly decreases over the fist day after acute venous occlusion. Erythrocyte aggregation occurred in capillaries, postcapillary vessels, and venules. Aggregated erythrocytes and individual leukocytes closed the lumen of vessels at the site of branching. Hence, blood flow became jerky. Stasis was observed in several segments of the vascular bed. The density of capillaries and venous vessels increased. Venules with a diameter of 40-50 μ looked twisted. Diapedesis of erythrocytes and extravasates were seen along capillaries and venules. The arterial segment of the intraorgan vascular bed was characterized by polymorphism, but signs of spasm prevailed and included a decrease in the diameter of small vessels (<80 μ), increase in the degree of sinuosity, and appearance of dilations and constrictions along these vessels. These changes reflect spasm of small resistive vessels. The observed signs for angiospasm confirm the assumption that phlebohypertension caused by occlusion of the internal TV is associated with the venoarterial response in scrotal organs.

The vascular bed of the testis and epididymis tended to be polymorphic over the 1st week of the study. Capillaries formed networks of different density; their diameter varied from 6.4± 0.3 to 9-13 μ. The wall had irregular contours. Endotheliocyte nuclei were displaced and characterized by pyknosis. Zones with high density of the vascular bed mainly included functional capillaries. The count of plasma cells decreased. Postcapillary and collecting venules were curved and gained a corkscrew shape. The number of anastomoses between these vessels significantly increased. We found an increase in the number of arteriolovenular anastomoses with a diameter of 10-15 μ. Their lumen was filled with formed blood elements.

These data show that microcirculatory disturbances during the early stage of varicocele are manifested in spasm of the inflow compartment, dilation and sinuosity of the capillary-and-venular volume a3nd capacitive compartments, aggregation

in the lumen, and stasis. Blood circulation in anastomoses increased. We revealed a tendency toward the development of local changes in this period. These significant changes occurred in arterial vessels. By the end of day 3 we detected not only segments of microcirculation, but also zones with small number of vessels. They included spasmodic arteries, small number of primarily plasma capillaries, and sinuous and often anastomosing venules. Dilated arteriovenous anastomoses were often found at the boundary of these regions. Biomicroscopy demonstrated a significant decrease in the blood flow rate or stasis. The observed changes may be considered as transient compensation signs, which provide blood redistribution in collateral veins.

The local redistribution of blood flow progressively increased up to day 14 and was associated with the formation of new intervascular anastomoses in a direction from small to large venous vessels. Newly formed vessels (venovenous anastomoses) prevailed in this stage. Neovascularization is mediated by the following mechanism. Vasospasm results in the development of focal ischemia. Similarly to previous and concomitant venous stasis, this state is accompanied by hypoxia. Activation of leukocytes, platelets, and fibroblasts is related to focal circulatory ischemia in the scrotum during varicocele. Under these conditions activated cells produce fibroblast growth factors, which increase mitotic activity of the endothelium and smooth muscle cells. The conclusion about activation of these mechanisms for structural adaptation was derived from the data on muscular hypertrophy of the wall in an increasing number of veins. The arterial compartment of the microcirculatory bed did not undergo qualitative changes. The muscular wall was hypertrophic. The decrease in the diameter of arterioles (6.2±2.4 μ, $p<0.05$) in ischemic focuses was associated with the formation of a well-developed smooth muscle layer in the wall. The irregularity of vascular pattern increased in the follow-up period. However, many capillary anastomoses disappeared (decrease in the number of capillaries). We revealed a significant increase in the number of arteriovenous anastomoses with a diameter of 30±4 μ. Functional activity of these intervascular anastomoses during venous stasis reflects blood flow in great draining venous collectors of the microcirculatory bed. Blood flow in great vessels and the formation of anastomoses constitute the mechanism of adaptation to venous plethora. It should be emphasized that these signs form the structural basis for steal syndrome. This state has the following pathogenesis. Vasodilation in the ischemic focus induced by exogenous or endogenous stimulation (*e.g.*, hypoxia) causes a paradoxical response, which is manifested in an even greater decrease in blood supply to the damaged segment due to blood outflow in vessels of the intact segment with normal reactivity. The existence of this mechanism was confirmed in our study. Ischemic focuses are topographically similar to focuses of abnormal spermatogenesis and sites of morphological signs for interstitial edema and sclerosis. However, these signs were lower in segments with normal blood flow.

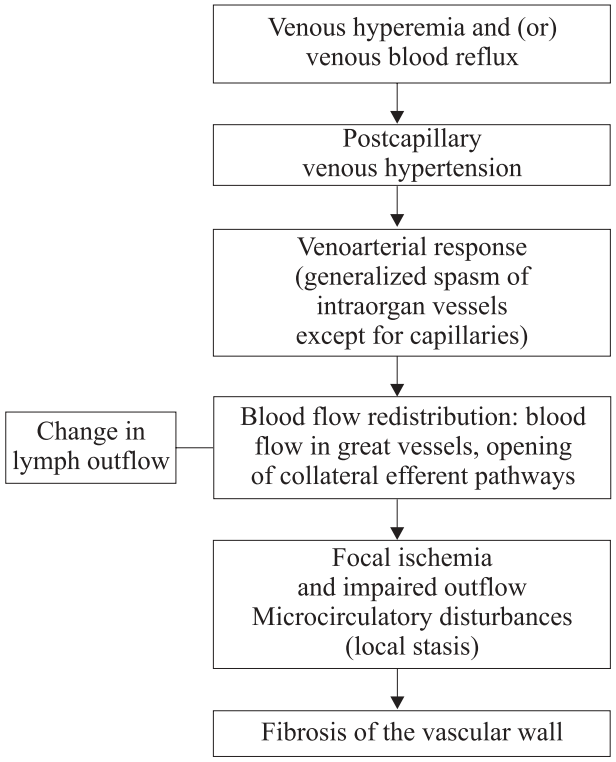

Fig. 1. Pathogenesis of circulatory disturbances in reproductive organs during varicocele.

Hence, local damage to spermatogenesis is mainly mediated by several mechanisms. Modular organization of the vascular bed in the testis contributes to this feature. Such vessels arise from the mediastinum testis and run along each convoluted tubule. Modular organization is not observed in the epididymis of the testis, which appears like a densely packed canaliculus. Local disturbances in vessels of this organ are accompanied by diffuse changes in the epithelium of the canaliculus and interstitium. Another cause of diffuse damage to the epididymis is that veins of this organ become involved in the system of intrasystemic and intersystemic anastomoses. These anastomoses impair endocrine and metabolic interactions between the epididymis and testis, which has a key role in regulation of epididymal function.

Local density of the vascular network progressively decreased on day 14 and in the follow-up period. These regions were mainly characterized by atrophy of capillaries and simplification of the capillary wall. Functioning capillaries became coiled. Their wall was sclerotized. Ultrastructural signs for capillary damage included vacuolization of the endotheliocyte cytoplasm, which formed protrusions in the lumen of capillaries. We also found irregular widening and fragmentation of the basal membrane, which lost continuity. Light microscopy revealed signs of progressive interstitial edema. Avascular zones were present. Local and total myoelastofibrosis, sclerosis, and atrophy were observed in the wall of arterial and venous vessels at the site of smooth muscle hypertrophy and hyperplasia. It should be emphasized that regions with preserved structure of the microcirculatory bed had normal characteristics, which were typical of the intact organ.

Signs of venous stasis were not revealed in the contralateral testis of male rats. However, signs for angiospastic ischemia of this testis (focal spasm of arterioles, increase in sinuosity of arterioles, and irregularity of the outer diameter) developed in the early period of the study. Hypertrophy of the muscular layer in afferent vessels and sclerosis were found in the late period. The number of ischemic focuses in this region was lower than in organs of the left scrotum. However, ischemic focuses was also irregularly distributed. The absence of signs for venous stasis in the right testis casts some doubt on the existence of venous anastomoses between the right and left testes that play a major role in the development of bilateral hypogonadism during varicocele. This is true at least for our model of internal TV occlusion.

In the present work we compared the results of experimental and clinical studies of blood flow in reproductive organs during varicose disorders. This pathological state is accompanied by disturbances of the complex pathogenesis. The major component is autoregulatory focal ischemia, which results from the venoarterial response to venous hypertension (Fig. 1).

Surgical treatment for varicocele suggests excision (ligation) of varicose veins, which results in blockade of blood outflow in great venous collectors. Postoperative abnormalities in the testes and epididymides are more severe than preoperative hemodynamic changes. Hence, surgical methods of vein ligation (vein excision) are etiopathogenetically unsuitable for the therapy of varicocele. Our data are consistent with the results of clinical studies performed in the andrology department over many years.

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