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## METHOD FOR EXPERIMENTAL MODELING OF DIABETIC ANGIOPATHY

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## **METHOD FOR EXPERIMENTAL MODELING OF DIABETIC ANGIOPATHY**

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### **ABSTRACT**

*These methods of modeling diabetic microangiopathy have their own distinctive aspects, separated by the mechanisms of their reproduction. Reproduction of the model of diabetes mellitus, taking into account the pathogenetic significance of the role of sorbitol in the development of angiopathy, allows us to obtain the possibility of studying microangiopathy at a reliable level.*

**Key words:** *diabetic angiopathy, hyperglycemia, experiment, model.*

Diabetic angiopathy is one of the most common organic manifestations of diabetes complications. According to the literature [5,8], at least 2 main factors are involved in the pathogenesis of angiopathies in diabetes mellitus: a genetic predisposition and an external factor, which is hyperglycemia and the associated cascade of metabolic, hormonal and rheological disorders, including end products of glycosylation .

In 1937, Jacobs first showed that intravenous administration of alloxan induced rabbits at a dose of 70 mg/kg causes transient hyperglycemia, followed by a drop in blood sugar and the development of seizures [2,12,14]. However, despite a fairly large amount of evidence of selective damage of  $\beta$ -cells by alloxan, the essence of this phenomenon is not fully understood.

The biochemical pathway for the formation of fructose from glucose occurs along the “pathway of sorbitol” [2,6,13]. Moreover, this pathway is activated in patients with diabetes with an increase in glucose concentration [4,11]. Glucose is restored before sorbitol. Sorbitol hardly penetrates through cell membranes and therefore accumulates in the cell [3,14]. All this indicates the high role of sorbitol in the development of diabetic microangiopathy, which means that the use of this substrate in the modeling process is of a natural biological character.

The aim of our study was to develop a new experimental model of diabetic angiopathy.

**Material and research methods.** In the experiment, outbred rabbits of both sexes were used, weighing 1500– 2500gr on a normal laboratory diet. By the beginning of the experiment, each group consisted of 10-12 rabbits. A total of 112 animals were used.

Diabetic microangiopathy in rabbits was modeled according to the original method developed by us (“A method for modeling diabetic angiopathy” License No. IAP 03642 PV RUz). The simulation was carried out as follows: 100-110 mg/kg of doxorubicin in 0.9% sodium chloride solution was intraperitoneally administered to rabbits weighing 1500– 2500 gr on an empty stomach under ether anesthesia, and after 48 hours after administration of doxorubicin, every day for 3 days, 0.2-0.4 ml of a 70% sorbitol solution was intraperitoneally administered. In dynamics, starting from the first day after the injection of the last dose of sorbitol, the development of diabetes mellitus and the manifestations of its angiogenic complications were observed. 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 3 days after the administration of doxorubicin. Over the next 30 days, rabbits developed diabetic microangiopathy. Animals with normal glycemia were removed from further studies, and the results obtained during the studies were canceled. Carbohydrate metabolism: blood glucose (mmol / L) was determined on a Vitros DT-60 automatic biochemical analyzer (Austria) using express dry chemistry reagents. For histological examination, pieces of muscle tissue of the studied area were fixed in neutral formalin, Carnoy’s fluids and embedded in paraffin. 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.

We assessed the state of regional blood flow of the vessels of the lower extremities using a Logidop 4 ultrasound dopplerography apparatus from

Kransbuchler (Germany) with sensors with frequencies from 8 MHz and higher. The state of the microvasculature was assessed by tissue biomicroscopy. For this, a Lumam I-3 luminescent microscope equipped with an OLK-2 photographic attachment was used. The resulting picture was recorded on video, which allowed us to evaluate the morphometric parameters of blood vessels. Partial oxygen pressure measurements through the skin were performed on the monitor using the TCO2M® model manufactured by NOVAMETRIX (USA). The summarizing indicators — average, percentiles, confidence intervals — were calculated, correlated with the graphic display, groups were compared by Student's *t*-test for dependent and independent samples with graphic display. Differences were considered significant at  $p < 0.05$ .

### **The results obtained and their discussion**

The first version of the diabetes mellitus model was a model in which, under aseptic conditions under ether anesthesia, the animals underwent laparotomy followed by resection of an even half of the pancreas from the tail pole (series 1). When removing relatively smaller volumes of the pancreas (1/3 of the tail) in animals in 75% of cases, diabetes did not occur - the animals recovered. There were no signs of hyperglycemia in the blood, and on biomicroscopy of the vascular bed of limb tissues, performed 7-10 days after modeling, they found in 40% of cases intravascular aggregation of red blood cells, in 45% of cases the absence of any vasomotor disorders. Only in those 10-15% in which the disease occurs, a decrease in the arterio-venular ratio to 1: 2 and extra-vascular inflammatory reactions in the form of edema were detected. When large volumes were removed (up to 2/3 of the free part of the pancreas) in 90% of cases, rabbits died from a hyperglycemic coma with high blood glycemia in the next 3-4 days (series 2). Biomicroscopy of these rabbits revealed intravascular stasis with hemorrhage in tissue structures. In a word, despite the fact that diabetes was formally reproduced, essentially we were dealing with hyperglycemic coma, the initial beginning of which was the removal of the main part of the pancreas, including its secretory zone. To clarify this issue, we conducted other series of experiments in which we used the chemical method for modeling diabetes

mellitus, that is, by reproducing the model as a result of the introduction of some organic substances that damage the  $\beta$ -cells of pancreatic islets. The value of chemical models lies in the fact that they can be reproduced in representatives of various species, in particular in small animals, as well as in animals with a diffuse pancreas [1,15]. Chemical shutdown of the islet apparatus is not accompanied by a violation of the exocrine pancreatic function [10,15]. Chemical models also deserve attention because some of the substances that cause diabetes are metabolic products in humans and animals [3,5,6]. The results of these 3 and 4 series of experiments as a whole turned out to be similar to those described above, namely, when alloxan was used at a dose of 200-300 mg/kg body weight in 85.7% of cases in animals, a picture of hyperglycemic coma developed (series 3) with fatal within 10 days after intravenous administration of a chemical agent. This was due to lightning damage to the pancreatic  $\beta$ -cells producing insulin, which corresponded to the development of type 1 diabetes mellitus (insulin dependent) and did not meet the requirements for reproducing diabetic angiopathy. In other words, diabetic angiopathy simply did not have time to develop in the given period of diabetes. In order to prolong the process of damage to  $\beta$ -cells, as well as to prevent the development of fulminant course of diabetes mellitus and to bring the process closer to clinical conditions, we were the first to use doxorubicin as a chemical substance with a side effect of selective exposure to pancreatic  $\beta$ -cells [6,11]. The drug was administered intraperitoneally at a dose of 100-110 mg/kg in a 0.9% NaCl solution (series 4). With intraperitoneal administration of doxorubicin, the outcomes were different: of 21 rabbits, 2 (9.5%) died from hyperglycemic coma, in 14 (66.7%) the process took an abortive course, and only 5 (23.8%) had a typical picture of diabetes with stable hyperglycemia. Comprehension of the stated facts led us to the following three conclusions. First, massive removal or chemical damage to the pancreas, regardless of the nature of the agent, does not lead to the development of diabetic angiopathy in the clinical sense of the term, but to hyperglycemic coma due to the lack of insulin in the body. Secondly, the severity of the condition is so great that diabetic angiopathy does not have time to develop. This logically led us to the third: diabetic angiopathy as a special form of

vascular complication of diabetes is possible only if there is a full link in the pathogenetic mechanism of its development. As is known, the mechanism of microangiopathies consists of non-enzymatic glycosylation of proteins of the capillary basement membranes under hyperglycemia and activation of the conversion of glucose to sorbitol under the influence of aldose reductase [11,13,15]. Excess sorbitol in the vascular bed leads to thickening and compaction of the vessels themselves [3,14]. This in turn leads to disruption of blood flow in the vessels of the microvasculature with the development of tissue ischemia [7,9]. There is a vicious cycle with glycosylation of proteins of the basement membranes and the accumulation of sorbitol in the walls of microvessels [6,14]. As a result of the course of this pathological process, the structure of the cells of the walls of the vessels is disrupted, the structures of the proteins of the intercellular substance of the vascular walls change with the acquisition of antigenic properties [6,8,9]. All this indicates the high role of sorbitol in the development of diabetic angiopathy, which means that the use of this substrate in the process of modeling this pathological condition is of a natural biological nature. Experimental confirmation of this was obtained in a new series of experiments (series 5), which differed from the previous one in that, in order to increase the reproducibility of the model and to bring the process closer to the clinical course under ether anesthesia according to our invention, 100-110 mg was administered intraperitoneally as a chemical kg of doxorubicin preparation in a 0.9% NaCl solution, and 48 hours after the administration of doxorubicin, 0.2-0.4 ml of a 70% sorbitol solution was injected intraperitoneally once daily for 3 days. In dynamics, starting from the first day after sorbitol injection, the development of diabetic microangiopathy was observed. Over the next 3-4 days, the rabbits developed a clinical picture of diabetes mellitus (hyperglycemia, polyuria, glycosuria), and on the 10-20th day of diabetic angiopathy: impaired permeability of the vascular walls, the formation of microaneurysms, the formation of microthrombi, the expansion of venules and postcapillaries, neoplasms of microvessels, micro hemorrhages, the formation of seals and scars in the perivascular tissue.

Conducted in vivo studies of microcirculation in the tissues of the limbs of this series of experiments revealed the presence of intravascular disorders in the form of thrombosis, an increase in arteriovenous relations to 1: 4-1: 6, with severe tissue infiltration of extravascular zones.

Such consequences (table 1) of the combined modeling method took place in 22 (73.3%) of 30 rabbits of this series; in 2 (6.7%) animals, only paralytic expansion of capillaries and an increase in vessel diameters were revealed, 2 (6.7%) of the rabbit died of hyperglycemic coma, and in 4 (13.4%) animals diabetes did not develop.

Table 1

The results of various methods for reproducing diabetes mellitus and diabetic microangiopathy

Signs	Series 1	Series 2	Series 3	Series 4	Series 5
N	20	20	21	21	30
Abortive course	15 (75%)	-	-	14 (66,7%)	4 (13,3%)
Hyperglycemic coma	-	18 (90%)	18 (85,7%)	2 (9,5%)	2 (6,7%)
Capillary expansion	5 (25%)	2 (10%)	3 (14,3%)	-	2 (6,7%)
Reproducibility	-	-	-	5 (23,8%)	22 (73,3%)

Perhaps the diabetes mellitus in these animals was of a hidden nature [6,11,12,14].

A comparative characteristic of changes in the total area of capillary lumen in the limb in the dynamics of reproduction of various methods of diabetes mellitus showed that the normal state of the area equal to 120 mm<sup>2</sup> progressively decreased with the administration of sorbitol against the background of doxorubicin utilized by the body. These changes were noted in all series of experiments and were characterized by their reliability both in relation to the control series and in relation to the group of animals with alloxan diabetes (Figure 1). Accordingly, morphometric indicators were also characterized by specific changes within the groups themselves, namely: counting and calculating the capillary / non-capillary ratio also indicated a decrease in capillary sections compared to areas of myocytes.

Moreover, the ratio of capillary/non-capillaries that took place in the control series of experiments was 1:4.2, and did not change significantly with alloxan diabetes. However, the administration of sorbitol led to a progressive decrease in the coefficient, which reached a ratio of 1: 36.7 by 40 days of modeling.

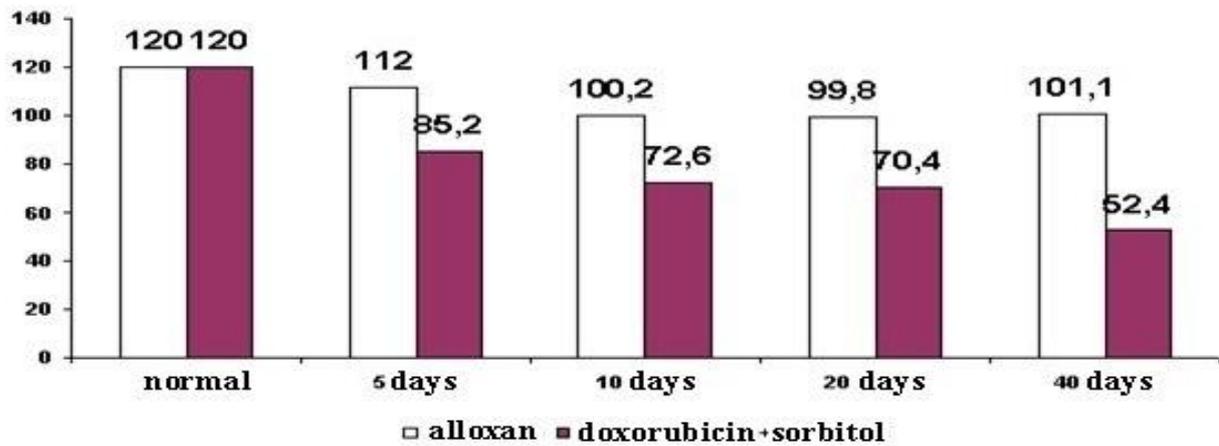


Figure 1. Comparative characteristic of changes in the total lumen area of capillaries in a limb in the dynamics of reproduction of various variants of diabetes

Through a skin study of the level of oxygen tension in limb tissues both at rest and after movement, it showed the development of signs of limb ischemia in models with a combined method of reproducing diabetes mellitus. Moreover, as shown in table 2, significant signs of changes were noted on the 40-80 days of the dynamics of the process (from  $14.3 \pm 1.2$  at rest to  $32.7 \pm 3.6$  after orthostasis;  $p < 0.05$ ).

Table 2

Comparative characteristics of changes in the level of oxygen tension in limb tissues at rest and during orthostasis in the dynamics of reproduction of various diabetes mellitus models

Diabetes options	Study Options	Observation dynamics				
		Control	10 days	20 days	40 days	80 days
Alloxan	Rest	$55 \pm 12,5$	$44 \pm 6,3$	$51 \pm 7,8$	$49 \pm 3,4$	$45 \pm 4,6$
	Orthostasis	$65 \pm 8,5$	$63 \pm 7,9$	$58 \pm 9,9$	$55 \pm 7,4$	$56 \pm 3,8$
Combined	Rest	$55 \pm 5,3$	$38 \pm 3,2^*$	$26 \pm 1,3^*$	$14,3 \pm 1,2^*$	$13 \pm 1,0^*$
	Orthostasis	$65 \pm 9,6$	$37 \pm 2,1^*$	$36 \pm 2,0^*$	$32,7 \pm 3,6^*$	$30 \pm 2,0^*$

\*  $p < 0.05$  - significant changes in relation to the control group

In cases of research of this indicator in animals with alloxan diabetes mellitus, ischemia of limb tissues was not reliable and quickly transient character.

## Conclusions

Reproduction of diabetic angiopathy, today, is becoming increasingly important. This is due, on the one hand, to the high specific gravity of the spread of this form of complication of type 2 diabetes mellitus in clinical practice [5,8,9], and on the other hand, to the need to develop adequate methods for drug correction of this pathology under experimental conditions [8,10].

Moreover, as you know, modeling of this complication is associated with the need to maximize the approximation of the conditions for its reproduction by the clinical [5,7,9]. Based on the foregoing, the primary consideration in reproducing diabetic angiopathy should be based on the modeling of diabetes mellitus.

The above methods for modeling diabetic microangiopathy have their own distinctive aspects, isolated by the mechanisms of their reproduction. Reproduction of the model of diabetes mellitus taking into account the pathogenetic significance of the role of sorbitol in the development of angiopathy allows to obtain microangiopathy research opportunities at a reliable level.

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