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Study of immunological activity and hemostasiological features in rats with experimental antiphospholipid syndrome

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Abstract

Antiphospholipid syndrome **(**APS) is a systemic autoimmune disease, which is characterized by thrombotic or obstetric events characterized by the presence in the blood of patients of a high titer of antibodies to natively charged membrane phospholipids, as well as to glycoproteins associated with them. The role of etiological factors, triggering mechanisms, especially at the initial stages of APS formation, is not clearly defined that's why the aim of our work was to study of immunological activity and hemostasiological features in rats with experimental antiphospholipid syndrome. In animals with simulated APS, a positive microprecipitation reaction with cardiolipin antigen was observed, which indicated the development of APS. The development of hypercoagulation due to both the vascular and platelet and coagulation links of the hemostasis system was also noted. Established changes in hemocoagulation indicators in APS occur due to the interaction of blood clotting factors with

APA.

Keywords: antiphospholipid syndrome; pathogenesis, hemostasis; immunological activity; antibodies.

Introduction. Antiphospholipid syndrome **(**APS) is a systemic autoimmune disease, which is characterized by thrombotic or obstetric events characterized by the presence in the blood of patients of a high titer of antibodies to natively charged membrane phospholipids, as well as to glycoproteins associated with them. APS is characterized by recurrent thrombosis associated with the synthesis of such antibodies as: AFA, to AKA, β2-HP-I, belongs to acquired thrombophilias and is a manifestation of autoimmune thrombosis. The prevalence of APS in the general population ranges from 1–5%, and according to some estimates, the frequency of APS is about 5 new cases per 100,000 people per year with a prevalence of about 40–50 cases per 100,000 people [1, 2, 3].

The role of etiological factors, triggering mechanisms, especially at the initial stages of APS formation, is not clearly defined; the biochemical markers of the disease are poorly studied, and effective treatment regimens for APS have not been developed. The problem of treating patients with APS has not been fully resolved, which is due to the heterogeneity of pathogenetic mechanisms, clinical manifestations and the lack of reliable clinical and laboratory indicators that allow predicting the recurrence of thrombotic complications [4, 5, 6].

The aim of work – to study of immunological activity and hemostasiological features in rats with experimental antiphospholipid syndrome.

Materials and methods. The research was conducted on 20 white male rats weighing 180-220 g, which were divided into 2 groups: 1st group – intact animals that were on a standard vivarium diet and received a physiological solution in the volume of 1 ml (n=20); 2nd group – rats that were simulated APS (n=20).

Antiphospholipid syndrome was modeled by subcutaneous administration of cardiolipin antigen (Sigma, USA) at a total dose of 0.2-0.4 mg per rat every other day for three weeks. The study lasted 8 weeks, during which the animals were monitored and/or treated [7, 8].

During the working with animals, 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 Law of Ukraine "On the Protection of Animals from Cruelty" No. 440 (dated 14.01.2020) were used [9].

Antibodies to cardiolipoin (AKA) were determined by the immunoenzymatic method using the Anti-Phospholipid Screen IgG/IgM kit (ORG 529, Orgentec Diagnostika GmbH, Germany) in accordance with the manufacturer's instructions. The sensitivity of the method is 0.5 Units/ml, the coefficient of variation is <10%. Normal range <10 U/ml, positive result ≥10 U/ml [10].

Blood clotting time was studied using the Duke method, the principle of which is based on determining the duration of bleeding from surface microvessels after their integrity has been violated. To do this, the tip of the tail was cut off from the rats, and 1 drop of blood was taken from the tail vein on a glass slide, and the stopwatch was turned on. Every 15-20 seconds, a drop of blood was passed through the needle, recording the appearance of the first fibrin threads [10].

The number of platelets was calculated according to the Fonio method in blood smears, which is based on counting the number of platelets in stained blood smears per 1000 erythrocytes, taking into account the amount of erythrocytes in this volume [10].

Determination of platelet aggregation was performed according to the express method of visual assessment by A. S. Shitikov. The method consists in adding aggregating agents to plasma and mixing, after which platelet aggregation develops. The formed aggregates reach large sizes and can be seen with the naked eye in the form of white grains - the "snowstorm" phenomenon [10].

Activated partial thromboplastin time (kaolin-kephalin time of plasma) (APTT) according to J. Caen is based on the study of the clotting time of recalcified platelet-poor plasma under the conditions of standard contact (kaolin) and phospholipid (kephalin) activation [10].

Prothrombin time (PT) was studied according to Quick A.J.'s method, the principle of which is based on determining the clotting time of recalcified plasma when tissue thromboplastin of a certain activity and sensitivity to the deficiency of prothrombin complex factors is added to it [10].

The level of fibrinogen was determined by the gravimetric method according to R. A. Rutberg. The fibrin formed after plasma clotting was dried and the fibrinogen content in the plasma was determined by the mass of the clot [10]. The fibrin clot mass was calculated according to formula.

Statistical processing of the obtained results was carried out with the help of the "Statistica 10.0" program. The probability of differences between the indicators of the control and experimental groups was determined by Student's and Fisher's tests. The level of

reliability was accepted at $p<0.05$ [11].

Results of study and their discussion. To establish the diagnosis of APS, it is necessary to have several positive results of laboratory tests, therefore, at the first stage of our study, we evaluated the microprecipitation reaction with cardiolipin antigen in rats with simulated APS. Additional confirmation of the pathology in laboratory animals was provided by parameters of the hemostasis system, namely: blood clotting time in phospholipid-dependent coagulation tests, aggregation ability of platelets, APTT, PT and fibrinogen level [12].

As shown by the results of the conducted studies, when determining the presence of AKA using the microprecipitation reaction with cardiolipin antigen, it was established that this reaction was negative in animals of the intact group. Precipitation was observed in animals with simulated APS, which indicated the development of APS [13]. However, the isolated measurement of AKA is considered insufficient to verify the diagnosis, so we studied the vascular-platelet and coagulation links of hemostasis.

The following results were obtained during the study of indicators of the hemostasis system in rats with experimental APS (Table 1).

Table 1

Indicators of the hemostasis system in rats with experimental antiphospholipid syndrome

Indicator	Intact animals, n=20	Rats with simulated APS, n=20
Blood clotting time, s	134,7±7,2	90,3±2,3*
Platelets, 10^9/l	568,4±15,1	215,1±8,4*
Platelets aggregation, s	13,1±1,3	64,2±3,1*
APTT, s	22,3±0,8	13,6±0,6*
PT, s	11,6±0,5	6,9±0,3*
Fibrinogeb, g/l	2,8±0,3	10,4±0,6*

Notes:

1. * - $p<0,05$ compared to the group of intact animals;
2. n – the number of animals in the group.

It was found that in rats with simulated APS, the blood clotting time was reduced on 1.5 times ($p<0.05$) compared to intact animals (90.3±2.3 s againts 134.7±7.2 s). The obtained data indicate an increase in the coagulation potential of platelets in rats with APS.

We found that animals with simulated APS had a probable decrease in the number of platelets on 2.6 times ($p<0.05$) compared to intact animals. The obtained results are explained by the fact that platelets play a key role in the development of thrombosis against the background of APS due to the presence of receptors that interact with antibodies to β2-HP-I with the subsequent release of procoagulant mediators - thromboxane A2 and platelet factor 4.

The decrease in platelets is likely the result of depletion of platelet potential, as APS-mediated thrombosis results from a hypercoagulable state caused by endothelial cell and monocyte activation. In addition, platelets are involved in the process of increased activation and generation of fibrin with the help of autoantibodies to the β2-GP-I complex. The formation of complexes on activated platelets contributes to their aggregation and thrombus formation, therefore thrombocytopenia is consequential. On the other hand, thrombocytopenia in APS may be the result of "immune-mediated clearance" of platelets [14, 15].

We noted ($p<0.05$) acceleration of platelet aggregation time compared to intact animals (this indicator was 64.2±3.1 s against 13.1±1.3 s), which is explained by the presence of receptors that are on the surface of blood platelets and enhance their procoagulant properties.

The study of the coagulation link of hemostasis included the study of APTT, PT and fibrinogen level. It was established that in animals with simulated APS, the APTT was reduced to 13.6±0.6 s and was significantly ($p<0.05$) different from the group of intact animals (22.3±0.8 s). Reduction of APTT indicates an active influence on the internal mechanism of activation of blood clotting and the possibility of action on the processes of formation of blood prothrombinase, acceleration of the I phase of hemocoagulation - generation of prothrombinase [13, 15-17].

It was also found that the reduction of PTT in animals with APS. This indicator was reduced on 1.7 times ($p<0.05$) compared to intact animals, which is associated with the risk of developing thrombosis.

When studying the level of fibrinogen, we observed a probable increase of 3.7 times ($p<0.05$) compared to intact animals (10.4±0.6 g/l against 2.8±0.3 g/l).

Experimentally obtained data (positive microprecipitation test with cardiolipin antigen and changes in the hemostasis system) confirm the development of APS in rats. Established changes in hemocoagulation indicators in APS occur due to the interaction of blood clotting factors with APA. Antibodies to prothrombin also act as a target in APS. Prothrombin is also a precursor of thrombin, which supports and modulates the thrombotic response. Prothrombin

antibodies induce increased formation of thrombin and fibrin, and promote the expression of tissue prothrombinase and E-selectin by endothelial cells.

Conclusion. In animals with simulated APS, a positive microprecipitation reaction with cardiolipin antigen was observed, which indicated the development of APS. The development of hypercoagulation due to both the vascular and platelet and coagulation links of the hemostasis system was also noted. Established changes in hemocoagulation indicators in APS occur due to the interaction of blood clotting factors with APA.

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