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PECULIARITIES OF THE CONDITION OF VASCULAR PLATELETIC HEMOSTASIS IN DIABETIC RETINOPATHY AND DIABETIC NEPHROPATHY IN RATS

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Abstract

The article presents experimental data on the study of changes in vascular platelet hemostasis in rats with experimental DR and DN on the background of type 2 diabetes. It is established that microcirculatory complications of type 2 diabetes are characterized by a decrease in platelet count, increased aggregation capacity. In the study of induced platelet aggregation, the polarity of the results of the study was proved, which indicates the dependence of complications on the functional state of vascular platelet hemostasis, primarily due to stimulated, depleted platelets and the corresponding number of intact platelets. More pronounced changes in vascular-platelet hemostasis on the background of type 2 diabetes in rats with DN compared with the group of animals that reproduced DR.

Keywords: diabetes mellitus; microangiopathy; vascular-platelet hemostasis; platelet aggregation.

Introduction. One of the global problems of modern medicine is diabetes mellitus (DM), and above all, type 2 diabetes, which accounts for about 90% of all cases and is pandemic worldwide [12]. According to the International Diabetes Federation (IDF), 425 million people with diabetes and 352 million people with impaired glucose tolerance at high risk of developing diabetes were registered in 2017. In 2045, the number of patients with diabetes is expected to increase to 629 million [11]. In Ukraine, according to the Center for Medical Statistics of the Ministry of Health of Ukraine, the number of registered patients with diabetes exceeds 1.8 million people, among whom type 2 diabetes predominates (90%) [10].

The process of blood clotting is provided by 3 main components: platelets, coagulation factors and the integrity of the vascular wall. If at least one of the components is damaged, the process is activated, which leads to thrombosis. Numerous studies have shown that in both types of diabetes there is damage to all components of the hemostasis system necessary to maintain normal blood clotting. The hemostasis system in diabetes has been studied for over 20 years. It has been established that diabetes is a prothrombotic condition, which is associated with a high risk of complications of the micro- and macrocirculatory system [8].

Today, some links in the pathogenesis of complications of the microcirculatory tract (diabetic retinopathy (DR), diabetic nephropathy (DN)), which account for the "lion's share" of all complications of diabetes, remain unclear, but it is believed that the mechanism of their development consists of two related pathophysiological processes: structural and functional disorders of the microvascular wall, disorders of vascular-platelet and humoral hemostasis [8, 9].

The aim of the study - to study the features of changes in the vascular-platelet link of hemostasis in rats with complications of the microcirculatory tract on the background of type 2 diabetes.

Materials and methods of research. Experimental studies were performed on 18 white nonlinear male rats weighing 250-300 g, which were divided into 3 experimental groups: 1 group - intact control - healthy animals kept on a standard diet of vivarium; Group 2 - control pathology - animals, which after the introduction of STZ and nicotinamide reproduced the model of DR; Group 3 - animals, which after the introduction of STZ and nicotinamide reproduced the model of DN [4].

A streptozotocin model was used to reproduce type 2 diabetes. To this end, rats were administered a single intravenous streptozotocin (SigmaAldrich Chemie GmbH, Germany) at a dose of 65 mg / kg [4]. The STZ solution was prepared in 0.1 M citrate buffer pH 4.5. In order to reduce the diabetogenic effect of STZ 15 minutes before its introduction,

intraperitoneally administered nicotinamide (Afton Pharma, India) at a dose of 230 mg / kg, which saves up to 40% of pancreatic insulin reserves in experimental rats, due to which, unlike other streptozotocin models, animals develop moderate and stable basal hyperglycemia. This model allows to reproduce the main pathogenetic signs of type 2 diabetes in humans, namely - impaired secretion and action of insulin and is characterized by the development of intolerance to carbohydrates,

After 1 week, a glucose tolerance test was performed to determine fasting blood glucose levels and 30, 60, 90 and 120 minutes after intragastric administration of 40% glucose solution at a dose of 3 g / kg and blood glucose levels from 9.0 to 14 mmol / l.

In order to reproduce DR, a solution of erythropoietin 3 was injected subcutaneously 3 times a week for 6 U per 100 g of body weight for 6 months. The choice of this model was based on the data of the authors, in whose works it was proved that erythropoietin is an inducer of angiogenesis and thus is a key link in the course of DR [3].

To reproduce DN, the diet of experimental rats was based on a high-fat diet. At 35-40 weeks, the animals showed signs of DN - proteinuria, decreased glomerular filtration rate [1].

To assess the state of the vascular-platelet link of hemostasis, the following indicators were selected: platelet count (g / l), the degree of platelet aggregation with ristomycin (%), the degree of platelet aggregation with adrenaline (%), the rate of platelet aggregation in 30 s (%), platelet aggregation time (min).

Platelet counts were performed by the Fonio method in blood smears using a Carson Advanced 400-1600x microscope (USA) [2]. Platelet aggregation was assessed by the express method of visual assessment of Shitikov A.S. (2008) [2]. Induced platelet aggregation was studied by the method of Pavlov S.B. (2017) [6].

When working with animals, they complied with the International Code of Medical Ethics (Venice, 1983), the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and the "General Ethical Principles for Animal Experiments adopted by the First National Congress of Bioethics" (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes, the Law of Ukraine "On protection of animals from cruel treatment" № 3477-IV of 21.02.2006 [7]

Statistical processing of the obtained results was performed using the program "Statistica 8.0". The probability of differences between the indicators of the control and experimental groups was determined by the criteria of Student and Fisher. The level of reliability was taken at $p < 0,05$ [5].

Results and discussion. The blood glucose level of rats of the 2nd and 3rd experimental groups on the 3rd day after STZ administration was significantly 4.2 times higher than that of intact animals. The development of type 2 diabetes was accompanied by polydipsia, polyuria, the animals were lethargic and apathetic.

It was studied that the number of platelets in the group of animals that reproduced complications of the microcirculatory tract on the background of type 2 diabetes was significantly lower than in the control group ($p < 0.05$), especially in the group of rats that reproduced DN (Table 1).

It can be assumed that this decrease is due to insufficient production of thrombopoietin or the elimination from the bloodstream of a large number of irreversibly activated platelets. It is known that hyperglycemia causes glycosylation of protein components of cell membranes, in particular platelets, which leads to a decrease in their number by reducing life expectancy.

Table 1

Indicators characterizing vascular platelet hemostasis in rats with experimental diabetic nephropathy and diabetic retinopathy ($X \pm Sx$, $n = 6$)

Indicator	Intact group	Animals with experimental DN	Animals with experimental DR
Platelet count, g / l	532.0 ± 11.0	$397.6 \pm 14.2 *$	$424.6 \pm 17.4 *$
The rate of platelet aggregation in 30 s, % / min	10.3 ± 1.1	$14.7 \pm 0.9 *$	$13.4 \pm 1.0 *$
Platelet aggregation time, min	12.3 ± 1.3	$15.5 \pm 1.1 *$	$14.7 \pm 1.2 *$

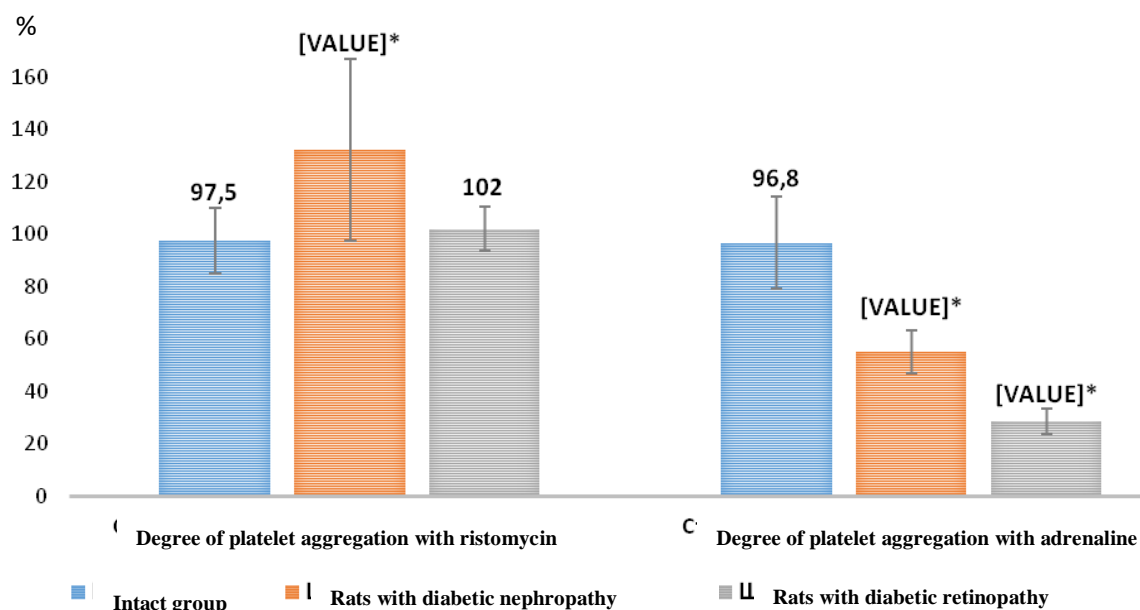
Note: $p < 0.05$ relative to the indicators of the intact group of animals.

When studying the rate of platelet aggregation for 30 seconds, it was found that in the group of rats, which reproduced DN, this figure increased 1.4 times ($p < 0.05$), and in the group of animals with DR - 1.3 times ($p < 0.05$). The time of platelet aggregation in the group of animals with DN increased 1.3 times ($p < 0.05$), in rats with DR - 1.2 times ($p < 0.05$).

The obtained results indicate hemostatic shifts of the vascular-platelet link of hemostasis, primarily due to increased aggregation capacity of platelets. This mechanism is due to the conditions of elevated blood glucose levels, including due to osmotic action, which results in glycosylation of platelet surface proteins with subsequent increase in the concentration of mediators that stimulate their activation, which leads to changes in platelet structure and increased aggregation properties [8].

The results of the study of the degree of platelet aggregation under the influence of

aggregation inducers (ristomycin / adrenaline) in rats with experimental DN and DR are presented in Fig. 1.



Note: $p < 0.05$ relative to the indicators of the intact group of animals.

Fig. 1. Platelet aggregation with the addition of aggregation inducers in rats with microcirculatory complications on the background of type 2 diabetes ($X \pm Sx$, $n = 6$)

It was found that the rate of platelet aggregation with ristomycin was equally significantly increased in the group of animals with experimental DN and with DR, while adrenaline-induced aggregation was significantly reduced in both groups of animals. The polarity of the results of the study depends on the functional state of vascular platelet hemostasis, primarily due to stimulated, devastated platelets and the appropriate number of intact platelets. In response to the addition of agonists, stimulated cells may not respond, and intact cells are activated, which creates a "bright" variety of indicators.

Conclusions:

1. In rats with DN on the background of type 2 diabetes, there were significant changes in vascular platelet hemostasis, in particular a decrease in platelet count and increase in platelet aggregation time.

2. In the study of induced platelet aggregation, the polarity of the results of the study was established, which indicates the dependence of complications on the functional state of vascular platelet hemostasis, primarily due to stimulated, depleted platelets and the corresponding number of intact platelets.

3. It was found that changes in the vascular-platelet link of hemostasis on the background of type 2 diabetes are more pronounced in rats with DN compared with the group of animals that reproduced DR.

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