Research Article

Reorganization of Intraorganic Blood Vessels of the Bladder in Experimental Diabetes Mellitus

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Abstract

Objective: To give histologic, morphometric and ultrastructural characteristics of intraorganic hemovessels of the urinary bladder of rats at the stages of streptozotocin diabetes.

Material and methods. There were used 70 Wistar male rats; diabetes mellitus was modeled with streptozotocin (60 mg/kg of body weight); material was taken on 14, 28, 42, 56 and 70-th day of experiment; histological, morphometric and electron microscopic research was performed.

Results. The microscopic, morphometric and ultrastructural peculiarities of transformation of intraorganic blood vessels of rats' bladder during streptozotocin diabetes were detected.

Conclusions: 1) the bladder diabetic microangiopathy is nonspecific process, the specificity of which is determined by the degree of expressiveness of vascular disorders which are characterized by these: a) the change of arteriolar vascular tone manifesting itself initially by dilatation, then by decrease of the lumen, then by secondary expansion; b) reconstruction of hemocapillar basal membrane, which becomes thicker 3.22-fold by the end of the experiment, disorganized and lamellar; c) blood rheological disturbances expressed in sludges in particular venules on the 14th day of experiment, on the 28th – in most venules, on the 42nd – also in capillaries, on the 56-70-th generalized sludge syndrome of all bladder layers appears; 2) diabetic angiopathy is accompanied with swelling of different genesis: interstitial one increases till the 28th day of experiment, since the 42nd it decreases; since the 42nd day plasma percolation of perivascular connective tissue increases; swelling of endotheliocytes appears on the 28-42th day of diabetes.

Keywords

urinary bladder; intraorganic blood vessels; microcirculation; streptozotocin diabetes

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Problem statement and analysis of the latest research

Specific lesions, which include diabetic nephropathy and diabetic cystopathy (DTS) are identified among the damages of the urinary system in diabetes. DC is still called diabetic urinary bladder (UB) dysfunction in the clinic [33]. Life threatening diabetic nephropathy is investigated comprehensively. Instead, DC received much less attention, despite the fact that it reduces the quality of life of patients, complicates the course of diabetes and requires an expensive treatment. Despite a large number of publications on the etiology and pathogenesis of DC, this issue is still far from being resolved [10, 19, 20, 23, 24, 26, 30, 31, 33, 37, 41, 44, 46].

It should be noted that the results of most research on the study of DC are based on clinical, pathophysiological, biochemical, biomechanical, electromyographic and sonographic data. However, the morphological confirmation of these results is absent. It should be mentioned that we have found very few works in which the morphology of CM in diabetes mellitus would be studied in our Ukrainian and Russian-language medical and biological literature. In addition, research findings presented in English-language sources are ambiguous and often controversial. Taking into account that now in Ukraine and in the countries of Europe and North America, diabetes mellitus is not an indication for CM biopsy in patients, the only and rational direction of studying morphology of CM in DC is the use of experimental models of diabetes mellitus, as mentioned by a number of authors [25, 32, 36, 42]. Among the large number of experimental models of diabetes mellitus, alloxane and streptozotocin models are most commonly used in scientific research. The streptozotocin model is twice often used of these two [27]. The latter is due to the fact that the alloxane is poorly dosed, is unstable in the solution and has significant neurotoxic and nephrotoxic effects [12, 35].

Diabetic microangiopathy with various manifestations and degrees of severity always develops during diabetes; it has a significant effect on the development of functional damage and reorganization of the structural components of various tissues and organs [2, 3, 5, 6, 8, 11, 14, 15, 16, 17, 21, 22, 28, 29, 34, 43, 45].

Despite the fact that diabetic microangiopathy was studied by many authors, today there is a little knowledge about the
peculiarities of its development in the UB wall. Only the results of a few clinical research, which are not systematized, contradictory and morphologically unverified, are available [9, 18, 20, 40]. There is also no data on the morphometric changes of intrathoracic blood microcirculation of UB on the stages of experimental diabetes mellitus, in particular streptozotocin diabetes (SD).

**Purpose:** to give histological, morphometric and ultrastructural characteristics of intraorganic blood vessels of UB of rats at the stages of SD.

### 1. Materials and Methods

The experiment was carried out according to the order of the Ministry of Education and Science of Ukraine No. 249 of March 1, 2012 “Procedure for holding scientific institutions of experiments, experiments on animals”, in accordance with the basic bioethical norms of the Helsinki Declaration adopted by the General Assembly, by Convention of Europe Council on Human Rights and Biomedicine (1977), the relevant provisions of the WHO, the International Code of Medical Ethics (1983), the Directive 2010/63/EU of the European Parliament and of the Council of Europe on the protection of animals used for scientific purposes.

The study was performed on 70-y year-old male Wistar rats: 50-y undergo modelling of diabetes mellitus (10 for each term) with intraperitoneally administered streptozotocin (60 mg / kg body weight) dissolved in 0.1 M citrate buffer; 20-y were control rats (4 for each term), which were administered only citrate buffer. Observations were performed on 14, 28, 42, 56 and 70 days of the course of the DM. Histologic sections were stained with hematoxylin and eosin, and half-thins with methylene blue. An electron microscopic study was carried out in accordance with generally accepted recommendations. Determination of the diameter of the lumen and the thickness of the walls of the blood vessels of the suburothelial blood microcirculation (SUBM) of UB was performed in ImageJ v. 1.47 (NIH, USA, http://imagej.nih.gov/ij) [39] using the original method developed by us [7]. We used the methods of non-parametric statistics (Wilcoxon-Mann-Whitney test) that were perfomed in R v. 3.0 [38].

### 2. Results and Discussion

On the 14th day of development of diabetes, there is enlargement of the arteries, arterioles and capillaries of both muscle and mucous membranes of the UB of different degree. At the end of the 2nd week of the SD course other researchers also observed widening of the lumen of the intrarenal arteries and afferent glomerular arterioles [21], capillaries of the submandibular gland [34], intralobular arteries and sinusoidal capillaries of the liver [16], capillaries of the hypothalamus and pituitary gland [6]. Enlargement of the lumen of the veins is also different: one of them extends moderately, others have an internal diameter greater than in 3 times comparing to this one in control group. Enlargement of vessels is filled with plasma and blood elements, mainly red blood cells. The edema of some areas of loose connective tissue of the own plate of the mucous membrane and submucosal layer is marked. Single small "coin columns" of red blood cells, indicating the beginning of the formation of a sludge-syndrome can be seen in individual venules in this terms of experiment on semi-thin cuts of UB. Yes, in Fig. 1b among 4 venules, which fell into a slice, a small sludge is visible only in one. Sludges are not observed in arteries and arterioles.

Morphometry shows that, the diameter of the lumen of the arterioles of the suburothelial blood microcirculation (SUBM) net increases by 1.38 times (p<0.001), of hemocapillaries - by 1.30 times (p<0.001), and of venules – only in 1.12 times (p<0.01). At the same time, the thickness of the wall significantly decreases, in 1, 14 times (p<0.01), only in hemocapillaries. Enlargement of the lumen of small arteries and arterioles in the initial stages of development of diabetes is associated with the development of endothelial dysfunction [17, 28], which appears because primary hyperglycemia stimulates endothelium to increase releasing of the arterioles nitric oxide (II), which is a key vasodilation mediator [1]. We associate the expansion of the lumen of the capillaries and venules and the thinning of capillary wall with an increase in the volume of circulating blood in the vessels, that is, with their overdriving due to increased hydrodynamic pressure [13].

Arterioles, venules and capillaries differ in ultrastructure a little bit from those vessels of control and intact animals. Basal membrane (BM) of capillaries has usual structure, its dense plate is slightly loose in some areas. The thickness of BM is (85.76±30.52) nm and is 1.15 times (p<0.001) wider, compared to the norm.

Arteries with enlarged lumen of different degrees are observed at the 28th day from the onset of induction of diabetes mellitus (Fig. 2). At the same time, there are spasmed arterioles. The dilatation of the veins is also diverse. Sites with interstitial edema are often detected. Aggregation of erythrocytes increases, but sludges do not appear in arterioles. On a half-thin section (see Fig. 1c) it is clear that erythrocytic slides are found in 6 veins of 7. Morphometrically, in this period the diameter of the lumen of arterioles and capillaries of suburothelial microcirculatory net remains larger than in control group, respectively in 1.34 and 1.18 times (p<0.001), and the thickness of the wall of arterioles increased in 1.26 times (p<0.001) comparing with the previous term and becomes 1.31 times larger than in control (p<0.001). The capillary wall doesn’t differ from norm and control (p>0.05). Venules continue to expand and the diameter of their lumen becomes larger than in control in 1.13 times (p<0.001), and their wall thickens 1.15 times (p<0.05).

Erythrocytic sludges in venules are also found at the ultrastructure level (Fig. 3a). The luminal plasmalema of the capillaries of the suburothelial microcirculatory net (Fig. 3b) is characterized by increasing in the number of thick short inclusions, some of which have a thinned leg indicating the be-
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Figure 1. Blood vessels of the UB wall of the rats in norm (a) and on the 14- (b), 28- (c), 42- (g), 56- (d), 70-th (e) days of the experiment. Semi-thin slices. Staining: methylene blue. Microphotos. Magnification: ×400. Signs: 1 - arteries; 2 - arterioles; 3 - venules; 4 - veins; 5 - mast cells.

Beginning of microlazmatosis. Mitochondria are not numerous, they are homogenized and vary in electron density. In endothelial cells of capillaries is well expressed KG, GrER. Free ribosomes are enough. There are many pinocytotic bubbles. Interendothelial connections are well visible. The thickness of BM of capillaries is (113.70±58.36) nm, it is larger than normal one 1.53-fold (p<0.001).

Polymorphism of the restructuring of blood vessels of UB continues to increase on the 42nd day of the experiment. Spasmed arteries with swollen wall often appear in adventitia and in the muscular membrane (Fig. 4). Spasmed small arteries and arterioles are also predominantly observed on half-thin sections on the 42th day of the experiment (see Fig. 1g). At the same time, individual vessels with normal diameter of the lumen are visualized.

At this term of observation, the diameter of the lumen of arterioles decreases in 1.27 times (p<0.001) and does not differ from the control (p>0.05) comparing to the previous term, but the thickness of their wall remains larger than in control group in 1.40 times (p<0.001). The diameter of the lumen of the capillaries is 1.15 times (p<0.001) smaller, and also does not differ from the control (p>0.05), as compared to the 28th day of the experiment. The thickness of their wall increases, comparing with the previous term, in 1.19 times (p<0.001) and becomes larger than control in 1.13 times (p<0.01). At the same time, the thickness of the wall of the venules remains greater than the control in 1.24 times (p<0.01). We consider that the cause of wall thickening of arterioles, capillaries and venules is the swelling of their endothelium. This is explained by the fact that transport of glucose in the endothelial cells is carried out by carriers of glucose, which work does not depend on the effects of insulin. That’s because the endothelium
of vessels at SD is overloaded with glucose [4]. We found that the level of glucose in the blood of diabetic rats at 42nd day of the experiment increased in 4.77 times and is \(24.98 \pm 2.16\) mmol/liter. The sorbitol pathway of glucose conversion is activated under such conditions, leading to the accumulation of osmotically active sorbitol in the endothelium, to delaying of Na+ and to the swelling of endothelial cells [10].

The venules continue to expand, the diameter of their lumen increases comparing to that in previous term in 1.13 times \( (p < 0.001)\) and becomes greater than in control in 1.33 times \( (p < 0.001)\). Such a restructuring of the vessels of the suburothelial blood circulating net with the simultaneous extension of the lumen of the venules and the narrowing of the capillaries could only take place with the involvement of arterio-venular anastomoses, which expanded and dumped blood into the venules. I. I. Savka [15] observed such vascular anastomoses on the 42nd day of the SD in the testicle, while other researchers - in the skin and the sciatic nerve on the earlier stages of development of diabetes [3, 11, 18].

The lumen of the arterioles begins to narrow since the 28th till the 42nd day of the experiment, spasmed arterioles and small arteries are often observed - this is caused by high hyperglycemia, which reduces the ability of the endothelial cells to synthesize vasodilators [1, 22, 29]. On the 28th and 42nd day after the beginning of the development of diabetes, a decrease in the diameter of the lumen of the interlobular arteries and afferent glomerular arterioles of the kidney was observed by P.B. Pokotylo [14], and of arterioles and capillaries of various layers of skin – by R. Ya. Boris [3].

The cytoplasm of the majority of endothelial cells of the spasmed arterioles is electron-dense at the ultrastructural level (Fig. 5a). The heterochromatin of their nuclei is located marginally. BM of endothelium is winding, spread, with local destruction. Erythrocytes are found in expanded capillaries (Fig. 5b). Ultrastructurally among capillaries there are functioning capillaries and capillaries with marked dystrophic phenomena. Mitochondria, GrER, KG, free ribosomes are found in the zone of organelles of endothelial cells of functioning capillaries. There are many pinocytotic bubbles, BM is thickened, in some areas loose. Capillaries with considerable endothelial cell dystrophy are characterized by electron-dense cytoplasm, marginal heterochromatin, organelles, moderate amounts of pinocytotic bubbles. At this term there is a microclasmatism of different degrees in many capillaries. BM of haemocapillaries becomes 1.63 times wider than the norm one \( (p < 0.001)\) and is \(120.90 \pm 44.85\) nm.

Moderately enlarged arteries and excessively dilated veins overfilled with blood are often found in the wall of the UB on 56-th day of observation (Fig. 6a). The lumen of both the first and the others is often occupied with erythrocytic masses and a small amount of plasma. There is a generalization of sludge syndrome in venules and arterioles in the majority of sites of the lamina propria of the mucous membrane and submucosal layer of UB. The veins overfilled with blood and erythrocytic sludges in venules appear on half-thin slices (Fig. 1d).

It is seen morphometrically that on the 56th day of the experiment the lumen of the arterioles of suburothelial blood microcirculation net increases in 1.11 times \( (p < 0.05)\), the lumen of capillaries - decreases in 1.08 times \( (p < 0.01)\), and of the venules - does not change \( (p > 0.05)\) comparing to the lumen on previous term. At the same time, the lumen of arterioles and venules becomes larger, comparing to the one of control, respectively in 1.16 times \( (p < 0.05)\) and in 1.31 times \( (p < 0.001)\), and the lumen does not differ in capillaries.
the thickness of the wall of arterioles and venules decreases respectively, in 1.28 times (p<0.01) and in 1.21 times (p<0.01) and ceases to differ from the one of control (p>0.05), comparing to the one on previous term. At the same time, the thickness of the capillary wall remains 1.14 times larger than control (p<0.05).

At the electron microscopic level in this period among the capillaries of suburothelial blood microcirculation net there are two types of capillaries: one - with the superiority of dystrophic and the other - with superiority of destructive phenomena. The first has cytoplasm of different electron density, it has a lot of electron-dense areas. Mitochondria with destroyed cristae, vacuoles, and pinocytotic bubbles can be identified in the cytoplasm. Nuclear shell forms invaginations. A small number of local thickenings of the cytoplasm and microovergrowths are present. The greatest difference between these capillaries is the lamellarity of their BM, comparing to the capillaries in the previous terms of observation period. The phenomenon of destruction prevails in other capillaries. Their cytoplasm is electron-dense, organelles are not determined, there are pinocytotic bubbles and vacuoles of different sizes. The wall of capillaries has different width, massive local thickenings of the cytoplasm and the microovergrowths of different thickness and length predominate. The nucleus of the endothelial cell is destructively altered, electron-dense, it significantly protrudes into the lumen of the capillary with the part of the cytoplasm. BM capillaries is very widespread, lamellar, intermitted. Marked clasmatosis is noticed in most capillaries. In many vascular areas there is a considerable plasmatic infiltration. The thickness of BM is (137.67±79.99) nm and is 1.85 times bigger than the norm (p<0.001). Although it thickens in 1.14 times, comparing to the one in previous term, but this increase is not significant (p>0.05), as a result of the considerable variability of this morphometric feature.

After 70 days from the onset of SD induction, the generalization of the sludge syndrome occurs in the arterioles and venules of all UB membranes. Sludges in expanded venules and the arteries overfilled with blood are found in half-thin sections (see Figs 1 and 6b). It is known that aggregation of erythrocytes causes the development of circulatory hypoxia [2].

It is found morphometrically that on the 70-th day of the experiment the lumen of arterioles of suburothelial blood microcirculation net becomes 1.15 times larger than control one (p<0.01), venules become larger 1.44-fold (p<0.001), and the thickness of their walls does not differ from those of the control (p>0.05). The diameter of the lumen of the capillaries does not change (p>0.05), but the thickness of their wall becomes greater than control one 1.24-fold (p<0.05).

Moderate expansion of arterioles and venules on the 56-70-th day of diabetes is associated with a decrease in vascular tone of sympathetic nerves [4], where hyperglycemia causes
Figure 5. Spasmed arteriole (a) and capillary with erythrocytic sludge (b) on the 42-nd day of the experiment. Electronic Microphotography. Magnification: ×9600 (a); ×4800 (b). Signs: 1 - narrowed lumen of arteriole; 2 - nucleus of the endothelial cell; 3 - winding basal membrane of endothelium of arteriole with local destruction; 4 - sludge in the lumen of the capillary; 5 – vacuolar dystrophy of smooth myocyte.

excessive accumulation of glucose, activation of aldose-reductase and formation of sorbitol, leading to the death of neurocytes and axonal transport disorder [4, 10].

It is seen ultrastructurally that BM of capillaries becomes lamellar in many parts (Fig. 7a). Its thickness in this period is (239.36±115.75) nm. It thickens, comparing to one the previous term in 1.74 times (p<0.001), and comparing to norm – in 3.22 times (p<0.001). The destructive processes in blood vessels are increasing, and many of the capillaries undergo total destruction (Fig. 7b). Increasing in plasmatic infiltration of perivascular connective tissue is observed.

One of the main components of the development of diabetic microangiopathy is the reorganization of BM of haemocapillaries [5, 43, 45], which correlates with the level and duration of hyperglycemia. We found that the slight thickening of BM is already observed on the 14-th day of the experiment, and till the 70-th it becomes 3.22 times larger than normal one. Since the 42-nd day of the experiment, the disorganization of the structure of BM appears, and on the 56-70-th day BM becomes lamellar. These changes of BM are associated with non-enzymatic glycosylation of its proteins, triggered and intensified by hyperglycemia during development of diabetes [8, 9, 45], and an increasing in level of glycosylated hemoglobin since the 14-th day till the end of the experiment indicates on this.

We found considerable dystrophic changes in the endothelial cells at the ultrastructural level since the 42-nd day of diabetes; they increased until the end of the experiment, when the signs of their destruction appeared. A number of researchers noticed such processes that manifest themselves since the 42-nd day of development of diabetes [2, 3, 34]. The vasoconstriction of arterioles and small arteries and circulatory hypoxia, the presence of which is confirmed by erythrocytic sludges leads to increasing of these pathological processes. The above changes, as well as changes in BM of capillaries, are considered by researchers as manifested diabetic microangiopathy [1, 2, 34].

3. Conclusions

1. The development of diabetic microangiopathy of UB in rats is a non-specific universal process that develops similarly to this one in other organs. The specificity of the development of streptotsotocin-induced diabetic angiopathy is determined by the degree of expression of non-specific vascular disorders and the peculiarities of their chronological course:

- one of the main components of the development of diabetic microangiopathy is the change of vascular tone of its arterioles, which is manifested on the 14-th day of the experiment by dilatation
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Figure 7. Lamellar basal membrane of the capillary (a) and total destruction of the wall of the capillary (b) on the 70-th day of the experiment. Electronic Microphotography. Magnification: ×8000. Signs: 1 - microovergrowths and local thickening of the cytoplasm; 2 - lamellar BM of capillary; 3 - areas of plasmatic infiltration.

of arterioles, on the 28-42-nd it manifests as a gradual decrease in the lumen, up to the spasm of separate arterioles, and on the 56-70-th as their secondary expansion;
- the second of the main units is the restructuring of BM of haemocapillaries. Its insignificant thickening is observed already on the 14-th day of the experiment, and till the 70-th it becomes larger than normal one in 3.22 times; since the 42-nd day of the experiment the disorganization of BM is revealed, and the lamellar structure becomes the most characteristic feature in its reorganization on the 56-70-th day;
- rheological disturbances of blood are the third one of the main components of the development of diabetic microangiopathy of UB, on the 14th day of the experiment they are expressed by small “coin columns” of red blood cells in separate venules, on the 28-th day they manifest as sludges in most venules, and on the 42-nd - also in capillaries; on the 56-70-th days there is a generalization of the sludge syndrome in the arterioles and venules, initially of the submucosal layer of UB, and on the 70-th it appears in all its layers.

2. Edemas of different genesis are typical manifestations of diabetic microangiopathy of UB. Interstitial edema of its wall increases until the 28-th day of the experiment, and it decreases on 42-nd day. The phenomena of plasmatic infiltration of perivascular connective tissue increase during the period from the 42-nd to the 70-th day. The swelling of endothelial cells is most visible on the 28-th and the 42-nd day of the development of diabetes.

4. Prospects of Further Researches

We plan further studying of intraorganic blood vessels of UB in experimental diabetes mellitus of type II.

References


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