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ANALYSIS OF CHANGES IN PROTEOLYTIC AND FIBRINOLYTIC ACTIVITY OF BLOOD PLASMA IN PATIENTS WITH RHEUMATOID ARTHRITIS DURING TREATMENT DEPENDING ON THE ENOS GENE

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Abstract

Despite significant progress in establishing the causes, mechanisms of occurrence, approaches to diagnosis and treatment, rheumatoid arthritis remains one of the most widespread and prognostically unfavorable diseases. In recent years, scientists have discovered dozens of new areas in the human genome associated with rheumatoid arthritis and found out that certain candidate genes play an important role in the development and progression of rheumatoid arthritis, which requires the search for new approaches to the prevention and treatment of this disease. **Purpose:** to study changes in the state of the proteolysis and fibrinolysis system in patients with rheumatoid arthritis after personalized treatment depending on the T-786C genotypes of the eNOS gene. **Materials and methods:** To study the T-786C polymorphism of the eNOS gene promoter, 2 groups of patients were selected: a control group - 20 practically healthy individuals and an experimental group - 60 patients with rheumatoid arthritis. The state of the proteolysis system was assessed by lysis of azoalbumin, azocasein, and azocol. The fibrinolytic activity of the blood plasma was evaluated by the lysis of azofibrin with subsequent determination of the total, non-enzymatic and enzymatic fibrinolytic activity of the blood plasma. **Results:** Positive dynamics of changes in fibrinolytic activity of blood plasma were noted in all studied groups of patients, regardless of polymorphic variants of the eNOS gene (rs 2070744): increase in enzymatic fibrinolytic activity among carriers of the TT genotype - by 18.06% ($p<0.05$), carriers TC-genotype – by 11.39% and CC-genotype carriers – by 13.24% ($p<0.05$). When evaluating the proteolytic system of the blood after treatment, a decrease in azoalbumin lysis and azocasein lysis was found in carriers of the TT genotype - by 16.6% ($p<0.05$) and 15% ($p<0.05$), in carriers of the TC genotype - by 23.57% ($p<0.05$) and 13.26% ($p<0.05$), and in CC genotype carriers - by 17.36% ($p<0.05$) and 10.77%, respectively. Azocol lysis decreased only in CC-genotype carriers - by 15.38% ($p<0.05$), with no significant difference in T-allele carriers.

Keywords: fibrinolysis, proteolysis, rheumatoid arthritis, T-786C polymorphism of the eNOS gene promoter.

Introduction. Despite significant progress in the study of the pathogenesis of rheumatoid arthritis (RA) in recent years, in the developed countries of the world there is a significant increase in morbidity, which prompts the search for the causes of this pathology and the development of effective treatment methods [1,2].

The interaction of genetic and environmental factors leads to a cascade of immune reactions that ultimately lead to the development of synovitis, joint destruction, and structural bone damage. This, in turn, leads to pain, disability, and emotional, social, and economic problems [3, 4].

In the literature today there are data on changes in the proteolytic and fibrinolytic activity of blood plasma in patients with stable angina pectoris II functional class (FC) compared to healthy participants, which causes, in the opinion of the author, a more pronounced progression of hemodynamic disorders and negatively affects the state of the microcirculatory bed and in general, on the prognosis and course of the pathology [5].

In the context of considering the links of the pathogenesis of RA, it is worth paying attention to the proteolysis system, which normally underlies many vital physiological processes, such as hemocoagulation, fibrinolysis, complement activation, and maintenance of homeostasis. However, individual components of the system of nonspecific proteinases and their inhibitors can play the role of damaging factors, the action of which determines the activation of hemocoagulation, which leads to direct damage to the vessels of the microcirculatory bed [6,7]. Therefore, the assessment of proteolytic and fibrinolytic activity of blood plasma in patients with RA allows us to clearly define its role in the pathogenesis of these pathologies.

Our research is consistent with a number of foreign studies, which investigate the *T-786C eNOS* gene polymorphism in patients with RA, which gives us the opportunity to determine the genetic and molecular effects of this gene on the development of RA, determine the risk group, carry out early diagnosis, prevention and correction of the treatment of this diseases [8].

Taking into account the above, the study of genetic aspects of the development of RA and changes in the proteolytic and fibrinolytic activity of blood plasma will allow qualitative diagnosis, prevention of possible complications, and a personalized approach to the treatment of RA.

Material and methods. To study the proteolytic and fibrinolytic activity of blood plasma, a set of reagents from "Danish Ltd" (Ukraine) was used using traditional methods [9]. The state of the proteolysis system was assessed by lysis of high-molecular and low-

molecular proteins and collagen. The fibrinolytic activity of the blood plasma was assessed by the lysis of azofibrin followed by the determination of the total (SFA), non-enzymatic (NFA) and enzymatic fibrinolytic activity of the blood plasma (FFA), which was determined by the formula: FFA=SFA-NFA.

The clinical diagnosis of RA was verified according to the criteria of the American College of Rheumatology and the European Antirheumatic League (ACR/EULAR 2010) [10]. Medical care for a patient with RA was provided in accordance with the Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical care and medical rehabilitation "Rheumatoid Arthritis", approved by the Order of the Ministry of Health of Ukraine No. 263 of April 11, 2014 [11].

The patients were divided into 2 groups: a control group - 20 practically healthy individuals and an experimental group - 60 patients with rheumatoid arthritis.

To determine polymorphic variants of the *eNOS* gene (*T-786C*) (rs2070744) [12], modified protocols with oligonucleotide primers using the PCR method and subsequent analysis of restriction fragment length polymorphism (RFP) were used.

Statistical processing was carried out using Microsoft Office Excel® 2007™, IBM SPSS Statistics® 23.0 applications. In the statistical analysis of the quantitative results of the study, arithmetic mean values (M) and standard error (m) were calculated.

Research results and discussion.

Changes in fibrinolytic activity of blood plasma depending on polymorphic variants of the *eNOS* gene (rs 2070744) are shown in table 1.

It was found that in all studied patients with RA, depending on the polymorphic variants of the *eNOS* gene (rs 2070744), the fibrinolytic activity of the blood plasma was reduced. The level of SFA, NFA and FFA in carriers of the *TT* genotype decreased almost equally by 23% ($p<0.05$), in carriers of the *TC* genotype - by 18.23% ($p<0.05$), 23.21% ($p<0.05$) and 12.66%, and in *CC* genotype carriers - by 34.32% ($p<0.05$), 36.63% ($p<0.05$) and 30.88% ($p<0.05$) relative to the control group.

In all studied groups of patients, positive dynamics of changes in the fibrinolytic activity of blood plasma after treatment were noted, regardless of the polymorphic variants of the *eNOS* gene (rs 2070744) (Table 1): an increase in FFA among carriers of the *TT* genotype - by 18.06% ($p<0.05$), carriers of the *TC* genotype – by 11.39% and carriers of the *CC* genotype – by 13.24% ($p<0.05$) with indicators approaching the level in the control group. It should be noted that against the background of treatment, the NFA content increased in carriers of the *TT* genotype by 16.96% ($p<0.05$), in carriers of the *TC* genotype by 14.16% ($p<0.05$) and in carriers of the *CC* genotype - by 15.84% ($p<0.05$), respectively. In turn, the level of SFA increased by 17.39%, 13.02% and 14.65% ($p<0.05$), respectively.

Table 1

Dynamics of indicators of fibrinolytic activity after treatment in patients with rheumatoid arthritis depending on polymorphic variants of the *eNOS* gene (rs 2070744)

Genotypes of the *eNOS* gene in patients		FFA E_{440}/ml/h	*SFA* E_{440}/ml/h	*NFA* E_{440}/ml/h
Control		0,89±0,11	2,27±0,14	1,38±0,08
TT, n=21	before treatment	0,72±0,09	1,84±0,15 p=0,052	1,12±0,08
	after treatment	0,85±0,11 p_1<0,05	2,16±0,17 p_1<0,05	1,31±0,12 p_1<0,05
TC, n=15	before treatment	0,79±0,12	1,92±0,09 p=0,005	1,13±0,13
	after treatment	0,88±0,12	2,17±0,11	1,29±0,13 p_1<0,05
CC, n=6	before treatment	0,68±0,14	1,69±0,23	1,01±0,13
	after treatment	0,77±0,14 p_1<0,05	1,94±0,23 p_1<0,05	1,17±0,14 p_1<0,05

Note: p is the probability of differences in indicators with the control group; p1 - the probability of differences between indicators before and after treatment.

Evaluating the blood proteolytic system in carriers of the *TT* genotype, an increase in the level of azoalbumin lysis was observed by 1.45 times ($p<0.05$), in carriers of the *TC* genotype by 1.64 times ($p<0.05$), and in carriers of pathological *CC* -genotype - 1.72 times ($p<0.05$) compared to the control group. The level of azocol and azocasein lysis was significantly higher in *CC* genotype carriers by 25% ($p<0.05$) and 22.03% ($p<0.05$) compared to the control group (Table 2).

When evaluating the proteolytic system of the blood after the use of telmisartan, rosuvastatin, and L-arginine against the background of disease-modifying antirheumatic therapy (DMRT), a decrease in the lysis of high-molecular and low-molecular proteins and collagen was revealed (Table 2). Azoalbumin lysis and azocasein lysis decreased in *TT*-genotype carriers - by 16.6% ($p<0.05$) and 15% ($p<0.05$), in *TC*-genotype carriers - by 23.57% ($p<0.05$) and 13.26% ($p<0.05$), and in *CC* genotype carriers – by 17.36% ($p<0.05$) and 10.77%, respectively. Azocol lysis after treatment decreased only in *CC* genotype carriers - by 15.38% ($p<0.05$), without a significant difference in *T* allele carriers.

Table 2

Dynamics of indicators of the proteolytic system after treatment in patients with rheumatoid arthritis depending on polymorphic variants of the *eNOS* gene (rs 2070744)

Genotypes of the *eNOS* gene in patients		Azoalbumin E440/ml/h	Azocasein E440/ml/h	Azocol E440/ml/h
Control		1,97±0,41	1,77±0,15	1,08±0,12
TT, n=21	before treatment	2,88±0,30	2,07±0,13	1,23±0,06
	after treatment	2,47±0,27 p, p_1<0,05	1,80±0,12 p_1<0,05	1,14±0,06
TC, n=15	before treatment	3,25±0,24	2,05±0,15	1,19±0,08
	after treatment	2,63±0,25 p_1<0,05	1,81±0,14 p_1<0,05	1,10±0,08
CC, n=6	before treatment	3,38±0,54	2,16±0,28	1,35±0,11
	after treatment	2,88±0,48 p_1<0,05	1,95±0,21	1,17±0,10 p_1<0,05

Note. p – probability of differences in indicators with the control group; p1 - the probability of differences between indicators before and after treatment.

It is known that the fibrinolytic system consists of a wide range of proteolytic enzymes that participate in many physiological and pathophysiological processes, in particular, in maintaining hemostatic balance, tissue remodeling, tumor growth, angiogenesis and reproduction, blood coagulation, metabolism, and inflammation. The main enzyme of the plasminogen activator system is plasmin, which causes the degradation of fibrin with the formation of soluble products of its breakdown. When activated, the proteolysis system is activated in the body's response to damage and leads to cell death and the development of significant functional and organic changes in systems and organs [13].

Conclusion. The most pronounced changes in proteolytic (increased lysis of azoalbumin, azocasein, azocol) and fibrinolytic (decrease in total and enzymatic) activities of blood plasma in patients with rheumatoid arthritis with SS genotype were established. However, the results of our study indicate that carriers with the T-allele had a better response to treatment.

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