Preys Nataliya, Savytskyi Ivan. The role of hemostatic changes in the pathogenesis of diabetic retinopathy. Journal of Education, Health and Sport. 2022;12(6):437-443. eISSN 2391-8306. https://dx.doi.org/10.12775/JEHS.2022.12.06.043
https://apcz.umk.pl/JEHS/article/view/56347
https://zenodo.org/records/14199660

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 1, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).

Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 1 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe:Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 16.05.2022. Revised: 31.05.2022. Accepted: 30.06.2022.

THE ROLE OF HEMOSTATIC CHANGES IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

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Abstract

Introduction: The paper presents the results of the hemostasis system of rats with experimental diabetic retinopathy.

Material and methods: Experimental studies were performed on white Wistar rats, which were divided into 2 groups: 1st group – intact animals; 2nd group – 60 animals with modelling of diabetic retinopathy without correction (control pathology). Analysis of the hemostasis system was performed according to the following indicators: plasma recalcification time, activated partial thromboplastin time, prothrombin time, thrombin time, heparin time, thermostable thromboplastin inhibitor, thermolabile thromboplastin inhibitor and fibrinogen level.

Results: Under the conditions of simulated diabetic retinopathy, the time of plasma recalcification was significantly reduced by 1.2 times ($p<0.05$) compared to intact rats; prothrombin time – 1.4 times ($p<0.05$); thrombin time – 1.1 times; heparin time – 1.1 times; thermostable thromboplastin inhibitor – 1.0 times; thermolabile thromboplastin inhibitor – 1.5 times ($p<0.05$), respectively. There was also a significant increase in the level of activated partial thromboplastin time by 1.3 times ($p<0.05$) compared to the intact group of animals and the level of fibrinogen by 4.9 times ($p<0.05$), respectively. Such violations of hemostasis at

the initial stage of the development of diabetic retinopathy R are associated with inhibition of anticoagulation mechanisms, since the contact phase of hemocoagulation did not change significantly.

On the 120th day of the experimental studies, the plasma recalcification time decreased by 1.3 times ($p<0.05$) compared to intact rats; prothrombin time - 1.5 times ($p<0.05$); thrombin time – 1.3 times ($p<0.05$); heparin time – 1.2 times ($p<0.05$); thermostable thromboplastin inhibitor - 1.1 times; thermolabile thromboplastin inhibitor - 2.0 times ($p<0.05$), respectively. It was established that the activated partial thromboplastin time also increased significantly by 1.4 times ($p<0.05$) compared to intact rats; there was a 4.0-fold increase in the concentration of fibrinogen ($p<0.05$), respectively, in intact animals.

Conclusions: DM is characterized by a number of changes in the hemostasis system, which determine the risk of thrombotic complications. One of the main reasons for these changes is: insulin resistance, hyperinsulinemia and insufficient compensation of carbohydrate metabolism. Hypercoagulation in DR conditions is manifested by an increase in the concentration and activity of factors VII and fibrinogen.

Key words: experimental diabetic retinopathy; experimental diabetes; hemostasis; thrombosis.

Introduction. Nowadays the problem of diabetes mellitus (DM) is one of the most relevant in the world due to its prevalence, severity and complications. The International Diabetes Federation (IDF) notes that in 2023, diabetes will be diagnosed in 463 million people. people, of which 91% have type 2 diabetes, and by 2045, the incidence of diabetes is predicted to increase to 700 million. people, which will make up more than 10% of the entire population [1, 2].

It is important that most metabolic processes are disturbed under the conditions of DM, which leads to an increase in the risk of damage to body tissues, and in the long run can cause serious diabetic secondary complications. Due to the lack of timely referral of patients and late diagnosis of type 2 diabetes, at the time of diagnosis, 50% of patients already have complications related to the development of micro- and macroangiopathy, among which diabetic retinopathy (DR) is quite common. This pathology is spreading more and more in the world, taking on the signs of a non-infectious epidemic. To date, a large number of scientific works devoted to the study of pathogenetic links of DR have been accumulated, but some mechanisms. still remain unstudied, which complicates the diagnosis and approaches to the correction of this disease. The risk of developing blindness in patients with diabetes is 2.4

times higher than in people without diabetes. Almost 94 million people have eye damage caused by diabetes [1, 3].

Despite numerous experimental studies, literature data on the pathogenesis of DR and treatment methods do not fully reflect the modern spectrum of pathogenetic mechanisms, as well as ways of correction aimed at the mechanisms of damage in this pathology [4].

The aim of the of work to investigate the changes in hemostasis in experimental animals, which were simulated DR.

Material and methods. The study was conducted on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 2 groups: 1st group – intact animals; 2nd group – 60 animals with modelling of DR without correction (control pathology).

Diabetes Type II and DR were modeled by intraperitoneal administration of streptozotocin (Sigma, USA) dissolved in 0.1 M citrate buffer with pH 4.5. Dose of streptozocin of 55 mg/kg of animal weight was divided into two administrations [5].

Administration of streptozocin was preceded by a high-fat diet for 28 days [5].

Analysis of the hemostasis system was performed according to the following indicators: plasma recalcification time, activated partial thromboplastin time, prothrombin time, thrombin time, heparin time, thermostable thromboplastin inhibitor, thermolabile thromboplastin inhibitor and fibrinogen level. All indicators studied according to generally accepted methods.

During the work with animals, we considered 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 protecting animals used for scientific purposes, the Law of Ukraine “On protection of animals from cruel treatment” No. 440-IX of 14 January 2020 [6, 7].

The value $p<0.05$ was chosen as the reliability criterion. An analysis was performed to see if the means differed. The results of determining the t-test give an answer about the equality or difference of the mean values, but they do not provide an opportunity to accurately measure the difference between the mean values. Note that this difference is quite conditional. This difference was calculated as a percentage. Thus, we demonstrated a comparison of mean values between different groups of animals.

Results. Since the hemocoagulation system is complex, cascade and multi-component, in order to achieve our goals, we chose a comprehensive approach to study each stage of blood coagulation.

The results of the study of coagulation hemostasis in rats with experimental DR are shown in Table 1.

Table 1 – Study of indicators of coagulation hemostasis in rats with experimental diabetic retinopathy (X±Sx)

Indicator	Intact group (n=10)	Control group (n=20)	
		on 60th day	on 120th day
Plasma recalcification time, s	45,7±1,3	38,4±1,2*	35,7±1,1*
Activated partial thromboplastin time, s	22,1±1,1	28,5±1,3*	31,3±1,5*
Prothrombin time, s	13,6±0,42	9,4±0,38*	9,2±0,31*
Thrombin time, s	34,8±1,3	30,7±1,4	26,2±0,8*
Heparin time, s	7,3±1,1	6,8±0,7	6,2±0,5*
Thermostable thromboplastin inhibitor, s	8,3±1,5	8,0±1,2	7,8±1,1
Thermolabile thromboplastin inhibitor, s	31,4±3,3	20,4±1,8*	15,7±1,6*/**
Fibrinogen, g/l	4,4±0,7	21,5±1,3*	17,7±1,1*/**

Notes:

1.* – $p<0.05$ compared to the intact group of animals;

2. ** – $p<0.05$ relative to the indicators obtained on the 60th day;

3. n – the number of animals in the group.

On the 60th day of the experiment, the following changes were observed: the plasma recalcification time was significantly reduced by 1.2 times ($p<0.05$) compared to intact rats (38.4±1.2 s vs. 45.7±1.3 s) ; prothrombin time - 1.4 times ($p<0.05$) (9.4±0.38 s versus 13.6±0.42 s); thrombin time – 1.1 times (30.7±1.4 s vs. 34.8±1.3 s); heparin time – 1.1 times (6.8±0.7 s versus 7.3±1.1 s); thermostable thromboplastin inhibitor – 1.0 times (8.0±1.2 s versus 8.3±1.5 s); thermolabile thromboplastin inhibitor - 1.5 times ($p<0.05$), respectively (20.4±1.8 s vs. 31.4±3.3 s).

There was also a significant increase in the level of activated partial thromboplastin time by 1.3 times ($p<0.05$) compared to the intact group of animals (28.5±1.3 s vs. 22.1±1.1 s) and the level of fibrinogen – by 4.9 times ($p<0.05$), respectively (21.5±1.3 g/l vs 4.4±0.7 g/l). Therefore, the results obtained on the 60th day indicate a reliable acceleration of the formation of prothrombinase by an internal mechanism (increased activated partial thromboplastin time level). Such disturbances of hemostasis at the initial stage of the development of DR are probably associated with inhibition of anticoagulation mechanisms, since the contact phase of hemocoagulation did not change significantly.

On the 120th day of the experimental studies, the plasma recalcification time decreased by 1.3 times ($p<0.05$) compared to intact rats (35.7±1.1 s vs. 45.7±1.3 s); prothrombin time - 1.5 times ($p<0.05$) (9.2±0.31 s vs. 13.6±0.42 s); thrombin time – 1.3 times ($p<0.05$) (26.2±0.8 s versus 34.8±1.3 s); heparin time – 1.2 times ($p<0.05$) (6.2±0.5 s versus 7.3±1.1 s); thermostable thromboplastin inhibitor – 1.1 times (7.8±1.1 s vs. 8.3±1.5 s); thermolabile thromboplastin inhibitor - 2.0 times ($p<0.05$), respectively (15.7±1.6 s vs. 31.4±3.3 s). It was established that the activated partial thromboplastin time index also increased significantly by 1.4 times ($p<0.05$) compared to intact rats (31.3±1.5 s vs. 22.1±1.1 s). There was also a 4.0-fold increase in fibrinogen concentration ($p<0.05$) in intact animals (17.7±1.1 g/l vs. 4.4±0.7 g/l).

Discusion. It is worth noting that on the 60th and 120th day there were significant differences only between the level of thermolabile thromboplastin inhibitor and the level of fibrinogen. A high level of fibrinogen is associated with the risk of developing micro- and macrovascular complications, but more pronounced changes were observed on the 60th day (the initial stage of the development of DR). In addition to normal polymerization, the formed fibrin monomers have the ability to interact with fibrinogen and its breakdown products. They are massively formed in the blood plasma during the activation of the clotting process and increase excessively during changes in the fibrinogen pool. An increase in the concentration of the latter in plasma is characteristic of the development of DR [8, 9].

Thus, it can be concluded that DM is characterized by such pathophysiological factors as: tissue hypoxia, a decrease in cytosolic calcium content, dyslipoproteinemia, which in turn inhibit the synthesis of endothelial relaxing peptide and cause vasospasm, plasmarrhagia and transudation, decrease blood disaggregant activity and increased thrombogenic potential [3, 8, 9].

Conclusions. 1) Under the conditions of simulated diabetic retinopathy, the time of plasma recalcification was significantly reduced by 1.2 times ($p<0.05$) compared to intact

rats; prothrombin time – 1.4 times ($p<0.05$); thrombin time – 1.1 times; heparin time – 1.1 times; thermostable thromboplastin inhibitor – 1.0 times; thermolabile thromboplastin inhibitor – 1.5 times ($p<0.05$), respectively. There was also a significant increase in the level of activated partial thromboplastin time by 1.3 times ($p<0.05$) compared to the intact group of animals and the level of fibrinogen by 4.9 times ($p<0.05$), respectively. Such violations of hemostasis at the initial stage of the development of diabetic retinopathy R are associated with inhibition of anticoagulation mechanisms, since the contact phase of hemocoagulation did not change significantly.

2) On the 120th day of the experimental studies, the plasma recalcification time decreased by 1.3 times ($p<0.05$) compared to intact rats; prothrombin time - 1.5 times ($p<0.05$); thrombin time – 1.3 times ($p<0.05$); heparin time – 1.2 times ($p<0.05$); thermostable thromboplastin inhibitor - 1.1 times; thermolabile thromboplastin inhibitor - 2.0 times ($p<0.05$), respectively. It was established that the activated partial thromboplastin time also increased significantly by 1.4 times ($p<0.05$) compared to intact rats; there was a 4.0-fold increase in the concentration of fibrinogen ($p<0.05$), respectively, in intact animals.

3) DM is characterized by a number of changes in the hemostasis system, which determine the risk of thrombotic complications. One of the main reasons for these changes is: insulin resistance, hyperinsulinemia and insufficient compensation of carbohydrate metabolism. Hypercoagulation in DR conditions is manifested by an increase in the concentration and activity of factors VII and fibrinogen.

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