Morphological Characteristics of Intestinal Vessels of Animals with an Experimental Model of Diabetes Mellitus Type 2 Complicated by Microangiopathy

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Abstract

Diabetes mellitus and its complications are considered far from unresolved problems of modern medicine. An important aspect in solving fundamental problems is experimental research aimed at assessing the state of change of pathogenetic important key positions in the development of such a formidable complication of diabetes as angiopathy. On the other hand, surgeons' great interest in bariatric and metabolic surgery, as method of surgical treatment of type 2 diabetes mellitus, requires in-depth studies to assess the state of angiogenic changes in the organs of the gastrointestinal tract, namely in the intestine. The article describes the morphological changes in the vascular capillary bed in the dynamics of the development of a new experimental model of diabetes mellitus, namely the chemical method of diabetic angiopathy. The development of an experimental model of diabetic angiopathy showed the staged nature of the changes in the form of the initial signs of angiopathy, with perivascular infiltration and edema in the form of congestive plethora in the flesh until the development of vascular hyalinosis, which indicates the irreversibility of the processes of pathological transformation. Thus, the results of morphological studies in the dynamics of modeling of diabetic angiopathy indicate the development of diabetes mellitus with its complication in the form of microangiopathy.

Keywords: *Diabetes mellitustype 2, microangiopathy, vascular morphology, diabetic angiopathy, bariatric surgery, obesity.*

Introduction

One of the important world problems of drug therapy for type 2 diabetes, along with the prevalence of this disease, is its lack of effectiveness. It is known, that compensation of type 2 diabetes mellitus, through its traditional treatment, reaches only 30% of patients, and remission in a minimal proportion of patients [1]. Nowadays, it has been proven, that a clinically significant alternative in the treatment of type 2 diabetes is bariatric surgery, which is used in patients with obesity[2], i.e. with a BMI (body mass index) above 35 kg/m2 and providing attainment of diabetes compensation in 76.8% of cases[14].

The great clinical interest are the possibilities of surgical treatment[3] of type 2 diabetes mellitus in patients with normal or reduced body mass index[15]. However, the effectiveness of such operations[4] requires fundamental research in the experiment, proving the safety and effectiveness of this procedure, including taking into account the existing pathomorphological transformations as a form of diabetic gastropathies and enteropathies[5]. The basis of their pathogenesis[7], of course, is the development of diabetic angiopathy[8].

The literature presents the results of experimental studies of the effect of bariatric and metabolic operations on the condition of the mucous membrane of the stomach and intestines, in particular, changes in its cellular balance[9]. Nevertheless, there is no information regarding the characteristics of angiogenic changes in the intestinal walls and stomach in the background of progression of type 2 diabetes. In turn, this would make possible to evaluate objectively the positive and negative aspects of the effect of these operations in the treatment of type 2 diabetes with a normal body mass index, but with the presence of diabetic angiopathy.

Materials and Method

In the experiment were used laboratory Wistar rats weighing 150-250 g on a normal laboratory diet. By the beginning of the experiment, each group consisted of 10-12 rats. 112 animals were used overall.

Diabetic microangiopathy in animals was modeled according to original technique developed by us ("A method of modeling diabetic angiopathy" Patent No. IAP 03642 PV RUz of 2006). The simulation was carried out as follows: laboratory rats weighing 150-250 grams on an empty stomach under ether anesthesia were injected 100-110 mg/kg of doxorubicin with 0.9% sodium chloride solution intraperitoneally, and 48 hours later, they were once daily injected intraperitoneally for 3 days 0.2-0.4 ml of a 70% sorbitol solution. In dynamics, starting from the first day after the injection of the last dose of sorbitol, we observed the development of diabetes mellitus and the manifestations of its angiogenic complications. The main criterion in the reproducibility of the disease was the presence of a stable level of glycemia (at least 9 mmol/l on an empty stomach) starting from the 3rd day after doxorubicin injection. Over the next 30 days, the animals developed diabetic microangiopathy. Animals with normal glycemia level were removed from further researches, and the results obtained during the studies were canceled. Carbohydrate metabolism: blood glucose (mmol/l) was determined on a VitrosDT-60 automatic biochemical analyzer (Austria) using reagents express medical chemistry.

For histological examination, Pieces of the studied intestinal zone were fixed in neutral formalin, Carnoy fluids and embedded in paraffin. Cross-sections were stained with hemotoxylin-eosin according to Van Gieson. Light microscopy and morphometry were carried out on a XSZ-20 trinocular microscope (China) with an optical resolution of 4x to 400x with a direct electronic nozzle in digital format.

Results

With a 5-day period of the experimental model, there were noted mild manifestations of diabetic microangiopathy.

They were manifested a certain increase in proliferation and swelling of arteriole endothelial cells, and areas of plasma impregnation of their walls, mainly in subendothelial zones with plasma proteins, detected in the form of small foci.

Swelling of endotheliocytes in some places was accompanied by destructive changes in some of them. Sometimes perivascular edema and weak lymphohistiocytic infiltration around individual arterioles were detected.

On the 10th day of observation in the capillaries and venules, were noted along with the phenomena of stagnant flushing, with a tendency to hemolysis, thinning of the walls of vessels containing, in addition to red blood cells, a lot of neutrophils.

On the 20th day of the reproduction of diabetic microangiopathy morphologically, more significant disorders of most capillaries and arterioles were observed, compared with those of the previous observation period. They manifested themselves in a marked swelling of endotheliocytes, flattening of the nuclei with a thickening of their basement membrane, and in some cases, their desquamation into the lumen of the vessels. Plasma impregnation with fixation of plasma components in the subendothelium was observed in the vessel wall. In addition to thickening of the basement membranes in arterioles, thickening of their layers and the presence of pericytes in small vessels were observed. Around the blood vessels revealed edema and perivascular infiltration.

On the 30th day of the reproduction of diabetic microangiopathy, the lumen of the capillaries looked wide with the phenomena of stagnant flushing. Some of them were in a state of thrombosis (Fig. 3). Arterial capillaries were characterized by changes in blood cells in the form of adhesion of red blood cells to the surface of endotheliocytes, and in some places, to the basement membrane. In addition to narrowing the lumen of some arterioles, due to thickening of the walls, as a result of plasma soaking, swelling and desquamation of endothelial cells in their lumen, sections of thinning of the walls were found (Fig. 4) with the release of red blood cells.

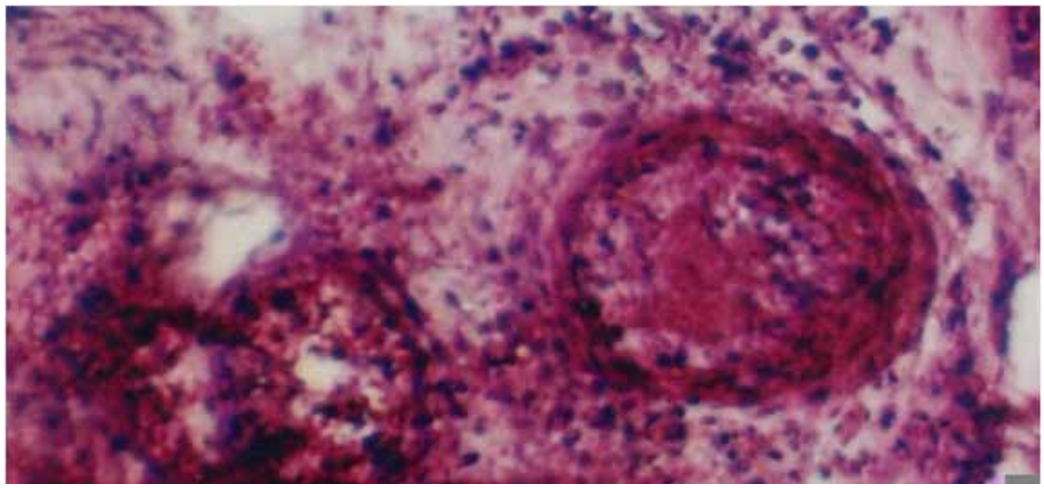

Figure 1. 30 days of modeling diabetic microangiopathy. Flushing of capillaries with signs of thrombosis and adhesion of red blood cells to the basement membrane. Hemotoxylin and eosin. Enlarged 400x.

Venules were characterized by widened lumen and severe perivascular infiltration.

40 days of modeling diabetic microangiopathy were manifested pronounced swelling of endothelial cells, its hypertrophy, destruction, detachment from the basement membrane, and their rejection into the lumen of the vessel.

Discussion

Sometimes, processes of pericytes or smooth muscle cells were immersed in the formed cracks. The marked thickening of the walls of most arterioles was accompanied by hyalinosis of their walls, which was both segmental and circulatory nature. A more significant, compared with the early observation period, plasma impregnation of the vessel walls was revealed, moreover, it was observed in all layers. A significant thickening of the walls of the arterioles occurred, mainly due to an increase in the thickness of the endotheliocytes' basement membrane. In some cases, it's profusion into the lumen of the vessel was determined, leading, together with the above changes, to a significant narrowing of it or complete obliteration (Fig. 6). In separate observations, on the 50th day of modeling, re-calibration was detected in arterioles, leading to occurence of "vasa vasorum". (fig 3).

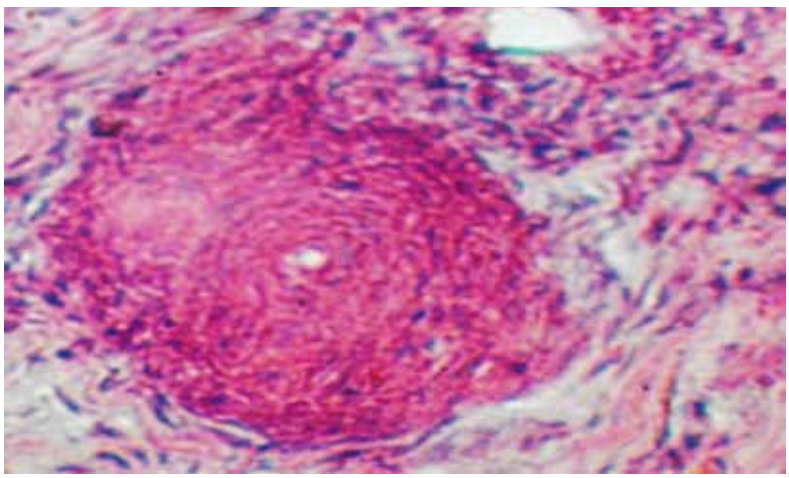

Figure 2. 40th day of modeling diabetic microangiopathy. Hyalinosis, thickening of the arteriole wall with a sharp narrowing of the lumen (obliteration). Hemotoxylin and eosin. Enlarged 400x.

In the later stages of the experiment (60-80th day), maintaining all of the above morphological signs of microangiopathy, the phenomena of complete loss of the endothelial lining by the hyalinized vessels are noted. In view of this, they were represented by acellular tubules which lumen was filled with nests of red blood cells, with the occurrence of thrombosis. These terms were characterized by significant problems of blood cells, in particular, the phenomena of hemolysis, and areas of thrombosis. As a result of this, erythrocytes redistributed to the surface of endotheliocytes (Fig. 4).In addition to the accumulation of erythrocyte aggregates resembling the shape of mulberries, swollen and torn away from the basement membrane endotheliocytes clogged the lumen of the vessels. Moreover, in some cases, perivascular pronounced proliferation of connective tissue, forming a kind of connective tissue "couplings", was observed.

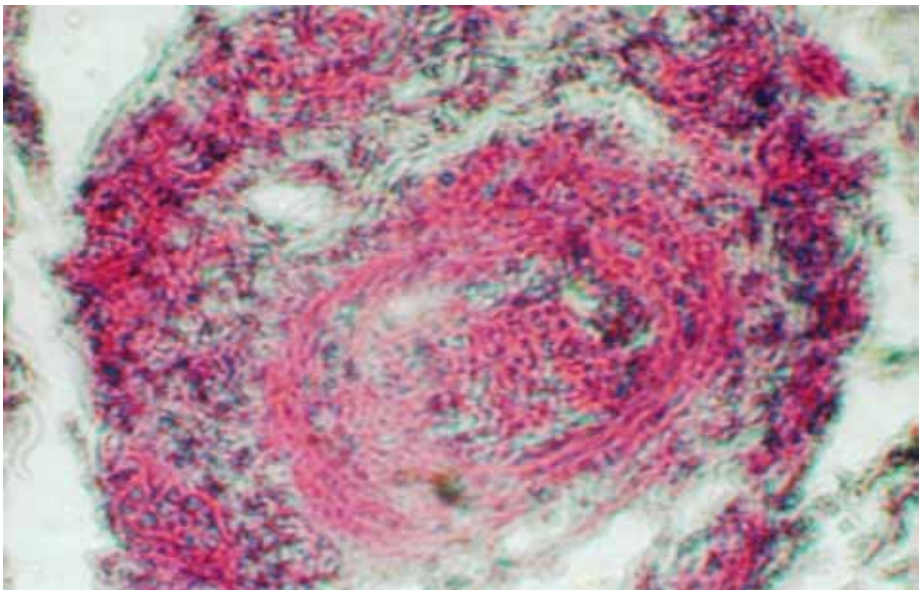

Figure 3. 50th day of diabetic microangiopathy. The phenomenon of percolibration with the advent of "vasa vasorum". Hemotoxylin and eosin. Enlarged 400x

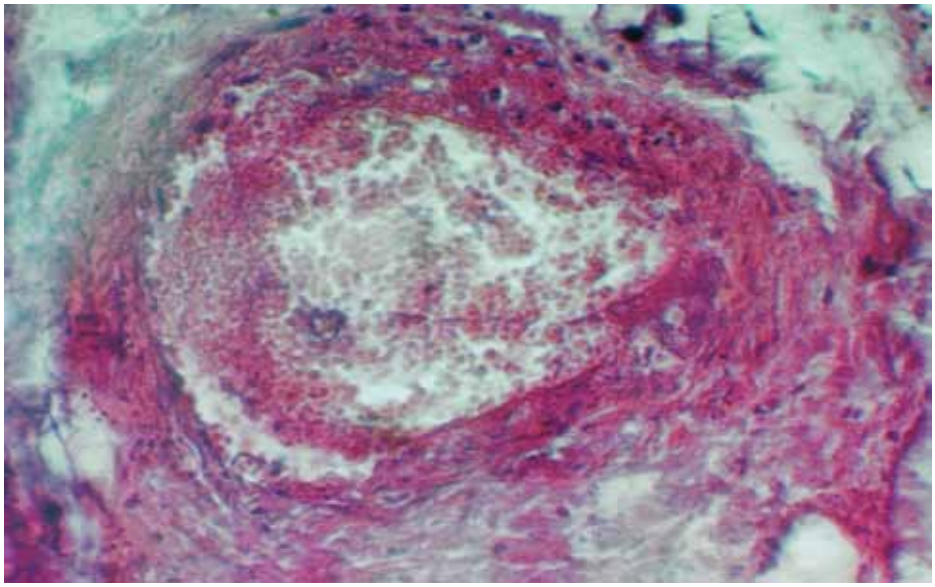

Figure 4. 60th day of modeling diabetic microangiopathy. Hyalinosis of the wall of the arterioles with a complete absence of the endothelial layer, the phenomenon of hemolysis of red blood cells with their location at the basement membrane. Hemotoxylin and eosin. Enlarged 400x

Conclusion

Thus, the results of our morphological studies in dynamics from 5 to 80 hours show that diabetic microangiopathy leads to the diabetes mellitus with its complications, which indicates the presence of damaged vessels when a distinct degree of microangiopathy is seen. This is due to pathomorphological changes in blood vessels. It is characteristically, that changes in experimental diabetes mellitus were exposed to all structural components of the vascular wall.

According to a number of authors, diabetes mellitus is a metabolic disease, called diabetic microangiopathy [10]. For this, the most characteristic morphological sign is the presence of a pronounced degree of severity of thickening of the basement membranes, plasma impregnation[11], hyalinosis of the walls of capillaries and arterioles, narrowing of lumen of blood vessels, proliferation of endothelial cells, perithelium, their destruction and rejection, changes in the rheological characteristics of blood [12].

You know, that diabetes mellitus with diabetic microangiopathy for 80 years is the result of all pathogenetic changes:

I period up to 10 hours: the allocation of mild signs of angiopathy with swelling of the endothelium, arterioles, soaking their walls, plasma protein, perivascular infiltration and edema in the form of stagnant congestion. Thinning of the walls of the capillaries was observed.

In the II period of experimental modeling (days 20-30), progressive processes are observed, which manifest themselves in deeper structural changes in the main vessels. In addition to plasma impregnation of the subendothelial departments of arterioles, proliferation of endothelial and perithelial cells, as well as their desquamation, enlightenment due to leakage of the walls was observed. It was also noted the expansion of the lumen of small vessels, their stagnant congestion, infiltration of adventitia layers.

III period (40-80th day) was characterized by a more significant development of morphological signs of diabetic microangiopathy. Along with morphological changes identified in the early stages, the phenomenon of hyalinosis of the majority of the walls of the arterioles, as well as their marked plasma soaking, were noted. Swelling and detachment of endotheliocytes with intercalation of smooth muscle cells into the vessel wall with their perical calibration and the formation of vasa vasorum, as well as a significant narrowing of the lumen of arterioles. At the same time, perivascular edema, infiltration and proliferation of connective tissue were observed. Changes in blood cells were manifested in their tendency to hemolysis, adhesion on the lumenal surface, the appearance of red blood cell aggregates with the development of thrombosis. Moreover, a pronounced lesion of endotheliocytes, their detachment from the basement membrane led to an increase in the clearance of arterioles, and the reaction of smooth muscle cells and pericytes contributed to the thickening of the basement membranes. Prolonged plasma impregnation causes the development of hyalinosis in microvessels.

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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